Centre for Integrated Research into Musculoskeletal Ageing

Elucidating the role of selenium in musculoskeletal ageing using different study designs

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Declaration of Authorship

All analyses and manuscript preparations involved the contribution of the main researchers for data collection, statistical help and my supervisors since this thesis was largely based on secondary analyses. I solely contributed to the database organisation, statistical analyses, data interpretation and manuscript preparation and submission with feedback from my supervisors and co-authors. This thesis was solely written by me with feedback from my supervisors, Prof. Tom Hill and Prof. John Mathers. There are no conflicts of interest to report.

COVID 19 Impact Statement

Due to the pandemic and the unknown ability for laboratory work during the lockdown, my supervisors and I decided it would be most appropriate to change one of the studies in the PhD thesis. This was a change from in-house laboratory work using the BORICC study, to external analyses using The Newcastle 85+ Study. Due to the lockdown and associated restrictions my ability to send off the serum samples for these analyses in the desirable time was affected. This delay majorly reduced the time available for receiving the results, analysing the data, and writing these up into three chapters, forming two thirds of my PhD. Furthermore, after submitting the 3-month COVID 19 extension in hopes of receiving the analyses in May, I did not receive the data until mid-September, which further delayed my schedule. In addition to these time constraints, I also struggled a lot with my mental health during the lockdown due to the social isolation and daily restrictions in addition to the increased stress and workload to complete my thesis on time. Ideally, I was planning to submit two manuscripts prior to my submission, however, due to the time constraints this was not feasible but are currently in progress.

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Abstract

Introduction: There is limited information on the role of selenium in MSK ageing and function. The overall aim of this PhD thesis was to elucidate the role of selenium in human MSK ageing using different study designs.

Methods: The Newcastle 85+ Study was used to assess biomarkers of selenium status (serum selenium, GPx3 activity, SePP) in 757 participants. The associations between these biomarkers and MSK function, and its rate of change up to 5 years was assessed using linear correlations and linear mixed models. The PRECISE Study was used to explore the effects of long-term selenium supplementation on bone turnover markers (BTMs) (OC, PINP, CTX, BALP) measured in non-fasted samples at baseline, 6 months and 5 years. Data were analysed using ANCOVA to investigate the shape of the dose-response relationships.

Results: In The Newcastle 85+ Study 82 %, 30 % and 83 % of the population had suboptimal selenium status when using selected cut-offs for serum selenium, GPx3 activity and SePP respectively. Low (tertile 1) and medium (tertile 2) selenium concentrations, compared to high (tertile 3) were associated with a greater rate of change in TUG performance, and severe sarcopenia respectively, and low (tertile 1) SePP concentrations, compared to high (tertile 3) were associated with a higher prevalence of disability. In The PRECISE Study, using a 70 µg/L selenium cut-off, 12 % of participants were classified as having suboptimal selenium concentrations. Plasma selenium concentrations increased in a dose-dependent manner with selenium supplementation after 6 months and remained elevated at 5 years. There was no significant effect of selenium supplementation on any of the BTMs.

Discussion: This PhD thesis shows that that while very old adults have suboptimal selenium status which may be associated with muscle health, there was no impact of selenium supplementation on BTMs.

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List of Abbreviations

24 h MPR 24 h Multiple Pass Recall
ANOVA Analysis Of Variance
ApoER2 apolipoprotein E receptor 2
ARH1 Heterogeneous first-order autoregressive
ATP Adenosine triphosphate
BADL Basic Activities of Daily Living
BALP Bone Alkaline Phosphatase
B beta
BMC Bone Mineral Content
BMD Bone Mineral Density
BMI Body Mass Index
BMP Bone Morphogenetic Protein
BTM Bone Turnover Marker
BORICC The Biomarkers Of RIsk of Colon Cancer
Ca^{2+} Calcium
cfDNA cell-free DNA
CI Confidence Interval
COMA Committee On Medical Aspects of food policy
CRP C-reactive Protein
CTX Carboxyterminal Cross-linking Telopeptide of Type 1 Collagen
CV coefficient of variation
CVD Cardiovascular disease
d day
DIO Deiodinases
DNA Deoxyribonucleic Acid
DRV Dietary Reference Values
DXA Dual-energy X-ray Absorptiometry
EAR Estimated Average Requirement
EDTA Ethylenediamine Tetra-acetic Acid
EFSA European Food Safety Authority
EI Energy Intake
ELISA Enzyme Linked Immunosorbent Assay
EPIC European Prospective Investigation into Cancer and Nutrition
ER Endoplasmic reticulum
ESPEN European Society for Clinical Nutrition and Metabolism
EWGSOP European Working Group of Sarcopenia
FAD Flavin adenine dinucleotide
FFQ Food frequency questionnaire
FRAX Fracture Risk Assessment Tool
g gram
GP General practitioner
GPx Glutathione peroxidase
h hour
Hba1c Glycated haemoglobin
HDL High density lipoprotein
HGS Hand grip strength
HNRC Human nutrition research centre
HR Hazards ratio
hsCRP high-sensitivity C-Reactive Protein
IADL Instrumental activities of daily living
IDS Immunodiagnostic Systems Limited
IFCC International Federation of Clinical Chemistry and Laboratory Medicine
IGF-1 Insulin growth factor-1
IL-6 Interleukin 6
IOF International Osteoporosis Foundation
IoM Institute of medicine
IQR Interquartile range
l litre
kDa kilodaltons
kg Kilograms
LC/MS-MS Liquid chromatography with mass spectrometry
LDL Low density lipoprotein
LGI Low grade inflammation
LOAEL Low observed adverse effect level
LRNI Lower reference nutrient intake
MCSF Macrophage Colony-Stimulating Factor
min minutes
mo months

MPR Multiple Pass Recall
MPS Muscle Protein Synthesis
mRNA Messenger Ribonucleic Acid
MSCs Mesenchymal Stem Cells
MSK musculoskeletal
mTOR mammalian Target Of Rapamycin
Na^+ sodium
NADPH Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NAN_3 Sodium azide
NDNS National Diet and Nutrition Survey
NF-Kβ Nuclear Factor Kappa Light Chain Enhancer of Activated β Cells
NHANES National Health And Nutrition Examination Survey
NOAEL No Observed Adverse Effect Level
NS-SEC National Statistics Socioeconomic Classification
NTX Amino terminal cross-linking telopeptide of type 1 collagen
OC Osteocalcin
OP Osteoporosis
OPG Osteoprotegerin
OR Odds Ratio
PINP Amino Terminal Propeptide of type 1 Procollagen
PPAR Peroxisome Proliferator-Activated Receptors
PTH Parathyroid Hormone
RANK Receptor Activator of Nuclear Factor kappa B
RANK-L Receptor Activator of Nuclear Factor kappa B ligand
RBC Red Blood Cell
RCT Randomized Controlled Trial
RDA Recommended Daily Allowance
RNI Reference Nutrient Intake
ROS Reactive oxygen species
RyR Ryanodine receptor
s seconds
SACN Scientific Advisory Committee on Nutrition
SD Standard deviation
Se Selenium
Sec Selenocysteine
SELENO"X" Selenoprotein "X"
SeMet Selenomethionine
SePP Selenoprotein P
SFA Saturated fatty acids
SMMSE Standardised Mini-Mental State Examination
SNP Single Nucleotide Polymorphism
SOD Superoxide dismutase
SPPB Short Physical Performance Battery
SPS2 Selenophosphate synthetase
SPSS Statistical package for the social sciences
TNF-α Tumour necrosis factor α
tRNA transfer ribonucleic acid
TXNRD Thioredoxin reductase
TUG Timed up and go
T_3 Triiodothyronine
T_4 Thyroxine
µg Microgram
UK United Kingdom
UL Upper Intake Level
Vit. Vitamin
WMD White muscle disease
y year

Chapter 1. General Introduction

1.1 Introduction

This literature review will focus on musculoskeletal (MSK) function in older adults. Topics will include definitions, measurements, aetiology and nutritional factors that play a preventative role in MSK disease. The nutritional focus of the literature review will be on the trace element, selenium (Se). Selenium has the potential to play an important role in MSK function through its role as an antioxidant; this will be expanded on further within this chapter. The chapter will conclude by summarising the gaps in the current literature and outlining the hypotheses and aims of this PhD thesis.

1.2 MSK Ageing and Function

Ageing Populations

An increasing proportion of the global population are adults aged 80 years and above (Age UK, 2019). This section of the population is the fastest growing and is expected to increase more than threefold between 2017 and 2050 (United Nations, 2017) to comprise 5 % of the world's population (United Nations, 2012). Within the UK, the proportion of very old adults, defined as adults aged 85 years and over, is projected to increase from 1024 K in 2018 to 3278 K by 2050 (ONS, 2022b) and to comprise 4.3 % of the UK's population by 2045 (ONS, 2022a). Despite this increase in life expectancy, older populations are not necessarily living in optimal health (Murray *et al.*, 2015). This is due to the increased risk of disease, disability and illness leading to increased social care and health care costs (CRPD 2006-2015). Data from the English Longitudinal Study of Ageing (ELSA) (Steptoe *et al.*, 2013), a representative survey of 12 K community-dwelling participants, and the Clinical Practice Research Datalink (CPRD), a database of GP records, found that adults aged 75 and over with long-term conditions are on the rise. For example, of those aged 85, only 14 % reported having no long-term conditions (CRPD 2006-2015; ELSA, 2018). Up until the age of 85, the limitations to activities of daily living (ADL), consisting of, but not limited to, eating, food prepping, walking, washing, are less common, affecting around 10 % of adults aged 65-69 years;

however, approximately 40 % of adults aged 85 years and above report requiring help with ADLs, suggesting a moderate decline in daily function and independence (Raymond, 2021).

Diseases affecting the MSK system are of significant importance due to their prevalence and devastating impacts including economic, social and personal burdens. Beyond the fifth decade of life, there is an inevitable decline in muscle mass (1-2 %/ year) and strength (1.5-5 %/ year) (Cruz-Jentoft *et al.*, 2019). This loss of muscle mass, strength and function is termed "sarcopenia" meaning "loss of flesh." The European Working Group for Sarcopenia (EWGSOP) declared that when all three aspects (loss of muscle mass, strength and function) are present, sarcopenia is considered severe (Cruz-Jentoft *et al.*, 2019). Currently, muscle quantity and quality are difficult to assess, therefore muscle performance and strength are the preferred measurements for sarcopenia diagnosis. Sarcopenia is associated with an increased risk of falls, fractures, lower quality of life and, ultimately, an increased mortality rate (Moylan and Reid, 2007; Cruz-Jentoft *et al.*, 2019). There are different contributors to sarcopenia, and categorisations including primary sarcopenia, determined by ageing, or secondary sarcopenia determined by other factors such as surgery, inflammation, inactivity or, inadequate nutritional intake (Morris *et al.*, 2020). These factors can contribute to sarcopenia development as excessive oxidative stress in muscle can create a catabolic environment that leads to atrophy over time (Baumann *et al.*, 2016; Powers, Radak and Ji, 2016). These processes can occur through reduced muscle protein synthesis (MPS) and adenosine triphosphate (ATP) production and increased reactive oxygen species (ROS) production, DNA damage and myofibrillar protein degradation (Cohen, Nathan and Goldberg, 2015). Chronic low-grade inflammation (LGI), such as higher levels of interleukin 6 (IL-6) and C-reactive protein (CRP), correlates with sarcopenia (Bian *et al.*, 2017) and reduced functional capacity such as loss muscle strength and muscle mass (Schaap *et al.*, 2006; Beck *et al.*, 2007; Churchward-Venne, Breen and Phillips, 2014).

Another major MSK disease is osteoporosis (OP). OP is a polygenic disorder, characterised by a decrease in bone architecture, strength, and density, which increases fracture prevalence (WHO, 1994). Globally, it is estimated that 200 million women suffer with osteoporosis (Shen *et al.*, 2022) and in the EU, by 2025, the annual OP fractures are estimated to reach 4.5 million (Hernlund *et al.*, 2013). Fragility fractures (wrist, hip, spine)

occur in one in three women and one in six men over 50 years (Kanis *et al.*, 2021). These fractures have a wide range of consequences ranging from pain, loss of independence, fear, healthcare burdens and increased mortality rates (Willers *et al.*, 2022). Risk factors for OP development include age and postmenopausal status, sex, genetics, ethnicity, body mass index (BMI), alcohol intake, smoking, thyroid function, parathyroid status, sunlight, calcitonin, exercise and certain nutritional intakes (NICE, 2017; Wade *et al*., 2014; Alswat *et al*., 2017). Throughout the life-course, bone is remodelled via a tightly coupled process that involves osteoclasts resorbing mineralised bone and removing old or damaged bone, in addition to the formation of new bone by osteoblasts (Kenkre and Bassett, 2018). In OP, there is an imbalance between bone resorption and bone formation, leading to overall bone loss (Kenkre and Bassett, 2018). Osteoclasts are synthesised from hematopoietic monocyte precursor cells forming multinucleated cells, this synthesis is dependent upon receptor activator of nuclear factor kappa β ligand (RANKL), macrophage colony-stimulating factor (MCSF) release from osteoblasts and ROS-activated signalling pathways (Rucci, 2008). ROS and inflammatory markers, such as IL-6, are secondary messengers in these signal pathways during osteoclast differentiation (Lean *et al.*, 2005), can facilitate RANK-L-mediated osteoclastogenesis (Kenkre and Bassett, 2018) and inhibit osteoblast differentiation (Mody *et al.*, 2001; Bakker and Jaspers, 2015). Since bone metabolism can be regulated via ROS and inflammatory markers, it is important to ensure there is balance between bone resorption and bone formation.

Mechanisms of Ageing

Ageing is often associated with increased oxidative damage, ROS and inflammation leading to an increased risk of chronic disease (Chrousos, 2009). Oxidative processes become impaired, causing an imbalance between oxidant and antioxidant status that can increase ROS (Giorgi *et al.*, 2018). Some levels of ROS are required for optimal functioning, for example, ROS are produced at low concentrations in healthy muscle to aid in protein oxidation, excitation-contraction coupling, glucose uptake, mitochondrial processes, and gene expression (Powers, Radak and Ji, 2016; Arbogast *et al.*, 2009). However, excessive levels are detrimental, ultimately leading to cellular senescence that accumulates over time and can contribute to the ageing phenotype (Di Micco *et al.*, 2021; Chrousos, 2009). Usually,

the negative effects of ROS are counterbalanced by protective antioxidants but when ROS levels exceed their protective capacity lipid peroxidation and protein oxidation occur leading to “oxidative stress” and eventually functional decline (Poljsak, Šuput and Milisav, 2013). Decreasing concentrations of ROS may mitigate the effects of excessive oxidative stress. This can be achieved by antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), thioredoxin reductase (TXNRD), protein disulphide isomerase and nutrients including selenium, vitamin D, E and C, among others (Poljsak, Šuput and Milisav, 2013). Selenium will be discussed later in this chapter.

1.3 Approaches for Assessing MSK Function in Humans

It is important to measure MSK function, including strength, power and mass, in older adults since it correlates strongly with disability, quality of life, hospital admission, care and mortality rates (Beaudart *et al.*, 2019; Cruz-Jentoft *et al.*, 2010). Some of the commonly used approaches for measuring muscle function are detailed in Table 1.1. The EWGSOP2 has noted that the choice of assessment method can vary depending on healthcare setting, the patient and monitoring purpose. Some of the tests listed in Table 1.1 have non-linear relationships, for example there were correlations between gait speed and leg strength in weaker, but not stronger individuals (Buchner *et al.*, 1996). This is important to consider as the efficacy of some functional tests may be limited to frailer individuals due to the vast heterogeneity in older adults (Lowsky *et al.*, 2014; Nguyen *et al.*, 2021). Table 1.2 describes the main techniques used to assess bone health in humans and summarises the strengths and limitations of each method, as well as the clinical interpretation. The gold standard for diagnosing osteoporosis is dual x-ray absorptiometry (DXA) which determines bone mineral density (BMD) whilst risk of fractures can be estimated using the Fracture Risk Assessment Tool (FRAX) questionnaire (Kanis *et al.,* 2008). On a molecular level, concentrations of bone turnover markers (BTMs) can be measured in urine or plasma. BTMs can either represent bone resorption, such as C-terminal cross link telopeptide (CTX), or bone formation, such as osteocalcin (OC). Generally, BTMs are responsive to antiresorptive treatment, and have a shorter response time compared to BMD which can take 2 years to detect noticeable changes (Ahn *et al.*, 2019), see Hlaing and Compston, (2014) for a review.

For some of the techniques described in Tables 1.1 and 1.2, clinically relevant cut-offs have been determined for specific populations, such as older or frail populations. However, for other techniques, consensus or cut-offs are lacking, such as those for muscle power (Beaudart *et al.*, 2019) or they are extrapolated from younger, healthier populations. Other issues with these techniques include specific limitations such as costs, availability, accessibility, interpretation, time and reliability (Mijnarends *et al.*, 2013) and, on a wider scale, the heterogeneity of functional capacity in older adults (Francis *et al.*, 2017).

Table 1.1: Selection of commonly used techniques to measure muscle function including strength and performance. Strengths, limitations and cut-offs are summarised (adapted from Beaudart *et al.*, 2019)

Measurement	Overview	Pros	Cons	Cut-offs
		Muscle Strength		
HGS	Hand grip dynamometer; usually measured during muscular isotonic contraction	Widely used, quick, correlates well with lower limb strength, activities of daily living and mortality (Lauretani *et al.*, 2003). Portable, simple, good test-retest reliability and excellent inter-rater reliability	Floor effects for upper extremity impairments and other complications, may use pneumatic dynamometer instead	EWGSOP proposed general values for grip strength (< 30 kg for men and < 20 kg for women) but also BMI dependent value (Cruz-Jentoft *et al.*, 2010)
Chair Stand	Measures lower body power, balance and endurance and relates to most demanding daily life activities; count number of sit-stand-sit cycles in 30 s. Developed by (Rikli and Jones, 1999)	Useful in standard and clinical settings, minimal training, widely accessible, good test-retest reliability and very strong inter-rater reliability, chair rises related to fall and hip fracture risk (Cawthon *et al.*, 2008), no floor effect	Limited data on inter-rate reliability and responsiveness	Normative value for Hong Kong older adults 70–74 years for example is a mean of 10.1 ± 3.8 stands during 30 s and 13 stands during 30 s for US norms (Macfarlane *et al.*, 2006)
		Physical Performance		
SPPB	Measures gait, balance, strength, endurance through monitoring the ability to walk 8 ft and rise from a seated position 5 times, stand feet together side by side, semi tandem and tandem (Guralnik *et al.*, 1994)	Useful in standard and clinical settings, minimal training, widely accessible, associated with inflammation (Cesari *et al.*, 2004), can predict disability and mortality (Guralnik *et al.*, 1994), good to excellent test-retest reliability, excellent inter-rater reliability in admitted patients	Ceiling effect for high functioning older adults, floor effects for those unable to walk	≤ 10 strong predictor to lose ability to walk 400 m (Vasunilashorn *et al.*, 2009) ≤ 8 associated with mobility related disability Low score 0-6 associated with risk of death (Guralnik *et al.*, 2000)
Gait Speed	Timing usual gait speed to predict disability or adverse health events, often time to walk 4 m in 2 minutes	Useful in standard and clinical settings, minimal training, widely accessible, good predictor of falls, hospitalization and mortality (Studenski *et al.*, 2011). Excellent test–retest reliability 4- and 10-m distance and very strong inter-rater reliability	Floor effect for frail adults unable to perform to get minimum score or ceiling effect: physically active older adults may surpass maximum score	Cut-off < 0.8 m/s for 4 m (Lauretani *et al.*, 2003) and < 1 m/s for 6 m indicates poor performance (Cesari *et al.*, 2009)
TUG	Time taken to rise from a seated position, walk a specific distance and return to the seat	Useful in standard and clinical settings, minimal training, widely accessible, excellent inter-rater reliability, moderate to good test-retest reliability no floor or ceiling effect	Responsiveness to change not well defined in older populations	14 s risk for falls (Shumway-Cook, Brauer and Woollacott, 2000)

HGS: hand grip strength; EWGSOP: European Working Group on Sarcopenia in Older People; SPPB: short physical performance battery; TUG: Timed Up and Go

Table 1.2: Selection of commonly used techniques to measure bone health. Strengths, limitations and cut-offs are summarised.

Measurement	Description	Pros	Cons	Cut-offs
Bone turnover markers (BTMs)	Products of collagen degradation by osteoclasts in urine (C-terminal and N-terminal cross link telopeptides of type 1 collagen and deoxypyridinoline), matrix proteins made by osteoblasts (osteocalcin, procollagen type 1 N-terminal and C-terminal propeptides), found in serum	Reliable and precise, used in clinical setting, rapid response to antiresorptive treatment, can monitor compliance and predict fracture risk (Ahn *et al.*, 2019)	Invasive, no indication of bone mineral density, fasting status. Poor within-subject and between-lab reproducibility (Ahn *et al.*, 2019)	IOF, IFCC, WG-BMS: data on BTMs insufficient to be included in clinical practice (Vasikaran *et al.,* 2011). PINP and CTX: reference markers to access clinical performance (Bauer *et al.,* 2012). Reference intervals: PINP IDS automated assay: Belgium and UK 18–50 ng/l 13.7–71.1 ng/l (Morovat *et al.*, 2013); CTX IDS automated assay: Germany 30–54 50–670 ng/l mean 230 ng/l (Michelsen *et al.*, 2013; Morris *et al.*, 2017)
DXA	Weak x-rays, scanner passes over body, measures how easily x-ray passes though body (T score); x-rays pass easily through brittle, less dense bones	Gold standard, non-invasive, precise, safe	Not portable, density does not always correlate with fracture, less sensitive to changes in fracture risk (Hendrickson *et al.*, 2018), fragility fractures don't always show as T scores below 2.5 (Wainwright *et al.*, 2005)	T score lumbar spine (antero-posterior), femoral neck, total hip, or 1/3 radius: between -1 and -2.5: osteopenia; < 2.5: osteoporosis (Cosman *et al.*, 2014)
FRAX score QFracture	Online calculators to predict fracture risk (Kanis *et al.*, 2008) https://www.sheffield.ac.uk/FRAX/tool.aspx?country=9	Correlates well with fracture, inexpensive, ease of application, can determine who needs OP treatment, available worldwide	Frequent falls not included (Siris, Baim and Nattiv, 2010), restricted to one bone mineral density site, racial and ethnic differences (fracture reference based on Caucasians from NHANES), only for untreated patients (Silverman and Calderon, 2010)	FRAX 10-year risk scores of ≥ 3 % for hip fracture or ≥ 20 % for major osteoporotic fracture (Cosman *et al.*, 2014)

BTM: bone turnover markers; CTX: C-terminal cross link telopeptide; PINP: procollagen type 1 N-terminal propeptide; IOF: International Osteoporosis Foundation; IFCC: International Federation of Clinical Chemistry and Laboratory Medicine; WG-BMS: Joint Working Group on Bone Marker Standards; IDS: Immunodiagnostic Systems Limited; NHANES: National Health and Nutrition Examination Survey; FRAX: Fracture Risk Assessment Tool

1.4 Nutrition and MSK Function: The Case for Selenium

Prolonging physical function through a maintenance of MSK function is crucial to mitigate MSK disease and this can be achieved via nutrition and physical activity (Clegg and Williams, 2018). There is a large amount of literature discussing the importance of vitamin D, calcium (Sharkey *et al.*, 2003; Rizzoli *et al.*, 2009), and protein intakes (Kanis *et al.*, 2021; Robinson, Cooper and Sayer, 2012). For some of these nutrients, such as vitamin D and calcium, the Daily Recommended Values (DRVs) are based on the relationship between these nutrients and MSK function (SACN, 2016). However, these are not the only nutrients required for MSK function; others include antioxidants such as selenium. Selenium was discovered in 1817 as a by-product of sulphuric acid synthesis. Selenium is a nonmetal, of atomic number 34, atomic mass 78.96 and exists in the same family as oxygen. Initially selenium was seen as a carcinogenic, toxic element but its importance for health was realised in 1957 when it was learned that selenium deficiency in animals was associated with heart, liver and muscle disease (Schwarz and Folz, 1957). Research continued into humans, leading to the discovery of the association between selenium deficiency and Keshan disease, an endemic cardiomyopathy (Delmas *et al.*, 1983). More details on Keshan disease are provided in Section 1.7. Other discoveries were the associations between supranutritional doses of selenium and reduced cancer incidence (Clark *et al.*, 1996) and the findings that selenium was important for biological functions such as thyroid hormone function, fertility and immunity (Appendix Table 1.1).

Selenium is an essential trace element with antioxidant and anti-inflammatory properties (Table 1.3 and Appendix Table 1.1). These effects are attributed to selenium's presence in selenoproteins as the 21st amino acid, selenocysteine (Sec) (Rayman, 2002; Reeves and Hoffmann, 2009). The major functional groups of selenoproteins are either antioxidants involved in redox control; endoplasmic reticulum (ER)-localised; thyroid hormone function or selenium metabolism. These can be from different families such as glutathione peroxidases (GPx), thioredoxin reductases (TXNRD) and iodothyronine deiodinases (DIOs). In 1973 the first selenoprotein, glutathione peroxidase 1 (GPx1) was identified in the rat liver. GPx's maintain membrane integrity and catalyse peroxide reduction which can cause cellular damage (Köhrle, 2000). In 1977 selenoprotein P (SePP) was discovered in blood

plasma (Bellinger *et al.*, 2009) and further research led to the discovery of more selenoproteins (Lescure *et al.*, 2009; Toppo *et al.*, 2008). To date, eight isoforms of GPx have been identified (Labunskyy, Hatfield and Gladyshev, 2014) however, GPx 5, 7 and 8 are not selenoproteins (Gladyshev *et al.,* 2016). The remaining GPx selenoproteins differ depending on their selenium content, cysteine versus Sec composition or, location (Toppo *et al.*, 2008). DIO's were the second family of selenoproteins to be characterised, of which there are three forms (DIO1-3). DIO's are required to catalyse thyroxine (T_4) into triiodothyronine (T_3), the active thyroid hormone (Labunskyy, Hatfield and Gladyshev, 2014). Another family of selenoproteins includes thioredoxin reductases (TXNRD) which provide reducing power for several biochemical processes. TXNRD's plays a regulatory role in metabolic activity, defend against oxidative stress and aid in DNA synthesis by reducing thioredoxins. The selenoproteins and their functions are listed in Table 1.3. All selenoproteins contain one Sec residue per polypeptide, with the exclusion of SePP (Papp *et al.*, 2007). SePP contains ten residues of selenocysteine (Sec) (Reeves and Hoffmann, 2009) and it contains histidine-rich regions that may be used in molecule binding for selenium transport, see Persson-Moschos *et al.*, (2000) for a review. The location of this residue in each selenoprotein determines the selenoproteins fate. Some selenoproteins have their residue in the C-terminal (TXNRD, SELENOK, SELENOS, SELENOO, and SELENOI) whilst the remaining selenoproteins have residues in the N-terminal (Lobanov, Hatfield and Gladyshev, 2009), which may serve in oxidant defence and selenium transportation (Fairweather-Tait *et al.*, 2010; Labunskyy, Hatfield and Gladyshev, 2014; Reeves and Hoffmann, 2009; Sunde et al., 2009) (Figure 1.1).

1.5 Selenium Content of Tissues and Hierarchy of Selenoproteins

For efficient functioning, an optimal amount of dietary selenium is required to maximise selenoprotein synthesis, such as GPx3 (Table 1.3) (Thomson, 2004a). Increasing selenium concentrations are seen in the liver, spleen, pancreas, heart, brain, lung, bone, with highest levels in the skeletal muscle, up to 25-50 % (Zachara *et al.*, 2001; Oster, Schmiedel and Prellwitz, 1988). Selenium retention follows a hierarchy which is generally conserved across species with highest concentrations in the kidney, liver and pancreas, followed by cardiac and skeletal muscle whilst in deficiency, the brain, reproductive and endocrine organs are prioritised (Schomburg and Schweizer, 2009; Burk and Hill, 2009). For example, whole-blood of selenium-deficient mice dropped to 13 % of selenium-replete mice, however, brain

selenium content only dropped to 56 %, suggesting selenium retention in the brain (Burk and Hill, 2009). This has been further corroborated with the connection between the apolipoprotein E receptor 2 (ApoER2) and SePP. ApoER2 transports SePP to the brain and is thought to exist in a two-tier mechanism whereby initially SePP crosses the blood-brain barrier, moving from circulation into the brain through the brain capillaries then once in the brain, SePP is transported via endocytosis by ApoER2 expressed in the neurons, to help the brain reserve SePP during deficiency (Burk *et al.*, 2014).

In addition to the tissue hierarchy there is also a selenoprotein hierarchy (Behne *et al.*, 1988; Brigelius-Flohé, 1999). GPx1 is more sensitive to selenium deficiency than GPx2 and GPx4 and, in addition, DIO1 is generally retained during deficiency (Schomburg and Schweizer, 2009; Fairweather-Tait *et al.*, 2010). The less responsive selenoproteins are thought to be more essential for selenium homeostasis and are termed “housekeeping” selenoproteins. Other sensitive selenoproteins, in addition to GPx1 are MSRB1, SELENOW and SELENOH (Labunskyy, Hatfield and Gladyshev, 2014). The responsiveness of GPx to selenium deficiency is mostly due to the quick turnover of its messenger ribonucleic acid (mRNA) (Low *et al.*, 2000), and this in turn makes it effective for monitoring a population’s response to selenium supplementation. It is thought that during selenium deficiency, translation at the UGA codon is prevented, and when the UGA codon is 50-55 nucleotides upstream of an exon-exon junction, the exon junction complex remains in place causing nonsense-mediated decay (Low *et al.*, 2000). However, this hypothesis does not hold true for all selenoproteins; SELENOW’s UGA is 15 nucleotides upstream, yet SELENOW is affected by selenium deficiency. An alternative hypothesis is that within 3’-UTR regions, selenoprotein mRNAs are altered, suggesting a modification to translation efficiency, although the details are still unclear (Sunde and Raines, 2011). Other ideas revolve around Sec-transfer ribonucleic acid (tRNA) since these are dependent on selenium concentrations which can lead to differences in recognition of UGA as stop or Sec codons (Touat-Hamici *et al.*, 2018). More details on selenoprotein hierarchy are summarised in Table 1.3.

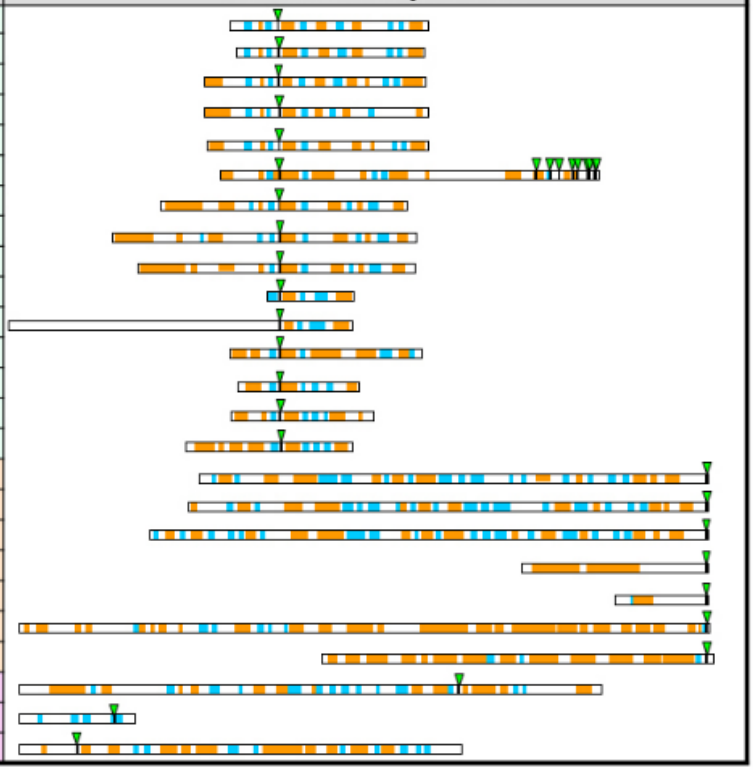
Selenoprotein	Sec-motif	Protein secondary structure
GPX1	UxxT	
GPX2	UxxT	
GPX3	UxxT	
GPX4	UxxT	
GPX6	UxxT	
SelP	UxxC	
DI1	SxxU	
DI2	SxxU	
DI3	SxxU	
SelW	CxxU	
SelV	CxxU	
SelT	CxxU	
SelH	CxxU	
SelM	CxxU	
Sep15	CxU	
TR1	U	
TR2	U	
TR3	U	
SelS	U	
SelK	U	
SelO	U	
SelI	U	
SelN	U	
SelR	UxxS	
SPS2	UxC	

Figure 1.1: Human selenoproteome. Thioredoxin fold selenoproteins are shown on the blue background. Selenoproteins evolved by C-terminal extension mechanism are shown on the orange background. Secondary structure end Sec insertion sites are shown at the right part of the table. β-sheets are shown in blue and α-helices in orange. Taken from http://genomics.unl.edu/RBC_EDU/sp.html

Table 1.3: Selenoprotein potential function, tissue distribution, expression, and sensitivity to selenium status (adapted from Fairweather-Tait *et al.*, 2010; Labunskyy, Hatfield and Gladyshev, 2014; Reeves and Hoffmann, 2009; Sunde *et al.*, 2009).

Selenoprotein	Potential Function	Tissue Distribution	When Se adequate Highly Expressed In…	Sensitivity to Se Status
Cytosolic glutathione peroxidase (GPx1)	Maintain membrane integrity and catalyse reduction of peroxidases	Lung, kidney, spleen and heart (rats), ubiquitous, intracellular, cytosolic	Liver and kidney	Highly sensitive, but recovers rapidly compared to other selenoproteins
Gastrointestinal glutathione Peroxidase (GPx2)	Protects from oxidative damage in GI and liver induced by gut microbiota or ingested prooxidants	Cytoplasmic, ER, whole gastrointestinal tract, liver	Highest in the crypt grounds of intestine, liver	Moderately resistant
Plasma glutathione peroxidase (GPx3)	Antioxidant, reduces lipid hydroperoxides	Secreted plasma, liver, kidney, heart, lung, thyroid, GI, breast EC fluid. Represents 10-30 % of selenium in plasma	Kidney, heart, third most abundant selenoprotein mRNA, thyroid	Sensitive
Phospholipid hydroperoxide glutathione peroxidase (GPx4)	Antioxidant, membrane protection from peroxidative degeneration; enzymatic and functional enzyme; main component of phospholipid hyperoxides in membranes	Cytoplasmic, ubiquitously expressed subcellular localization between cytosol, nuclear, and mitochondria differs between tissues, testes	Testes	Moderately resistant, only decreaes 40–50 % of maximal activity in selenium-deficient animals and mRNA are not largely affected
Glutathione peroxidase (GPx6)	Close homologue to GPx3 (Brigelius-Flohé and Maiorino, 2013)	Restricted in expression to the developing embryo and olfactory epithelium in adults	Bowman's glands and mouse embryos (Brigelius-Flohé and Maiorino, 2013)	Unknown
Thioredoxin reductase I (TXNDR1)	Antioxidant, regulation of intracellular redox state, DNA repair	Cytoplasmic, nuclear, ubiquitous	Detected at higher levels than TXNRD2	Decreased activity, but mRNA unchanged
Thioredoxin reductase 2 (TXNRD2)	Antioxidant, regulation of intracellular redox state	Mitochondria, widely expressed	Unknown	Increases with increased status (Reszka *et al.*, 2012). Higher resistance to deficiency compared to TXNRD1 (in rat liver and kidney)
Thioredoxin reductase 3 (TXNDR3)	Antioxidant and sperm maturation	Cytosol, ER, nucleus	Testes	
Iodothyronine deiodinase I (DIO1)	Converts T_4 into T_3	Membrane associated	Thyroid, liver, kidney, CNS, brown adipose tissue	mRNA affected in chicken in liver and muscle (Liu *et al.*, 2014; Lescure *et al.*, 2009) but better retained than GPx1 (Bermano *et al.*, 1995)
Iodothyronine deiodinase 2 (DIO2)	Thyroid hormone maturation	Membrane associated	Pituitary, brain, thyroid, placenta, heart, skeletal, CNS, brown adipose tissue	Retained under low status
Iodothyronine deiodinase 3 (DIO3)	Deactivates thyroid hormones	Membrane associated	Brain, placenta, CNS, skin	Higher stability
Selenoprotein F (SELENOF, SEP15)	Protein folding and secretion	ER, widely expressed	Brain, thyroid, liver, kidney, prostate and testis	Sensitive
Selenoprotein H (SELENOH, C11orf31)	Transcription factor, DNA-binding protein, upregulate selenoproteins in response to stress (Bellinger *et al.*, 2009)	Nuclear-localised, ubiquitous (Bellinger *et al.*, 2009)	Relatively high in early stages of embryonic development	Highly dependent of adequate status

Selenoprotein I (SELENOI)	Mammalian form of phospholipid-synthesising enzyme: ethanolamine phosphotransferase (Bellinger *et al.*, 2009)	Transmembrane, ubiquitous		
Selenoprotein K (SELENOK)	Protein folding in ER, inflammation, immunity, antioxidant activity	ER, plasma membrane, liver, placenta, pancreas (Papp *et al.*, 2007)	Heart and skeletal muscle	Moderately sensitive (Yao *et al.*, 2013a), muscular dystrophy induced by selenium-deficiency (Huang *et al.*, 2011)
Selenoprotein M (SELENOM)	Coincides with osteocalcin and alkaline phosphatase expression (Grosch *et al.*, 2013); protein folding in ER, antioxidant	Localised ER	SELENOM: Staining in mice in bony skeletal structures (Grosch *et al.*, 2013), liver, prostate, kidneys, testis, Dep15: brain	Sep15 moderatley sensitive, less so brain and testis
Selenoprotein N (SELENON, SEPN1)	Ca^{2+} release, slow muscle fibre growth in embryos (Jurynec *et al.*, 2008; Arbogast *et al.*, 2009)	ER-resident transmembrane glycoprotein, ubiquitous; 2 isoforms, skeletal muscle, brain, lung and placenta (Moghadaszadeh *et al.*, 2001)	Muscle in foetal (Petit *et al.*, 2003), Brain, lungs	Moderately sensitive (Yao *et al.*, 2013a), muscular dystrophy induced by selenium-deficiency (Huang *et al.*, 2011)
Selenoprotein O (SELENOO)	Unknown (Bellinger *et al.*, 2009)	Mitochondria, widely distributed (Kryukov, 2003; Bellinger *et al.*, 2009)		Muscular dystrophy induced by selenum-deficiency (Huang *et al.*, 2011)
Selenoprotein P (SELENOP, SEPP)	Selenium transporter taking selenium from liver to reproductive organs and brain, redox function with metal binding properties	Plasma, broad distribution	mRNA highest in brain, testes, liver (Burk and Hill, 2009) and one of higher levels of selenoproteins	Moderately sensitive, kidney and liver least affected, brain and testes most affected in mice, maintained in bone during deficiency (Pietschmann Nicole *et al.*, 2014)
Methionine sulfoxide reductase B1 (MSRB1, SELR)	Reduces sulfoxymethyl groups, aids in methionine metabolism (Bellinger *et al.*, 2009)	Cytoplasmic, nucleus, widely distributed	Liver and kidney	Unknown
Selenoprotein S (SELENOS, VIMP)	Inflammatory response, cytokine regulation, removal of misfolded proteins (Bellinger *et al.*, 2009)	Transmembrane ER protein, ubiquitous (Grumolato *et al.*, 2008)	Unknown	Moderately sensitive in chicken pectoral (Yao *et al.,* 2013a)
Selenoprotein T (SELENOT)	Ca^{2+} regulation	ER, Golgi, widely distributed in foetal and adult tissue	Adrenal gland during development (Wistar rat (Grumolato *et al.*, 2008)	Moderately sensitive in chicken pectoral muscle (Yao *et al.*, 2013a)
Selenoprotein V (SELENOV)	Unknown, potentially redox	Cytosol (Dikiy *et al.*, 2007)	Testes (Bellinger *et al.*, 2009)	Unknown
Selenoprotein W (SELENOW)	Antioxidant, muscle development, upregulated in response to external stressors (Vendeland *et al.*, 1995; Lescure *et al.*, 2009)	Cytoplasmic, skeletal muscle, heart, brain, ubiquitous	Skeletal muscle, heart and brain (Yeh *et al.,* 1997), rats, proliferating myoblasts, long bone (Kim *et al.*, 2021)	Highly dependent on adequate levels, decreases in deficiency especially in heart, lungs, prostate, SI, skin, liver, but retained in the brain (in sheep) (Yeh *et al.,* 1997).
Selenophosphaste synthetase 2 (SEPHS2)	Synthesises selenophosphate from selenide and ATP	Cytoplasmic, ubiquitous	mRNA in liver	SePP deleted mice had lower mRNA levels for all selenoproteins in brain and testes, except SPS2

GI: gastrointestinal; ER: endoplasmic reticulum; EC: extracellular; mRNA: messenger ribonucleic acid; T_3: Triiodothyronine; T_4: thyroxine; Ca^{2+}: calcium; CNS: central nervous system

1.6 Selenium Dietary Sources, Assessment of Intake and Status and Recommendations

Selenium Concentrations in Soil and Food Sources of Selenium

Within the earth's crust, selenium concentration ranges from 0.05 to 0.10 mg/kg (Lopes *et al.,* 2017). Selenium is formed as by-product of metal, such as copper-mining which occurs largely in the USA, Japan and Canada (Haug *et al.*, 2007). Other processes such as sulphuric acid-rich polluted rain, elemental extraction and petroleum and coal combustion release selenium into the atmosphere (Haug *et al.*, 2007). Selenium is also present in fossil fuels, soils, plants, and water (Lopes *et al.*, 2017). Within soil, selenium concentration averages at 0.40 mg Se/kg (Fordyce, 2007), although concentrations vary widely even within the same soil types and regions (Fordyce, 2007). The USA, China, and India present large areas where soil selenium is high enough to cause toxicity (Lopes *et al.*, 2017). However, most soils on the Earth's surface, especially in UK, Australia, central Siberia, New Zealand, Thailand, Africa, Finland, Turkey, Nepal, northeast to south central of China, Denmark, and parts of Bangladesh and India, as well as tropical zones have suboptimal concentrations of selenium (0.05-0.09 mg Se/kg) (Lopes *et al.*, 2017). For example, in the UK more than 95 % of soil samples contain less than 1 mg Se/kg (Broadley *et al.*, 2006). These suboptimal soil concentrations have led to selenium deficiency disorders in livestock and suboptimal selenium status in humans (Combs, 2001; Rayman, 2000). Many factors affect soil selenium availability and distribution. These vary from soil drainage, temperature, pH, selenium oxidation state, soil compounds and microorganisms to agricultural practices and atmospheric deposition (Lopes *et al.*, 2017; Fordyce, 2007; Broadley *et al.*, 2006; Combs, 2001).

Humans obtain selenium primarily through the consumption of food, followed by supplements (Fairweather-Tait *et al.*, 2010). The content of selenium in food varies from 5-690 µg Se/100 g (Rayman, 2008a; Fairweather-Tait *et al.*, 2010) and is mostly determined by its origin due to the concentration of soil selenium (Kieliszek and Blazejak, 2016). Selenium-rich foods are generally higher in protein, including meats, organ products (liver, heart, kidney), fish, seafood, cereals, and Brazil nuts (Outzen *et al.*, 2015; Fairweather-Tait *et al.*,

2010; Kieliszek and Blazekaj, 2016). Seasonality can alter the selenium content, for example, milk concentrations were higher in winter compared to summer (Roca-Perez *et al.*, 2010). Furthermore, cooking methods can reduce selenium content due to volatilisation (Dumont, Vanhaecke and Cornelis, 2006) or absorption to utensils (Bratakos *et al.*, 1988) and these factors can depend on the species (Lu *et al.*, 2018). For example, up to 50 % of selenium is lost during the boiling of vegetables, cereals (Khanam and Platel, 2016) and dairy produce (Dumont, Vanhaecke and Cornelis, 2006). However, Higgs, Morris and Levander (1972) found that selenium concentrations remained consistent with different cooking methods whilst Navarro-Alarcon and Cabrera-Vique (2008) reported selenium concentrations to increase with processing.

Animal produce often provides a reliable source of selenium with high bioavailability (Finley, 2006) and due to animal feed often been supplemented with selenium, this holds true in areas with lower soil selenium concentrations (Fairweather-Tait *et al.*, 2010). While selenium is essential for all animals, it is not essential for all plants (White, 2016) although plants do accumulate selenium in different amounts (White, 2016). Selenium-accumulating species (*Asteraceae, Brassicaceae* and *Fabaceae*, Brazil nut) contain higher concentrations of sulphur, which is similar to selenium, allowing selenium to use the same uptake, translocation and metabolism pathways thereby increasing the concentration (White, 2016). With the exception of onions, brassicas and asparagus, vegetables do not contain high levels of selenium (0.001-0.022 µg Se/g) (Kieliszek and Blazejak, 2016) while legumes contain higher concentrations (Pappa, Pappas and Surai, 2006). Cereal products contain some selenium (10-550 µg Se/kg fresh weight) and are classified as having sufficient bioavailability (Fairweather-Tait *et al.*, 2010; Tamas *et al.*, 2010) and furthermore, cereals equate to more than 50 % of intakes in some populations, especially in low selenium areas (Tamas *et al.*, 2010).

Bioavailability of Selenium Sources

This section will provide details on the bioavailability of selenium, introducing the different forms and their metabolism. Bioavailability is the amount of an element that is absorbed through the intestinal membrane to reach the systemic circulation, where it is distributed to

organs and tissues to become bioactive (IoM, 2000). The form of selenium is important to consider as it can influence selenium bioavailability, absorption, and ultimately selenium status (Schümann *et al.*, 1997). Factors that affect bioavailability are bioaccessibility (transportation within intestines or lungs in soluble form) (Reeder, Schoonen and Lanzirotti, 2006) and bioactivity (increased activity of some selenoproteins due to assimilation of specific selenium forms) (Bodnar *et al.*, 2016). Other influential factors are genetic variation in selenoprotein genes that can influence selenoprotein activity (Hu and Diamond, 2003) and responses to dietary selenium (Méplan *et al.*, 2007). There are two types of selenium, organic (including selenomethionine (SeMet), Sec, Se-methyl-selenocysteine, Se-yeast) and inorganic forms (selenate, selenite, selenide). Organic forms such as SeMet, contribute greatly to the selenium content in meat products, whilst Sec and Se-methyl-selenocysteine are found in cereals, nuts, meat, and fish (Rayman, 2008a). In general, bioavailability is higher for organic forms of selenium compared to inorganic (Fairweather-Tait, 2010; Bodnar *et al.*, 2016; Thomson, 1986; IoM, 2000). Furthermore, organic forms maintain selenium status for longer (Rayman, 2004; Levander *et al.*, 1983; Luo *et al.*, 1985; Thomson *et al.*, 1982) and have been more effective at increasing selenium concentrations. For example, SeMet increased plasma selenium 1.6 times more than sodium selenite (Burk *et al.*, 2006) and half the dose of SeMet was required for selenoprotein plateau compared to selenite (Rayman, 2008c).

Dietary selenium, most commonly in the form of SeMet and Sec, is digested and released from selenoproteins and absorbed by the small intestine, often in the form of selenide (Fairweather-Tait and Collings, 2010). Most species share sulphur analogues in the small intestine during absorption, which may lead to competition for absorption (Thomson, 1986). SeMet uses sodium (Na^+) dependent pathways and can be added non-specifically into proteins by replacing methionine (Fairweather-Tait and Collings, 2010). The similarity in ionic radius means that SeMet can use the same active transport mechanism as methionine and can substitute sulphide atoms (Vendeland *et al.*, 1994). Unlike SeMet, Sec is not inhibited by sulphur compounds (Navarro-Alarcon and Cabrera-Vique, 2008) but incorporation into selenoproteins is highly regulated (Combs, 2015). Selenite, on the other hand, is absorbed passively and metabolised into selenide (Navarro-Alarcon and Cabrera-Vique, 2008). Once absorbed, the selenium species are transported to the liver; ingested

selenium is taken up from SeMet or portal vein blood and added to Sec-specific tRNA for selenoprotein synthesis during translation (Rotruck *et al.*, 1973; Labunskyy, Hatfield and Gladyshev, 2014). The alternative route, in the case of large quantities, is excretion via urine or breath (Fairweather-Tait and Collings, 2010; EFSA, 2014). Studies suggest that excretion rather than absorption is the key in regulating selenium homeostasis (EFSA, 2014).
In the subsequent sections, I will summarise dietary assessments and the selenium intakes of older adults. After this, I will move on to discuss the measurements of selenium status using biomarkers, providing insight into their use, and then discuss the current selenium status of adults. Lastly, I will discuss the DRVs for selenium that are used to dictate selenium requirements.

Dietary Assessment and Selenium Intakes in Older Adults

Dietary assessment can be undertaken using a variety of methods. These can be prospective methods, such as food records (weighted or estimated), checklists, and more recently, photographic apps or, retrospective methods, such as multiple pass recalls (MPR), diet history and food frequency questionnaires (FFQ). However, individuals differ in the quantity and types of food consumed, therefore accurately measuring dietary intakes can be challenging and all methods have errors (Naska, Lagiou and Lagiou, 2017; Ahmed and Haboubi, 2010). One major issue with self-reporting dietary data is unintentionally under- or over-reporting, in addition to the “Hawthorne effect” whereby individuals alter their diet due to the perception others may have of their habitual diet (McCambridge, Witton and Elbourne, 2014). There are also practical challenges, such as levels of literacy to perform the assessments, willingness, and cognitive ability, which can be affected by older age. Furthermore, there can be analytical problems such as missing data, measurement errors, collinearity in nutrients, recipes, fortified foods, and unreliable estimations of intakes from databases. This is especially important for selenium intakes due to the varied food origin and can be complicated in countries that rely on imports from higher soil selenium countries (Fairweather-Tait *et al.*, 2010; Keck *et al.,* 2006). Therefore, certainty surrounding selenium intakes are poor, especially in older adults.

Generally, with an increase in age, there is a decrease in food intake including micronutrients such as selenium (González *et al.*, 2006). Selenium intakes vary widely globally by orders of magnitude (Combs, 2001), for example in North America, intakes range from 60-220 µg Se/d, whilst in the Europe, average intakes range from 30-50 µg Se/d (Rayman, 1997; Navarro-Alarcon and Cabrera-Vique, 2008), whereas in China, where Keshan disease occurs, intakes range from 7-11 µg Se/d, (Combs, 2001), see Section 1.7 for more details on Keshan Disease. European populations have shown declines in selenium intake over 25 years (Rayman, 2002; MoA, 1997), some of which have fallen below the Reference Nutrient Intake (RNI) (75 µg/d for males and 60 µg/d for females) and in some cases, below the Lower Reference Nutrient intake (LRNI) in certain populations such as the very old (Roberts *et al.*, 2018; Flynn *et al.*, 2009; Rayman, 2008a; Perri *et al.,* 2020). In the UK, according to the National Diet and Nutrition Survey (NDNS), selenium intake averaged at 41-43 µg/d and for adults aged above 75 years, 39 % of men and 76 % of women did not meet the LRNI of 40 µg/d (Roberts *et al.,* 2018; SACN, 2013). These declines are most likely from a drop in selenium- and protein-rich wheat imports often from North America (Rayman, 2002). This issue has been amplified in the UK since the 1980s which has coincided with a reduction in plasma selenium (MoA, 1997; Rayman, 1997; Ysart *et al.*, 1999; Sunde *et al.*, 2008; Fordyce *et al.*, 2009). This was exacerbated due to the use of sulphur fertilisers that competed with selenium or, the change from single superphosphate to triple superphosphate (Fordyce *et al.*, 2009), along with other environmental factors described in earlier in this Chapter. Data relating to micronutrient intakes often come from surveys, such as the NDNS which looks at intakes of adults aged 19-64 years, then ≥ 65 years (Bates *et al.*, 2002) excluding detailed data from very old adults who are at greater risk (Ahmed and Haboubi, 2010). However, in the MRes component of my PhD programme, I contributed to this understanding by exploring the selenium intakes in a population of very old adults aged 85 years and over (Perri *et al.*, 2020). As suspected, the average selenium intake was suboptimal, with more than 50 % consuming below the LRNI (Perri *et al.,* 2020).

Selenium Status Assessment and Selenium Status in Older Adults

As previously discussed, measuring nutritional intakes can be unreliable, therefore assessing biomarkers of nutrient status can help overcome this (Kuhnle, 2012; Ashton *et al.*, 2009; Combs, 2015; Fairweather-Tait *et al.*, 2010). Status is the amount of (potentially) biologically

active nutrient in the body. Intake, tissue concentration, excretion and retention form a nutrient status which can be used to determine requirements for deficiency, toxicity, and disease-prevention (Combs, 2015). The association between dietary selenium intakes and status are described in the subsequent paragraphs in addition to these studies (Yang, 1989; Burk *et al.*, 2006).

Selenium is present in various fluids and tissues in different forms (Combs, 2015; Ashton *et al.*, 2009; Thomson, 2004a), for example, blood samples can provide measurements of whole-blood, serum, plasma and erythrocytes, whereas urine, the selenite-pool, saliva, hair or toenails can provide other measurements (Longnecker *et al.*, 1996). Assessment of biomarkers can be short-term or long-term; short-term status is derived from plasma, serum, platelet and urinary excretion (Nève, Vertongen and Molle, 1985), whilst long-term status is derived from whole-blood, toenail, hair and erythrocytes (Ashton *et al.*, 2009; Oakes *et al.*, 2008). Within the measurements of selenium, there are two forms of selenoproteins, structural and enzymatic (Elsom *et al.*, 2006; Rayman, 2008b). A well-studied selenoprotein is GPx3 which represents 10-25 % of selenium in plasma, with the remainder likely bound to albumin as SeMet (Reszka *et al.*, 2012; Deagen *et al.*, 1993; Burk, Hill and Motley, 2001). Some studies have suggested that for maximum expression of GPx activity, where enzyme function is optimised, plasma/serum selenium concentrations of 70-100 µg/l are required (Nève, 1995; SACN 2013; Combs, 2001; IoM, 2000; Daniels, 2004), equating to intakes of 40-50 µg/d (Xia *et al.*, 2005; Yang, 1987; Duffield *et al.*, 1999). However, this concentration is not consistent as other studies have suggested higher selenium concentrations are required, ranging from 80-122 µg/L (Xia *et al.*, 2010; Lyons *et al.,* 2004; Rayman, 2005; Thomson *et al.*, 1993; Thomson *et al.*, 1977; Rea *et al.* 1979; Rayman, 1997) with overall levels ranging from 40-200 µg/L (Nève, 1991). Another, more recently quantified selenoprotein, is SePP, which can be used as a short-term indicator because of its shorter half-life (Burk, Read and Bellew, 1991). SePP represents 40-70 % of selenium in plasma (Burk, Hill and Motley, 2001; Deagen *et al.*, 1993) and requires higher concentrations of serum/plasma selenium in the range of 90-125 µg/L (Hurst *et al.*, 2010; Xia *et al.*, 2010; EFSA, 2014) which equates to selenium intakes of 100-150 µg/d (Burk *et al.*, 2006; Brodin *et al.*, 2020; Combs, 2015; Hurst *et al.*, 2010). However, in selenium-deficient populations, 20-90 µg/L of selenium was sufficient for optimisation of SePP (Xia *et al.*, 2010).

These discrepancies emphasise the need for a standardised protocol using the same research design to determine selenium requirements. Studies suggest sensitivity can be limited in those with higher baseline intakes or status, for example, SePP concentrations did not significantly increase when supplementing with 200 µg/d of sodium selenite in participants with a habitual intake of 100 µg/d (Persson-Moschos and Alfthan, 1998), nor when supplementing with 200-600 µg/d with either Se-yeast, sodium selenite, or selenate in those with a baseline plasma of 122 µg/L (Burk *et al.*, 2006). However, therapeutic dosages of selenite (1.1 to 15.3 m^2 intravenous) in participants with baseline serum selenium of 59 µg/L led to increased SePP concentrations, independent of age or sex, indicating there may be a higher upper threshold than anticipated (Brodin *et al.*, 2020).

After considering these points, it is important to assess the habitual intake of a population, as this will influence the suitability of a particular biomarker. For example, plasma selenium can be more responsive than whole-blood GPx activity when selenium status is suboptimal, making it suitable for studying older populations in the UK (Ashton *et al.*, 2009; Bates *et al.*, 2002). Meanwhile, hair and toenail concentrations are suitable options for non-invasive measures and correlate well with blood or plasma concentrations (Yang *et al.*, 1989). Nevertheless, each biomarker has limitations, for example, the assumption that hair and toenail selenium indicate selenium status is not validated, and contamination can occur with selenium sulphide shampoo. Likewise, the enzymatic activity of selenoproteins can plateau at lower serum selenium concentrations depending on an individual's threshold (Thomson *et al.*, 1982) making certain biomarkers such as GPx3 activity less suitable in populations with higher intakes. Inflammation has also been noted to underestimate selenium concentrations (MacDonell *et al.*, 2018), or to be associated with lower concentrations (Tseng *et al.*, 2013; Walston *et al.*, 2006). This could be due to cytokines increasing capillary permeability so that albumin and bound proteins are redistributed into the interstitium, leading to lower serum selenium concentrations (Oakes *et al.*, 2008). Therefore, measuring a combination of these biomarkers, including selenoprotein concentrations and activity, can be more useful in determining selenium status (Fairweather-Tait *et al.*, 2010; Thomson, 2004a).

Despite the knowledge built upon biomarkers of selenium status over the years, there are few studies determining the selenium status of very old adults. This is important as ageing is associated with increased heterogeneity, disease risk and micronutrient deficiencies, including selenium. The earlier sections summarised the importance of selenium in the human body by incorporation into selenoproteins and later sections will detail the importance of selenium in MSK ageing, laying the rationale for the importance of assessing selenium status. Following this, the rationale will be further highlighted by revealing the suboptimal intakes of selenium in older adults which raises the concern that selenium status of older adults will also be suboptimal. The subsequent section will now summarise the available literature on selenium status of older adults.

A review in 2019 compiled the results of older adults across the globe and found that serum selenium varied greatly from 50.0 µg/L in Brazilian 71–83-year-olds to 147.4 µg/L in Chinese adults aged 65 years and above (Robberecht *et al.*, 2019; Rita Cardoso *et al.*, 2016). Another review by Fairweather-Tait *et al.*, (2011) compiled studies exploring the associations between selenium status and health outcomes and concluded that a selenium concentration of 60-140 µg/L is adequate. In New Zealand, older adults (mean 84.6 years) had selenium concentrations of 63.2 µg/L (MacDonell *et al.*, 2018) and, in Australia, participants aged over 81 years had lower selenium status compared to younger adults (Lymbury *et al.*, 2008). In Sweden, adults aged between 70-80 years had selenium concentrations of 67.1 µg/L (Alehagen *et al.*, 2016) and in Turkey (mean 73 years), the average selenium concentration was 65.5 µg/L (Koç *et al.*, 2015). Compiling selenium concentrations from 10 European countries, the EPIC-Europe cohort study (25-70 years) reported a mean concentration of 85.6 µg/L (Hughes *et al.*, 2015), however, there was no data on the age range from 85-90 years old. Nonagenarians and centenarians had selenium concentrations ranging from 37.3 µg/L in Polish 101–105-year-olds to 102.6 µg/L in Chinese 90–99-year-olds, however, these populations are unique and likely have additional lifestyle and dietary patterns helping them live such long lives, and therefore not generalisable to other older adults (Robberecht *et al.,* 2019). One of the few studies looking at selenium status over time, a 9-year longitudinal study in France, found that 65-year-olds who survived the duration of the follow-up had a higher baseline serum selenium of 1.10 µmol/L (86.5 µg/L) compared to those who died (1.01 µmol/L; 79.5 µg/L) (Akbaraly *et al.*, 2005).

Furthermore, a study in Italian adults (> 65 years) found mean selenium concentrations of 0.94 µmol/L (74.0 µg/L), whilst institutionalised adults of > 85 years had lower concentrations of 0.8 µmol/L (62.9 µg/L) (Olivieri *et al.*, 1995); similarly, using NDNS data from the UK, Bates *et al.*, (2002) found that free-living older adults (> 85 years) had higher concentrations of 0.94 µmol/L (74.0 µg/L) than institutionalised older adults (0.89 µmol/L; 70.0 µg/L). As seen above, despite some studies reporting selenium status in older adults, very few studies assess selenium status in very old adults (> 85 years) or use multiple biomarkers. This PhD thesis will address this gap in using The Newcastle 85+ Study in Chapter 3.

Dietary Selenium Recommendations

The previous sections revealed that selenium intakes and status in older adults are suboptimal. Determining whether these intakes and status are suboptimal is based on dietary recommendations. A nutritional requirement is defined as the minimum amount of a nutrient requirement to avoid deficiency; this can be clinical, biochemical or physiological. Dietary recommendations have been in place for hundreds of years and most likely date back to the 1860s where recommendations were used to help unemployed populations due to the economic hard times. Following this, many other recommendations have been devised to help plan food supplies during war and food shortages, or to prevent deficiency.

Across the globe, many countries have devised their own recommendations for national policies, nutritional programmes and food regulations; these often change across age-ranges, life-stages and gender. In 1993, the European Commission (EC) devised the population reference intake to aid in food labelling throughout Europe. In 1994, dietary reference intakes (DRIs) for the US and Canada were created by the Food and Nutrition Board of the Institute of Medicine (IoM), including aspects from the UK report. DRIs added a new level to the recommendations by also considering optimisation of health, as opposed to solely focusing on nutrient deficiency. Within DRIs there are four different recommendations: estimated average requirement (EAR), recommended daily allowance (RDA), adequate intake (AI) and tolerable upper intake level (UL). As mentioned above, there is a lack of consistency between countries in their recommendations. For example, the

UK recommendations pool adults aged 51 years and above, whilst other countries have recommendations for adults up to 75 years. Population reference intakes (PRIs), akin to RNIs and RDAs, in the EC are established for adults aged over 18 years, the rationale being that despite the lower intakes in older adults, there is a lack of evidence suggesting older adults have different nutrient requirements.

Nutritional recommendations for selenium were initially based on extrapolations from experimentally-derived requirements in animals (NRC, 1980). Following this, Keshan disease was identified (see Section 1.7) and selenoproteins with enzymatic functions were characterised. In the UK, initial dietary recommendations for selenium established by the Committee on Medical Aspects of Food and Nutrition Policy (COMA) were based on the selenium intake required to maximise the activity of GPx3. It was suggested that 100 µg/L of selenium whole-blood was the level at which GPx3 activity plateaued and was therefore saturated (Thomson *et al.*, 1977). At the time of setting this DRV in 1991, selenium concentrations in the UK were above 100 µg/L and the RNI (1 µg Se/kg/BW) was set to maintain these levels (DoH, 1991). In 2013, the Scientific Advisory Committee on Nutrition (SACN, 2013) produced a position statement reiterating the use of the same DRVs for selenium, with no change in criteria. This was due to insufficient data to indicate a public health issue with selenium status in the UK, or sufficient rationale to justify a full risk assessment. Other organisations have also used GPx3 activity saturation as a basis for recommendations (Table 1.4). The majority of recommendations (IoM, World Health Organisation/Food and Agriculture Organisation, WHO/FAO, Scientific Committee for Food (SCF)) are based on a study exploring the effect of selenium supplementation on GPx3 activity in Chinese participants where the recommendations were based on a graph in the published article (Yang, 1987). In this Chinese population (mean intake 11 µg/d), L-SeMet supplementation to reach a daily total selenium intake of 41 µg/d (including supplementation) was sufficient to optimise GPx3 activity; this was then weight-adjusted for the desired population equating to 0.87 µg/kg/BW. The WHO/FOA firstly adopted a requirement using estimates for normative selenium concentrations from that study (Yang, 1987). This was later revised to account for different diets and selenium intakes from various populations across countries by increasing the coefficient of variation (CV) from 16 % to 25 %. The IoM incorporated another study into their recommendations, using a New

Zealand population (Duffield *et al.*, 1999) where mean intakes were 28 µg/d, plus 10 µg of supplementation creating an EAR of 38 µg/d. These two studies averaged at an EAR of 45 µg/d, the RDA is 120 % of the EAR and rounded to the nearest 5 µg, creating an RDA value of 55 µg/d. New Zealand and Australia derived their average requirements from two studies, these were Duffield *et al.*, (1999) and another dose-response study, Xia *et al.*, (2005) where L-SeMet supplementation for 20 weeks led to a plateau of GPx3 activity at 40 µg/d and 2/3rd of this concentration was used to devise the RDI. The Australian and New Zealand committee disregarded the Yang, (1987) study due to a difference in the population and dietary components, a lack of peer preview process and the low quality study with missing details or statistical analyses. More recently, other organisations have used SePP, although initially there was insufficient data or validated techniques to use SePP concentrations to devise recommendations in the earlier statements such as IoM, COMA and SCF. However, D-ACH, the combination of Germany, Austria and Switzerland, revised their recommendations in 2015 considering a dose-response relationship between selenium intakes and SePP (Kipp *et al.,* 2015; Xia *et al.*, 2010). That study used individuals from a selenium-deficient area of China where SePP saturation occurred with a daily intake of 49 µg/d, equating to approximately 1 µg/kg/BW, which was then adapted for average European weights. Finally, in their review of studies, EFSA, (2014) considered the adequate intake to be 70 µg/d of selenium.

Table 1.4: Review of selenium dietary reference values from different organisations including the type of recommendation and values, and studies used to derive the recommendation.

Organisation	Recommendation		Type	Study	Reference
	Men	Women			
Committee of IoM National Academy of Sciences Food and Nutrition Board 2000 (USA and Canada)	55	55	RDA	Dose-response and graph estimation Saturation of GPx3 at 41 µg/d in Yang and 38 µg/d averaged at 45 µg for EAR. Then added twice CV of 10 % US re-analysis suggested plateau at 10 µg/d without statistical confirmation of plateau Didn't consider additional health benefits from higher intakes as evidence was lacking	(Yang, 1987; Duffield *et al.*, 1999)
COMA/Department of Health 1991 (UK)	75 40	60 40	RNI LRNI		(Yang, 1987)
FAO/WHO 2002/2004	33	25	NR RNI	Derived from average Se normative requirements + 2 x assumed standard error (of 12.5 %) Adult men 19-65 y: 0.42 µg/kg/day; > 65 y: 0.41 µg/kg/day. Adult women 19-65 y and > 65 y: 0.37 µg/kg/day Normative requirement from Yang 1987(Yang, 1987), but 2/3rd of this (24.3 µg) then adjusted for weight, equating 40 and 30 µg	(Walzel, 1988)
NHMRC Australia and New Zealand	70 60	60 50	RDI EAR	Dose-response L SeMet 20 wk 10-40 µg/d GPx3 plateaued 40 µg; SePP increased more than Se and GPx. Upper estimation of 90 µg to maximise plasma GPx3. RDI was set assuming CV of EAR of 10 %, then rounded to nearest 5 µg. Xia et al showed plateau at 47 µg/d SeMet or 76 with selenite. Averaged EAR of 58 and 49 µg/d for men and women, rounded to 60 and 50 µg/d	(Duffield *et al.*, 1999) (Xia *et al.*, 2005)
NNR 2014 (Nordic)	60 35 70 20	50 30 60 20	RNI AR RI LI	Dose-response Reference body weights for Western populations: estimated 60 µg/d men; 50 µg/d women	(Xia *et al.*, 2010)
Scientific Committee for Food 1992	40 55 20	40 55 20	AR PRI LI	Saturation of GPx3 at 41 µg/d	(Yang, 1987)
D-A-CH Germany (D), Austria (A) and Switzerland (CH) 2015 (Kipp *et al.*, 2015)	70	60	RNI	Dose-response Reference body weights for D-A-CH: estimated 70 µg/d men; 60 µg/d women	(Xia *et al.*, 2010)

IoM: Institute of Medicine; RDA: Recommended Daily Allowance; GPx3: glutathione peroxidase 3 activity; EAR: Estimated Average Requirement; COMA: the Committee on Medical Aspects of Food and Nutrition Policy; RNI: Reference Nutrient Intake; LRNI: Lower Reference Nutrient Intake; FOA/WHO: World Health Organisation/ Food and Agriculture Organisation; NHMRC: National Health and Medical Research Council; NR: normative requirement; L SeMet: L-selenomethionine; SePP: selenoprotein P; Se: selenium; CV: coefficient of variance; NNR: Nordic Nutrition Recommendations; AR: Average Requirement; RI: Recommended Intake; LI: Lower Intake; D-A-CH: Germany (D), Austria (A) and Switzerland (CH)

1.7 Selenium Deficiency and Toxicity

This section will summarise selenium deficiency and toxicity, including associated diseases and symptoms. Due to its narrow ranges between toxicity and deficiency, selenium has been termed as a "double-edged sword" element (Levander and Raymond, 2006; Kieliszek, 2013; Navarro-Alarcon and Cabrera-Vique, 2008). However, globally, it is estimated that 15 % of the world's population are selenium deficient (Fordyce, 2013; White, Broadley and Gregory, 2012; Tan *et al.*, 2016). Signs of deficiency occur at intake levels below 30 µg/d and have been characterised as impaired immune and fertility function, cancer, cardiovascular disease (CVD) risks, hepatitis B infections, asthma, muscle disease, thyroid issues and mortality (EFSA, 2014). Low intakes can occur for a range of reasons, some of which are mentioned in Section 1.4; others include malabsorption, metabolic disease and parenteral nutrition (Bodnár *et al.*, 2016). Severe selenium deficiency has been linked to the endemic Keshan disease and Kashin-Beck disease, a cardiomyopathy and osteoarthropathy, respectively. Keshan disease is endemic to rural areas of China (Northeast to Southwest) and was given the name due to its unknown occurrence in a Keshan country of Heilongjiang Province in Northeast China. Keshan disease generally affects young children and disadvantaged women with low socioeconomic backgrounds (Combs, 2001) and symptoms range from congestive heart failure, cardiac arrhythmias, necrotic lesions and myocardium calcification (Lescure *et al.*, 2009). Aetiology is thought to occur via infections by the enterovirus Coxsackie, intoxication, and nutrient deficiency (Lescure *et al.*, 2009). Another endemic condition is Kashin-Beck disease which occurs in Northeast to Southwest China, N Korea and Southeast Siberia (Yao *et al.*, 2011). Kashin-Beck is an osteoarthropathy and presents as swelling, joint pain, stiffness and myalgia leading to shortened fingers and toes and dwarfism (Guo *et al.*, 2014). Various hypotheses for its development have been created such as *Fusarium* mycotoxin poisoning, imbalances between macro and trace elements, genetic factors, fulvic acid in drinking water and a combined iodine and selenium deficiency (Yao *et al.*, 2011). Although the mechanisms behind selenium's effect on Kashin-Beck disease are unclear, supplementation can reverse the disease (Guo *et al.*, 2014; Yao *et al.*, 2011).

Detailed above, and as with any other micronutrient, deficiency and toxicity are associated with health-related problems. The safe upper level of selenium intake has recently been updated to 255 µg/d for adults (ESFA, 2023) (previously it was estimated at 400 µg/d (IoM, 2000)). A peer-reviewed article quoted a safety intake recommendation at 800 µg/d for no observed adverse effect level (NOAEL), 1540 to 1600 µg/d for low observed adverse effect level (LOAEL) and 5000 µg/d for a toxic level, where selenosis occurs (Whanger, 2004). Some geographical locations are exposed to excessive environmental selenium, such as Central China where high soil concentrations can lead to intakes of 1600 µg/d (Combs, 2001). Symptoms are characterised by a loss of hair and nails, hepatomegaly, garlic odour breath, gastrointestinal issues, irritability, acute respiratory distress syndrome, myocardial infarction, renal failure, fatigue and mild nerve damage (Fordyce, 2007; EFSA, 2014). Selenium toxicity is thought to occur due to the production of superoxide and hydrogen peroxide when selenocompounds react with glutathione and thiols (Spallholz, 1994; Suzuki *et al.*, 2006). This can alter protein functions involved in DNA repair causing free radicals and oxidative stress (Letavayová, Vlčková and Brozmanová, 2006). There is still controversy over how much selenium is required for optimal functioning but, more than often, excessive amounts lead to disruption of energy metabolism, growth hormone, IGF-1, and thyroid hormone (Navarro-Alarcon and Cabrera-Vique, 2008). This can be a risk for individuals who supplement with excessive amounts of selenium to help with disease prevention (Rayman, 1997). However, deficiency is more prominent than toxicity as the safe upper limit is often far greater than the average intake in most populations (Whanger, 2004).

Outside of the clinical symptoms of selenium deficiency and toxicity lies suboptimal selenium status. Research has highlighted the U-shape relationship between selenium intake or status (Rayman, 2012). For example, serum selenium above 100 µg/L has been associated with improved cognition and reduced all-cause mortality (Giovaninni *et al*., 2018), and plasma concentrations of 120 µg/L have been associated with protection of some cancers (Combs, 2001). However, another review found that concentrations beyond 120 µg/L led to no further protection from cancer (Rayman, 2012). Furthermore, selenium concentrations between 130-150 µg/L were associated with minimal mortality (Bleys *et al.,* 2008), however, beyond this, there has been an increased risk of mortality and type 2 diabetes (Stranges *et*

al., 2010). Thus, this U-shaped relationship needs to be considered when exploring selenium status and its impact on health.

1.8. Selenium and MSK Function

The subsequent sections of the PhD thesis will now focus on selenium and its role in MSK function, first summarising the mechanisms behind selenium's role in MSK function, then summarising selenium's role in muscle in animals and humans. The following sections will repeat this order but instead focus on the role of selenium in bone health. Additional details of studies for this section are summarised in Table 1.5, 1.6 and Appendix Table 1.3. There have been various hypotheses created to determine the mechanism between selenium deficiency and MSK function, ranging from altered calcium signalling, oxidative stress, inflammation, apoptosis and selenoprotein disruption (Lescure *et al.*, 2009; Rederstorff, Krol and Lescure, 2006). Suboptimal selenoprotein levels may upregulate inflammatory cytokines, leading to muscle weakness and oxidative damage and higher levels of IL-6 have been associated with low selenium status, suggesting a bi-directional pathway (Prystupa *et al.*, 2017; Tseng *et al.*, 2013). Selenium deficiency can target muscles leading to dystrophy through oxidative stress and apoptosis via downregulation of selenoprotein genes involved in muscle (Huang *et al.*, 2011; Yao *et al.*, 2013a; Yao *et al.*, 2013b) which may affect MPS and muscle function, although the mechanisms are still unclear (Rederstorff, Krol and Lescure, 2006; Chariot and Bignani, 2003; van Dronkelaar *et al.*, 2018). Skeletal muscle requires calcium for signalling, muscular contraction, and enzymatic sites (Berchtold, Brinkmeier and Müntener, 2000). Different calcium channels control the influx and efflux of calcium ions; these include ryanodine receptor (RyR) and calcium pump channels for intracellular calcium release. RyR1 (ryanodine receptor 1) is a calcium channel required in muscle for excitation-contraction coupling; disruption in calcium channels has been related to selenoprotein interference, suggesting a potential mechanistic role of selenoproteins in MSK function (Lescure *et al.*, 2009; Jurynec *et al.*, 2008; Reeves, Bellinger and Berry, 2010).

Muscle and Selenium: Evidence from animal models

There is a wealth of evidence to suggest how selenium deficiency causes muscle dysfunction in animals (Table 1.5). Selenium's potential role in muscle function was first noted in animals

grazing on low selenium soils which led to white muscle disease (WMD) (Whanger, 2009). Selenoprotein W (SELENOW) was the first selenoprotein associated with muscular function and has a conserved gene sequence 10CXXU13 (C is cysteine and U is Sec) across mammalian species (Whanger, 2009). Selenoprotein N (SELENON) mRNA and its associated protein are in most human and murine tissues. Alterations of SELENON gene expression have been associated with early onset, autosomal, recessive neuromuscular disorders termed SEPN1-related myopathy (Castets *et al.*, 2009; Petit *et al.*, 2003). SEPN1-related myopathies have been characterised by weakness of the trunk and neck muscles and global muscle atrophy which can cause spinal rigidity, severe scoliosis, and respiratory insufficiency during younger years. SELENON concentrations are generally higher in embryonic tissues than adult tissues, such as somites, precursors of muscular tissues, and SELENON expression decreases during myoblast differentiation, implying SELENON's role in muscle development (Castets *et al.*, 2009; Petit *et al.*, 2003; Thisse *et al.*, 2003). Research alludes to SELENON interacting with ryanodine receptors responsible for calcium release in the sarcoplasmic reticulum (SR) (Jurynec *et al.*, 2008). Without SELENON, calcium concentrations can increase impairing mitochondrial homeostasis leading to stress and apoptosis as well as a conversion to slower twitch muscle fibres and sarcomeric disorganisation through cellular proteolysis (Berchtold, Brinkmeier and Müntener, 2000) which can then increase protein breakdown (Michelucci *et al.*, 2021). Selenium may also play a role in other areas of muscle function, for example, old rats given an antioxidant-rich diet containing selenium had an improved MPS response (Marzani *et al.*, 2008) and selenium supplementation in muscle contusion of rats led to lower inflammatory markers (creatine kinase in muscle, IL-6 and IL-1β) and improved gait performance compared to those not receiving supplementation (Goenawan *et al.*, 2022).

Muscle and Selenium: Evidence from Human Epidemiology

In humans, selenium-deficient patients can have elevated serum levels of creatine kinase, muscle fatigue, pain and proximal weakness associated with skeletal muscle disorders (Chariot and Bignani, 2003). In older women, higher intakes of dietary selenium plus other antioxidants was associated with enhanced performance in chair rises and walking (Roberts *et al.*, 2011). Low dietary intakes of selenium, among other micronutrients, were associated with poor muscle strength, frailty and physical performance (Bartali *et al.*, 2006). In an

observational study, higher nail selenium concentrations were associated with improved Timed-Up-and-Go (TUG) performance (Islam *et al.*, 2007). However, when intakes were double the RDA, Chaput *et al.*, (2007) did not find any associations between selenium intakes and muscle mass. Focusing on selenium status, community-living women (≥ 65 years) with low serum selenium and other antioxidant concentrations, compared to those with high intakes, had poor physical performance (Martin *et al.*, 2011) and in another observational study, plasma selenium status was positively associated with hand grip strength (HGS) (Beck *et al.*, 2007). Plasma selenium in the lowest quartile, when compared to the highest, was associated with worse hip, knee and grip strength (Lauretani *et al.*, 2007) and in community-dwelling adults, serum selenium has been associated with muscle strength (Lauretani *et al.*, 2007), sarcopenia prevalence (van Dronkelaar *et al.*, 2018) and muscle mass (Chen *et al.*, 2014). Aside from clinical case reports, often in those receiving parenteral nutrition, selenium's role in MSK function is generally not considered and there are even fewer studies looking at long-term selenium supplementation. In one study, males using selenium supplementation for 1-24 years were compared to non-supplemented males where supplementation was associated with higher muscle selenium in thigh biopsies (Behne, Alber and Kyriakopoulos, 2010).

Muscle and Selenium: Evidence from Case Control Studies and Randomised Controlled Trials

There are few studies exploring the effects of selenium supplementation on muscle function in humans using randomised controlled trials (RCT). Ten selenium-deficient patients provided with selenium supplementation (200 μg/d) showed improvements in plasma selenium and type 1 muscle fibre diameter (Rannem *et al.*, 1995). Likewise, selenium supplementation improved muscle pain and tenderness in case control supplementation studies (Robinson, 1981; Robinson *et al.*, 1978; Reinhard *et al.*, 1998), For example, intravenous selenious acid (400 μg/d for 6 weeks) provided to a parenteral nutrition patient (severely selenium-deficient female, 33 years) was associated with enhanced proximal muscle strength (Brown *et al.*, 1986).

Table 1.5: Evidence for the associations between selenium intake or status and muscle function in animals and humans, including species, sample size, study design, selenium status, and/or dosage and outcomes.

Animal	Demographics, Sample Size	Study Design, Duration	Dosage, Form	Outcomes
Equine (Delesalle *et al.*, 2017)	New-born foals WMD (n = 8)	Case report		Increased serum muscle enzymes, low GPx; muscle necrosis in non-survivors
(White *et al.*, 2016)	Adult, untrained thoroughbred (n = 12)	Experimental: 2h submax. training 36 d	Control Se+: sodium selenite 0.3 µg/kg DM	Se+: increased serum Se and TXNRD; no difference in plasma GPx activity, RBC, serum creatinine kinase; lipid hydroperoxides reduced immediately Se may reduce oxidative muscle damage
Goat (Tian *et al.*, 2022)	3 groups (n = 6/group)	Experimental 60 d	Control Se+: 2.4 µg/kg Se-yeast High Se+: 4.8 µg/kg Se-yeast	Both Se+: no differences in growth and muscle composition High Se+: increased total antioxidant capacity, GPx and scavenging activity; reduced shear force and oxidative stress
Turkey (Fischer, Bosse and Pallauf, 2008)	1-day old male, 8 groups (n = 18/group)	Experimental 35 d	Control < 0.010 µg/kg Se+: 0.10 to 0.40 µg/kg sodium selenite Vitamin E in all diets	Control: muscle damage markers increased; reduced liver GPx activity
Fish (Wang *et al.*, 2020)	Juvenile rainbow trout (n = 600)	Experimental 6 wk	Control Se+: 4 µg/kg Se-yeast	Se+: increased muscle protein content and retention, mTOR activity, postprandial MPS
(Jurynec *et al.*, 2008)	14-18 somite stage embryo Zebra fish	Experimental	SELENON-	Reduced fibres, calcium influx and defective slow muscle cells. Se+: ryanodine binding and oxidative response normalised
Chicken (Gao *et al.*, 2018)	24 wk, Maternal Se+, (n = 720) 3 treatments, 6 replicates (n = 40) male offspring	Experimental 8 wk	Control Organic (Se/O) 0.5 mg/kg Inorganic (Se/I) 0.5 mg/kg) Offspring: sodium selenite 0.15 mg/kg	Se+: increased muscle, insulin, muscle markers and SELENOW mRNA; reduced serum uric acid, skeletal muscle Atrogin-1 and MuRF1 mRNA. Se/O vs Se/I: no difference in muscle development
(Wu *et al.*, 2014)	1d old male chicks (n = 250) 2 groups (125/group)	Experimental Euthanized from 15-55 d	Se-: 0.033 mg Se/kg Se+: sodium selenite 0.15 mg Se/kg	Se-: increased inflammatory-related genes; reduced SELENOW mRNA in muscles
Guinea pig (Hill, 2001)	Weanling, male 4 groups	Experimental	Control, Se- diet, Vitamin E- Combined Se + Vitamin E- Se+: 0.5 mg/kg sodium selenate	Se and Vitamin E-: 54 % euthanised: severe weakness; muscle damage increased with highest levels in Se- diet. Fatal myopathy (VESD) lipid peroxidation, muscle affection, low GPx
Rats (Marzani *et al.*, 2008)	Old (20 mo) and young (8 mo) Wistar	Experimental 7 wk	Control Se+: Antioxidant-rich	MPS lower in older compared younger rats Se+: improved MPS response
(Goenawan *et al.*, 2022)	Male, 3 groups (n=5/group) Wistar	Experimental	Control Contusion Se+: 0.0513 µg/kg/d 3d	Se+: IL- 1β and 1L-6 lower and improved step gait in Se+ compared to contusion; no difference in serum CK-MM
Mice (van Dijk *et al.*, 2016)	18 mo C57/BL6J, male 2 groups: control (n = 33) diet (n = 133)	Experimental 7mo	Control Se+: casein-based antioxidant + leucine enriched protein	Antioxidant- 7mo: increased fatigue; reduced muscle strength compared to control Protein+: improved grip strength, muscle power; reduced fatigue Antioxidant+: improved mitochondrial dynamics, oxidative status and grip strength; reduced fatigue

(Bodnár *et al.*, 2016)	25 wk, BDF1 male	Experimental 3 wk	Control: 0.3 ppm Se Se+: Selenate + NanoSe (equivalent 4 and 40 µg/kg/BW/d)	Se+: grip force unaffected; increased amplitude of EDL and SOL single twitches Higher Se+: increased voluntary running speed and distance Lower Se+: increased SOL fatigue resistance, calcium release and SELENON expression in EDL
(Fodor *et al.*, 2020)	Old (22 mo) and young (4 mo) C57BL/6 mice 5 groups	Experimental 2 mo	Control + running Young: control Old: control, compact mutation mice or NanoSe (equivalent 40 µg/kg/BW/d)	Older mice: reduced voluntary running, maximal tetanic and twitch force, calcium release, SELENON and RyR1; increased ROS and degraded RyR1 Se+ and running: Improved muscle force, fatigue resistance, SR calcium and SELENON content
Human	**Demographics, Sample Size**	**Study Design, Duration**	**Dosage, Form**	**Outcomes**
(Arbogast *et al.*, 2009)	Patients with SELENON deficient myotubes (n = 6)	Experimental		SELENON- and oxidative activity and protein oxidation: higher compared to controls
(van Rij *et al.*, 1979)	28-72 y Total parental nutrition (10-40 d) (n = 22)	Review of Case Studies	IV SeMet 100 µg/d	24h Se+: increased erythrocyte and GPx activity 1wk Se+: improved muscle tenderness, pain in thighs and walking ability
(Brown *et al.*, 1986)	33 y female Short bowel syndrome Home parental nutrition 4 y	Case Study 6 wk	IV selenious acid 400 µg/d 6 mo Plasma Se: 5 ng/ml (normal: 60-140) GPx3 activity: 0.02 U/ml (normal: 0.19-0.33)	Se+: reversed muscle weakness and fatigue; improved Se and GPx activity with increases to 117 ng/ml and 0.21 U/ml by 4 mo
Human	**Demographics, Sample Size**	**Study Design, Duration**	**Dosage, Form**	**Outcomes**
(Robinson *et al.*, 1978)	Mean 35 y (n = 24) muscular complaints, low soil area Tapanui, NZ CC, 3 subjects supplemented	Case Control	1. 100 µg SeMet for 12 wk 2. 100 µg SeMet or sodium selenite 10-11 wk or 65 µg Se in mackerel 4 wk	1. Blood Se increased; decreased 2-mo post supplementation; 50% symptom relief 2. Whole-blood, plasma and erythrocyte Se, urinary and faecal excretion increased SeMet and mackerel Se: better absorbed than sodium selenite
(Reinhard *et al.*, 1998)	Mean 47 y, fibromyalgia patients (9 men, 59 women, n = 68) Female controls (n = 57), Germany	Observational	Serum Se patients: 71 µg/l Se Controls: 77 µg/l	Se: difference between groups
(Helmersson *et al.*, 2005)	50 y, men (n = 615) Sweden	Longitudinal 27 y follow-up	urinary 8-iso-PGF2α, 15-keto-dihydro-PGF2α, hsCRP, serum amyloid A protein, IL-6	Serum Se highest quartile at 50 y: lower oxidative stress and lipid peroxidation
65 y (Maggio *et al.*, 2010)	≥ 65 y men and women (n = 951) InCHIANTI study, Italy	Observational	Plasma Se: 0.95 ± 0.15 µmol/L IGF-1: 113.4 ± 31.2 ng/ml	Se and IGF-1: positive association
(Lauretani *et al.*, 2007)	≥ 65 y men and women (n = 981) InCHIANTI study, Italy	Observational	Plasma Se: 0.95 ± 0.15 µmol/L Q1 < 0.839; Q2 0.839–0.934; Q3 0.935–1.037; Q4 > 1.037	Lower Se quartile and hip, knee and GS: negative association
(Bartali *et al.*, 2006)	≥ 65 y women (n = 643) Women's Health and Aging Study, US	Longitudinal 6 mo	Serum Se Incidence Rate: Lowest Q: 21.6; Upper 3 Q: 10.8	Lower Se quartile and disability risk: positive association
(Semba *et al.*, 2006)	≥ 65 y women (n = 766:250 frail, 516 non-frail) Women's Health and Aging Study, US	Observational 3 y	Se Frail: 112 µg/L (109-114 95% CI) Se Non-frail: 118 (116-120 95% CI)	Se and frailty: negative association Upper vs lower quartile: no difference in frailty incidence rates
(Beck *et al.*, 2007)	≥ 65 y women, moderately to severely disabled (n = 676) Women's Health and Aging Study, US	Observational	Mean plasma Se: 1.49 µmol/L (0.23 SD) Mean HGS: 18.2 kg (4.9 SD)	Serum Se and HGS: positive association

(Islam *et al.*, 2007)	62-67 y, adults (n = 507) Freemasons Health North Island, New Zealand	Cross sectional	Hair and toenail Se, 24 h dietary recall Se intake µg/d: Men 48.8; Women 37.6 Hair Se µg/g: Men 0.47 Women 0.40 Nail Se µg/g: Men 0.59 Women 0.60	Se and TUG: negative association
(Martin *et al.*, 2011)	63-73 y men (348) and women (280) (n = 628) Hertfordshire cohort study	Observational	Men Se: 52.5 µg/d Women Se: 52.1 µg/d	Se intake and 3MWT: negative association in women
(Chen *et al.*, 2014)	Mean 71 y (n = 327) Taipei	Cross sectional	Serum Se quartiles	Estimated Se and muscle mass: negative association
(de Jong *et al.*, 2001)	Mean 75 y, women (n = 103) Nonusers of Se-supplementation (n = 80) Dunedin, Urban, New Zealand	Cross sectional	Mean Se: 34 ± 10 µg/L 80 % of non-supplement users Se < 1 µmol/L 83 % consuming below 42 µg/L	Se tertile and physical performance (HGS and TUG): positive association Plasma Se and GPx activity: positive association
(Heffernan *et al.*, 2019)	128 studies, Se (n = 5) 124 participants, males	RCT Systematic Review Mineral + trace elements in exercise performance	1. 200 µg/d, trained cyclists, 4 wk (Shafiei Neek, Gaeini and Choobineh, 2011) 2. SeMet 180 µg/d, 24 males, untrained endurance, 10 wk (Margaritis *et al.*, 1997) 3. Organic Se 240 µg, 24 non-smoking males, mean 23 y (Tessier *et al.*, 1995) 4. Se, 24 males, 10 wk endurance training (Zamora *et al.*, 1995) 5. 200 µg sodium selenite (Savory *et al.*, 2012)	1. No effect on testosterone or lactate accumulation 2. No effect on mitochondrial activity, aerobic performance, myosin heavy chain expression 3. Increased GPx in exercise compared to placebo 4. Reduced exercise-induced mitochondrial density and biogenesis 5. Reduced lipid hydroperoxide in OW
RCT (Robinson, 1981)		RCT	Control Se+ 3 dosing trials: 2 double blinded 100 µg/d sodium selenite or SeMet	Se+: increased blood Se and GPx1 activity; 50 % improved muscular symptoms
(Rannem *et al.,* 1995)	25-65 y, Se- (n = 10) Short bowel syndrome Long-term parental nutrition	RCT 5-7 times/wk 4 mo	Placebo IV sodium selenite 200 µg/d Plasma Se increased 0.21 to 1.25 µmol/L	Se+: improved serum Se and mean diameter of type 1 muscle fibres; no improvement in quadriceps strength

WMD: white muscle disease; GPx: glutathione; Se+: selenium-supplemented; DM: dry mass; Se: selenium; TXNRD: thioredoxin reductase; RBC: red blood cell; BW: body weight; Se-: selenium-deficient; mTOR: mammalian target of rapamycin; MPS: muscle protein synthesis; DIO: deiodinase; T3: Triiodothyronine; FT3: free T3; TSH: thyroid stimulating hormone; GH: growth hormone; IGF-1: insulin-like growth factor 1; T_4: thyroxine; FT_4: free T_4; dio1: type 1 deiodinase; SELENON: selenoprotein N; AkT: Protein kinase B (PKB); P70S6K: p70 ribosomal S6 kinase; Myf5: Myogenic factor 5; MyoD: Myogenic Differentiation Antigen; MyoG: Myogenic Factor; SELENOW: selenoprotein W; FOXO: forkhead box transcription factors; MuFRI: NF-kB: Nuclear factor kappa B; TNFα: Tumour Necrosis Factor alpha; iNOS: Inducible nitric oxide synthase; COX-2: Cyclooxygenase-2; PTGES: Prostaglandin E Synthase; VESD: vitamin E selenium deficiency; IL-1β: Interleukin 1 beta; IL-6: interleukin 6; CK-MM: Creatine Kinase, muscle; ppm: parts per million; ROS: reactive oxygen species; RyR1: Ryanodine receptor 1; SR: sarcoplasmic reticulum; SeMet: selenomethionine; IV: intravenous; EMG: electromyography; WBC: white blood cell; 8-iso-PGF2α: 8-iso-prostaglandin F2α; hsCRP: high sensitivity c reactive protein; SAA: Serum amyloid A; ADL: activities of daily living; GS: hand grip strength; TUG: timed up and go; RCT: randomised controlled trial; FFQ: food frequency questionnaire; 3MWT: 3 m walking test; BIA: body impedance analysis; SMM: skeletal muscle mass; OW: overweight; FA: fatty acid

Selenium and MSK Function: Bone

The role of selenium in bone metabolism has been demonstrated in numerous studies (Table 1.7). Furthermore, bone holds the second highest proportion of selenium content (16 %), followed by muscle stores (27.5 %) (Zachara *et al.*, 2001; Oster, Schmiedel and Prellwitz, 1988) suggesting a requirement of selenium in bone health. Additionally, at least 9 selenoproteins are identified in foetal osteoblasts or osteoclasts, the bone formation and resorption cells, respectively (Zhang, Zhang and Xiao, 2014; Jakob *et al.*, 2002; Zeng, Cao and Combs, 2013; Pietschmann *et al.*, 2014; Ebert *et al.*, 2006; Dreher *et al.*, 1998; Köhrle *et al.*, 2005). Selenium supports bone formation through the TXNRD1 selenoprotein which is expressed in the osteoblast differentiation pathway (Zhang, Zhang and Xiao, 2014) and requires $1,25(OH)_2D_3$ upregulation. Without selenium, $1,25(OH)_2D_3$ fails to improve TXNRD1 activity indicating that selenium deficiency would be detrimental to osteoblastic differentiation (Jakob *et al.*, 2002). Osteoclast apoptosis is induced at high levels of selenium (5-10 µM sodium selenite) and osteoblast defence systems are improved (through a reduction in oxidative stress (Zeng, Cao and Combs, 2013; Chung *et al.*, 2006; Liu *et al.*, 2012; Dreher *et al.*, 1998; Cao, Gregoire and Zeng, 2012). As described in Section 1.2, inflammatory molecules, such as IL-6, and ROS, both of which stimulate bone resorption (Garrett *et al.*, 1990; Evans and Ralston, 1996; Key *et al.,* 1994) and increase RANK-L signalling respectively, (Zhang, Zhang and Xiao, 2014) are neutralised by selenoproteins, thereby reducing bone resorption and maintaining redox balance (Kim *et al.*, 2021; Prabhu *et al.*, 2002). Additionally, selenium, as mentioned, is also involved in thyroid hormone function. DIO selenoproteins are required to convert the inactive T_4 to the active T_3; when selenoprotein concentrations are suboptimal less T_3 is synthesised and free T_4 levels remain high which has been associated with hip BMD and non-vertebral fracture risk and, interestingly, inversely associated with serum selenium and SePP (Hoeg *et al.*, 2012). Despite selenium's involvement in bone, only a few other studies have examined the effect of selenium experimentally (Table 1.6).

Selenium and Bone: Evidence from animal models

Experimental studies in animals indicate the role of selenium in bone metabolism. For example, an osteo-chondroprogenitor-specific deletion in the Sec tRNA gene in transgenic mice showed impaired skeletal growth and ossification, further suggesting selenium's importance in bone health (Downey *et al.*, 2009). In mice, increasing glutathione reduced oestrogen-deficiency bone loss whilst glutathione depletion increased bone loss (Lean *et al.*, 2005) and, at supranational doses, GPx1 was capable of degrading hydrogen peroxide and TXNRD1 prevented nuclear factor kappa light chain enhancer of activated B cells (NF-kβ) activation (Prabhu *et al.*, 2002; Kim *et al.*, 2007; Christensen *et al.*, 2007). Overexpressing GPx in mouse osteoclasts resulted in prevention of osteoclast formation and suppression of RANK-L which included NF-kβ activation (required for osteoclast formation) with enhanced oxidative stress resistance (Lean *et al.*, 2005; Zhang, Zhang and Xiao, 2014). Regarding bone quality and growth, compared to selenium-supplemented mice, selenium-deficient mice had poorer bone microarchitecture due to improper mineralisation with higher inflammatory and bone resorption markers (Cao, Gregoire and Zeng, 2012). In selenium-deficient rats, studies have found abnormal skeletal growth and poor bone health (Moreno-Reyes *et al.*, 2001; Hurt, Cary and Visek, 1971; Thompson, Haibach and Sunde, 1995), lower BMD and femur ash weight (Sasaki *et al.*, 1994), shortening of epiphyseal plates (Min *et al.*, 2015) and combined selenium and iodine deficiency (< 0.02 μg/g) reduced cartilage and bone growth (Ren *et al.*, 2007). In second generation selenium-deficient rats, trabecular bone volume, surface, and diameter decreased with fewer, thinner trabeculae. These experimental studies in animal models provide further evidence for selenium's role in MSK function.

Selenium and Bone: Evidence from human epidemiology

There is a growing body of evidence from human observational studies that selenium is involved in bone metabolism. One potential indicator of selenium's importance in bone metabolism is that even during selenium deficiency, a supply of selenium to the bone is maintained and transported by SePP (Pietschmann *et al.*, 2014). In observational trials, selenium, either as dietary intakes, or status, has been associated with osteoporosis prevalence, BMD and fractures which will be described in the following text below and in Table 1.6. Selenium

intake was negatively associated with osteoporotic hip fracture risk in US adults (> 20 years) (Zhang *et al.*, 2006) and in a case control study of elderly Chinese adults (Sun *et al.*, 2014). Using the National Health And Nutrition Examination Survey (NHANES) selenium intakes were positively associated with total femur BMD, especially in postmenopausal women and, negatively associated with FRAX scores (Wu *et al.*, 2021). A cross-sectional study found a negative association between selenium intakes and osteoporosis prevalence assessed using phalange BMD (Wang *et al.*, 2019) and hair selenium levels were positively associated with BMD (Park *et al.*, 2020). Similarly, serum selenium was positively associated with estimated heel and forearm BMD (Qu *et al.*, 2021). Likewise, in elderly men (≥ 70 years), selenium concentrations (mean 92 μg/l) and SePP (3.4 mg/L) were positively associated with total and femoral trochanter BMD (Beukhof *et al.*, 2016) and in a cohort of postmenopausal women there was a negative association between concentrations of selenium and SePP and BTM whilst there was a positive association between selenium concentrations and BMD (Hoeg *et al.*, 2012).

Despite these associations between selenium intakes, or status, and BMD (Beukhof *et al.*, 2016; Hoeg *et al.*, 2012; Wu *et al.*, 2021; Park *et al.*, 2020; Pedrera-Zamorano *et al.*, 2012; Wang *et al.*, 2019) and fractures (Sun *et al.*, 2014; Galvez-Fernandez *et al.*, 2021; Zhang *et al.*, 2006), some studies have not found significant associations between selenium intakes or status, and osteoporosis, BMD or fractures (Odabasi *et al.*, 2008; Wolf *et al.*, 2005; Arikan *et al.*, 2011; Galvez-Fernandez *et al.*, 2021; Wang *et al.*, 2015; Liu *et al.*, 2009; Ilich *et al.*, 2009; Chan *et al.*, 2009). There was no association between selenium status and BMD (Arikan *et al.*, 2011), nor any differences between selenium status in healthy, osteoporotic or, osteopenic Chinese women (Liu *et al.*, 2009). Other studies have suggested both low and high concentrations of selenium can be detrimental to bone, for example, a U-shaped dose-response was found with higher baseline selenium (84.9 μg/l) and OP fractures (Galvez-Fernandez *et al.*, 2021); plasma selenium below 105 μg/l was associated with lower BMD, whilst levels above 105 μg/l were associated with an increased fracture risk. Plasma selenium beyond 105 μg/L may allow selenium to be non-specifically incorporated into proteins as SeMet (Monsen, 2000) which could alter osteoblast differentiation and osteoclast activity (Zeng, Cao and Combs, 2013). These variances between studies could be from differences in methodology, measurements of osteoporosis, biomarkers of selenium status, geographical

regions, age ranges of participants and genetic variation in selenium-related genes (Hesketh and Méplan, 2011). In addition to these nutritional associations, there have also been associations between genetic polymorphisms and selenoproteins whereby there is an inter-individual variation in micronutrient metabolism (Hesketh and Méplan, 2011). Furthermore, there is genetic variation associated with bone metabolism (Cusack and Cashman, 2003; Tranah *et al.*, 2008). The arachidonate 12-lipoxygenase (ALOX12) gene aids in oxygen insertion into polyunsaturated fatty acids and is part of the arachidonate lipoxygenase enzyme super-family (Deng *et al.*, 2002; Devoto *et al.*, 1998). A by-product of ALOX12 activity, 12-hydroperoxyeico-satetraenoic acid (12-HPETE) acts as a ligand for peroxisome proliferator-activated receptors (PPARs). PPARs inhibit osteogenesis and increase adipogenesis from bone-marrow derived mesenchymal stem cells (MSCs) (Gimble *et al.*, 2006; Lecka-Czernik *et al.*, 2002). ALOX12 activity could increase PPAR binding and decrease osteoblast generation, leading to a reduction in BMD (Kawaguchi *et al.*, 2005). Single nucleotide polymorphisms (SNPs) within ALOX12 have been associated with femoral neck BMD (Al-e-Ahmad, 2018). Other SNPs associated with bone health are between GPx1 and BMD (Mlakar *et al.*, 2010), osteoporosis prevalence postmenopausal women (Ozgocmen *et al.*, 2007) and between SELENOS and osteoarthritis risk (Bos *et al.*, 2009).

Selenium and Bone: Evidence from randomised controlled trials

To my knowledge, there is only one RCT exploring the effect of selenium supplementation on bone health. This RCT measured the effect of sodium selenite supplementation (50 and 200 µg/d) on BTMs, BMD and inflammatory markers in postmenopausal women with osteoporosis or osteopenia. Supplementation over 6 months had no significant effect on these outcomes, although the mean baseline status was 79.4 µg/L suggesting this population was selenium replete, which may explain the lack of results (Walsh *et al.*, 2021).

These last sections of this chapter have clearly shown that more research is required, using different study designs to improve the understanding of selenium's role in MSK ageing and function. Section 1.9 below presents a summary of the aims and objectives of each experimental chapter in this PhD thesis.

Table 1.6: Evidence for associations between selenium intake or status and bone metabolism in animals and humans, including species, sample size, study design, selenium status, and/or dosage and outcomes.

Animal	Demographics, Sample Size	Study Design, Duration	Dosage, Form	Outcomes
Mice (Cao, Gregoire and Zeng, 2012)	18 wk, male c57BL/6J (n = 33)	Experimental 4 mo	Se-: 0.9 μg Se/kg Se+: SeMet or pinto 100 μg Se/kg	Se-: reduced GPx1 activity, femoral trabecular bone volume; increased trabecular bone separation and serum CRP, TRAP and PTH
Rats (Sasaki *et al.*, 1994)	3-11 mo, 2nd generation Se-	Experimental	Se-	Se-: reduced whole and distal femur and tibia BMD and BMC
(Moreno-Reyes *et al.,* 2001)	12 wk, female Wistar Pups Se- diet (n = 24)	Experimental 72 d	Se-: 0.005 mg/kg Se+: 0.19 mg/kg	Se+: PTH and 1,25(OH)2D_3 doubled; reduced weight, tail length, plasma calcium, osteocalcin, femur and tibia BMD
(Yao *et al.,* 2012)	Weaned, Wistar (n = 96)	Experimental 12 wk	Control KBD diet 0.031 mg/kg Se+: 0.14 mg/kg Se + Iodine+	Se+ and Se + Iodine+: increased trabecular number, thickness, bone: tissue volume; reduced trabecular separation KBD diet: reduced total serum protein, albumin; increased tibial growth plate, chondrocyte necrosis
(Min *et al.*, 2015)	8-12 wk, Dark Agouti (n = 8-10/group)	Experimental 2 mo	Se-: 0.018 μg/g Se+: 0.288 μg/g	2nd generation Se- rats: reduced GPx4, GPx1 type II collagen, GPx1 activity, epiphyseal plate lesion
(Ren *et al.*, 2007)	Weanling Sprague-Dawley (SDR) (n = 48)	Experimental, 4 groups 3 mo	Se-: Se <0.02 μg/g, iodine 0.4-0.5 μg/g Se + iodine +: Se 0.1–0.3 μg/g, iodine 0.4-0.5 μg/g Iodine-: Se 0.1-0.3 μg/g, iodine 0.04 μg/g Combined-: Se 0.01 μg/g, iodine 0.04 μg/g	Se+: increased PTHrP expression; reduced ColX expression irrespective of Iodine Combined-: reduced tibial length and growth plate cartilage thickness
Human	**Demographics, Sample Size**	**Study Design, Duration**	**Dosage, Form**	**Outcomes**
Younger Adults (Rivas *et al.*, 2012)	≥18 y, women (n = 280) Spain	Cross Sectional 24hr FFQ, Hip and LS BMD	Control 104.0 ± 27.1 μg/d OP group: 96.5 ± 33.8 μg/d	Se and BMD: no association
(Chan *et al.*, 2009)	≥ 20-35 y, women (n = 441) China	Cross Sectional 5d food record, Hip, LS and FN BMD	Beijing Se: 46.4 ± 14.5 μg/d Hongkong Se: 80.6 ± 27.2 μg/d	Se and BMD: no association
(Zhang *et al.*, 2021)	≥ 20 y , adults (n = 17150) China	Longitudinal 3d food record Self-reported fracture	Se quartile: Q1: 20 ± 5 Q2: 31.8 ± 3 Q3: 42.5 ± 4 Q4: 71.2 ± 45 (μg/d)	Se and fracture: non-linear association U shaped response: varied by gender and urbanization
(Park *et al.*, 2020)	≥20 y, mean 53 y, adults (n = 1167) Korea	Cross Sectional	Control: Hair Se 0.06 μg/g OP: Hair Se 0.05 μg/g	Lower hair Se (quartiles) and BMD: positive association
(Wang *et al.*, 2019)	Mean 52 y, adults (n = 6267) China	Cross Sectional Validated, SQ FFQ, Phalanges BMD	Control: 44.0 ± 23.3 μg/d Se OP: 39.1 ± 31.1 μg/d Se	Lower Se and OP prevalence: positive association OR 0.72 (0.55–0.94)
PMW (Odabasi *et al.*, 2008)	Mean 61 y, OP: 77, control: 61 (n = 138) Turkey	Case Control BMD	Control: 79.0 ng/ml Se OP: 77.0 ng/ml Se	Se: no difference between groups
(Arikan *et al.*, 2011)	48-60 y, OP: 35; Osteopenia: 37; Control: 35 (n = 107) Turkey	Case Control BMD	Control: 67.12 ± 11.6 μg/L OP: 66.16 ± 12.1 μg/L Osteopenia: 66.89 ± 15.5 μg/L	Se: no difference between groups
(Liu *et al.*, 2009)	45-65 y, OP: 123; Osteopenia: 127; Control: 31 (n = 290) China	Cross Sectional BMD	Control: 0.065 ± 0.01 mg/L OP: 0.067 ± 0.02 Osteopenia: 0.069 ± 0.02	Se: no difference between groups Se and BMD: no association

(Wolf *et al.*, 2005)	50-79 y (n = 11068) Women's Health Initiative Observational Study, USA	Cross Sectional Semi quantitative FFQ, total BMD	Se antioxidant: 85.9 ± 38.6 µg/L Total group Se: 94.1 ± 43.2 µg/d	Se and BMD: no association, after adjustments
(Ilich *et al.*, 2009)	Mean 60 y (n = 120) Croatia	Cross Sectional 3d food record, FFQ, LS and hip BMD	Control group: 104.0 ± 27.1 µg/L OP: 96.5 ± 33.8 µg/L	No association between BMD and Se deficiency
(Pedrera-Zamorano, 2012)	Mean 61 y (n = 335) Spain	Cross Sectional 7d food record, Ad-SoS phalanges, calcium intake	Se: 95.5 µg/L	Higher Se intake and Ad-SoS score: negative association when Ca^{2+} lower
Older Men (Beukhof *et al.*, 2016)	75-807, men (n = 387) Netherlands	Cohort Total and femoral BMD	Se: 91.9 µg/ SePP: 3.4 mg/L 0.5 % selenium-deficient	Se + SePP + BMD: positive association
(Wang *et al.*, 2015)	50-80 y, men, OP: 30; Osteopenia: 31; Control: 30 (n = 91) China	Case Control BMD	Control: 133.97 ± 29.0 ppm OP: 125.53 ± 22.8 Osteoporosis: 144.88 ± 26.8	Se: no difference between groups Se and BMD: no association
Older Adults (Ale-Ahmad *et al.*, 2018)	≥ 60 y, OP: 90; Control: 90 (n = 180) Iran	Case Control 2 ALOX12 SNPs, BMD	Control: 81.09 ± 25.6 µg/L SeFs OP: 57.58 ± 25.5 µg/L Se	Se: different between groups Se and BMD: positive association
RCT (Walsh *et al.*, 2021)	≥ 55 y, post-menopausal women (n = 120) with OP or osteopenia	RCT 6 mo uNTX:Cr, BMD, BTM, antioxidant and inflammatory markers	1:1:1 placebo: 50: 200 µg sodium selenite	Se: no difference in outcome measures
Systematic Review (Xie *et al.*, 2018)	KBD patients (n = 2931 from 15 RCT), UK	Systematic Review Efficacy of supplementation	Se salt, sodium selenite + vitamin E Se-yeast, sodium selenite and sodium selenite + vitamin C from high to lowest	Se+: all increased repair of metaphyseal lesions compared to placebo Overall evidence quality: low

Se: selenium; BM: body mass; CAT: catalase ; PTH: parathyroid hormone; TRAP: Tartrate-resistant acid phosphatase; CRP: c-reactive protein; SeMet: selenomethionine; BMD: bone mineral density; BMC: bone mineral content; BW: body weight; KBD: Kashin-Beck disease; PTHrP: Parathyroid hormone related protein ; ColX: type X collagen ; OC: osteocalcin; IGF-1: insulin-like growth factor 1; PMW: postmenopausal women; FFQ: food frequency questionnaire; LS: lumbar spine; OP: osteoporosis; CS: cross-sectional; FN: femoral neck; HR: hazard ratio; CC: case control; Ad-SoS: amplitude dependent speed-of-sound; TSH: thyroid stimulating hormone; T_4: thyroxine ; BMI: body mass index; T2DM: type 2 diabetes mellitus; SNP: single nucleotide polymorphism; uNTX:Cr: urinary cross-linked N-telopeptide of type 1 collagen corrected for urine creatinine.

1.9 Summary

Using a comprehensive, integrated approach, this PhD thesis will help elucidate the role of selenium in MSK ageing, using two different study designs, observational and experimental. This will be achieved by assessing biomarkers of selenium status in very old adults, exploring this association with MSK function cross-sectionally and prospectively, and determining the clinical effects of selenium supplementation on BTMs. This PhD thesis combines both epidemiological and experimental data and therefore provides a greater understanding of the effects of selenium on MSK function, combining different age populations and study designs.

1.10 Hypotheses, Aims and Objectives

The evidence collated over the past three decades indicates that selenium is essential for optimal biological functioning, as well as MSK function. Experimental results from animal models suggest that selenium supplementation can modulate bone and muscle metabolism. In human epidemiology studies, selenium status has been associated with BMD and fracture risk. However, despite the evidence so far, further studies are required to explore the relationship between selenium status and a wider panel of MSK measures such as TUG, HGS, disability, sarcopenia and BTMs. Therefore, this PhD thesis aims to address this in three chapters using two different study designs, which will be discussed in more detail in Chapter 2. These different skills and expertise will be gained through different universities; Sheffield University will provide the metabolic aspect through analysis of BTMs; Newcastle University will enhance skills related to nutritional assessments and dietary analysis; and Charité Universitätsmedizin Berlin will provide the molecular analysis for assessing the biomarkers of selenium status.

The outputs and knowledge generated from this PhD thesis could help build a robust evidence base that will inform and influence public health nutrition policy regarding selenium nutrition, as well as providing underpinning knowledge to relevant stakeholders and industrial partners.

Chapter 3: Selenium Status in Very Old Adults: The Newcastle 85+ Study

Research Question: Are there any associations between the biomarkers of selenium status (serum selenium, GPx3 activity and SePP) and selenium intakes? What are the determinants of the biomarkers of selenium status? Is there a relationship between serum selenium and the selenoproteins, GPx3 activity and SePP?

Hypothesis: It was hypothesised that there will be high prevalence of suboptimal selenium status in this population-based cohort of very old adults. Serum selenium will predict the selenoproteins (GPx3 activity and SePP) in a linear relationship due to the estimated limiting concentrations of serum selenium thereby not allowing for full expression of selenoproteins.

Objectives

1. Assess the selenium status (by measuring concentrations of serum selenium, GPx3 activity and SePP) and prevalence of suboptimal selenium status in baseline samples
2. Compare The Newcastle 85+ Study population selenium status with the DRVs for selenium
3. Explore the associations between selenium status (serum selenium, GPx3 activity and SePP) and dietary selenium intakes
4. Identify the determinants of the biomarkers of selenium status including socioeconomic, health and lifestyle factors
5. Quantify the relationship between serum selenium concentrations and GPx3 activity and SePP

Chapter 4: Selenium Status and MSK Function in Very Old Adults: The Newcastle 85+ Study

Research Question: Do biomarkers of selenium status (serum selenium, GPx3 activity and SePP) predict baseline MSK function (HGS, TUG, sarcopenia)? Do biomarkers of selenium status (serum selenium, SePP, GPx3 activity) predict the change in MSK function over 5 years?

Hypothesis: Participants in The Newcastle 85+ Study with optimal selenium status, compared to those with suboptimal selenium status, will have better MSK function, and a slower rate of change in MSK function over 5 years.

Objectives

1. Explore associations between selenium status (serum selenium, GPx3 activity and SePP) and MSK function (HGS, TUG, sarcopenia)
2. Identify the determinants of MSK function including biomarkers of selenium status, socioeconomics, health and lifestyle factors
3. Determine the relationships between selenium status and MSK function at baseline and the rate of change in MSK function over 5 years

Chapter 5: Selenium Status and Disability in Very Old Adults: The Newcastle 85+ Study

Research Question: Do biomarkers of selenium status (serum selenium, GPx3 activity and SePP) predict baseline disability? Do biomarkers of selenium status (serum selenium, SePP, GPx3 activity) predict the change in disability over 5 years?

Hypothesis: Participants in The Newcastle 85+ Study with optimal selenium status, compared to those with suboptimal selenium status, will have lower disability and a slower decline over 5 years.

Objectives

1. Explore associations between selenium status (serum selenium, GPx3 activity and SePP) and disability
2. Identify the determinants of disability including biomarkers of selenium status, socioeconomics, health and lifestyle factors
3. Determine the relationships between selenium status and disability at baseline and the rate of change in MSK function over 5 years

Chapter 6: The Effect of Selenium Supplementation on Biomarkers of Bone Turnover

Research Question: What are the long-term effects of Se-yeast supplementation on BTMs?

Hypothesis: Long-term selenium supplementation will influence BTMs in older people.

Objectives

1. Assess concentrations of biomarkers of BTMs in serum, at baseline, 6 months, and 5 years
2. Investigate the short-term (6 months) and long-term (5 years) effects of selenium supplementation (placebo, 100, 200, 300 μg Se-yeast/d) on the following BTMs: osteocalcin (OC), procollagen type 1 N-terminal propeptide (PINP), collagen type 1 cross-linked C-telopeptide (CTX) and bone alkaline phosphatase (BALP)

1.11 Appendix

Table 1.1: Selenium's role in the human body (adapted from Rayman, 2012; SACN, 2013; Köhrle *et al.,* 2005).

Physiological Area	Impact of Selenium
Thyroid gland	Synthesis and function of thyroid hormones Iodothyronine deiodinases (DIO) 1-3: Regulates and synthesises 3,3'5-triiodothyronine (T_3, active form) via conversion of thyroxine (T_4). DIO1 and DIO2 modulate the synthesis of active T_3 from T_4. Thioredoxin reductase: Regenerates antioxidant systems, nucleotide reduction in DNA production, maintains intracellular redox state, protects thyroid from reactive oxygen species (ROS), required for cell proliferation and gene expression regulation
Brain	Protective of lipid peroxidation and modulates neurotoxicity
Cardiometabolic	Involvement in metabolic syndrome, cardiovascular disease, blood pressure, haemorrhage
Antioxidative properties	Glutathione peroxidase (GPx) 1-4: Neutralises ROS (i.e. hydrogen peroxide) and modifies inflammation and oxidative damage
Antimutagenic and anticancerogenic	Reduces cancer risks and DNA damage
Reproduction	Improves sperm membrane and fertilizing ability Sperm mitochondrial capsule: Produced through GPx4: aids sperm maturation and protects against oxidative damage
Growth and development	Regulates development and differentiation Selenophosphate synthetase, SPS2: Helps produce donor for selenocysteine (Sec) synthesis (Fordyce, 2007)
GI tract	Aids rumen microorganisms and digestive enzymes, anti-inflammation and suppression of cytotoxic cytokines protects intestinal mucosa during chemotherapy
Liver, lungs, kidney	Protects liver from hepatic steatosis damage, inflammation and oxidative stress protection in the lungs and heavy metal detoxification in kidneys Selenoprotein P: selenium transporter between liver and other organs
Antimicrobial and antiparasitic	Antiviral, antibacterial, antifungal and anti-helminthic effects, immunity (Fordyce, 2007)
Musculoskeletal	Selenoprotein N: Located in endoplasmic reticulum (ER) membrane: first to be associated with inherited disorders including muscular dystrophy; gene mutations at multiple loci cause the diseases (Rederstorff, Krol and Lescure, 2006) Selenoprotein W: 10 kDa muscle protein required for muscle function (Whanger, 2009) Some selenoproteins buffer against oxidative damage proving protection against muscle weakness and bone deterioration (Beck *et al.*, 2007; Kim *et al.*, 2021)

Table 1.2: Summary of muscular diseases induced by selenium deficiency (adapted from Rederstorff, Krol and Lescure, 2006)

Animal	Disease	Symptoms
Calves, lambs	White muscle disease associated with low selenoprotein W concentration	calcification and stiffening of skeletal and cardia muscle tissue due to improper calcium regulation of muscle sarcoplasmic reticulum, weakness and recumbency
Foals	White muscle disease and yellow fat disease	Degradation of adipose tissue replaced by connective tissue
Pigs	Vitamin E and Selenium Deficiency (VESD) associated with low intakes (Rammell, Pearson and Bentley, 1988)	Mulberry heart disease, hepatosis dietetica, nutritional myopathy
Chickens, turkey, salmon	Nutritional muscular dystrophy associated with low selenium intakes	Cardiac and skeletal muscle affections
Chickens	Exudative diathesis (Huang *et al.*, 2015) associated with vitamin E and selenium deficiency	Greenish enema, subcutaneous haemorrhage
Lambs	Rigid lamb syndrome associated with vitamin E and selenium deficiency	Spine and limb rigidity
Guinea pig	Fatal myopathy associated with vitamin E and selenium deficiency	Lipid peroxidation, muscle affection, low glutathione peroxidase

Table 1.3: Additional details from studies providing evidence for the involvement of selenium in muscle and bone function including species, sample size, study design, selenium status, and/or dosage and outcomes.

Species	Demographics, Sample Size	Study Design, Duration	Dosage, Form	Outcomes
			Muscle	
Chicken (Huang *et al.*, 2015)	1 d, (n = 40/group) Nutritional muscular dystrophy (NMD)	Experimental 6 wk	Control: 10 µg Se/kg Se- Vit. E+: all-rac-α-tocopheryl acetate 50 mg/kg Se+ Vit. E-: sodium selenite 0.3 mg/kg Se+ and Vit. E+	Control 3 wk: 93 % NMD, 36 % mortality Se-: increased muscle damage; decreased antioxidant and GPx activity, selenoprotein mRNA
(Wu *et al.*, 2014)	1d, male (n = 180)	Experimental 55d 25 selenoprotein mRNAs	Se-: 0.033 mg Se/kg Se+: sodium selenite 0.2 mg/kg	Se-: downregulated 11 antioxidative selenoproteins
(Yao *et al.*, 2013a)	12 d embryo	Experimental isolated myoblasts	SELENOW overexpression	Increased selenoprotein expression and ROS interactions Reduced oxidative damage
Humans (Fedacko *et al.*, 2013)	Statin-associated myopathy treatment patients (n= 60)	Experimental 0-3 mo	CoQ10 + selenium	Se+: improved CoQ10 and SAM; reduced muscle pain weakness, cramps, fatigue Additional Se+: no effect on SAM
			Bone	
Rat (Skrajnowska *et al.*, 2022)	Sprague Dawley, male (n = 41)	Experimental Healthy femur or LNCaP prostate cancer cells	Se+: 0.5 mg Se/kg Long-term Se and Cu supplementation	Se+: no reduction in Se or Cu in femur of cancer rats; Fe and K increased (bone health importance) Se may slow osteolytic changes from metastasis
Human BMSC, hMSC-TERT (Ebert *et al.*, 2006)	Media for cell culture from foetal calf serum	Experimental	Standard cell cultures (5-10 % foetal calf serum) 5-10 nM selenite	Higher Se+: restored basal selenoprotein activity and mRNA and expression; reduced ROS accumulation Lower Se+: reduced expression GPx in osteoblasts; increased chromosome damage
Human (Galvez-Fernandez *et al.*, 2021)	> 20 y adults (n = 1365), Spain	Cross Sectional	Low BMD 82.8 µg/L High BMD 85.7 µg/L	Se and BMD: negative association Se < 105 µg/L; positive association Se > 105 µg/L Se and Fracture: positive association Se > 100 µg/L
(Wu *et al.*, 2021)	≥ 40 y (n = 2938) NHANES, US	Cross Sectional	Se Intake: 102 µg/d Whole-blood Se: 197 µg/L Serum Se: 131 µg/L	Se intake and total femur BMD: positive association, especially post-menopausal women Se intake and whole-blood and FRAX score: negative association All Se markers and fracture history: negative association
(Hoeg *et al.*, 2012)	Post-menopausal women (n = 1144), OPUS, Europe (from 5 cities)	Cohort, Prospective	Se: 94.3 µg/L SePP: 3.2 mg/L	Se and SePP and BTM: negative association Se and total hip and lumbar BMD: positive association Se and SePP and non-vertebral and vertebral fractures: no association

NMD: nutritional muscular dystrophy; Se+: selenium-supplemented; Se-: selenium-deficient; Vit.E+: vitamin E supplemented; Vit.E-: vitamin E deficient; GPx: glutathione peroxidase; SePP: selenoprotein P; SELENOW: selenoprotein W; CoQ10: co enzyme Q10; SAM: statin-associated myopathy; LNCaP: Lymph Node Carcinoma of the Prostate ; Cu: copper; Fe: iron; K: phosphorus; BMSC: bone marrow stromal cell; hMSC-TERT: telomerase-immortalized human mesenchymal stem cells; BMD: bone mineral density; NHANES: national health and nutrition examination survey; FRAX: fracture risk assessment tool

Chapter 2. General Methodology

2.1 Overview of Study Designs for each Experimental Chapter

The framework for the experimental chapters (Chapters 3-6) of this PhD thesis, including both study designs, observational and experimental can be seen in Figure 2.1. Briefly, Chapters 3-5 involved cross-sectional and longitudinal aspects from an observational study, The Newcastle 85+ Study. These analyses were the first known to assess three biomarkers of selenium status (serum selenium, GPx3 activity and SePP) in a very old population and explore the baseline associations with MSK function and disability, as well as the rate of change in these outcomes over time. Chapter 6 involved performing secondary analyses using a RCT, The PRECSE study (Prevention of Cancer by Intervention with Selenium). This final experimental chapter of the thesis explored the effect of selenium supplementation on BTMs, analysed as per protocol. In accordance with my focus on bone health, as part of my PhD programme, I visited the Bone Biochemistry Laboratory in the first year of my PhD programme to shadow the laboratory technician. The analyses I shadowed used the Cobas E411 analyser to measure BTMs (CTX, PINP, OC) in serum plasma and formed part of a RCT that explored the effects of selenium supplementation on BTM's in osteoporotic, post-menopausal women (Walsh *et al.*, 2021), see Chapter 1, Section 1.8.

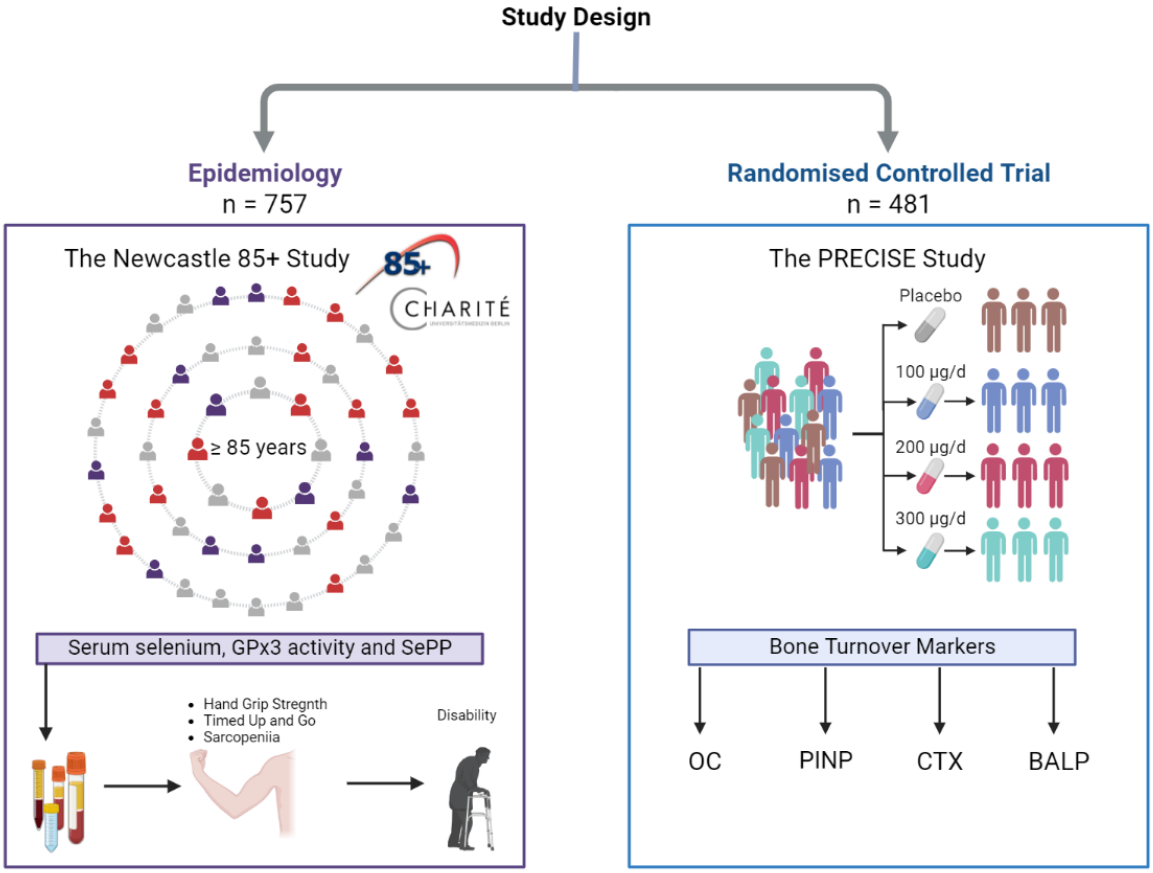


Figure 2.1: A schematic depicting the experimental chapters in this PhD thesis, created in Biorender.com. The epidemiology study design used The Newcastle 85+ Study, whilst the randomised controlled trial used The PRECISE Study (Prevention of Cancer by Intervention with Selenium). Biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity and selenoprotein P) were measured in serum selenium of 757 participants aged 85 years and older. These were used to explore associations with musculoskeletal measures (hand grip strength, Timed-Up-and-Go, sarcopenia and disability) at baseline and up to 5 years. In The PRECISE Study, selenium yeast supplementation was provided as a placebo, or 100-300 µg/d for 5 years and plasma selenium and bone turnover markers (osteocalcin, procollagen type I N-terminal propeptide, collagen type I cross-linked C-telopeptide and bone alkaline phosphatase) were measured in 481 non-fasted samples at baseline, 6 months, and 5 years.

2.2 Study Methodology

2.2.1 The Newcastle 85+ Study

2.2.1.1 The Newcastle 85+ Study Details

The Newcastle 85+ Study is a longitudinal study of health outcomes and trajectories which consists of 1042 participants born in 1921. Participants were registered with GPs from North Tyneside or Newcastle upon Tyne primary care trusts and were recruited from 64 centres (Northeast England) allowing the cohort to be sociodemographically representative of the general UK population. The only exclusions were individuals with end-stage terminal illness and those who could not be visited by a lone nurse without posing risks. Of the suitable individuals, a letter was sent out and initial assessment was undertaken between 2006 and 2007 (Figure 2.2A). The aims of The Newcastle 85+ Study were to 1) assess the spectrum of health in the very old (85 years and over); 2) explore health outcomes and trajectories through time and determine any associations between these outcomes and the psychosocial, biological, and clinical measures taken; 3) identify factors contributing to the maintenance of health and independence; and 4) advance understanding of the biological nature of human ageing (Collerton *et al.*, 2007). A timeline and overview of the study, including attrition can be seen in Figure 2.2B. Sample size was based on a pilot study estimating the expected recruitment number of those turning 85 years old within a single year and considering blood samples and analyses, statistical calculations from the pilot study indicated sufficiency in estimating main effects and, a similar study in 85-year-olds (Leiden 85+ Study) recruited 599 participants which proved to be sufficient to draw statistically significant conclusions (von Faber *et al.*, 2001; Collerton *et al.*, 2007).

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and The Newcastle and North Tyneside local research ethics committee (06/Q0905/2) approved the research and all participants provided written and informed consent. For those who lacked capacity, a carer or relative provided consent in line with the UK Mental Capacity Act 2005. Capacity was also gauged by the research nurse during the first visit using a consent checklist and pathway, and information from the participant, family and other close members.

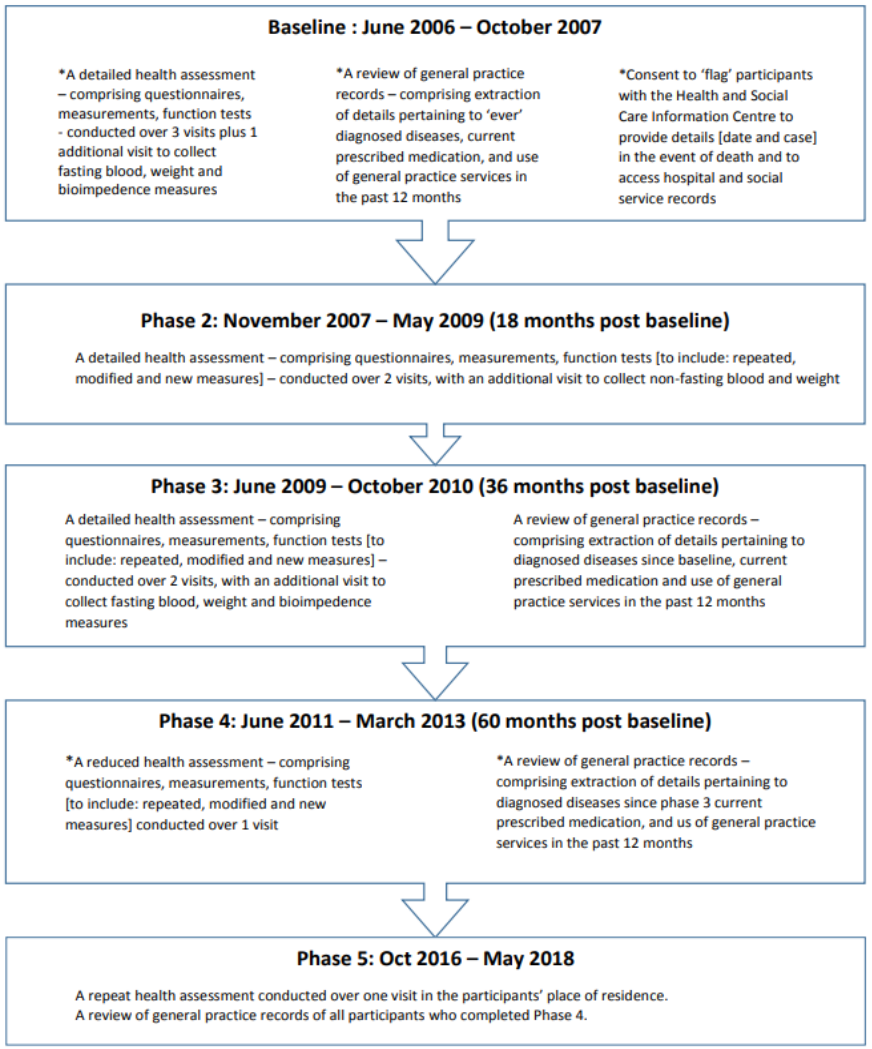
2.2.1.2 Socioeconomic, Lifestyle and Health Measures

Assessments were undertaken in each participant's place of residence (home or an institution) by research nurses who underwent 6 weeks of training (Collerton *et al.*, 2009). Participants who were temporary hospital patients were assessed following discharge. Questionnaires, functional tests, fasting blood samples, medical record reviews, dietary intakes and body weight measurements were taken at the initial health assessment and three other visits (1.5, 3, 5 year), excluding dietary intakes which were only taken at baseline (Table 2.1) (Collerton *et al.*, 2007; Martin-Ruiz *et al.*, 2011). General practice medical records were analysed to obtain information on current medication, service usage and disease information. Assessments took place over one month, unless participants were particularly frail in which assessments could be spread over a longer duration. GPs were informed about the participants involvement, giving consent was provided, and abnormal assessment findings were reported. The inter-reliability was assessed in GP record extraction between the trained nurses and indicated a moderate-to-good agreement (Collerton *et al.*, 2007).

Participants were classified into the National Statistics Socio-Economic Classification (NS-SEC) three class scheme based on their previous main occupation, these were: higher managerial, administrative and professional occupations (Class 1); intermediate occupations (Class 2); and routine and manual occupations (Class 3) (Chandola and Jenkinson, 2000). Education was determined by the duration of full-time higher education. Self-rated health was assessed from baseline through to 5 years, and was categorised as excellent/very good, good, fair/poor. Cognitive impairment was classified as scores ≤ 25 points out of 30, on the Standardised Mini-Mental State Examination (SMMSE). This questionnaire is a dementia-screening instrument and asks questions related to the performance of daily activities of living (ADL), basing these on time and place orientation, attention, recall and language (Vertesi *et al.*, 2001). The SMMSE questionnaire was taken at baseline, 3 and 5-year follow-ups. BMI was calculated as kg weight/m^2 height at baseline through to 5 years. Fat-free mass (FFM) (kg) was calculated using the Tanita-305 body fat bioimpedance instrument (Tanita Corp., Tokyo, Japan) and was available at baseline and 3 years. Medication use, including non-prescribed medication was determined from baseline through to 5-year follow-ups using GP records and packaging at participant interviews. Smoking and alcohol

questionnaires were taken at baseline and involved habit status, type of product, frequency, past habits and duration. Physical activity was assessed from baseline through to 5 years, using a purpose-built questionnaire. The questionnaire was validated in this population by trialling a pilot study, in addition to comparing outcomes to an objective measure using accelerometery (Innerd *et al.*, 2015). Participants answered questions regarding how regularly they participated in mild, moderate, and highly energetic activities, such as light gardening, heavy housework, and swimming, respectively. The available answers and corresponding scores were as follows: ≥ 3 times per week (3); 1-2 times per week (2); 1-3 times per month (1); and hardly ever (0). Overall scores out of 18 were calculated using the following formula: (3 × highly energetic activity score) + (2 × moderately energetic activity score) + mildly energetic activity score. This was then categorised as low/moderate/high (score 0–1/score 2–6/score 7–18, respectively). Total energy intake (kcal) and protein intake (g) were determined using the 24 h MPR. Disease count was calculated using a select list of chronic diseases (Table 2.2). If a participant had a disease, a score of 1 was given, otherwise a score of 0 was given (Collerton *et al.,* 2009). A molecular marker of inflammation, high sensitivity CRP (hsCRP), which is known to affect MSK function was included in fasted samples at baseline through to 3 years (Koh *et al.*, 2005; Kim *et al.*, 2018).

A)



B)

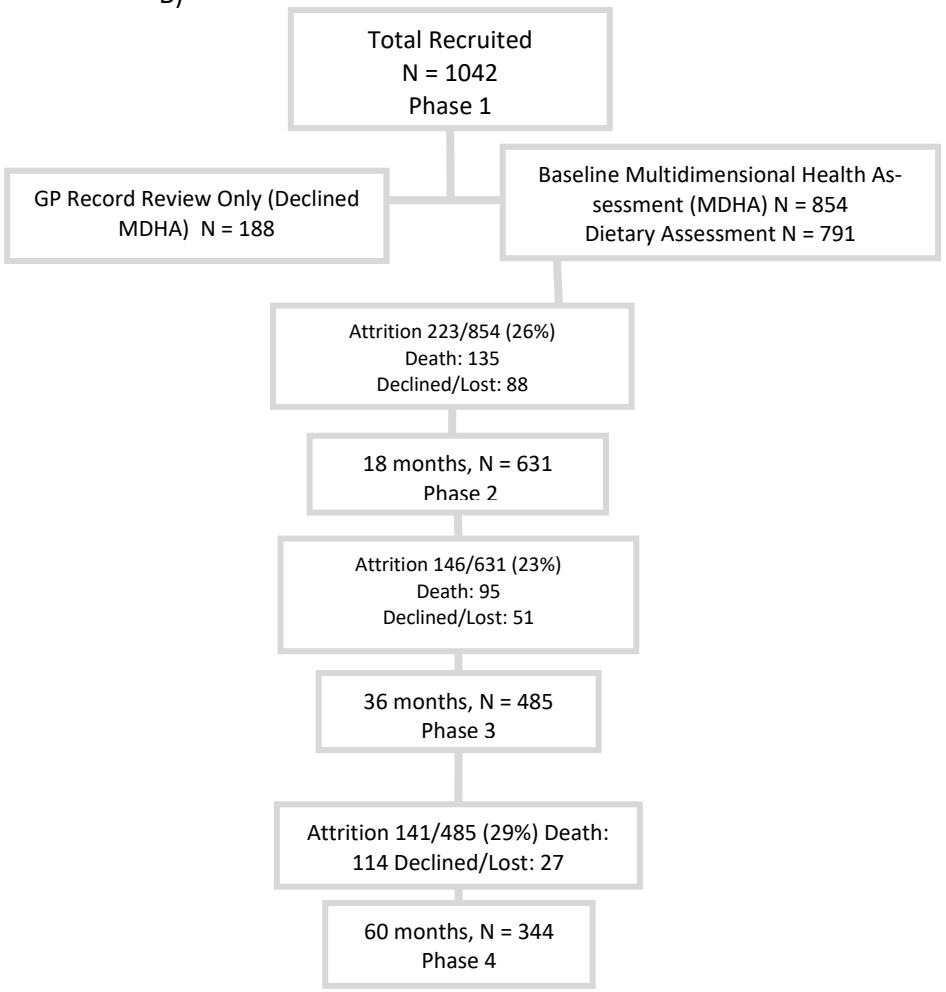


Figure 2.2: A) Study timeline and overview. Health assessments and reviews of general practice records were conducted by trained research nurses in participants' usual place of residence. **B)** Flow diagram of participant recruitment and sample size at each stage.

Table 2.1: Assessments including questionnaires, measurements and blood tests taken at baseline by trained nurses (Martin-Ruiz *et al.*, 2011; Collerton *et al.*, 2007).

Assessment	
Questionnaires	• Socio-economic status (National Statistics Socio-Economic Classification (NS136 SEC) three class scheme [Higher managerial, administrative and professional occupations (Class 1); intermediate occupations (Class 2); and routine and manual occupations (Class 3) based on past main occupation. • Family data • Physical health (global health status, longstanding illness, angina, shortness of breath, falls, generalised pain, joint pain, fractures, incontinence, vision and hearing) • Psychological health (depression) • Disability • Diet • Oral health • Lifestyle (smoking, alcohol and exercise) • Social support and participation • Use of health and social care
Measurements and Function Tests	• Weight • Bio-impedance (body composition-fat and water) • Waist and hip circumference • Tooth count • Blood pressure • Hand-grip strength • Walking test (TUG; timed 'up and go' test) • Cognitive function (mini-mental state examination and computerised assessment of memory and attention (CDR battery)) • Electrocardiogram (ECG) • Spirometry and oximetry • Demi-span
Blood Tests	Overnight fast, if possible, to analyse: • Routine haematology and biochemistry: full blood count; creatinine and electrolytes; liver panel; bone panel; glucose; glycosylated haemoglobin • Lipid profile: cholesterol, triglycerides, high- and low-density lipoproteins, apolipoproteins (A1 and B) • Thyroid function: free T_4, free T_3, reverse T_3, TSH and TPO antibodies • Inflammatory markers: High sensitivity CRP, rheumatoid factor, cytokines (TNF-α and Interleukin 6) • Cortisol • Nutritional markers: Vitamins B_2, B_6, B_{12}, C and D, ferritin, red cell folate and homocysteine • Biomarkers: DNA repair capacity, telomere length, F2-isoprostane (marker of oxidative stress) • Markers of immunosenescence: T cell oligoclonality and lymphocyte subpopulation distributions (senescent T-cells, memory T-cells and NKcells).

CDR: Clinical Dementia Rating; Free T_4: free thyroxine; Free T_3: free triiodothyronine; TSH: Thyroid Stimulating Hormone; TPO: thyroid peroxidase antibodies CRP: c-reactive protein; TNF-α: Tumour Necrosis Factor-alpha NK cells: Natural Killer cells

Table 2.2: Diseases included in the disease count variable (Collerton *et al.*, 2009).

Diseases Included[a]
Hypertension
Ischaemic Heart Disease
Cerebrovascular disease
Peripheral vascular disease
Heart failure
Atrial flutter or fibrillation
Arthritis (osteoarthritis or cervical or lumbar spondylosis or rheumatoid arthritis or other arthritis or non-specified arthritis)
Osteoporosis
Chronic obstructive pulmonary disease or asthma
Other respiratory disease
Diabetes
Hypothyroidism or hyperthyroidism
Cancer diagnosed within past five years (excluding non-melanoma skin cancer)
Eye disease (cataract or age-related macular degeneration or glaucoma or diabetic eye disease or registered blind or partially sighted)
Dementia
Parkinson's disease
Renal impairment

[a]General practice diagnoses or health assessment defined ischaemic heart disease, diabetes, and thyroid disease; electrocardiogram was used to diagnose atrial fibrillation or flutter; estimated glomerular filtration rate <30 ml/min/1.73 m^2 diagnosed renal impairment; haemoglobin concentration of < 11.5 g/dl (< 115 g/l) diagnosed anaemia. For the remaining diseases presence was derived from record review data.

2.2.1.3 Dietary Assessment

At baseline (2006/2007) 24 h MPRs were used to assess dietary intake in 791 participants (62 % females, 38 % males) within their usual residence. These were performed on two separate weekdays (Monday-Thursday, excluding Fridays and weekends), separated by a week. A structured interview was used to perform these analyses in a retrospective manner to collect information on habitual consumption of food, beverages and supplements over 24 h. Data was entered twice, independently to reduce errors and the Photographic Atlas of Food Portion Sizes (Nelson, 1997) was used to estimate portion sizes. Using the 2-day mean intakes, energy, macronutrient and micronutrient intakes were predicted using The McCance and Widdowson's Composition of Food (McCance, 2002) alongside a Microsoft Office Access database containing nutrient compositions of frequently consumed foods. A pilot study using the same cohort found that 24 h MPR was more reliable than a FFQ (Adamson *et al.*, 2009) where most participants (85 %) revealed that they felt the 24 h MPR replicated their habitual intakes of food and drink (Mendonça *et al.*, 2016b). As with all dietary assessments, misreporting is a limitation. Using cut-off values derived from energy intake (EI) estimations divided by estimated basal metabolic rates (BMR_{est}) (EI:$BMR_{\mathbf{est}}$) can detect misreporters (Goldberg *et al.*, 1991). In older participants, a more accurate technique is the Fredrix equation, which was used in this study (Mendonça *et al.*, 2016a); EI:BMR_{est} <

1.05 and < 2.0 indicate under and over reporters, respectively (Siervo *et al.,* 2014). Additionally, supplement data was obtained from individuals and was created as a binary variable where a score of 1 indicated supplement use, whilst a score of 0 indicated no supplementation use. Selenium (µg) was estimated as a daily intake; values were based on DRVs of selenium including those consuming below the LRNI (40 µg/d), between the LRNI and up to the RNI (60 µg/d for females and 75 µg/d for males) and, the RNI and above (IoM, 2000).

2.2.1.4 MSK Function

All MSK measures described in this section were assessed at baseline, 1.5, 3 and 5 years, except for sarcopenia and severe sarcopenia prevalence, which were available at baseline and 3 years. To assess muscle strength, HGS was measured using a hand-held dynamometer (Takei A5401 digital 0-100 kg x 0.1 kd LCD) (Figure 2.3). Participants stood with their arm hanging beside their body whilst their elbows were at 180 ° angles and squeezed the dynamometer as hard as possible in each hand. Two measurements were taken (kgF) for each hand and the mean measurement of all four measurements was calculated and used for analyses. In the TUG test (Mathias, Nayak and Isaacs, 1986; Greene *et al.*, 2013), participants were asked to rise from a chair (46 cm from the floor with armrests), and as quickly and safely as possible, walk 3 m, turn 180° and return to be seated (Figure 2.3). To standardise the results, time was recorded (s) with a stopwatch from the first attempt to rise from the chair and ended when the participants returned and sat on the seat.

Muscle mass was estimated via bioelectrical impedance analysis (BIA) using a Tanita-305 body fat analyser (Tanita Corp., Tokyo, Japan). BIA strongly correlates with magnetic resonance imaging (MRI) which is considered a gold standard measure for skeletal muscle mass (SMM) (Janssen, Heymsfield and Ross, 2002). During the assessment, participants stood barefoot on the metal sole plates of the device. Bioimpedance data (Ω) was input into the equation from Janssen *et al.*, (2000) to estimate SMM (kg), which was then adjusted for height to calculate SMM index values (SMMI) ($kg.m^2$). To assess sarcopenia status, SMMI, HGS and TUG performance were interpreted according to the EWGSOP2 cut-offs (Cruz-Jentoft *et al.*, 2019). Gait speed (m/s) was estimated from TUG performance, using the

following formula: 6/[TUG time]) * 1.62; a gait speed of < 0.8 m/s indicated slow gait speed (Cruz-Jentoft *et al.*, 2019). Cut-offs of < 8.87 kg.m^2 and < 6.67 kg.m^2 were used to identify low SMMI (Dodds *et al.*, 2017), and values of < 27 kg and < 16 kg (Cruz-Jentoft *et al.*, 2019) were used to determine low HGS for men and women, respectively. Severe sarcopenia was established in subjects demonstrating poor TUG performance, below the EWGSOP2-derived cut-off of ≥ 20 s (Cruz-Jentoft *et al.*, 2019). Thus, sarcopenia was identified in participants demonstrating both low SMMI and HGS, below predetermined, gender-specific cut-offs.

Figure 2.3: Takei Hand dynamometer used to test hand grip strength and examples of Timed Up and Go tests (TUG). TUG schematic taken from Dzhagaryan *et al.*, (2015).

The disability score was produced by summing the scores of 17 activities that comprised of self-reported ability to perform activities that were predominately derived from the Groningen Activity Restriction Scale (Kempen *et al.*, 1996). Activities included basic activities of daily living (BADL), instrumental activities of daily living (IADL), including mobility, lower limb mobility, chair rises, stair climbing, grocery shopping and walking 370 m (Table 2.3). The questionnaire was worded to assess a participant's maximal capacity of these activities by asking "can you", rather than, "do you" (Glass, 1998). Participants then answered the questionnaire where a score of 1 indicated difficulty in performance or inability of an activity, and a score of 0 indicated no difficulty in performance; a maximum score of 17 indicated greatest disability. The total number of activities that were difficult to perform or required personal help or a walking aid/appliance were summed to determine the disability score, thus difficultly with any activity classified the participant as having a disability (Jagger *et al.*, 2011; Kingston *et al.*, 2012).

Table 2.3: The 17 activities of daily living used in the self-reported questionnaire to assess disability.

Response (Score)	BADL	IADL	Mobility
Can do on own without difficulty (0)	Feeding self – including cutting up of food	Light housework	Getting around the house
Can do on own but with difficulty (1)	Washing face and hands	Heavy housework	Going up and down stairs
Can do on own but with aid/appliance, unable to without personal help (1)	Washing all over	Grocery shopping	Walking at least 400 m
	Getting in and out of bed	Preparing and cooking hot meals	
	Getting on and off the toilet	Taking medication	
	Getting in and out of a chair	Managing finances	
	Dressing and undressing		
	Cutting own toenails		

Edited from (Kingston *et al.*, 2012) BADL: basic activities of daily living; IADL: instrumental activities of daily living

2.2.1.5 Biomarkers of Selenium Status

Baseline blood samples from 2006/2007 (n = 757) that had been stored at -80 °C were analysed for the biomarkers of selenium status (serum selenium, GPx3 activity and SePP). The literature was reviewed by exploring similar studies with long storage durations to ensure the stability of serum selenium for the appropriate analyses. There were few studies storing samples for as long as 16 years, however, the studies available did suggest that

selenium was stable over time (up to 10-15 years) (Appendix Table 2.1). Likewise, serum SePP is stable during repeated freezing over long periods of time and is preferred over plasma SePP due to improved consistency potentially due to the interactions of SePP with plasma proteins or, reduced SePP proteolysis (Saito, 2004). Furthermore, upon a personal communication with Prof. Lutz Schomburg at Charité Universitätsmedizin Berlin as part of a collaboration, I was reassured of the stability of the biomarkers. Prof. Schomburg noted that other samples of serum stored for long durations retained linear correlations between the biomarkers of selenium status suggesting stability: "We are convinced that the different selenium status biomarkers are stable in frozen samples, which applies to total selenium, SePP and GPX3 activity. This notion is supported by the good correlation we measure with samples that are very old, often more than 10 years. We have measured samples that had been taken many years before analysis, and which still yielded nice and linear correlations between the different selenium status biomarkers (Demircan *et al.,* 2021; Cabral *et al.,* 2021; Schomburg *et al.,* 2019; Hughes *et al.,* 2015). In case one of the biomarkers would not survive longer storage, such linear correlations would get lost."

Following this, serum samples were sent for analyses. These included serum selenium (µg/L), glutathione peroxidase 3 activity (U/L) and selenoprotein P (mg/L). I was also invited to visit the laboratory where the analyses took place; this was an insightful and invaluable experience that enhanced my understanding and improved my PhD programme experience (Figure 2.4). During the visit I was able to view the equipment used for the analyses and perform analyses on the bench top total reflection x-ray fluorescence (TXRF) machine using my own blood sample to compare to a normative sample from a healthy population. This experience, along with the Sheffield lab visit (Section 2.1), helped fill the gaps in my PhD programme around laboratory exposure and techniques.

Total serum selenium was measured using a bench-top TXRF (T-Star, Bruker Nano GmbH, Berlin, Germany) for 2000 s per sample. As a standard, a gallium buffer (1000 µg/L) was used to dilute participant serum to a 1:2 ratio (selenium:buffer). Eight µl of the diluted solution were applied to polished quartz glass slides (Bruker Nano GmbH, Berlin, Germany) and these were dried overnight in an incubator at 37 °C. As a control, serum standard Seronorm was used (Sero AS, Billingstad, Norway). The inter- and intra-assay CVs were below 10 %

(Demircan *et al.*, 2021). GPx3 activity was analysed using coupled-enzyme reaction measuring nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) consumption. Serum samples were incubated at 20 °C with 0.27 mg/ml NADPH, 1 mM sodium azide (NaN_3), an enzyme buffer containing 3.4 mM reduced glutathione and 0.3 U/ml glutathione reductase. The reaction was initiated using hydrogen peroxide. At 340 nm, reductions in UV absorption were proportional to NADPH consumption, which reflected the activity in the 5 µl of serum. The mean activity was reported, as the measurement was triplicated, and a control was provided using standard serum measured in triplicates. The inter-assay CV was below 15 % and intra-assay CV was below 10 % (Schomburg *et al.*, 2003). Serum SePP was analysed using a validated immunoluminometric, commercial enzyme-linked immunosorbent assay (ELISA) (selenOtestTM, selenOmed GmbH, Berlin, Germany). A sandwich ELISA technique using 5 µl of serum and human SePP-specific monoclonal antibodies was used in addition to three controls that represented the assay's working range. A luminometer LB952T (Berthold Technologies, Oak Ridge, TN, USA) measured chemiluminescence for 1 s and, based on standards of known concentration that were measured in each assay run, a standard curve was fitted to the data. Each sample was measured in triplicate, and the mean SePP concentrations were calculated. The inter- and intra-assay CVs were below 10 % for low and medium SePP concentrations and below 20 % for the high SePP concentration control (Hoeflich *et al.,* 2010; Hollenbach *et al.,* 2008).

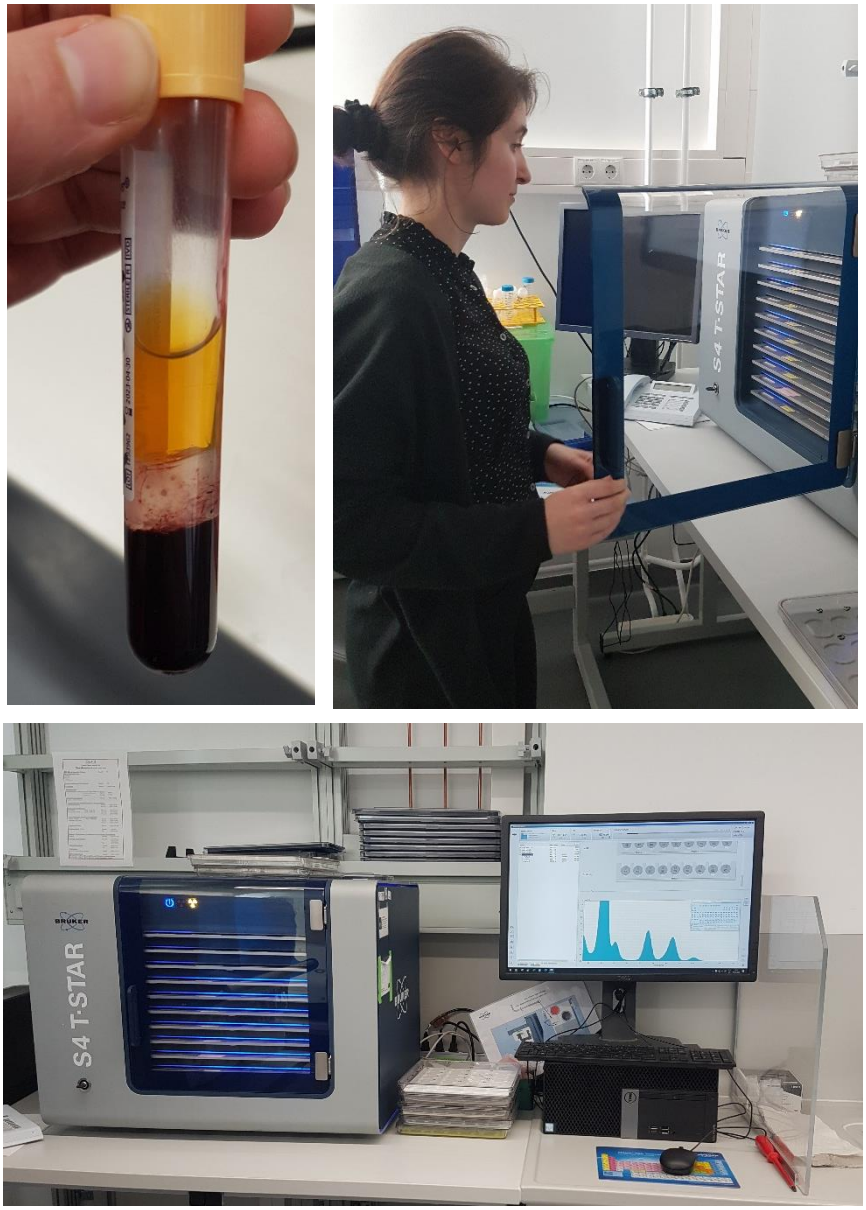


Figure 2.4: The visit to Prof. Lutz Schomburg's laboratory at the Charité Universitätsmedizin Berlin, to experience using and understanding the bench-top total reflection x-ray fluorescence (TXRF) used for the serum selenium analyses.

2.2.2 The PRECISE Study (Prevention of Cancer by Intervention with Selenium)

2.2.2.1 PRECISE Study Details

This part of the PhD thesis uses the second study design, a RCT which formed another collaboration with a Danish research team. As a secondary analysis, funding was acquired to analyse BTMs in this population. The data was obtained through a collaboration between my PhD supervisors based in Sheffield who have expertise in BTM analyses and in knowing

this, I thought that using a RCT would provide invaluable information to explore the effects of selenium supplementation on BTMs. I agreed with the Danish research team that I would analyse the data and interpret the results as part of my PhD thesis.

The primary aim of the initial pilot study, Prevention of Cancer by Intervention with Selenium (PRECISE), was to assess the viability for a full-scale, international, randomised trial. It was hypothesised that selenium supplementation would reduce cancer risk in healthy adults during a 5-year intervention. The trial was registered (ClinicalTrials.gov ID: NCT01819649) and began in 1998 and ended in 2004 and was organised by the Selenium Centre, Odense University Hospital, Denmark. The regional Data Protection Agency and Scientific Ethical Committees of Vejle and Funen counties approved the study (Journal number. 19980186). Follow-up studies included thyroid function, lipid biomarkers, cardiovascular health, mortality at 10 years, and BTMs at 5 years (Rayman *et al.*, 2018; Cold *et al.*, 2015; Rayman, 2011). This section will focus on the overall study methods including BTM measurements whilst Chapter 6 will focus on the statistical analyses for my PhD programme.

Participants

Using a random sample from the Danish Civil Registration, invitation letters were sent out and 2897 60–74-year-old participants from the County of Funen were invited between November 1998 to June 1999. Of these, 630 accepted and were screened for inclusions (Table 2.4). Exclusion criteria were: i) a Southwest Oncology Group performance status score greater than 1; ii) active liver or kidney disease (alanine -aminotransferase, alkaline phosphatase, bilirubin or urea two standard deviations above the normal reference range); iii) previous diagnosis of cancer (excluding non-melanoma skin cancer); iv) diagnosed HIV infection; v) receiving immunosuppressive therapy; vi) unable to understand written and spoken information; vii) receiving ≥ 50 μg/d of selenium supplements in the previous 6 months (by patient report). A non-fasted blood sample was collected from those meeting the inclusion criteria and placebo yeast tablets were provided during a 4-week run-in phase to determine compliance. At the second visit, participant satisfaction and adherence (> 80 % of tablets taken were assessed using tablet counts (Cold *et al.*, 2015). Following this, 491 participants

met the inclusion and adherence criteria for continuation and all participants provided written informed consent.

Table 2.4: Inclusion and exclusion criteria for The PRECISE Study.

Inclusion	Exclusion
60-74 years	A Southwest Oncology Group performance status score > 1, indicating impairment in general well-being and activities of daily life
Any sex	Active liver or kidney disease (alanine aminotransferase, alkaline phosphatase, bilirubin or urea two standard deviations above the normal reference range)
Healthy volunteers	Previous diagnosis of cancer (excluding non-melanoma skin cancer)
World Health Organization performance status 0 or 1	Diagnosed HIV infection
No active liver- or kidney disease (serum alanine aminotransferase (ALAT), alkalic phosphatase, bilirubin, creatinine or urea within 2 S.D of laboratory reference range)	Receiving immunosuppressive therapy
No previous cancer diagnosis	Unable to understand written and spoken information
No known HIV-infection	Receiving ≥ 50 µg/d of selenium supplements in the previous 6 months (by patient report).
Participant must understand oral and written information	
Participant must not use selenium supplementation of above 50 µg/d	
Participant must give written consent prior to inclusion	

S.D: standard deviation; HIV: human immunodeficiency virus

2.2.2.2 Randomisation

The eligible 491 suitable participants were enrolled into a randomised, double-blinded, non-stratified, single-centre, parallel clinical trial with four experimental arms distributed as 1:1:1:1 placebo (yeast tablet; n = 126), 100 µg selenium/d (n = 124), 200 µg selenium/d (n = 122) or 300 µg selenium/d (n = 119). One participant was removed (see Chapter 6, Section 6.3.6 for details on rationale), therefore, participants with BTMs at baseline were n = 124, 122, 118 and 117 for placebo, 100, 200, 300 µg selenium/d, respectively, giving a total of 481 participants (Figure 2.5). The study used computer-generated, blocked and non-stratified randomisation conducted by the Division of Epidemiology and Biostatistics, Arizona Cancer Centre, University of Arizona. Couples living at the same address were provided with the same intervention supplementation dose for practical reasons i.e. to prevent mixing of selenium dosages. The responsibility of distributing tablets was placed with pharmacists at

Odense University Hospital. Participants, research staff and investigators were blinded to supplementation doses (Cold *et al.*, 2015).

Intervention

The selenium was provided in tablet form as Se-enriched yeast in 100, 200 and 300 µg/d doses. These doses were suggested to be safe as the tolerable upper intake level for adversity, set by the Institute of Medicine was 400 µg/d (IoM, 2000) (although this has since been updated to 255 µg/d, ESFA, 2023). The SelenoPrecise© tablets (prepared by Pharma Nord ApS) contained 54-60 % of total selenium as SeMet with unknown selenocompounds providing the remainder (Larsen *et al.*, 2004). This form of selenium was selected based on the reduction in cancer risk in the Nutritional Prevention of Cancer (NPC) trial (Clark *et al.*, 1996) and has absorption and retention properties similar to wheat-based selenium (Levander *et al.*, 1983). The placebo was identical to the supplementation tablets and consisted of inactive spray-dried baker's yeast (250 µg yeast placebo, 80 µg cellulose, 65 µg dicalcium phosphate and ≤ 5 µg of inactive ingredients). Smell and taste were matched by coating all tablets in titanium oxide and tablets were packaged in 28 tablet blister packs.

2.2.2.3 Socioeconomic, Lifestyle and Health Measures

Participant characteristics were determined at baseline and collected during visits with trained research nurses. Further evaluations were performed at 6, 12, 18, 24, 36 and 60 months which included assessment of medical status, tablet count, records of side effects and the provision of new tablets, as previously described in Cold *et al.*, (2015). BMI was calculated as kg weight/m^2 height. Participants were classified into education status using surveys based on time spent in education after public school (0 = no, 1 = 1-3 years, 2 = 3-4 years, 3 = > 4 years). Living status was determined as living alone (0 = no, 1 = yes). Smoking status was determined at baseline (0 = never 1 = previous 2 = current). Alcohol intake was reported as standard drinks per week. Medication usage (thyroid, antiresorptives (AR), glucocorticoids (GC), hormone replacement therapy (HRT)) was classified as a binary variable depending on the medication (0 = no, 1 = yes), with thyroid medication having three categories (0 = none, 1 = levothyroxine, 2 = antithyroid drugs). Supplementation use was classified

as a binary variable (0 = no, 1 = yes) for each supplement type (calcium, vitamin D, multivitamins).

2.2.2.4 Plasma Selenium

Non-fasting blood samples were collected at baseline, 6 months and 5 years. Plasma was prepared and stored at -80 °C. Total selenium in plasma (µg/L) was assessed using LGC Limited inductively-coupled-plasma mass spectrometry (ICP-MS) with external calibration, as described in Cold *et al.*, (2015). A micro-flow quartz concentric nebuliser functioning at 0.1 rpm in a pumping mode and a Scott double-pass spray chamber cooled to 2 °C was used to introduce sample dilutions into plasma. To reduce the interferences on the selenium isotopes (77Se, 78Se and 82Se), collision cell ICP-MS (7700 × ; Agilent Technologies) was used and the isotopes were measured in He-mode and H^2-modes with 3 replicate measurements. Industrial drift was corrected for by using an internal standard of germanium, which was added online. In addition, 2 % methanol (Optigrade; LGC) was added online and mixed to compensate for carbon content discrepancies between samples and standards that may alter the efficiency of ionisation. A stock solution of 1000 mg/kg Se (Romil) was used to prepare fresh calibration standards (0–50 ng/g Se) by gravimetric dilution in 0.5 % (v/v) nitric acid (UltraPure; Romil). Quality control of total selenium measurements was determined using a matrix-certified reference material, BCR-637 human serum, with a certified selenium concentration of 81.0 ± 7.0 µg/L Se (density corrected 79.1 ng/g). The Se concentration for BCR-637 was 78.3 ± 2.7 µg/L Se (sixteen independent replicates), indicating good accuracy of the method. The inter-assay CV was 3.4 %. High-selenium concentrations had an intra-assay CV of 0.5 % whilst low-selenium concentrations had an intra-assay CV of 3 %.

2.2.2.5 Biomarkers of Bone Turnover

Non-fasting blood samples were collected at baseline, 6 months and 5 years and the serum was analysed from at the Bone Biochemistry Laboratory, Department of Oncology and Metabolism, University of Sheffield (England). The BTMs: N-MID OC (measuring the large 1-43 N-mid and the intact OC); PINP (measuring the trimer only); CTX; and BALP were measured

using the immunodiagnostic systems (IDS-iSYS) automated immunoassays (Immunodiagnostic Systems, Boldon, UK). OC, N-midfragment was measured in a reproducible manner with a fully automated assay requiring 50 µl per sample. PINP requires a sample size of 20 µl and is often referred to as the most sensitive and specific marker of bone formation, likely coinciding with its recommendation by IOF and IFCC. Finally, BALP was measured using the Ostase assay, requiring 50 µl and provides high sensitivity and reproducibility of results independent of fasting state or kidney function. The inter-assay CVs were 5.0, 7.2, 6.5 and 3.5 % for OC, PINP, CTX and BALP, respectively. The BTMs were analysed in 2017, with study recruitment occurring from 1998 to 1999 until 2004. Thus, the oldest study samples were 19 years old, and the most recent ones were 13 years old; studies have suggested that BTMs, when stored at -80 °C, are stable for longer periods of time (Eastell *et al.*, 2018; Ahn *et al.*, 2019).

The rationale for choice of the selected BTMs in this study is described in Table 2.5 (Burch *et al.*, 2014). PINP and CTX were selected as they are the two reference markers recommended by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) for inclusion in all studies using BTMs (Vasikaran *et al.*, 2011). Additionally, PINP and OC have been associated with selenium and SePP in the OPUS study (Hoeg *et al.*, 2012) where OC was more closely associated with SePP than PINP (Hoeg *et al.,* 2012). Studies suggest BALP can help identify changes in bone mineralisation such as osteomalacia and Paget's disease (Nizet *et al.*, 2020).

Table 2.5: Bone turnover markers and evidence for feasibility to estimate bone health (adapted from Burch *et al.,* 2014).

Marker	Function	Evidence	Reference
OC	Formation Characterises osteoblastic activity Bone specific marker	More closely associated with SePP than PINP Increases with growth and fracture recovery Low within person variability Lower in those using corticosteroids	(Hoeg *et al.,* 2012; Burch *et al.,* 2014)
PINP	Formation Characterises osteoblastic activity Precursor of collagen type 1 made by osteoblasts	Associated with selenium and SePP Recommended by IOF and IFCC More sensitive than other BTMs and less affected by feeding status and diurnal variation	(Hoeg *et al.,* 2012; Burch *et al.,* 2014; Vasikaran *et al.,* 2011)
CTX	Resorption Characterises osteoclastic activity and hydrolysis of collagen	Associated with selenium and SePP Recommended by IOF and IFCC More responsive that other BTMs with bisphosphonates	(Hoeg *et al.,* 2012; Burch *et al.,* 2014; Vasikaran *et al.,* 2011)
BALP	Formation Characterises osteoblastic activity Marker of mineralisation Plays role by promoting hydroxyapatite crystal growth Corresponds to high osteoblastic activity	Review of usefulness of BALP; low variability, useful for follow-up studies Low biological and diurnal variation Increases during fracture recovery, Paget's disease, rickets, osteocalcin, osteoporosis, vascular calcification, chronic kidney disease	(Burch *et al.,* 2014; Nizet *et al.,* 2020)

OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase; SePP: selenoprotein P; IOF: International Osteoporosis Foundation; IFCC: International Federation of Clinical Chemistry and Laboratory Medicine.

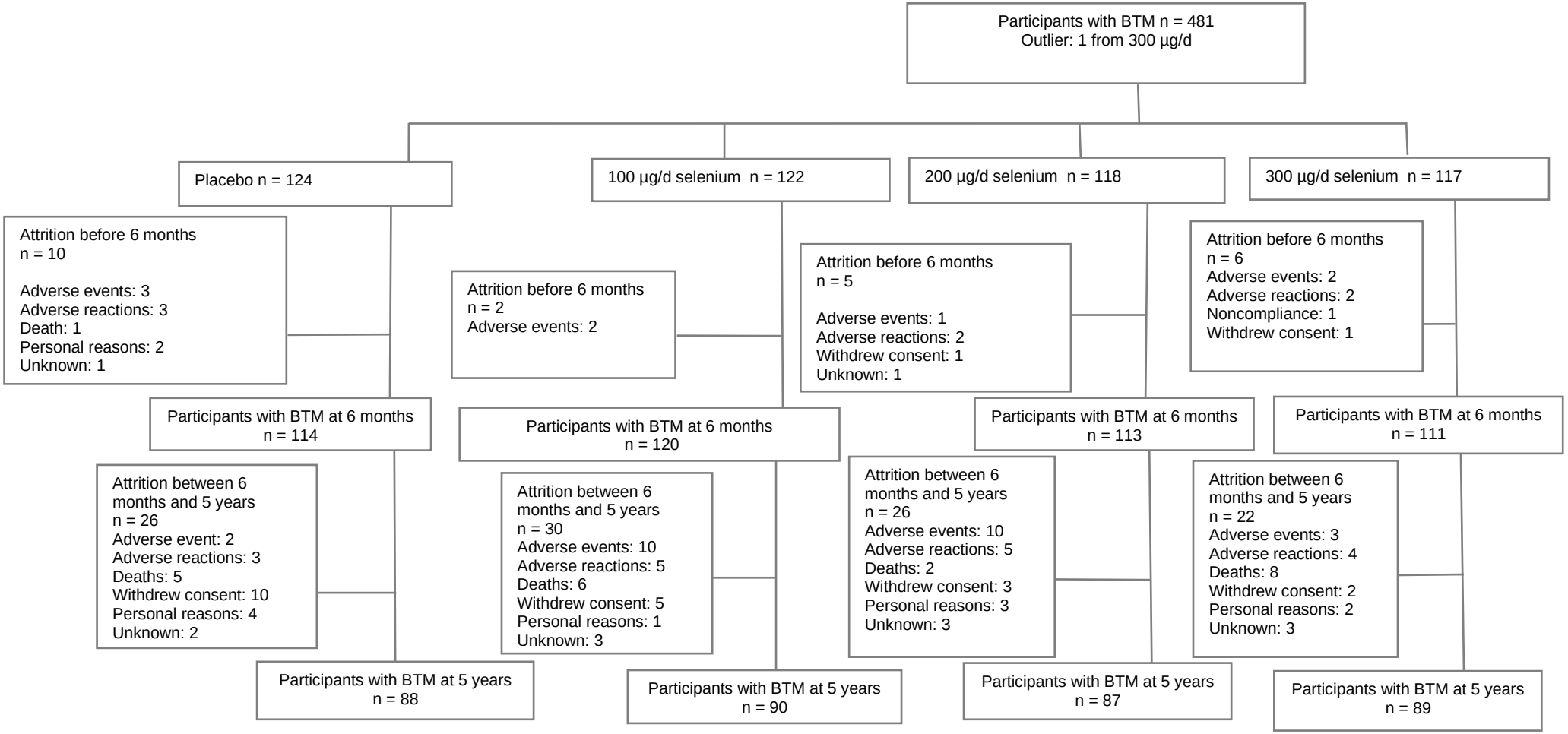


Figure 2.5: Flow diagram of participants with bone turnover markers and treatment randomisation at stage of The PRECISE Study.

2.3 General Statistics

Here I will provide some brief details of the general statistics I will use for each study design, cross-sectional and prospective, including both observational and RCT studies. I will then provide further details including justifications of each statistical approach in the preceding chapters.

2.3.1 Epidemiological Data Handling (The Newcastle 85+ Study)

The Newcastle 85+ Study data was analysed cross-sectionally in Chapter 3 and 4. In Chapter 3, Pearson correlations and linear regressions were used to determine the relationships between the biomarkers of selenium status (serum selenium, GPx3 activity and SePP) and determine the predictors of these biomarkers of selenium status, respectively. In addition to these analyses, in Chapter 3 I quantified the concentration of serum selenium that was required for a plateau of GPx3 activity and SePP, indicating optimisation of these selenoproteins. This replicated some of the studies that have used linear or quadratic equations to set the selenium recommendations, although other studies used experimental data with selenium supplementation to determine selenium DRVs. The analyses in Chapter 3 were performed to bridge the knowledge gap in selenium recommendations for very old adults since the current DRVs are based on younger populations. In Chapter 4, Pearson correlations were used to determine the relationships between the biomarkers of selenium status and MSK function (HGS, TUG, and sarcopenia). Regressions were employed to explore the predictors of MSK function (linear regression for HGS, TUG, and logistic regression for sarcopenia) including each biomarker of selenium status. These analyses for Chapter 4 were based on the rationale that selenium has been associated with MSK function (HGS, sarcopenia, BMD, fracture risk) in adults, however, there is limited research in very old adults, who are at greater risk of poor MSK function and prevalence sarcopenia. In Chapter 5, the same analyses were repeated as in Chapter 4, but here, I explored the relationships between the

biomarkers of selenium status and disability scores, and the predictors of disability scores. This was performed as the results from Chapter 4 suggested that selenium status may play a role in MSK function, especially MSK measures involving cognition, like TUG, and therefore disability scores were analysed as it combines MSK function, motor function and cognition, through IADLs, in a complete assessment. In addition to the cross-sectional analyses of Chapter 4 and 5, prospective analyses, using linear mixed models were performed to determine the associations between the biomarkers of selenium status and MSK outcomes and their rate of change over time. In Chapter 4, the models included time points at baseline, 1.5 and 3 years for sarcopenia prevalence, and time points at baseline, 1.5, 3 and 5 years for HGS and TUG. In Chapter 5, these were carried out at baseline, 1.5, 3 and 5 years for disability scores.

2.3.2 RCT Data Handling (The PRECISE Study)

The final experimental chapter, Chapter 6, utilised The PRECISE Study, which was a RCT. This chapter explored the dose-response relationships between selenium supplementation and BTMs using ANCOVA with polynomial contrasts. This was used to determine if there was a specific supplementation dosage of selenium at which BTMs best responded best.

2.4 Appendix

Table 2.1: Stability of selenium during storage including different durations, measuring techniques, analytes and temperatures.

Storage Temp (°C)	Biomarkers	Storage	Analysis	Outcome
4, 25 °C (Hollenbach *et al.*, 2008)	SePP	Up to 1 d	Immunoassay	• SePP: initial value to 90.4–108.1 % of control 25 °C and 88.4–108.4 % at 4 °C • 5 identical serum samples: 6 freeze-thaw cycles, 25 °C for 2 h between cycles. Mean values: 91.4 % of control • Stability of SePP in EDTA-, heparin- and citrate-plasma: mean SePP: 89.6–90.3 % of control 25 °C and 94.2–107.3 % 4 °C; no consistent decline • Plasma SePP varied more than serum; independent of time and temp
-20 °C (Arnaud, *unpublished*)	Serum	3 y	Unknown	• Se: no significant differences from baseline to 3 years
-20, 4°, 20, 40 °C (Muñoz Olivas, Quevauviller and Donard, 1998)	Sec, SeMet, $TMSe^+$	Up to 12 mo	HPLC-ICP/MS	• HDPE 20°C and 40 °C: severe losses $TMSe^+$ between 80 % and 90 % 15 d; 4 °C: $TMSe^+$ decreased with time • Pyrex + Teflon 20 °C (Pyrex superior to Teflon) and 4°C: excellent stability > 3 mo with all conditions and species • Pyrex and Teflon 40 °C: stable with some variation • HDPE 20 °C: rapid loss TMSe+, variable • Best stability > 1 year: Pyrex 4 °C + 20 °C in dark, especially organic species
-20, 20, 40°C (Quevauviller *et al.*, 1995)	Selenite Selenate	2, 6 mo	PTFE and polyethylene	• 2 mo: selenite + selenate stable • 6 mo: selenite decreased in all conditions • -20 °C: satisfactory stability for both species
–18°, 4, 20 °C Dark (Moreno *et al.*, 2002)	Sec SeMet	1-365 d	atomic absorption ICP coupled with chromatographic systems	• All conditions and containers: all species stable for at least 12 mo • Total Se in non-soluble fraction: stable 15 d 4 °C Pyrex • SeMet in enzymatic extract 4 and –18 °C**:** stable in both containers for 10 d • $TMSe^+$ in enzymatic extract 4 and –18 °C: stable in both containers > 15 d
-15, 4 °C (Zhang *et al.*, 1986; Sheppard and Millar, 1981)	Swine, GPx activity	0-3, 7, 14, 28, 56 d	Coupled assay with Beckman DU/ Gilford spectrophotometer	• 4 °C and –15 °C: activity decreased 1-56 d post-collection, greater at 4 °C • Contrasts Sheppard and Miller: ovine plasma GPx activity maintained at least 2 wk at –15 °C but not 4°C
-20, -70 °C (Persson-Moschos *et al.*, 1995)	SePP	10-15 years	Radioimmunoassay	• 5 different serum samples: -20 °C up to 15 y or –70 °C for 10 y • SePP: did not degrade during storage • Normal human serum: 21 °C 36 h: no decrease compared to serum –20 °C
-70 °C (Hill *et al.*, 1996)	Plasma, SePP	6 mo 6 y	Radioimmunoassay	• No discernible effect at 6 mo, some stored up to 6 y
-20, 4 °C (Sabé, Rubio and García-Beltrán, 2003)	Plasma	1 y	Single-beam atomic absorption spectrometer with Zeeman background correction	• -20 °C: stable relative to Se content for at least 1 y • Se content: no significant differences between temps at 2 d

SePP: selenoprotein P; EDTA: ethylenediaminetetraacetic acid; Sec: selenocysteine; SeMet: selenomethionine; HPLC-ICP/MS: high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry; HDPE: high density polyethylene; PTFE: polytetrafluoroethylene; TMSe+: trimethylselenium ion; GPx: glutathione peroxidase.

Chapter 3. Selenium Status in Very Old Adults: The Newcastle 85+ Study

3.1 Abstract

Background: Selenium is a trace element required for human health. It is known that selenium intakes are suboptimal in older adults in the UK, but less is known about selenium status and its determinants in very old adults (≥ 85 years).

Objectives: To assess biomarkers of selenium status among participants, and to determine predictors of selenium status. To quantify the relationship between serum selenium and selenoproteins, GPx3 activity and SePP.

Methods: Biomarkers of selenium status (serum selenium, GPx3 activity and SePP) at baseline were measured using standard laboratory techniques in 757 participants from The Newcastle 85+ Study. Biomarkers of selenium status concentrations were assessed and compared to cut-offs based on selenium DRVs. Linear regressions were used to explore the determinants of the biomarkers and a linear equation was plotted between serum selenium and the two selenoproteins to assess the relationships.

Results: The median concentrations were serum selenium 53.6 μg/L, GPx3 activity 142.1 U/L, and SePP 2.9 mg/L. There was a strong, positive association between serum selenium and GPx3 activity, ($r(755)= 0.363$, $P < 0.001$) and SePP ($r(757)= 0.497$, $P < 0.001$). There was a strong, positive relationship between serum selenium and GPx3 activity ($y=90.79+0.97*x$; $R^2 = 0.132$ $P < 0.001$) and SePP ($y=0.99+0.04*x$; $R^2 = 0.247$ $P < 0.001$). Using serum selenium cut-offs from the literature, GPx3 activity was estimated to plateau at 158.7 U/L, whilst SePP was estimated to plateau at 4.6 mg/L.

Conclusion: In this population of very old adults, selenium status was suboptimal, especially the biomarkers, serum selenium and SePP. There were linear associations between serum selenium and the selenoproteins, suggesting expression was not optimised. It would be interesting to explore whether these deficiencies are associated with adverse MSK function.

3.2 Introduction

It is becoming apparent that a large proportion of adults within older populations are not reaching the recommended selenium intake. This was discussed in Chapter 1, Section 1.6; in summary, older adults generally have suboptimal intakes, with a large proportion consuming below the LRNI. For example, using the UK NDNS data (2008/09 and 2009/10) median selenium intakes were 43 µg/d (47 µg/d for males, and 38 µg/d for females) (Roberts *et al.*, 2018). This equated to 30 % of males, and 52 % of females consuming below the LRNI of 40 µg/d (SACN, 2013). Likewise, in The Newcastle 85+ Study, over half of the intakes were below the LRNI (Perri *et al.*, 2020). The challenges associated with dietary intakes including selenium have also been discussed in Chapter 1, Section 1.6 and include issues around geographic dependency (Keck and Finley, 2006), incomplete data (McCance and Widdowson's, 2019), cognitive impairment and IT accessibility.

Establishing the selenium status of very old adults through effective measurements can help to understand and address these issues. Measurement of selenium status can be achieved in a variety of ways, as detailed in Chapter 1, Section 1.5, and in the review by Ashton *et al.*, (2009). Two well studied plasma selenoproteins are GPx3, where activity plateaus at serum selenium concentrations of 70 µg/L (Nève, 1995; Combs, 2001; IoM, 2000), and SePP, which plateaus at serum selenium concentrations of 90-122 µg/L (Xia *et al.*, 2010; Hurst *et al.*, 2010; Burk *et al.*, 2006; Persson-Moschos *et al.*, 2000; Hill *et al.*, 1996). These plasma/serum biomarkers are suited to older populations with lower intakes due to their sensitivity to low to moderate selenium status (Bates *et al.*, 2002). Few studies have explored selenium status in very old adults. To recap some studies from Chapter 1, Section 1.6, The EPIC-Europe cohort (20-70 years) merged selenium concentrations from 10 European countries, revealing a mean concentration of 82 µg/L, although adults aged 85-90 years were not included in the study (Hughes *et al.*, 2015). A review comparing the selenium status of nonagenarians and centenarians found concentrations to range from 37 µg/L to 138 µg/L (Robberecht *et al.*, 2019), although this has limited comparability to adults aged 85–90-years as these long-living adults likely have a healthy survivor bias. Therefore, in addition to selenium DRVs lacking specific age groups, another gap in the literature is lack of assessment of selenium status in adults aged ≥ 85 years and this will form one of aim of this chapter.

Previous studies have explored the determinants of selenium status, some of which have been discussed in a review (Fairweather-Tait *et al.*, 2010). Age has been a debatable determinant, where some studies suggest selenium status is higher (Bates *et al.*, 2002; Stranges *et al.*, 2010), similar (Rayman *et al.*, 2008c), or lower in older adults, compared to younger adults (Dubois, 1990; Robberecht and Deelstra, 1984; Olivieri *et al.*, 1994). Likewise, gender has been a debatable determinant, where some studies suggest women have lower serum selenium than men (Arnaud *et al.*, 2006; Niskar, Paschal and Kieszak, 2003; Lopes *et al.*, 2004; Al-Mubarak *et al.*, 2020), whilst others do not (Monget *et al.*, 1996; Imai *et al.*, 1990). A UK NDNS study in older adults (≥ 65 years) found season, location, living status, medication usage, income, education and smoking to predict selenium status (Bates *et al.*, 2002). Some of these determinants have been noted in other studies such as living status (Löwik *et al.*, 1992), income and education (Stranges *et al.*, 2010; Bates *et al.*, 2002; Laclaustra, 2010), location (Arnaud *et al.*, 2006; Niskar, Paschal and Kieszak, 2003) and smoking (Lloyd and Lloyd and Clayton, 1983; Bates *et al.*, 2002; Arnaud *et al.*, 2006; Thomson, 2004b; Stranges *et al.*, 2010), however, see Hughes *et al.*, (2015). Smoking may be a determinant due its association with inflammation, which is another determinant of selenium status (Al-Mubarak *et al.*, 2020). Physical activity has not been a strong determinant of selenium status in some studies (Tessier *et al.*, 1995; Arnaud *et al.*, 2006), although an observational study found higher concentrations of selenium in sedentary males (Letsiou *et al.,* 2014). Likewise, some studies have failed to find any associations between selenium status and alcohol intake (Koyama *et al.*, 1995; Kafai and Ganji, 2003), albeit in younger populations. However, in a French population, alcohol, meat and fish consumption were associated with higher serum selenium, whilst lower selenium was associated with smoking and obesity (in women) (Arnaud *et al.*, 2006). Furthermore, in alcoholics, serum selenium can be lower (Robberecht and Deelstra, 1984) which may be associated with malnutrition and poor liver function (Robberecht and Deelstra, 1984; Alfthan and Neve, 1996; Borawska *et al.*, 2004). Leading on from this, other determinants can include markers of liver and kidney function, such as plasma creatinine, gamma-glutamyl transferase and alkaline phosphatase (Alfthan and Neve, 1996; Al-Mubarak *et al.*, 2020). Overall, there appears to be a range of determinants of selenium status which vary

between populations and therefore, I will also assess the determinants of biomarkers of selenium status in this chapter.

Very little is known about the recommendations for selenium status in very old adults. As discussed extensively in Chapter 1, Section 1.6, dietary selenium recommendations have evolved over the years. Initially these selenium DRVs were based on animal studies (NRC, 1980) and then on requirements to prevent Keshan disease and later focused on the intakes required for the plateauing of selenoproteins, specifically GPx3 activity (Yang, 1987; Xia *et al.*, 2005; Duffield *et al.*, 1999) and later on SePP (Xia *et al.*, 2010). When a plateau is reached, it is assumed that the selenoprotein is optimised and fully expressed, and therefore, the selenium intake required for this would be deemed adequate. The studies that formed the basis of these recommendations were experimental whereby increasing amounts of selenium supplementation, often L-SeMet, would be provided and the plateau of the selenoprotein would be measured on a graph. There has been controversy over these selenium recommendations regarding their appropriateness, whether they are sufficient to prevent disease or, in some cases, if they are too high, since there is no clinical evidence to support that lower selenium concentrations are detrimental. These discrepancies have been applied in different organisations recommendations. For example, SACN, (2013) updated their report in 2013 and concluded there would be no changes to the DRV since there was insufficient data to indicate a public health issue with selenium status in the UK; conversely, DA-CH merged recommendations from multiple countries to update the DRV of selenium to account for higher intakes for the plateauing of SePP (Kipp *et al.*, 2015).

A striking concern is that most of these dietary recommendations are extrapolated from younger populations, or do not include recommendations for adults aged 85 years and above. For example, the recommendations from WHO/FAO (WHO, 1987) have estimations for adults up to 65 years, the SCF (IoM, 2000) recommended a PRI for adults without consideration of older adults, and ESFA, (2014) pointed out that estimations for selenoprotein optimisation comes from studies involving adults aged 18 up to 64 years

(EFSA, 2014). This emphasises the knowledge gap in these recommendations and poses the question whether older adults have the same requirements as younger adults. It would not be farfetched to suggest that older adults have different requirements due to alterations in metabolism and physiology (Russell, Rasmussen and Fada, 1999; Clegg and Williams, 2018). This chapter aims to address this knowledge gap by quantifying the relationship between serum selenium and selenoproteins to determine whether there are differences in the concentrations required for selenoprotein plateau and therefore, the selenium requirements of older populations.

As explained in Chapter 1, Section 1.6, the evidence from nutritional surveys suggests suboptimal selenium intake in the UK; however, despite this, research is limited on the assessment of selenium status in very old adults, or only includes few biomarkers (Roberts *et al.*, 2018; Hughes *et al.,* 2015). Therefore, combining a variety of nutritional biomarkers of selenium status will help expand the knowledge and give a rounded approach to assessing selenium in very old adults. Based on the findings that over 50 % of participants in The Newcastle 85+ Study had selenium intakes that were below the LRNI (Perri *et al.*, 2020), I hypothesised that there will be a high prevalence of suboptimal selenium status in this population-based cohort of very old people. Serum selenium will predict the selenoproteins (GPx3 activity and SePP) in a linear relationship due to the limiting concentrations of serum selenium in this population, thereby not allowing for full expression and plateau of selenoproteins. The aims of this study were: 1) to assess the selenium status (by measuring concentrations of serum selenium, GPx3 activity and SePP) in participants from The Newcastle 85+ Study; 2) to explore the determinants of biomarkers of selenium status; and 3) to quantify the relationship between serum selenium concentrations and GPx3 activity and SePP.

3.3 Materials and Methods

Below is a summary of the Material and Methods, the full details are provided in Chapter 2, Section 2.2.1.

3.3.1 Study Population

Data and samples were obtained from The Newcastle 85+ Study, a longitudinal, population-based study of a single-year birth cohort in the Northeast of England that explored health outcomes and trajectories in adults aged 85 years and over. The study was initiated in 2006 recruiting 1042 participants born in 1921, for full details, see Chapter 2, Section 2.2.1.

3.3.2 Socioeconomic, Lifestyle and Other Covariates

Assessments included questionnaires, functional tests, fasting blood samples, medical record reviews, dietary intakes and body weight measurements which were taken at the initial health assessment (2006/2007) and three other visits (1.5, 3, 5 years), details of which are provided in Chapter 2, Sections 2.2.1.1 and 2.2.1.2. The covariates included were: selenium, energy and protein intake assessed via 24 h MPR; sex; occupational status; education level; self-rated health; medication use and disease count assessed via GP records; BMI calculated from weight and height measurements; FFM determined using BIA; waist:hip ratio; cognitive impairment determined using the SMMSE; smoking status and alcohol use; hsCRP. These covariates were selected based on the previous literature as described in Section 3.2 of this Chapter.

3.3.3 Biomarkers of Selenium Status

Baseline blood samples from 2006/2007 (n = 757) that had been stored at -80 °C were analysed for biomarkers of selenium status. A literature search was performed to ensure the selenium would have adequate stability for the analyses in addition to a personal

communication, overall, it was suggested that selenium would be stable over the sample storage duration as most studies reported minimal change in serum/plasma selenium (Appendix Table 2.1). Serum selenium was measured using TXRF, GPx3 activity was measured using a coupled-enzyme reaction measuring NADPH consumption and, SePP was measured using a commercial ELISA. For full details, including the literature search, please see Chapter 2, Section 2.2.1.5.

3.3.4 Statistical Analyses

IBM statistical software package version 27.0 (SPSS) was used to perform the exploratory and statistical analyses, where $p < 0.05$ was considered statistically significant. To determine normality of the continuous variables, the Shapiro-Wilk test and quantile–quantile (QQ) plots were used. Selenium intake and status were used as continuous variables in main analyses and categorised into biologically relevant cut-offs based on previous literature: serum selenium above and below 70 µg/L (Nève, 1995; Combs, 2001); GPx3 activity above and below 115 U/L (devised from 2.5th centile of GPx3 activity from SCAN-B cohort, Demircan *et al.*, 2021); selenoprotein P above and below 4.5 mg/L (the mean SePP concentrations devised from the EPIC-Europe cohort (Hughes *et al.,* 2015 mean 3.9 and 4.3 mg/L in males and females) and an American selenium supplementation study (Burk *et al.,* 2006, mean 5.5 mg/L). Other studies have suggested that higher concentrations of serum selenium, of 90 µg/L and upwards, are required for the plateauing of the selenoproteins (Xia *et al.*, 2010; Lyons *et al.*, 2004; Rayman, 2005), however, using this cut-off led to a disproportionate number of participants in each group and therefore, this cut-off was not used (97 % below 90 µg/L, 3 % at or above 90 µg/L, compared to 92 % below 70 µg/L, 18 % at or above 70 µg/L). Descriptive statistics were used to summarise the baseline characteristics of all participants and of those with serum selenium concentration above and below 70 µg/L. Serum selenium cut-offs were used, rather than the selenoprotein cut-offs, since serum selenium contains both selenoproteins, and more research is available for serum selenium cut-offs, compared to GPx3 activity and SePP that have little consensus.

Differences in characteristics between selenium cut-offs were assessed using Chi-square test (categorical) and Kruskal–Wallis (for ordered and non-normally distributed data). Pearson correlation was used to examine relationships between the each of the biomarkers of selenium status (serum selenium, GPx3 activity and SePP) and selenium intake.

3.3.4.1 Relationships and Predictors between Biomarkers of Selenium Status at Baseline

R Studio was used with the libraries ggplot, ggvenn and venndiagram to plot a Venn diagram indicating the participants who were suboptimal and optimal for each biomarker of selenium status using the same cut-offs described above. A linear regression was used to determine the predictors of each of the biomarkers of selenium status (serum selenium, GPx3 activity and SePP). The biomarkers were set as the dependent variables, while the independent variables were: selenium intake (continuous); sex (men/women, binary); occupational status (routine/manual, intermediate, managerial/professional occupations, categorical); education (0-9, 10-11, ≥ 12 years, categorical); self-rated health (excellent/very good, good, fair/poor, ordinal); energy intake (continuous); protein intake (continuous); medication use (continuous); BMI (continuous); FFM (continuous); waist:hip ratio (continuous); SMMSE (continuous); disease count (0-1, 2, ≥ 3, categorical); smoking status (current, former, never, categorical); alcohol drinker (yes/no binary); hsCRP (continuous).

3.3.4.2 Quantification of the Relationship between Serum Selenium and GPx3 Activity and SePP

To quantify the relationship between serum selenium and the selenoproteins (GPx3 activity and SePP), a linear regression was used. The regression equation was then used to determine the concentrations of each selenoprotein when using the literature derived cut-off of serum selenium concentration for the selenoprotein plateau i.e. 70 μg/L for GPx3 activity and 90 μg/L for SePP. A linear relationship was initially plotted and is presented in the main analyses, whilst a quadratic relationship is presented in the Appendix. The R^2 values did not differ largely between the linear or quadratic lines (0.132 versus 0.141 for

GPx3 activity and 0.247 versus 0.250 for SePP) and thus, the linear model was selected for the main analyses as the most parsimonious relationship.

3.3.5 Sensitivity Analysis

As a comparison, analyses were repeated using the serum selenium cut-off of 90 μg/L. This analysis was selected as other studies have suggested that 90 μg/L of serum selenium was associated with a plateauing of either GPx3 activity, or both GPx3 activity and SePP (Rayman, 2005; Nève, 1991; Burk *et al.*, 2006; Xia *et al.*, 2010; Thomson *et al.*, 1977; Thomson *et al.*, 1993; Rea *et al.*, 1979; Rayman, 1997; Hill *et al.*, 1996; Lyons *et al.,* 2004). An outlier analysis was also performed by excluding those who had serum selenium concentrations in the 75th percentile plus 1.5 multiplied by the interquartile range (IQR). This cut-off was based on a previous study that found no differences between this outlier detection, or when using the mean plus 3 SD's (Schwiebert *et al.*, 2020). In this case, I opted for the former as the cut-off (102 μg/L) was lower (112 μg/L) and since this population had a suboptimal baseline selenium concentration, it was more appropriate to select the lower value to help distribute the sample size per group (Sensitivity Analyses Table 1-4). In addition to removing these participants, I also removed two participants who had serum selenium concentrations below the detection limit of 10.0 μg/L.

3.4 Results

3.4.1. Participant Characteristics and Baseline Selenium Status

Baseline characteristics of the 757 participants from The Newcastle 85+ Study for whom data were available are shown in Table 3.1. In addition, this table also summarises the characteristics of those with serum selenium concentration above and below 70 μg/L. Those with suboptimal serum selenium (< 70 μg/L) concentration were more likely to be male (P = 0.010), live in institutions (P = 0.002), have higher physical activity (P = 0.001), less likely to take selenium supplements (P < 0.001), have higher medication usage (P < 0.001), lower cognitive score (P = 0.007), higher hsCRP (P = 0.002), higher free T_4 (P = 0.025), higher BMI (P = 0.002) and higher FFM (P < 0.001).

Table 3.2 summarises the 5th to 95th percentiles of selenium intakes and biomarkers of selenium status; the median concentrations were: serum selenium 53.6 µg/L, GPx3 activity 142.1 U/L, SePP 2.9 mg/L and selenium intake 39.1 µg/d. Table 3.3 summarises the mean and median of selenium intakes and the biomarkers of selenium status, including those meeting the selected cut-offs. The median selenium intake for all participants did not differ significantly between those with concentrations < 70 µg/L or ≥ 70 µg/L (P = 0.057). Most participants (81.8 %, n = 619) had serum selenium concentrations < 70 µg/L. Likewise, most participants (82.8 %, n = 627) had suboptimal SePP concentration i.e., below 4.5 mg/L. Conversely, fewer participants (29.8 %, n = 255) had suboptimal GPx3 activity i.e., below 115 U/L. Participants with optimal selenium status were also more likely to be adequate for both SePP and GPx3 activity (P < 0.001).

Table 3.1. Characteristics of study participants represented by serum selenium cut-offs.

Characteristic	All Participants n = 757	Suboptimal Selenium < 70 µg/L	Optimal Selenium ≥ 70 µg/L	p
Socio-demographic factors				
Women % (n)	61.1 (461)	59.0 (364)	70.8 (97)	0.010
Men % (n)	38.9 (293)	41.0 (253)	29.2 (40)	
Years of education % (n) n = 743				
0–9	64.2 (477)	65.2 (395)	59.9 (82)	0.483
10–11	23.6 (175)	22.8 (138)	27.0 (37)	
≥ 12	12.2 (91)	12.0 (73)	13.1 (18)	
Occupational class % (n) n = 721				
Managerial and Professional	35.1 (253)	34.6 (204)	37.4 (49)	0.371
Intermediate	14.7 (106)	14.1 (83)	17.6 (23)	
Routine and Manual	50.2 (362)	51.4 (303)	45.0 (59)	
Living in Institutions % (n) n = 755				
Yes	8.9 (67)	10.4 (64)	2.2 (3)	0.002
No	91.1 (688)	89.6 (554)	97.8 (134)	
Diet-related factors				
Diet change in past year % (n) n = 733				
Yes	17.1 (117)	6.5 (39)	6.8 (9)	0.910
No	82.9 (568)	93.5 (561)	93.2 (124)	
Total energy kCal (M, SD) n = 732	1688.6, 511.0	1688.6, 524.1	1689.0, 450.9	0.824
Protein Intake g (M, SD) n = 732	64.2, 22.3	64.0, 22.5	65.1, 21.9	0.676
Misreporting food intake % (n) n = 685				
Yes	6.5 (48)	17.9 (99)	13.7 (18)	0.259
No	93.5 (685)	82.1 (455)	86.3 (113)	
Lifestyle factors				
Smoking % (n) n = 754				
Non-Smoker	35.0 (264)	35.5 (219)	32.8 (45)	0.616
Former Smoker	59.4 (448)	58.7 (362)	62.8 (86)	
Current Smoker	5.6 (42)	5.8 (36)	4.4 (6)	
Current alcohol intake % (n) n = 751				
Yes	62.3 (468)	62.4 (384)	61.8 (84)	0.883
No	37.7 (283)	37.6 (231)	38.2 (52)	
Physical activity (PA) % (n) n = 738				
Low (score 0–1)	21.7 (162)	24.0 (147)	11.0 (15)	0.001
Moderate (score 2–6)	43.0 (322)	42.8 (262)	44.1 (60)	
High (score 7–18)	35.3 (264)	33.2 (203)	44.9 (61)	
Selenium Supplement Use n = 755				
Yes	0.7 (5)	0.0 (0.0)	3.6 (5)	< 0.001
No	99.3 (750)	100.0 (618)	96.4 (132)	
Number of Medications (M, SD) n = 732	6.3, 3.8	6.6, 3.8	5.3, 3.7	< 0.001
Total Medication n % n = 753				
0-2	16.7 (126)	14.4 (89)	27.0 (37)	< 0.001
3-5	26.7 (201)	25.6 (158)	31.4 (43)	
≥ 6	56.6 (426)	59.9 (369)	41.6 (57)	
Health-related factors				
Self-rated health n = 738				
Excellent/Very Good	40.9 (302)	39.0 (235)	49.3 (67)	0.067
Good	37.7 (278)	38.4 (231)	34.9 (47)	
Fair/Poor	21.4 (158)	22.6 (136)	16.2 (22)	
SMMSE (M, SD) n = 753	26.1, 4.9	25.9, 5.2	27.2, 3.4	0.007
hsCRP mg/L (M, SD) n = 753	6.9, 14.2	7.4, 15.2	4.3, 9.5	0.002
Free T_4 pmol/L (M, SD) n = 742	15.6, 2.7	15.7, 2.7	15.1, 2.4	0.025
Free T_3 pmol/L (M, SD) n - 743	4.5, 0.5	4.5, 0.5	4.6, 0.5	0.059
Anthropometry				
BMI (M, SD) n = 674	24.4, 4.4	24.6, 4.4	23.4, 3.9	0.002
Fat Free Mass (M, SD) n = 689	45.2, 9.0	45.7, 9.0	42.9, 8.6	< 0.001
Waist:Hip Ratio (M, SD) n = 685	0.89, 0.08	0.9, 0.08	0.9, 0.07	0.056
Height (M, SD) n = 712	1.6, 0.08	1.6, 0.07	1.6, 0.07	0.107

SMMSE: standardised mini mental state examination; hsCRP: high sensitivity C-Reactive protein; Free T_4: free thyroxine; Free T_3: free triiodothyronine; BMI: body mass index; M: mean; IQR: interquartile range

Table 3.2: Biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) and selenium intake of study participants represented by percentiles (5-95th).

Biomarker of Selenium Status N = 755	Percentiles						
	5	10	25	50	75	90	95
Selenium Intake	13.26	18.06	27.21	39.07	56.37	76.30	93.56
Serum Selenium	26.64	32.90	42.80	53.60	66.44	77.10	84.64
GPx3 Activity	63.78	79.56	107.4	142.10	179.30	210.42	231.96
Selenoprotein P	0.90	1.33	2.04	2.93	3.97	5.08	5.66

GPx3: glutathione peroxidase 3 activity

Table 3.3: Biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) of study participants represented by serum selenium cut-offs.

Characteristic	All Participants	Suboptimal Selenium < 70 µg/L	Optimal Selenium ≥ 70 µg/L	p
Selenium Intake (M, IQR) n=732	39.1, 29.2	44.7, 28.1	48.4, 29.0	0.057
Serum Selenium (M, IQR) n=757	53.6, 23.6	50.1, 19.2	78.2, 12.1	< 0.001
Selenoprotein P (M, IQR) n=757	2.9, 1.9	2.8, 1.7	4.3, 2.5	< 0.001
GPx3 Activity (M, SD) n=755	144.1, 50.7	138.3, 48.5	170.1, 52.4	< 0.001
Selenium at 70 µg/L % (n)				
Yes	18.2 (138)			
No	81.8 (619)			
SePP at 4.5 mg/L % (n)				
Yes	17.2 (130)	53.1 (69)	46.9 (61)	< 0.001
No	82.8 (627)	87.7 (550)	12.3 (77)	
GPx3 Activity at 115 U/L % (n)				
Yes	70.2 (530)	78.3 (415)	21.7 (115)	< 0.001
No	29.8 (225)	90.2 (203)	9.8 (22)	

M: mean; IQR: interquartile range; SePP: selenoprotein P; GPx3: glutathione peroxidase 3

3.4.2 Relationships between Biomarkers of Selenium Status at Baseline

Using continuous variables of the biomarkers of selenium status there was a strong, positive association between serum selenium and GPx3 activity ($r(755)= 0.363$, $P < 0.001$), SePP ($r(757)= 0.497$, $P < 0.001$), and selenium intake ($r(732)= 0.103$, $P = 0.005$). Likewise, there was a strong, positive association between SePP and GPx3 activity ($r(755)= 0.625$, $P < 0.001$). In contrast, there was a non-significant association between selenium intake and GPx3 activity ($r(730)= 0.031$, $P = 0.396$) or SePP ($r(732)= 0.071$, $P = 0.054$) (Table 3.4). A Venn diagram (Figure 3.1) revealed the overlap between the participants who were considered suboptimal according to each biomarker of selenium status (Figure 3.2 A). There was a 78.8 % overlap in those who were suboptimal in serum selenium (< 70 µg/L) and SePP (< 4.5

mg/L), with around 21.2 % per biomarker where suboptimal selenium and SePP was incongruent. Fewer participants (31.7 %) were suboptimal for both SePP and GPx3 activity (< 115 U/L) and even fewer (29.2 %) that had both suboptimal serum selenium and suboptimal GPx3 activity. Almost 30 % of participants had suboptimal concentrations of all three biomarkers. In Figure 3.1 B, the opposite was displayed showing the overlap in those considered to have optimal concentrations. There was an 11.1 % overlap in those with optimal concentrations of all biomarkers of selenium status and an almost equal split between those with optimal serum selenium and GPx3 activity (20.7 %) and SePP and GPx3 activity (22.7 %).

Table 3.4: Correlations between biomarkers of selenium (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) and selenium intake at baseline.

		Serum Selenium µg/L	**SePP mg/L**	**GPx3 Activity U/L**	**Selenium Intake µg/d**
Serum Selenium µg/L	Pearson Correlation	1	0.497	0.363	0.103
	p		< 0.001	< 0.001	0.005
	N	757	757	755	732
SePP mg/L	Pearson Correlation		1	0.625	0.071
	p			< 0.001	0.054
	N		757	755	732
GPx3 Activity U/L	Pearson Correlation			1	0.031
	p				0.396
	N			755	730
Selenium Intake µg/d	Pearson Correlation				1
	p				
	N				732

M: Mean; IQR: interquartile range; SePP: selenoprotein P; GPx3: glutathione peroxidase 3

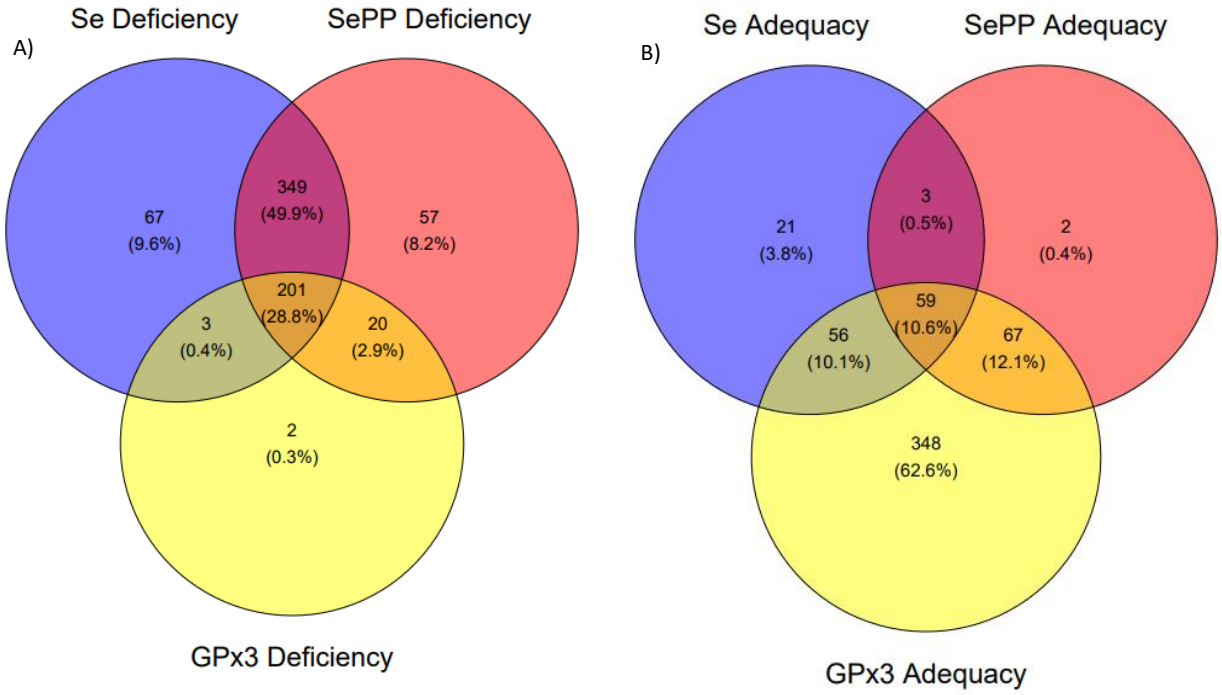


Figure 3.1: Venn diagram depicting the overlap between participants A) in those who had suboptimal serum selenium (< 70 µg/L), selenoprotein P (< 4.5 mg/L) and glutathione peroxidase 3 activity (< 115 U/L) B) in those who had optimal serum selenium (≤ 70 µg/L), selenoprotein P (≤ 4.5 mg/L) and glutathione peroxidase 3 activity (≤ 115 U/L).

The linear regressions for associations between serum selenium and each biomarker, dietary selenium intake and each biomarker, and between GPx3 activity and SePP are shown in Figure 3.2. Using continuous variables of the biomarkers of selenium status, there was a positive association between all biomarkers, with the strongest association between SePP and GPx3 activity ($y=76.63+21.93*x$; $R^2 = 0.390$, $P < 0.001$). The next strongest association was between serum selenium and SePP ($y=0.99+0.04*x$; $R^2 = 0.247$, $P < 0.001$), followed by serum selenium and GPx3 activity ($y=90.79+0.97*x$; $R^2 = 0.132$, $P < 0.001$) and then selenium intake and serum selenium ($y=52.16+0.07*x$; $R^2 = 0.011$, $P = 0.005$). There was a non-significant association between selenium intake and GPx3 activity ($y=114+0.05*x$; $R^2 = 9.910^{E-4}$, $P = 0.396$) and SePP ($y=2.92+3.46^{E-3}*x$; $R^2 = 0.005$, $P = 0.054$). These were repeated using a quadratic equation for serum selenium and the selenoproteins (GPx3 activity and SePP) and are presented in the Appendix Figure 3.1.

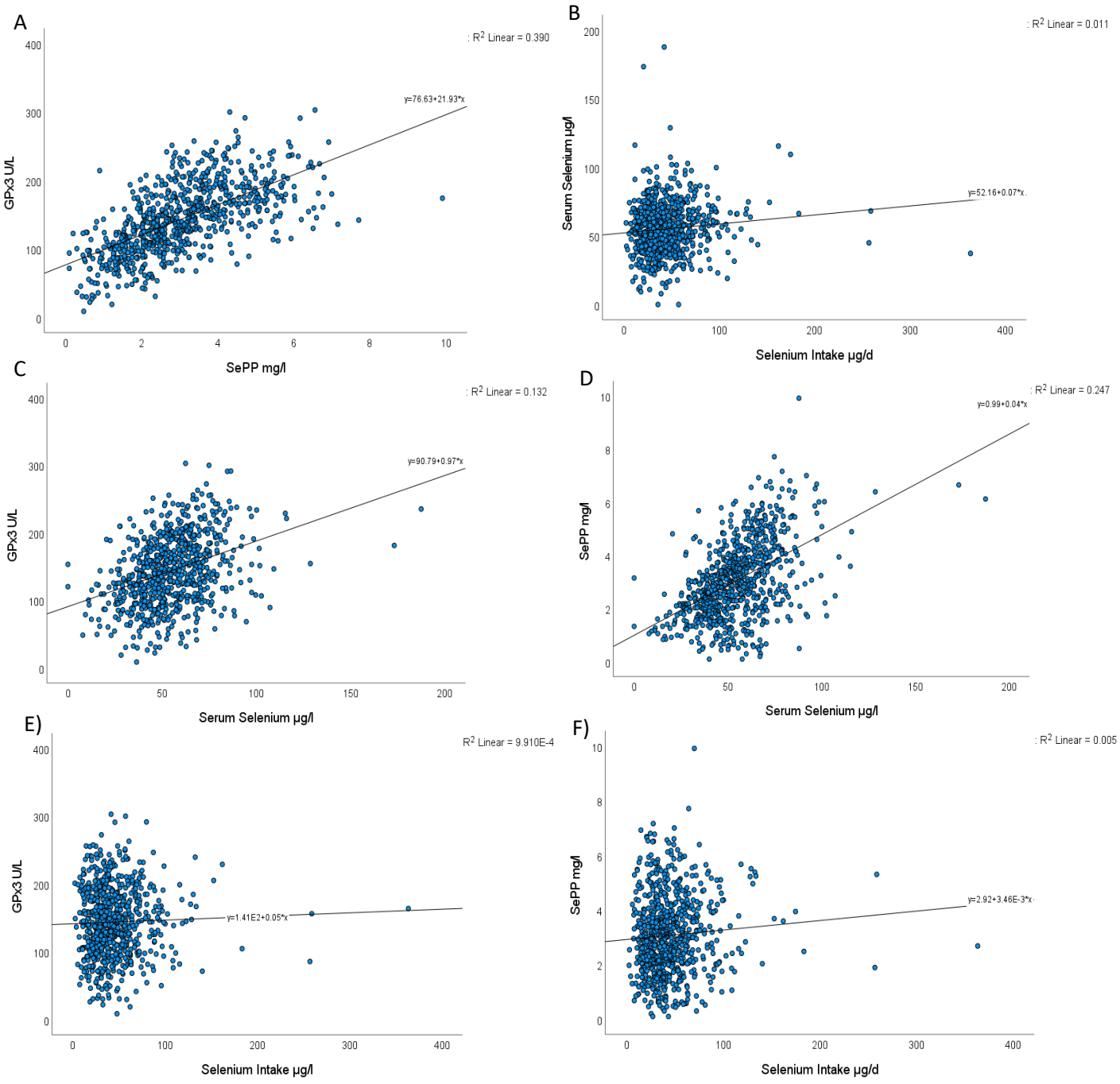


Figure 3.2: Linear correlation between selenium biomarkers. A) Glutathione peroxidase 3 activity and selenoprotein P y=76.63+21.93*x R^2: 0.390, P < 0.001; B) Selenium intake and serum selenium y=52.16+0.07*x R^2: 0.011, P = 0.005; C) Serum selenium and glutathione peroxidase 3 activity y=90.79+0.97*x R^2: 0.132, P < 0.001; D) Serum selenium and selenoprotein P y=0.99+0.04*x R^2: 0.247, P < 0.001; E) Selenium intake and glutathione peroxidase 3 activity y=114+0.05*x R^2: 9.910E^{-4}, P = 0.396; F) Selenium intake and selenoprotein P y=2.92+3.46^{E-3*}x R^2: 0.005; P = 0.054.

3.4.3 Predictors of Biomarkers of Selenium Status

The equations associated with the significant predictors from the fully adjusted models are displayed in Table 3.5; protein was a significant predictor of each biomarker of selenium status. Serum selenium was predicted to be higher in those who were male (β 8.04 ± 3.00, P = 0.008), having higher waist:hip ratios (β 24.08 ± 12.08, P = 0.047), higher protein intake (β 0.13 ± 0.05, P = 0.013) and lower in those living in institutions (β -10.15 ± 4.99, P = 0.042), poor self-rated health (β -2.13 ± 1.07, P = 0.046), higher disease count (β -0.99 ± 0.50, P = 0.048) and medication usage (β -2.40 ± 1.15, P = 0.037). GPx3 activity was predicted to be higher in those who were male (β 31.50 ± 8.19, P < 0.001), having higher protein intake (β 0.33 ± 0.07, P < 0.001) and lower in those with higher BMI (β -2.20 ± 0.73, P = 0.003). There was only one predictor for SePP, where concentrations were predicted to be higher in those with higher protein intakes (β 0.01 ± 0.004, P = 0.003). The full details are provided in Appendix Table 3.1, including p values and non-significant predictors.

Table 3.5: Equations derived from a fully adjusted linear regression for each selenium biomarker (serum selenium, glutathione peroxidase 3 activity and selenoprotein P) using the significant predictors and intercept values, β(SE).

Biomarker	Equation from linear regression
Selenium	= 45.60(14.35) + 8.38(3.04)Sex + 24.66(12.19)Waist:Hip Ratio + 0.13(0.05)Protein Intake – 2.13(1.10)SRH – 1.07(0.51)Disease Count
GPx3 Activity	=144.99(38.84) + 30.92(8.23)Sex – 2.17(0.74)BMI + 0.35(0.60)Protein Intake – 1.32(0.60)PA
SePP	=2.94(1.15) + 0.01(0.004)Protein Intake

GPx3: glutathione peroxidase 3; SePP: selenoprotein P; SRH: self-rated health; BMI: body mass index; PA: physical activity

3.4.4. Quantification of the Relationship between Serum Selenium and GPx3 Activity and SePP

Based on the literature cut-offs of 70 µg/L of serum selenium, the equations from the regressions (Figure 3.2) estimated GPx3 activity to plateau at 158.7 U/L. Conversely, using the GPx3 activity cut-off (115 U/L), derived from the SCAN-B cohort (Demrican et al. 2021), the equations from the regressions (Figure 3.2) estimated a serum selenium of 25.0 µg/L. Based on the literature cut-offs of 90 µg/L of serum selenium, the equations from the regressions (Figure 3.2) estimated SePP to plateau at 4.6 mg/L. Conversely, using the SePP activity cut-off (4.5 mg/L), derived from the EPIC-Europe cohort (Hughes *et al.*, 2015), the equations from the regressions (Figure 3.2) estimated a serum selenium of 87.8 µg/L. When using quadratic equations and the same serum selenium concentrations for each selenoprotein i.e., 70 µg/L for GPx3 activity, and 90 µg/L for SePP, GPx3 activity was estimated to plateau at 160.7 U/L (GPx3 activity = $71.4+1.65*x*-5.34^{E-3}*x^2$; R^2: 0.141) whilst SePP was estimated to plateau at 4.4 mg/L (SePP = $0.65+0.05*x*-9.39^{E-5}*x^2$; R^2: 0.250) (Appendix Figure 3.1). When using the serum selenium cut-off (< 70 µg/L and ≥ 70 µg/L), there was a stronger, positive association between those with suboptimal serum selenium concentrations and GPx3 activity (y=78.45+1.23*x R^2: 0.114, P < 0.001), and SePP (y=0.91+0.04*x R^2: 0.168, P < 0.001). The associations were weaker between those with optimal serum selenium concentrations and GPx3 activity (y=149+0.25*x R^2: 0.006, P = 0.283), and SePP (y=2.82+0.02*x R^2: 0.029, P = 0.253).

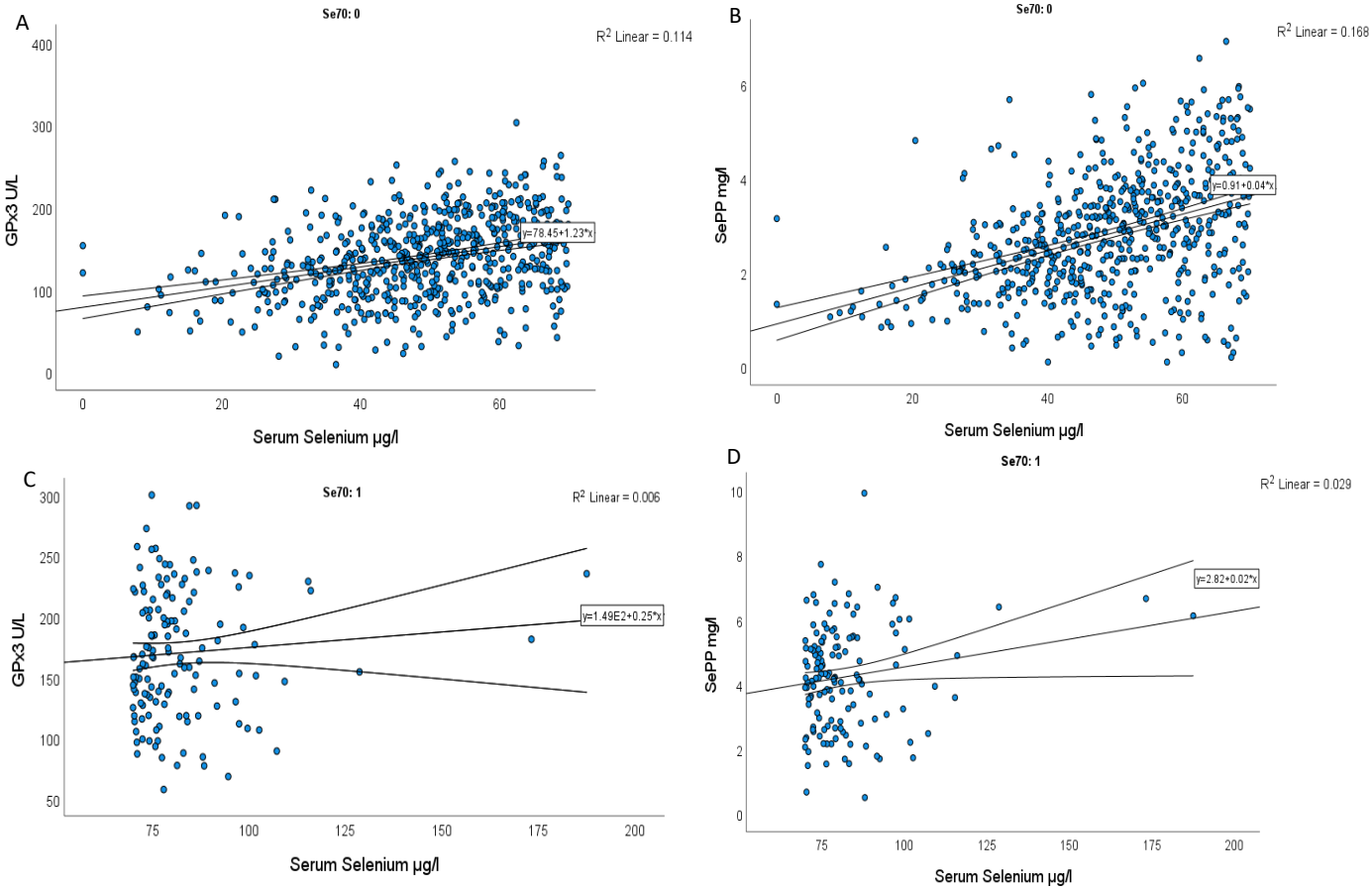


Figure 3.3: Linear correlation between serum selenium (< 70 µg/L and ≥ 70 µg/L) and selenoproteins: glutathione peroxidase 3 activity and selenoprotein P. A) Serum selenium < 70 µg/L and GPx3 activity y=78.45+1.23*X R^2: 0.114, P < 0.001; B) Serum selenium ≥ 70 µg/L and GPx3 activity y=149+0.25*X R^2: 0.006, P = 0.283 C) Serum selenium < 70 µg/L and SePP y=0.91+0.04*X R^2: 0.168, P < 0.001; D) Serum selenium ≥ 70 µg/L and SePP y=2.82+0.02*X R^2: 0.029, P = 0.253.

3.4.5. Sensitivity Analysis

The analyses were further repeated using a cut-off of 90 µg/L; the only significant differences were that those with optimal serum selenium were more likely to be using selenium supplements (P < 0.001), having better self-rated health (P = 0.021), lower hsCRP (P = 0.012) and higher SePP (P < 0.001) (Sensitivity Analyses Table 3.1 and 3.2). There were fewer predictors of each biomarker of selenium status, where serum selenium was predicted by disease count and medication, whilst the predictors of GPx3 activity and SePP predictors remained the same, as displayed in Table 3.5 (Sensitivity Analyses Table 3.3). Occupational class was significantly different between outliers (P = 0.006), as was selenium

supplement use ($P < 0.001$), total medication ($P = 0.012$) and BMI ($P = 0.050$) (Sensitivity Analyses Table 3.4). However, there were no significant differences between the biomarkers of selenium status or intake (Sensitivity Analyses Table 3.5). When using tertiles of the biomarkers of selenium status as determined by statistical derivation (Sensitivity Analyses Table 3.6), sex was no longer significantly different between low, medium or high (tertiles 1-3, respectively) selenium concentrations ($P = 0.253$), nor was BMI ($P = 0.051$) or FT_4 ($P = 0.177$). On the other hand, those with high (tertile 3) serum selenium had higher protein intakes ($P = 0.030$). Those with medium (tertile 2) serum selenium had higher alcohol intake and those with low (tertile 1) serum selenium were more likely to be non-drinkers ($P = 0.010$). Those with high (tertile 3) serum selenium were more likely to have higher FT_3 levels ($P < 0.001$), whilst those with low (tertile 1) serum selenium were more likely to misreport dietary intakes ($P = 0.049$). Those with high (tertile 3) serum selenium had higher selenium intake than those with low (tertile 1) serum selenium ($P = 0.002$). The differences in the adequacy of the biomarker of selenium status remained the same when using selected cut-offs or tertiles of serum selenium (Sensitivity Analyses Table 3.7).

3.5 Discussion

The majority of this population of older adults had suboptimal concentrations of serum selenium and SePP, however, GPx3 activity was optimal in over 70 % of the participants according to the selected cut-offs. There were strong, positive associations between the biomarkers of selenium status but not between selenium intake and both selenoproteins. Serum selenium predicted both GPx3 activity and SePP in a linear manner, indicating suboptimal expression of these selenoproteins. Each aspect of this summary will be reviewed below in more detail.

Baseline Selenium Status

Many studies have reported inadequate selenium intakes in older adults (de Jong *et al.*, 2001; Stoffaneller, Morse and Nancy, 2015; Combs, 2001), however, measurements of multiple biomarkers of selenium status are scarce in very old adults. My results indicate suboptimal selenium status (serum selenium and SePP), in older adults, lower than those in

other populations, albeit with a younger mean age (Brown *et al.*, 2000; Rayman *et al.*, 2008c; Broome *et al.*, 2004). For example, in a British cohort (50-64 years), SePP concentration was 4.9 mg/L (Hurst *et al.*, 2010), and one of the studies used for selenium DRVs reported a mean SePP concentration of 5.3 mg/L (Xia *et al.*, 2005). In adults aged > 70 years, those in the lowest quintile had serum selenium < 70 μg/L and SePP concentrations < 4.3 mg/L (Schomburg *et al.*, 2019) which are higher than the lowest tertiles from my analyses. In the EPIC-Europe cohort (mean 58 years, 70 % women), where the SePP cut-off from my analyses was based, the controls had mean serum selenium of 82 μg/L and SePP of 4.4 mg/L (Hughes *et al.*, 2015). A study in the US comprising a population (40-79 years) from low-income areas found a mean serum selenium of 117.6 μg/L, a mean GPx3 activity of 132 U/L and a mean SePP concentration of 4.7 mg/L. In comparison to my analyses, the GPx3 activity was lower, although that study included a younger age range and different ethnicities, which is known to affect selenoprotein concentrations (Hargreaves *et al.*, 2014). From these studies, it suggests that overall, my population had concentrations below the requirements for the plateauing of SePP, and therefore optimisation, although, GPx3 activity was optimal in over 70 % of participants. Similarly, this was also found in healthy and institutionalised adults (75-79 years) where despite the institutionalised adults having lower plasma selenium compared to free-living participants, the GPx3 activity did not differ significantly (Bunker *et al.*, 1988). The lower SePP concentrations appear to be contrary to the selenoprotein hierarchy, whereby SePP is usually prioritised over GPx (Behne *et al.*, 1988; Labunskyy, Hatfield and Gladyshev, 2014; Burk and Hill, 2009). This could be due to the tissue hierarchy, where organs lower in hierarchy such as the liver, where SePP is synthesised, are depleted first (Schomburg, 2022; Burk and Hill*,* 2015). However, the kidney is also compromised in times of deficiency, so one would expect GPx3 activity levels to also be lower, in addition to the fact that GPx3 is dependent on SePP (Schweizer *et al.*, 2005; Renko *et al.*, 2008). Out of the major glutathione peroxidases, GPx4 is prioritised as compared to GPx3 and, within other selenoproteins, DIOs are not essential, nor retained in times of deficiency (Schomburg and Schweizer, 2009; Becker *et al.*, 2014; Beck *et al.*, 2003)

although other studies suggest GPx4 and DIO are “housekeeping” selenoproteins and are retained (Brigelius-Flohé, 1999). In a study using a hypoxia model, selenium export via SePP was downregulated, whilst GPx4 was upregulated (Loeschner *et al.*, 2014); this contrasts other studies that suggest SePP is retained during deficiency as a transporter of selenium to priority organs, such as the brain (Schomburg, 2022; Burk and Hill, 2009; Burk *et al.*, 2014). This hypoxia model could explain the lower selenium status and SePP seen in my population where medication, illness (Forceville *et al.*, 1998) and infections (Beck *et al.*, 2003) are more common, akin to hypoxic conditions. An optimal baseline serum selenium concentration, defined using the selected cut-offs, was associated with a higher physical activity score, yet a lower FFM compared to those with suboptimal serum selenium concentration. Another study in younger adults (mean 40 years) found a similar association between low serum selenium and sedentary males (Letsiou *et al.,* 2014). However, in my analyses the interesting finding of a lower FFM may be explained by other factors, such as medication use, there were fewer participants with optimal baseline serum selenium using ≥ 6 medications compared to those with suboptimal selenium; polypharmacy and the side effects of many medications may affect FFM. It may be that the oxidative stress following intense physical activity is reduced in those optimal serum selenium concentrations due to selenoproteins role in redox balance; this downregulation may lead to a lowered mitohormesis response to exercise thereby accruing less lean mass in response to exercise, or improper recovery in older adults following exercise (Powers *et al.* 2012). The association between selenium and insulin resistance may also play a role leading to dysregulation in insulin sensitivity and muscle metabolism (Hauffe *et al.,* 2020; Chung *et al.,* 2023). Furthermore, research has suggested a link between SePP and exercise resistance through its muscle receptor low-density lipoprotein receptor–related protein 1 (LRP1) where SePP was associated with reduced responses to exercise endurance training (Misu *et al.,* 2017). More research in this area is required to further explore this relationship.

Serum selenium correlated strongly with both selenoproteins (GPx3 activity and SePP) and with selenium intakes. This has been observed in another study that found a strong correlation between serum selenium and SePP, and GPx3 activity (Hargreaves *et al.*, 2014) and, in a review of studies, SePP correlated strongly with selenium intakes and status (Persson-Moschos *et al.*, 2000). However, in my analyses, both selenoproteins did not correlate with intakes, although, selenium intakes significantly correlated with SePP in participants with serum selenium < 70 µg/L ($r(595)= 0.098$, $P = 0.016$, data not shown). In my analyses, it was found that correlations were stronger in those individuals with suboptimal serum selenium and, in some cases, some correlations, such as serum selenium and GPx3 activity, became non-significant in those with optimal serum selenium. Other studies have found similar results where suboptimal selenium status correlates well with suboptimal selenium intake (Levander, 1982; Thomson *et al.*, 1977) or selenoprotein concentrations, for example, GPx activity correlated with whole-blood when selenium concentration was 60 ng/ml compared to 200 or 400 ng/ml (Demircan *et al.*, 2021; Whanger *et al.*, 1988). An interesting observation was that selenium intake did not differ between those classified as having optimal selenium status ≥ 70 µg/L and those classified as having suboptimal status < 70 µg/L. This may be due to the uncertainty in food surveys in estimating selenium intakes (Keck and Finley, 2006).

Predictors of Biomarkers of Selenium Status

As expected, protein intake was a significant predictor of the biomarkers of selenium status. Protein-rich foods are generally also rich in selenium and have correlated with SePP in older women (Persson-Moschos *et al.*, 2000). Like my analyses, Bates *et al.*, (2002) found those with increased medication and poorer health to have suboptimal concentrations of serum selenium. This may be due to an increase in inflammation which is negatively associated with selenium status (Nichol *et al.*, 1998; Sempértegui *et al.*, 2003; Huang, Rose and Hoffmann, 2012; Ghayour-Mobarhan *et al.*, 2005). Similarly, waist:hip ratio predicted serum selenium and BMI predicted GPx3 activity and these anthropometric measures are often associated with increased inflammation and oxidative stress (Keaney *et al.*, 2003). Furthermore, in my results, sex (being male) was a positive predictor of serum selenium and GPx3 activity, sex differences have been reported in other studies (Gámez *et al.*, 1997; Arnaud *et al.*, 2006; Niskar, Paschal and Kieszak, 2003; Lopes *et al.*, 2004; Al-Mubarak *et al.*,

2020), but not others (Bates *et al.*, 2002; Monget *et al.*, 1996; Imai *et al.*, 1990). Some reasons may be due to altered hormonal status, such as estrogen (Karita *et al.*, 2008), although there are mixed results; Arnaud *et al.*, (2006) and Stoffaneller, Morse and Nancy, (2015) found higher selenium status in postmenopausal women, whilst others did not (Bureau *et al.*, 2002). Since the women in my population were 85 years and older and contributed to a large proportion of the participants (61 %), the menopausal state could affect the overall selenium status as reported in another study where selenium concentrations varied over 50 % (Hybsier *et al.*, 2017). Like my findings, other studies also reported women to have higher levels of GPx3 activity (Ha and Smith, 2003), which may be due to the disproportionate increase in visceral fat with menopause and increasing age leading to increased inflammation and therefore increased requirements of GPx3 (Steyn *et al.*, 2019). Higher levels of physical activity were negatively associated with GPx3 activity. In contrast, GPx3 activity increased in sedentary, postmenopausal women (65 years) undertaking a 12-week walking program (Rusip and Suhratini, 2020). Changes in GPx3 activity can be associated with oxidative stress whereby GPx3 functions to detoxify free radicals which are known to increase after intense physical activity (Powers *et al.*, 1999). However, in my population, higher physical activity was associated with lower GPx3 activity. Finally, the biomarkers of selenium status were negatively associated with free T_4, a marker of thyroid function; this is plausible in The Newcastle 85+ population where serum selenium concentrations are low. Selenoproteins, specifically the DIOs are required for the conversion of the inactive T_4 (thyroxine) to T_3. When selenium is inadequate, selenoprotein synthesis is limited, creating lower conversion rates and therefore higher concentrations of T_4 (Kobayashi *et al.*, 2021). This trend has also been seen in other studies where T_4 increases with selenium deficiency (Olivieri *et al.*, 1995; Bates *et al.*, 2002; Drutel, Archambeaud and Caron, 2013).

Quantification of the Relationship between Serum Selenium and GPx3 Activity and SePP

Using a linear regression and the reported cut-off for serum selenium concentration (70 µg/L) for GPx3 activity plateau, the estimated concentrations for GPx3 activity plateau were 158.7 U/L. Using the reported cut-off for serum selenium concentration (90 µg/L) for SePP plateau, the estimated concentrations for SePP plateau were 4.6 mg/L, which is in line with other studies suggesting 4.4-7 mg/L (Combs, 2015; Hurst *et al.*, 2010; Burk *et al.*, 2006;

Hughes *et al.*, 2015). Another study that also used linear and quadratic regressions to plot the relationship between serum selenium and SePP found that the quadratic, rather than the linear equation, led to a better estimation of SePP plateau (Marchaluk, Persson-Moschos and Thorling, 1995), although there were minimal differences between the R^2 of the graphs (0.46 linear, 0.50 quadratic). Quadratic equations were also tested in my analyses, but likewise, the R^2s were not largely different, therefore the most parsimonious model i.e. linear, was retained. When rearranging the equations to estimate the serum selenium concentration to reach the cut-offs used for my analyses, i.e. 115 U/L and 4.5 mg/L, GPx3 activity estimated a serum selenium of 25.0 µg/L, whilst SePP estimated a serum selenium of 87.8 µg/L. In my population, the linear relationships between serum selenium and the selenoproteins indicate suboptimal selenium status. The SePP cut-off fitted well with the suggested requirements for SePP plateau, however, the GPx3 activity cut-off appeared to estimate a considerably suboptimal serum selenium concentration (25.0 µg/L), below any of the requirements previously reported. This could suggest that SePP requirements of selenium in very old adults are similar to that of younger adults (> 65 years), however, it may be necessary to adapt the requirements in line with GPx3 activity. However, if we assumed 70 µg/L was also optimal for SePP plateau, SePP concentrations were estimated to be 2.3 mg/L which is also lower than that of other studies. These findings stress the need for future experimental studies using selenium supplementation to determine the DRVs in older adults.

Despite these potential similarities in selenium concentrations required for efficient functioning of selenoproteins, my population still had suboptimal selenium status. It may be that very old adults have adapted to lower selenium concentrations and have internal mechanisms to cope with these lower concentrations without a detriment to health, as there is no evidence to suggest impaired health or function with suboptimal concentrations of selenoproteins (WHO, 2004). These adaptations may be through lower excretion, or an upregulation antioxidant pathways, and thus have no adverse consequences (Duffield *et al.*, 1999; Robinson *et al.*, 1985). For example, in rats, GPx mRNA levels plateaued at half the levels required for GPx activity suggesting normative requirements could be lower and these lower levels may be compensated by other antioxidative systems (Sunde, 1997). Furthermore, in different populations, i.e. those with suboptimal selenium exposure and

status, lower concentrations of selenium may be required plateau; for example Hill *et al.*, (1996) found that maximal activity was achieved at a lower concentration (70 µg/L; 0.9 µmol/L) in a selenium-deficient population (Xia *et al.,* 1989) compared to the higher concentrations seen in other studies (86-118 µg/L; 1.05-1.5 µmol/L) (Duffield *et al.*, 1999; Marchaluk, Persson-Moschos and Thorling 1995; Persson-Moschos *et al.*, 1995) and this was also observed in other populations with suboptimal status (Olivieri *et al.*, 1995; Whanger *et al.*, 1988). The requirements may also change depending on the selenoprotein, for example, lower concentrations (equating to 0.82 µmol/L; 65 µg/L) led to an earlier plateau of DIO compared to GPx (Duffield *et al.*, 1999). On a different note, a rodent study by Yim *et al.*, (2019), proposed that selenium deficiency was associated with pro-longevity mechanisms. Despite the lower selenoprotein concentrations in the selenium-deficient rats, there was no negative effect on lifespan and, in fact, an increase in lifespan was observed. This may be due to a reduction in amino acid levels activating the mammalian target of rapamycin (mTOR) pathway associated with cellular ageing and an upregulation of nutrient sensing (Fontana, Partridge and Longo, 2010).

The overall strengths and limitations in terms of the study design are summarised in Chapter 7, Section 7.2. To summarise, this is the first, largest, cross-sectional study known to date that has measured selenium status using a range of biomarkers, in very old adults. However, as this was a cross-sectional study, unlike the studies used for the selenium DRVs, the derivations from the linear regression equations are to be taken with caution. Nonetheless, this is still an important step in exploring the relationships between biomarkers of selenium status in older adults. The lack of clustering in the biomarkers of selenium status may have influenced the lack of findings, further analyses could utilise unsupervised hierarchical clustering. Furthermore, a remarkable observation was that two participants from the 757-sample had serum selenium concentrations below 10.0 µg/L. This was never witnessed in the laboratory before and thus, during the outlier sensitivity analysis, these two participants were removed, however, there were no significant changes in the findings.

3.6 Conclusion

The selenium status of this population was suggested suboptimal, especially serum selenium and SePP concentrations. However, despite the suboptimal status in this population, these

older adults do not appear to be obviously compromised by these concentrations, due to the fact they are living beyond 85 years. However, in the following chapter, I will assess the relationships between these biomarkers of selenium status and MSK function to determine if there are any health consequences in those participants with suboptimal selenium status.

3.7 Appendix

Table 3.1: Predictors of the biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) determined using linear regression.

	Variable	Model	
		β (SE)	*p*
Serum Selenium	Constant	55.32 (15.72)	< 0.001
	Sex	8.04 (3.00)	0.008
	BMI	−0.31 (0.27)	0.250
	Fat Free Mass (kg)	-0.06 (0.18)	0.723
	Waist:Hip	24.08 (12.08)	0.047
	SMMSE	−0.09 (0.26)	0.746
	Free T_3 (pmol/L)	1.38 (1.54)	0.371
	Free T_4 (pmol/L)	-0.64 (0.30)	0.034
	hsCRP (mg/L)	-0.07 (0.06)	0.200
	Energy Intake (Kcal)	−0.003 (0.002)	0.204
	Protein Intake (g)	0.13 (0.05)	0.013
	Selenium Intake (g)	0.032 (0.03)	0.226
	NS-SEC	-0.22 (0.93)	0.817
	Education	0.09 (1.21)	0.940
	Living Status	-10.15 (4.99)	0.042
	Self-rated Health	−2.13 (1.07)	0.046
	Disease Count	-0.99 (0.50)	0.048
	Total Medications	-2.40 (1.15)	0.037
	Smoking Status	−0.004 (0.82)	0.997
	Alcohol Intake	1.88 (1.68)	0.262
Glutathione Peroxidase 3	Constant	194.46 (42.81)	< 0.001
	Sex	31.50 (8.19)	< 0.001
	BMI	−2.20 (0.73)	0.003
	Fat Free Mass (kg)	0.67 (0.49)	0.169
	Waist:hip	23.30 (32.92)	0.479
	SMMSE	-0.20 (0.72)	0.779
	Free T_3 (pmol/L)	-6.77 (4.21)	0.108
	Free T_4 (pmol/L)	-1.80 (0.83)	0.030
	hsCRP (mg/L)	-0.04 (0.15)	0.793
	Energy Intake (Kcal)	0.001 (0.006)	0.851
	Protein Intake (g)	0.33 (0.15)	0.024
	Selenium Intake (g)	-0.03 (0.07)	0.732
	NS-SEC	-4.87 (2.54)	0.056
	Education	-3.74 (3.31)	0.258
	Living Status	8.37 (13.56)	0.537
	Self-rated Health	-3.35 (2.90)	0.249
	Disease Count	-0.76 (1.36)	0.576
	Total Medications	-2.42 (3.13)	0.439
	Smoking Status	-1.87 (2.25)	0.404
	Alcohol Intake	3.78 (4.57)	0.409
Selenoprotein P	Constant	4.32 (1.27)	< 0.001
	Sex	0.46 (0.24)	0.059
	BMI	−0.006 (0.02)	0.776
	Fat Free Mass (kg)	-0.006 (0.01)	0.689
	Waist:hip	0.72 (0.98)	0.461
	SMMSE	−0.02 (0.02)	0.484
	Free T_3 (pmol/L)	-0.03 (0.13)	0.801
	Free T_4 (pmol/L)	-0.08 (0.02)	< 0.001
	hsCRP (mg/L)	-0.003 (0.004)	0.532
	Energy Intake (Kcal)	0.00 (0.00)	0.325
	Protein Intake (g)	0.01 (0.004)	0.003
	Selenium Intake (g)	0.001 (0.002)	0.701
	NS-SEC	-0.14 (0.08)	0.065
	Education	-0.18 (0.10)	0.070
	Living Status	-0.48 (0.40)	0.238
	Self-rated Health	0.03 (0.09)	0.691
	Disease Count	0.02 (0.04)	0.552
	Total Medications	-0.02 (0.09)	0.850
	Smoking Status	0.01 (0.07)	0.931
	Alcohol Intake	-0.20 (0.14)	0.134

SE: standard error; BMI: body mass index; SMMSE: standardised mini mental state examination; Free T_4: free thyroxine; Free T_3: free triiodothyronine; hsCRP: high sensitivity C-reactive protein; NS-SEC: National Statistics Socio-economic classification.

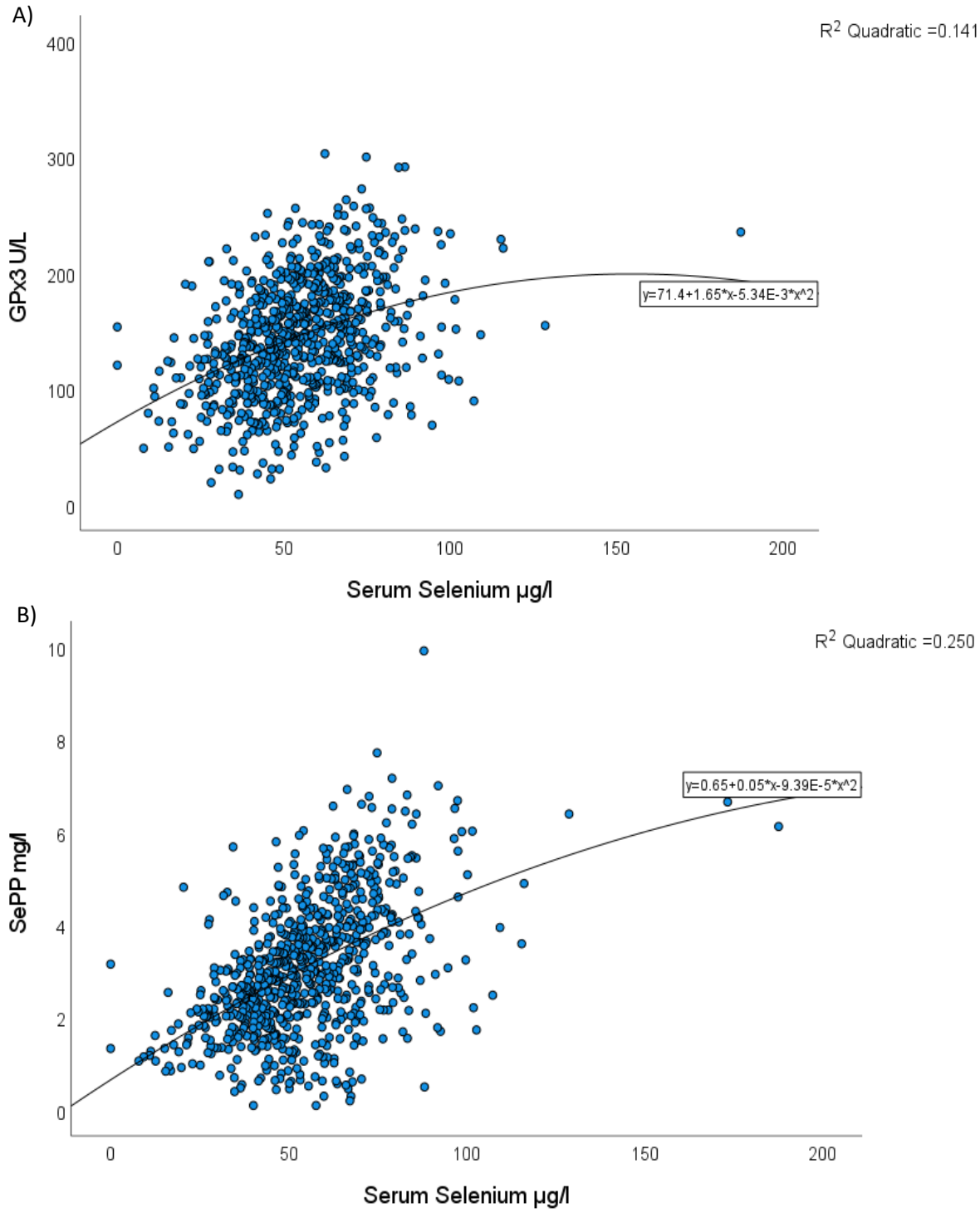


Figure 3.1: Quadratic correlation between serum selenium and selenoproteins. A) Serum selenium and glutathione peroxidase 3 activity $y=71.4+1.65*x-5.34^{E-5}*x^2$ R^2: 0.141 B) Serum selenium and selenoprotein P $y=0.65+0.05*x-9.39^{E-5}*x^2$ R^2: 0.250

3.8 Sensitivity Analyses

Table 3.1. Characteristics of study participants represented by serum selenium cut-offs.

Characteristic	All Participants n = 757	Suboptimal Selenium < 90 µg/L	Optimal Selenium ≥ 90 µg/L	p
Socio-demographic factors				
Women % (n)	61.1 (461)	60.9 (445)	69.6 (16)	0.400
Men % (n)	38.9 (293)	39.1 (286)	30.4 (7)	
Years of education % (n) n = 743				
0–9	64.2 (477)	64.4 (464)	56.5 (13)	0.672
10–11	23.6 (175)	23.5 (169)	26.1 (6)	
≥ 12	12.2 (91)	12.1 (87)	17.4 (4)	
Occupational class % (n) n = 743				
Managerial and Professional	35.1 (253)	35.0 (244)	39.1 (9)	0.189
Intermediate	14.7 (106)	14.3 (100)	26.1 (6)	
Routine and Manual	50.2 (362)	50.7 (354)	34.8 (8)	
Living in Institutions % (n) n = 755				
Yes	8.9 (67)	9.2 (67)	0.0 (0)	0.129
No	91.1 (688)	90.8 (665)	100.0 (23)	
Diet-related factors				
Diet change in past year % (n) n = 733				
Yes	17.1 (117)			0.910
No	82.9 (568)			
Total energy kCal (M, SD) n = 732	1688.6, 511.0	1707.0, 515.5	1774.4, 408.8	0.895
Protein Intake g (M, SD) n = 732	64.2, 22.3	65.0, 22.4	72.2, 27.5	0.464
Misreporting food intake % (n) n = 685				
Yes	6.5 (48)	84.6 17.9 (99)	15.4 13.7 (18)	0.259
No	93.5 (685)	80.1 82.1 (455)	19.9 86.3 (113)	
Lifestyle factors				
Smoking % (n) n = 754				
Non-Smoker	35.0 (264)	35.2 (257)	30.4 (7)	0.392
Former Smoker	59.4 (448)	59.1 (432)	69.6 (16)	
Current Smoker	5.6 (42)	5.7 (42)	0.0 (0.0)	
Current alcohol intake % (n) n = 751				
Yes	62.3 (468)	61.8 (450)	78.3 (18)	0.109
No	37.7 (283)	38.2 (278)	21.7 (5)	
Physical activity (PA) % (n) n = 738				
Low (score 0–1)	21.7 (162)	22.1 (160)	8.7 (2)	0.147
Moderate (score 2–6)	43.0 (322)	43.2 (313)	39.1 (9)	
High (score 7–18)	35.3 (264)	34.8 (252)	52.2 (12)	
Selenium Supplement Use % (n) n = 755				
Yes	0.7 (5)	0.1 (1)	17.4 (4)	< 0.001
No	99.3 (750)	9.99 (731)	82.6 (19)	
Number of Medications (M, SD) n = 732	6.3, 3.8	6.1, 3.5	4.8, 3.2	0.075
Total Medication % (n) n = 753				
0-2	16.7 (126)	16.4 (120)	26.1 (6)	0.100
3-5	26.7 (201)	26.3 (192)	39.1 (9)	
≥ 6	56.6 (426)	57.3 (418)	34.8 (8)	
Health-related factors				
Self-rated health % (n) n = 738				
Excellent/Very Good	40.9 (302)	40.2 (288)	63.6 (14)	0.021
Good	37.7 (278)	37.7 (270)	36.4 (8)	
Fair/Poor	21.4 (158)	22.1 (158)	0.0 (0)	
SMMSE (M, SD) n = 753	26.1, 4.9	27.0, 3.5	26.5, 4.1	0.625
hsCRP mg/L (M, SD) n = 753	6.9, 14.2	6.3, 14.2	2.7, 2.9	0.012
Free T_4 in pmol/L (M, SD) n = 742	15.6, 2.7	15.6, 2.6	15.1, 1.6	0.605
Free T_3 in pmol/L (M, SD) n = 743	4.5, 0.5	4.5, 0.5	4.5, 0.6	0.655
Anthropometry				
BMI (M, SD) n = 674	24.4, 4.4	24.5, 4.4	23.3, 2.8	0.112
Fat Free Mass (M, SD) n = 689	45.2, 9.0	45.5, 9.1	43.9, 10.2	0.166
Waist:Hip Ratio (M, SD) n = 685	0.89, 0.08	0.9, 0.07	0.9, 0.07	0.685
Height (M, SD) n = 712	1.6, 0.08	1.6, 0.08	1.6, 0.08	0.656

SMMSE: standardised mini mental state examination; hsCRP: high sensitivity C-Reactive protein; Free T_4: free thyroxine; Free T_3: free triiodothyronine; BMI: body mass index; M: mean; IQR: interquartile range; SePP: selenoprotein P; GPx3: glutathione peroxidase 3

Table 3.2: Biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) of study participants represented by serum selenium cut-offs.

Characteristic	All Participants	Suboptimal Selenium < 90 µg/L	Optimal Selenium ≥ 90 µg/L	p
Selenium Intake (M, IQR) n = 732	45.3, 29.2	39.7, 29.9	49.9, 40.5	0.178
Serum Selenium (M, IQR) n = 757	55.0, 23.6	54.0, 22.1	99.2, 13.6	< 0.001
Selenoprotein P (M, IQR) n = 757	3.1, 1.9	2.9, 1.9	4.3, 3.5	< 0.001
GPx3 Activity (M, SD) n = 755	144.1, 50.7	144.0, 50.2	158.5, 48.8	0.053
Selenium at 90 µg/L % (n)				
Yes	97.0 (23)			
No	3.0 (734)			
GPx3 Activity at 115 U/L % (n)				
Yes	70.2 (530)	69.9 (512)	78.3 (18)	0.391
No	29.8 (225)	30.1 (220)	21.7 (5)	
SePP at 4.5 mg/L % (n)				
Yes	17.2 (130)	15.9 (117)	56.5 (13)	< 0.001
No	82.8 (627)	84.1 (617)	43.5 (10)	

M: mean; IQR: interquartile range; SePP: selenoprotein P; GPx3: glutathione peroxidase 3

Table 3.3: Equations derived from linear regression for each selenium biomarker (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) using the significant predictors and intercept values, β(SE), for participants consuming below 90 µg/L.

Biomarker	Equation from linear regression < 90 µg/L
Selenium	= 41.38(12.10) – 1.13(0.43)Disease Count – 2.09(0.98)Medication
GPx3 Activity	=151.88(39.73) + 28.58(8.50)Sex – 2.03(0.76)BMI – 1.36(0.61)PA + 0.37(0.15)Protein
SePP	=2.59(1.14) + 0.02(0.004)Protein

GPx3: glutathione peroxidase 3; SePP: selenoprotein P; BMI: body mass index; PA: physical activity

Table 3.4: Baseline characteristics of participants and comparison between those classified as non-outliers and outliers of serum selenium using 75th percentile of serum selenium plus interquartile range x 1.5.

Characteristic	All Participants n = 757	Non-Outlier	Outlier	p
Socio-demographic factors				
Women % (n)	61.1 (461)	60.9 (453)	80.0 (8)	0.218
Men % (n)	38.9 (293)	39.1 (291)	20.0 (2)	
Years of education % (n) n = 743				
0–9	64.2 (477)	64.1 (470)	70.0 (7)	0.928
10–11	23.6 (175)	23.6 (173)	20.0 (2)	
≥ 12	12.2 (91)	12.3 (90)	10.0 (1)	
Occupational class % (n) n = 721				
Managerial and Professional	35.1 (253)	35.3 (251)	20.0 (2)	0.006
Intermediate	14.7 (106)	14.2 (101)	50.0 (5)	
Routine and Manual	50.2 (362)	50.5 (359)	30.0 (3)	
Living in Institutions % (n) n = 755				
Yes	8.9 (67)	8.9 (66)	10.0 (1)	0.900
No	91.1 (688)	91.1 (679)	90.0 (9)	
Diet-related factors				
Diet change in past year % (n) n = 733				
Yes	17.1 (117)	93.4 (676)	0.0 (0)	0.424
No	82.9 (568)	6.6 (48)	100.0 (9)	
Total energy kCal (M, SD) n = 732	1688.6, 511.0	1686.5, 510.6	1720.1, 537.2	0.986
Protein Intake g (M, SD) n = 732	64.2, 22.3	64.0, 22.1	76.3, 33.7	0.424
Misreporting food intake % (n) n = 685				
Yes	6.5 (48)	82.7 (558)	100.0 (10)	0.148
No	93.5 (685)	17.3 (117)	0.0 (0)	
Lifestyle factors				
Smoking % (n) n=754				
Non-Smoker	35.0 (264)	35.1 (261)	30.0 (3)	0.661
Former Smoker	59.4 (448)	59.3 (441)	0.0 (0)	
Current Smoker	5.6 (42)	5.6 (42)	70.0 (7)	
Current alcohol intake % (n) n = 751				
Yes	62.3 (468)	62.1 (460)	20.0 (2)	0.245
No	37.7 (283)	37.9 (281)	80.0 (8)	
Physical activity (PA) % (n) n = 738				
Low (score 0–1)	21.7 (162)	21.7 (160)	20.0 (2)	0.596
Moderate (score 2–6)	43.0 (322)	43.2 (319)	30.0 (3)	
High (score 7–18)	35.3 (264)	35.1 (259)	50.0 (5)	
Selenium Supplement Use % (n) n = 755				
Yes	0.7 (5)	0.4 (3)	20.0 (2)	< 0.001
No	99.3 (750)	99.6 (742)	60.0 (3)	
Number of Medications (M, SD) n = 732	6.3, 3.8	6.1, 4.0	4.6, 4.0	0.158
Total Medication % (n) n = 753				
0-2	16.7 (126)	16.8 (125)	10.0 (1)	0.012
3-4	26.7 (201)	26.4 (196)	50.0 (5)	
≥ 6	56.6 (426)	56.8 (422)	40.0 (4)	
Health-related factors				
Self-rated health % (n) n = 738				
Excellent/Very Good	40.9 (302)	40.6 (296)	66.7 (6)	0.176
Good	37.7 (278)	37.7 (275)	33.3 (3)	
Fair/Poor	21.4 (158)	21.7 (158)	0.0 (0)	
SMMSE (M, SD) n = 753	26.1, 4.9	26.3, 4.6	24.4, 9.2	0.962
hsCRP mg/L (M, SD) n = 753	6.9, 14.2	6.1, 4.4	2.7, 5.2	0.066
Free T_3 pmol/L (M, SD) n = 742	4.5, 0.5	4.5. 0.6	4.4, 0.7	0.373
Free T_4 pmol/L (M, SD) n = 743	15.6, 2.7	15.6, 3.0	15.8, 4.0	0.784
Anthropometry				
BMI (M, SD) n = 674	24.4, 4.4	24.4, 5.6	22.6, 4.5	0.050
Waist:Hip Ratio (M, SD) n = 685	0.88, 0.08	0.88, 1.0	0.87, 0.13	0.727
Height (M, SD) n = 712	1.62, 0.08	1.62, 0.12	1.6, 0.15	0.987
Fat Free Mass (M, SD) n = 689	45.2, 9.0	45.3, 13.8	43.9, 22.0	0.177

SMMSE: standardised mini mental state examination; hsCRP: high sensitivity C-Reactive protein; Free T_4: free thyroxine; Free T_3: free triiodothyronine; BMI: body mass index; M: mean; IQR: interquartile range

Table 3.5: Biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) of study participants using 75th percentile of serum selenium plus interquartile range x 1.5.

Characteristic	All Participants	Non-outlier	Outlier	p
Selenium Intake (Median, IQR) n=732	45.3, 29.8	39.7, 30.1	45.7, 113.8	0.231
Serum Selenium (Median, IQR) n=757	55.0, 19.0	54.2, 22.8	112.4, 55.1	0.001
Selenoprotein P (Median, IQR) n=757	3.1, 1.4	2.9, 1.9	3.8, 3.9	0.111
GPx3 Activity (M, SD) n=755	144.1, 50.7	144.5, 50.1	166.2, 58.4	0.264
Selenium 70 µg/L % (n)				< 0.001
Yes	97.0 (23)	17.4 (130)	80.0 (8)	
No	3.0 (734)	82.6 (617)	20.0 (2)	
GPx3 Activity at 115 U/L % (n)				0.495
Yes	70.2 (530)	70.1 (522)	80.0 (8)	
No	29.8 (225)	29.9 (223)	20.0 (2)	
SePP at 4.5 mg/L % (n)				0.054
Yes	17.2 (130)	83.1 (621)	40.0 (4)	
No	82.8 (627)	16.9 (126)	60.0 (6)	

M: mean; IQR: interquartile range; SePP: selenoprotein P; GPx3: glutathione peroxidase 3

Table 3.6: Baseline characteristics of participants separated by tertiles of serum selenium.

Characteristic	All Participants n = 757	Low Selenium ≤ 46.7 µg/L (Tertile 1)	Medium Selenium 46.73-62 µg/L (Tertile 2)	High Selenium ≥ 62.0 µg/L (Tertile 3)	p
Socio-demographic factors					
Women % (n)	61.1 (461)	33.2 (153)	31.5 (145)	35.4 (163)	0.253
Men % (n)	38.9 (293)	33.8 (99)	36.2 (106)	30.0 (88)	
Years of education % (n) n = 743					
0–9	64.2 (477)	34.0 (162)	34.4 (164)	31.7 (151)	0.463
10–11	23.6 (175)	34.3 (60)	29.1 (51)	36.6 (64)	
≥ 12	12.2 (91)	27.5 (25)	35.2 (32)	37.4 (34)	
Occupational class % (n) n = 721					
Managerial and Professional	35.1 (253)	32.0 (81)	32.0 (81)	36.0 (91)	0.161
Intermediate	14.7 (106)	25.5 (27)	33.0 (35)	41.5 (44)	
Routine and Manual	50.2 (362)	35.6 (129)	34.3 (124)	30.1 (109)	
Living in Institutions % (n) n = 755					
Yes	8.9 (67)	68.7 (46)	19.4 (13)	11.9 (8)	< 0.001
No	91.1 (688)	30.1 (207)	34.6 (238)	35.3 (243)	
Diet-related factors					
Diet change in past year % (n) n = 733					
Yes	17.1 (117)	33.3 (16)	33.3 (16)	33.3 (16)	0.999
No	82.9 (568)	33.0 (226)	33.6 (230)	33.4 (229)	
Total energy kCal (M, SD) n =732	1688.6, 511.0	1699.9, 514.2	1722.9, 575.4	1704.0, 440.8	0.658
Protein Intake g (M, SD) n = 732	64.2, 22.3	62.1, 20.0	65.8, 24.5	67.5, 22.4	0.030
Misreporting food intake % (n) n = 685					
Yes	6.5 (48)	39.3 (46)	35.0 (41)	25.6 (30)	0.049
No	93.5 (685)	29.9 170)	33.6 (191)	36.4(207)	
Lifestyle factors					
Smoking % (n) n = 754					
Non-Smoker	35.0 (264)	31.1 (82)	34.8 (92)	34.1 (90)	0.450
Former Smoker	59.4 (448)	34.4 (154)	33.5 (150)	32.1 (144)	
Current Smoker	5.6 (42)	38.1 (16)	21.4 (9)	40.5 (17)	
Current alcohol intake % (n) n = 751					
Yes	62.3 (468)	29.5 (138)	36.1 (169)	34.4 (161)	0.010
No	37.7 (283)	39.9 (113)	28.6 (81)	31.4 (89)	
Physical activity (PA) % (n) n = 738					
Low (score 0–1)	21.7 (162)	49.4 (80)	25.9 (42)	24.7 (40)	< 0.001
Moderate (score 2–6)	43.0 (322)	29.8 (96)	37.9 (122)	32.3 (104)	
High (score 7–18)	35.3 (264)	27.7 (73)	32.2 (85)	40.2 (106)	
Selenium Supplement Use % (n) n = 755					
Yes	0.7 (5)	0.0 (0)	0.0 (0)	100.0 (5)	0.006
No	99.3 (750)	33.7 (253)	33.5(251)	32.8 (246)	
Number of Medications M, SD n = 732	6.3, 3.8	6.7, 3.4	6.2, 3.6	5.3, 3.4	< 0.001
Total Medication % (n) n = 753					
0-2	16.7 (126)	23.8 (30)	29.4 (37)	46.8 (59)	< 0.001
3-4	26.7 (201)	25.9 (52)	37.8 (76)	36.3 (73)	
≥ 6	56.6 (426)	39.9 (170)	32.2 (137)	27.9 (119)	
Health-related factors					
Self-rated health % (n) n = 738					
Excellent/Very Good	40.9 (302)	34.6 (84)	41.4 (101)	47.0 (117)	0.074
Good	37.7 (278)	36.0 (100)	32.4 (90)	31.7 (88)	
Fair/Poor	21.4 (158)	37.3 (59)	34.8 (55)	27.8 (44)	
SMMSE (M, SD) n = 753	26.1, 4.9	26.6, 4.0	26.8, 3.7	27.5, 2.8	
hsCRP mg/L (M, SD) n = 753	6.9, 14.2	8.1, 17.8	6.2, 14.1	4.3, 9.0	0.001
Free T_3 pmol/L (M, SD) n = 742	4.5, 0.54	4.5. 0.55	4.6, 0.5	4.6, 0.5	< 0.001
Free T_4 pmol/L (M, SD) n = 743	15.6, 2.7	15.8, 2.7	15.5, 2.6	15.5, 2.5	0.177
Anthropometry					
BMI (M, SD) n = 674	24.4, 4.4	24.4 4.4	24.8, 4.5	24.1, 4.2	0.051
Waist:Hip Ratio (M, SD) n = 685	0.88, 0.08	0.88, 0.07	0.89, 0.07	0.9, 0.08	0.506
Height (M, SD) n = 712	1.62, 0.08	1.6 0.07	1.62, 0.08	1.6, 0.08	0.560
Fat Free Mass (M, SD) n = 689	45.2, 9.0	45.7, 8.6	46.5, 9.4	44.2, 9.1	0.024

SMMSE: standardised mini mental state examination; hsCRP: high sensitivity C-Reactive protein; Free T_3: free triiodothyronine; Free T_4: free thyroxine; BMI: body mass index. M: mean; IQR: interquartile range

Table 3.7: Biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) of study participants separated by serum selenium tertiles.

Characteristic	All Participants	Low Selenium ≤ 46.7 µg/L (Tertile 1)	Medium Selenium 46.73-62 µg/L (Tertile 2)	High Selenium ≥ 62.0 µg/L (Tertile 3)	p
Selenium Intake (Median, IQR) n = 732	45.3, 29.8	42.6, 36.3	45.3, 24.2	51.2, 33.4	0.002
Serum Selenium (Median, IQR) n = 757	55.0, 19.0	39.1 10.0	54.1, 4.4	75.4, 15.0	< 0.001
Selenoprotein P (Median, IQR) n = 757	3.1, 1.4	2.3 1.14	3.00, 1.24	3.9, 1.7	< 0.001
GPx3 Activity (M, SD) n = 755	144.1, 50.7	123.8 43.2	143.2 46.5	163.6 52.0	< 0.001
Selenium Tertile µg/L % (n)					
Low ≤ 46.7	33.4 (253)				
Medium 46.73-62.0	33.3 (252)				
High ≥ 62.0	33.3 (252)				
GPx3 Activity at 115 U/L % (n)					
Yes	70.2 (530)	26.4 (140)	34.7(184)	38.9 (206)	< 0.001
No	29.8 (225)	50.2 (113)	29.8 (67)	20.0 (45)	
SePP at 4.5 mg/L % (n)					
Yes	17.2 (130)	5.4 (7)	21.5 (28)	73.1 (95)	< 0.001
No	82.8 (627)	39.2 (246)	35.7 (224)	25.0 (157)	

M: mean; IQR: interquartile range; SePP: selenoprotein P; GPx3: glutathione peroxidase 3

Chapter 4. Selenium Status and MSK Function in Very Old Adults: The Newcastle 85+ Study

4.1 Abstract

Background: Selenium has been associated with MSK function in observational studies, however, these associations are not explored in very old adults (≥ 85 years).
Objectives: To explore the relationships between biomarkers of selenium status and MSK function among participants in The Newcastle 85+ Study at baseline and prospectively.
Methods: Biomarkers of selenium status (serum selenium, GPx3 activity and SePP) at baseline were measured using standard laboratory techniques in 757 participants from The Newcastle 85+ Study. HGS was measured using a hand-held dynamometer and averaging the two measurements from each hand. TUG measured how long it took to rise from a chair, walk 3 m and return, and sarcopenia prevalence was determined according to EWGSOP cut-offs. The relationship between the biomarkers of selenium status and MSK function (HGS, TUG, sarcopenia) were analysed at baseline and up to 5 years using linear mixed models that adjusted for appropriate covariates.
Results: At baseline, in the fully adjusted models, there were no associations between any of the biomarkers of selenium status and the MSK outcomes. Over time, in fully adjusted models, there was a negative association between GPx3 activity and the change in prevalence of sarcopenia (β 8.44^{E-4} ± 3.88^{E-4}, P = 0.030), and, when using tertiles, low (tertile 1) serum selenium (≤ 46.7 µg/L) was associated with greater change in TUG performance (β 0.06 ± 0.02, P = 0.013) and medium (tertile 2) serum selenium (46.7-62.0 µg/L) was associated with a greater prevalence of severe sarcopenia (β -0.16 ± 0.07, P = 0.020).
Conclusion: In cross-sectional analyses, serum selenium was associated with higher HGS, and serum selenium and SePP were associated with better TUG performance. Over time, low (tertile 1) or medium (tertile 2) concentrations of selenium were associated with greater rates of change in TUG and sarcopenia, respectively. It would be interesting to explore this further using a clinical endpoint of physical function, such as disability.

4.2 Introduction

Healthy ageing is a prominent concern due to the global increase in ageing populations (ONS, 2018). MSK function is important throughout life, especially during older age when reductions in muscular strength, muscle mass and function and the loss of BMD are greater (Faulkner *et al.*, 2007; Cruz-Jentoft *et al.*, 2019). These functional changes increase the risk of age-related MSK diseases including sarcopenia and osteoporosis (Cruz-Jentoft *et al.*, 2010) which are associated with an increase in falls, and fractures (von Haehling, Morely and Anker, 2010) and hospitalisation (von Haehling, Morely and Anker, 2010; Cruz-Jentoft *et al.*, 2010). Development of poor MSK function is multifactorial and both insufficient nutrient intake (Scott *et al.*, 2010; Robinson, 2008; Ganapathy and Nieves, 2020) and low physical activity (Freiberger, Sieber and Pfeifer, 2011; Beck *et al.*, 2017; Lee *et al.*, 2018; Taylor *et al.*, 2004), which are both common in older adults, exacerbate the risk of poor MSK function. Ageing is also associated with increased oxidative stress that is caused by an imbalance between oxidant and antioxidant status (Baumann *et al.*, 2016; Chrousos, 2009). This can lead to multiple detrimental impacts such as DNA damage and reduced MPS which can impair muscle function (Powers *et al.*, 2011). Evidence from observational studies has suggested that micronutrient deficiencies, especially in those with antioxidant properties, may contribute to increased risk of sarcopenia, disability and poor muscular function (van Dijk *et al.*, 2016; Arikan *et al.*, 2011; Lescure *et al.*, 2009; Chariot and Bignani, 2003; Rederstorff, Krol and Lescure, 2006).

Selenium deficiency has been associated with MSK function including cardiomyopathies, SEPN1-related disorders, muscle pain and weakness, BMD, fracture incidence, FRAX score and impaired thyroid function relating to bone metabolism (Chariot and Bignani, 2003; Rederstorff, Krol and Lescure, 2006). In an observational study in community-dwelling women (≥ 65 years), there was a positive association between serum selenium concentration and muscle strength, assessed by HGS (Beck *et al.*, 2007). There was also a positive association with serum selenium and BMD in postmenopausal women (Hoeg *et al.*,

2012) and older men (Beukhof *et al.*, 2016). Likewise, in a US population (NHANES) (≥ 40 years), serum selenium was associated with a lower incidence of previous fractures, lower FRAX scores and higher BMD (Wu *et al.*, 2021) and in a systematic review, selenium, magnesium, and calcium had the strongest, positive effects on sarcopenia prevalence (van Dronkelaar *et al.*, 2018).

Despite these associations between selenium and MSK function, research on these associations among very old people is limited (Lauretani *et al.*, 2003) and even fewer studies have used large sample sizes or utilised single-year birth cohorts. Other limitations of existing studies include incomplete follow-ups, use of self-reported health data, poor coverage on health domains and often only recruite community-dwelling individuals (Chen, 2014; Martin *et al.*, 2011; ter Borg *et al.*, 2015). These issues have been overcome in The Newcastle 85+ Study which included individuals born in 1921, regardless of their health status. This chapter builds on analyses reported previously that examined selenium intake and MSK function among participants in The Newcastle 85+ Study (Perri *et al.*, 2020) and from Chapter 3 of this thesis, to examine the relationships between the biomarkers of selenium status and MSK function.

I hypothesised that participants from The Newcastle 85+ Study with optimal selenium status will have better MSK function than participants with suboptimal selenium status. The aims of this study were: 1) to explore the associations between baseline selenium status (serum selenium, GPx3 activity and SePP) and baseline MSK function (HGS, TUG, sarcopenia) in very old adults; and 2) to determine the relationships between the biomarkers of selenium status and MSK function at baseline and the rate of change in MSK function up to 5 years.

4.3 Materials and Methods

4.3.1. Study Population

Data and samples were obtained from The Newcastle 85+ Study, a longitudinal, population-based study of a single-year birth cohort in the Northeast of England that explored health outcomes and trajectories in adults aged 85 years and over. The study was initiated in 2006 recruiting 1042 participants born in 1921, for full details, see Chapter 2, Section 2.2.1.

4.3.2. Socioeconomic, Lifestyle and Other Covariates

Assessments included questionnaires, functional tests, fasting blood samples, medical record reviews, dietary intakes and body weight measurements which were taken at the initial health assessment (2006/2007) and three other visits (1.5, 3, 5 years), details of which are provided in Chapter 2, Sections 2.2.1.1 and 2.2.1.2. Other covariates included in these analyses were: sex; occupational status; education; self-rated health; SMMSE; energy intake; protein intake; medication use; FFM; smoking; alcohol intake; presence of hand arthritis; and walking aids. These covariates were selected based on the results from Chapter 3 of this thesis, and previous research investigating the effects of vitamin D and protein on MSK function in the same cohort (Granic *et al.*, 2018; Mendonça *et al.*, 2016a). However, since writing Chapter 3, I removed the disease count and BMI covariates as these were redundant due to their similarities with medication use and FFM, respectively. I also removed the inflammatory markers as these did not improve the model fit, and selenium intake, since there was no difference in intakes amongst those with serum selenium above and below 70 µg/L and the results from my MRes did not find any associations between selenium intake and MSK function over time (Perri *et al.*, 2020).

4.3.3 Assessment of MSK Function (HGS, TUG, Sarcopenia)

Full details of MSK function are provided in Chapter 2, Section 2.2.1.4. In brief, HGS was measured using a hand-held dynamometer and two measurements (kg) were taken from each hand, where the average was taken from the four measurements. For the TUG test, the time in seconds was recorded for participants to rise from a chair, walk 3 m and return

(Podsiadlo and Richardson, 1991). Muscle mass was estimated via BIA, these values were input into the equation from Janssen *et al.*, (2000) to estimate SMMI ($kg.m^2$). To determine sarcopenia status, SMMI, HGS and TUG performance were interpreted according to the EWGSOP2 cut-offs for sarcopenia and severe sarcopenia (Cruz-Jentoft *et al.*, 2019). HGS and TUG were measured at baseline, 1.5, 3, and 5 years, whilst sarcopenia status was assessed at baseline and at 3 years.

4.3.4 Biomarkers of Selenium Status

Baseline blood samples from 2006/2007 (n = 757) that had been stored at -80 °C were analysed for biomarkers of selenium status. Serum selenium was measured using TXRF, GPx3 activity was measured using a coupled-enzyme reaction measuring NADPH consumption and SePP was measured using a commercial ELISA. For full details, see Chapter 2, Section 2.2.1.5.

4.3.5. Statistical Analyses

IBM statistical software package version 27.0 (SPSS) was used to perform the exploratory and statistical analyses; $p < 0.05$ was considered statistically significant. To determine normality of distributions of the variables, the Shapiro-Wilk test and quantile–quantile (QQ) plots were used. TUG performance was not normally distributed, and therefore, a log 10 transformation was applied, and this was used throughout the analyses. Full participant characteristics are described in detail in Chapter 3, Section 3.4.1. Biomarkers of selenium status were used as continuous independent variables in the initial analyses and subsequently categorised into statistically derived tertiles when modelling the associations with MSK outcomes. The tertiles 1, 2 and 3, of selenium status, hereafter, low, medium and high, were as follows: serum selenium concentration (μg/L): ≤ 46.7, 46.7-62.0, ≥ 62; GPx3 activity (U/L): ≤ 120.3, 120.3-166.1, ≥ 166.1; serum SePP concentration (mg/L): ≤ 2.32, 2.33-3.57, ≥ 3.58. I considered using the relevant thresholds from previous literature, as in Chapter 3 (above and below 70 μg/L (Nève, 1995; Combs, 2001) for serum selenium, 115 U/L for GPx3 activity (Demircan *et al.*, 2015) and 4.5 mg/L for SePP (Hughes *et al.*, 2015; Burk *et al.*, 2006), however, there was an unequal distribution of participants in each cut-off, with most participants having suboptimal concentrations of serum selenium and SePP

(82 % below 70 µg/L, 83 % below 4.5 mg/L) which limited the statistical power. Notwithstanding the limitations, I have included the latter as sensitivity analyses. Differences in MSK function according to serum selenium status (tertiles) were assessed using Chi-square test (categorical) and Kruskal–Wallis (ordered and non-normally distributed).

4.3.5.1 Relationships between Biomarkers of Selenium Status and MSK Function at Baseline

Relationships between each biomarker of selenium status (serum selenium, GPx3 activity, SePP) and MSK outcome (HGS, TUG) were investigated using linear regression. The biomarkers of selenium status (in continuous and tertile format) were first added into an unadjusted model (Model 1), followed by the additional covariates. These were: sex (men/women, binary); NS-SEC (routine/manual, intermediate, managerial/professional occupations, categorical); education (0-9, 10-11, ≥ 12 years, categorical); self-rated health (excellent/very good, good, fair/poor, ordinal); SMMSE (continuous); energy intake (continuous); protein intake (continuous); medication use (continuous); FFM (continuous); smoking (current/former/never); and alcohol intake (binary). Where applicable, the presence of hand arthritis or use of walking aids were added to HGS and TUG models, respectively. The same approach and covariates, including walking aids, were applied in logistic regressions to determine the predictors of sarcopenia and severe sarcopenia prevalence.

4.3.5.2 Relationships between Biomarkers of Selenium Status and the Change in MSK Function Over Time

Linear mixed models were used to determine the relationships between each biomarker of selenium status and each MSK outcome (HGS and TUG) at baseline, and the rate of change in each MSK outcome over 5 years. For each time point (baseline, 1.5, 3 and 5 years), time was treated as a categorical variable. The random effects were time and the intercept. Fixed effects were the variables of interest including the selenium status biomarkers (continuous and tertiles) and associated covariates (listed above in 4.3.5.1). I used two different models: (Model 1) time, sex, biomarker, time x biomarker, sex x biomarker interactions; (Model 2) adjustments made for presence of hand arthritis or use of walking aids (binary), sex, NS-SEC,

education, self-rated health, energy intake, protein intake, medication use, FMM, SMMSE, smoking and alcohol intake. Restricted maximum likelihood (RML) and unstructured or heterogeneous first-order autoregressive covariance matrixes were applied to derive parameter estimates (β). Negative β estimates for HGS and positive β estimates for TUG and sarcopenia, indicated poorer performance. The same process was repeated for sarcopenia and severe sarcopenia, with the exception that measures were only available at baseline and 3 years follow-up, allowing for two time points. Graphical outputs were created in Microsoft Excel 2010 using the equation: Intercept value + Time × (Time-beta + Time × selenium-beta interaction term) + selenium-beta.

4.3.6 Sensitivity Analyses

The analyses were repeated using the selected cut-offs (70 μg/L for serum selenium, 115 U/L for GPx3 activity and 4.5 mg/L for SePP) to make comparisons with the initial analyses, these consisted of baseline characteristics, correlations between the biomarkers of selenium status and MSK function, follow-up measurements of MSK function and the associations between the biomarkers of selenium status and MSK function at baseline and their rate of change, over time. These results are presented in the Sensitivity Analyses (Table 4.1-4.4, respectively).

4.4 Results

4.4.1. Participant Characteristics

Baseline characteristics relating to MSK function represented by tertiles of serum selenium concentration are summarised in Table 4.1 (see Chapter 3 for further baseline characteristics). There was a significant difference in HGS according to the selenium tertiles, where HGS was lower in those with medium (tertile 2) serum selenium (18.8 kg), compared to those with low (tertile 1) (20.7 kg) and high (tertile 3) serum selenium (19.3 kg) ($P = 0.030$). Similarly, there was a significant difference in TUG performance between those with medium (tertile 2) serum selenium (13.4 s) and high (tertile 3) serum selenium (11.5 s) ($P < 0.001$). There were 10 % more participants using walking aids in those with low (tertile 1) serum selenium, compared to those with high (tertile 3) serum selenium ($P = 0.007$), but no

significant differences with hand arthritis (P = 0.440). The prevalence of severe sarcopenia was greatest in those with low (tertile 1) serum selenium (P = 0.013), and the lowest occurrence in those with medium (tertile 2) serum selenium.

Table 4.1: Baseline musculoskeletal measures of participants represented by tertiles of serum selenium.

Characteristic	Participants	Low ≤ 46.7 µg/L (Tertile 1)	Medium 46.7-62.0 µg/L (Tertile 2)	High ≥ 62.0 µg/L (Tertile 3)	P
HGS Phase 1 (M, SD) n = 700	19.4, 8.3	20.2, 7.5	18.8, 8.2	19.3, 7.2	0.030
TUG Phase 1 (M, IQR)	12.5, 5.0	11.5, 5.5	13.4, 6.0	11.5, 5.5	< 0.001
Phase 1 Arthritis in hands % (n) n = 739					
Yes	6.9 (51)	8.2 (20)	5.3 (13)	7.2 (18)	0.440
No	93.1 (688)	91.8 (224)	94.7 (232)	92.8 (232)	
Phase 1 Walking Aids % (n) n = 700					
Yes	17.4 (122)	24.1 (53)	14.4 (34)	14.3 (35)	0.007
No	82.6 (578)	75.9 (167)	85.6 (202)	85.7 (202)	
Sarcopenia % (n) n = 675					
Yes	21.2 (143)	24.5 (52)	17.2 (39)	22.0 (52)	0.157
No	78.8 (532)	75.5 (160)	82.8 (188)	78.0 (184)	
Severe Sarcopenia % (n) n = 675					
Yes	11.0 (74)	14.6 (31)	6.2 (14)	12.3 (29)	0.013
No	89.0 (601)	85.4 (181)	93.8 (213)	87.7 (207)	

HGS: hand grip strength; TUG: Timed-Up-and-Go

4.4.2 Relationships between Biomarkers of Selenium Status and MSK Function at Baseline

Relationships between the three biomarkers of selenium status and disability scores are presented in Table 4.2. At baseline, when using the continuous biomarkers of selenium status, there was a positive association between serum selenium and HGS (r(732)= 0.074, P = 0.045), whereas there was a strong, negative association between serum selenium and TUG (r(700)= -0.134, P < 0.001) (Table 4.2). However, there were no associations between serum selenium and sarcopenia or severe sarcopenia. There were no significant associations between HGS and GPx3 activity or SePP, but there was a significant, negative association between SePP and TUG performance (r(700)= -0.075, P = 0.046). There were no significant associations between any of the MSK outcomes and GPx3 activity, or between any of the biomarkers of selenium status and sarcopenia or severe sarcopenia. When using tertiles of the biomarkers of selenium status, there was a positive association between serum selenium HGS (r(723)= -0.081, P = 0.030) and a negative association between serum

selenium and TUG performance ((r(700)= -0.085, P = 0.025) and SePP (r(700)= -0.093, P = 0.014). There were no associations between the remaining variables (Appendix Table 4.1).

Table 4.2: Baseline correlations between biomarkers of selenium (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) and hand grip strength, Timed-Up-and-Go, sarcopenia and severe sarcopenia.

		HGS kg	TUG s	Sarcopenia	Severe Sarcopenia
Serum Selenium µg/L	Pearson Correlation	0.074*	-0.134*	0.011	0.011
	P	0.045	< 0.001	0.785	0.777
	N	732	700	670	675
GPx3 Activity U/L	Pearson Correlation	-0.054	-0.060	-0.029	0.017
	P	0.146	0.112	0.451	0.667
	N	730	698	673	673
SePP mg/L	Pearson Correlation	-0.052	-0.075*	-0.011	0.029
	P	0.156	0.046	0.771	0.459
	N	732	700	675	675

GPx3: glutathione peroxidase 3; SePP: selenoprotein P; HGS: hand grip strength; TUG: Timed-Up-and-Go

* denotes significance

At baseline, the linear regressions revealed that there was a weak, positive association between serum selenium and HGS (y=16.18+0.03*x, R^2: 0.005, P = 0.045) and a strong, negative association with TUG performance (y=24.4-0.11*x, R^2: 0.018, P < 0.001). There was no association between GPx3 activity and HGS (y=19.05-8.29^{E-3}*x R^2: 0.003, P = 0.146) and TUG (y=21.02-0.02*x, R^2: 0.004, P = 0.112), or between SePP and HGS (y=18.72-0.28*x, R^2: 0.003, P = 0.156), however, there was a strong, negative association between SePP and TUG (y=20.83-0.77*x, R^2: 0.011, P < 0.001) (Figure 4.1).

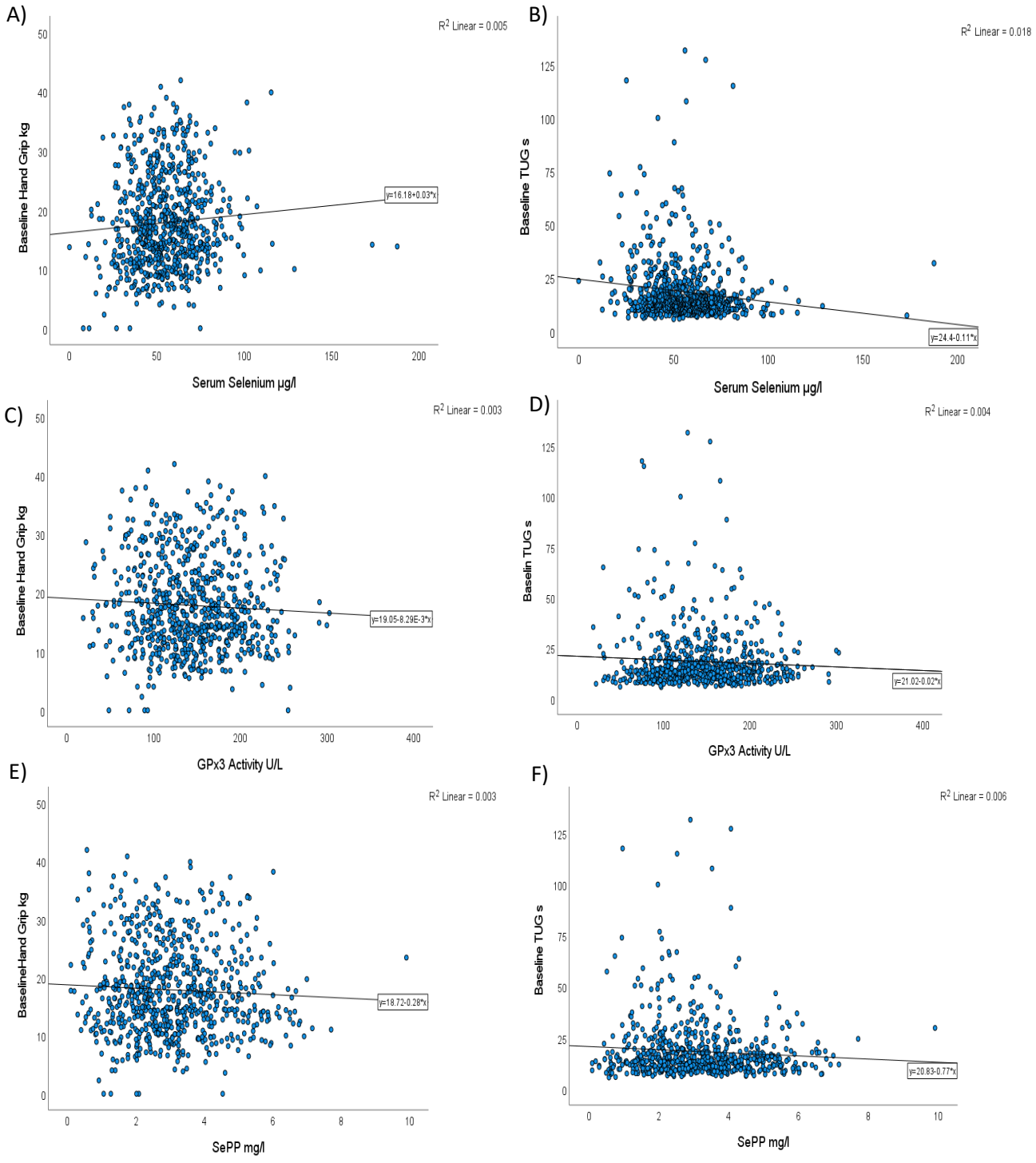


Figure 4.1: Linear relationships between each biomarker of selenium status and hand grip strength (HGS) and Timed-Up-and-Go (TUG). A) Serum selenium and HGS y=16.18+0.03*x, R^2: 0.005, P= 0.045; B) Serum selenium and TUG y=24.4-0.11*x, R^2: 0.018, P < 0.001; C) Glutathione Peroxidase 3 Activity and HGS y=19.05-8.29^{E-3}*x, R^2: 0.003, P = 0.146; D) Glutathione Peroxidase 3 Activity and TUG y=21.02-0.02*x, R^2: 0.004, P = 0.112; E) Selenoprotein P and HGS y=18.72-0.28*x, R^2: 0.003, P = 0.156; F) Selenoprotein P and TUG y=20.83-0.77*x, R^2: 0.011, P < 0.001.

4.4.3 Predictors of MSK Function at Baseline

At baseline, when using the continuous format of the biomarkers of selenium status, the significant predictors of HGS and TUG were the same regardless of the choice of biomarker of selenium status (serum selenium, SePP, GPx3 activity). The equations including β ± SD for the significant predictors are displayed in Table 4.3, whilst the full output can be found in Appendix Table 4.2. In the unadjusted model (Model 1), a 1 μg/L increase in serum selenium was associated with an increase in HGS (β 0.03 ± 0.12, P = 0.045). Serum selenium was also associated with an improvement in TUG performance (β -0.002 ± 0.000, P < 0.001). However, these associations were not maintained in the fully adjusted regression model (Model 2). Significant predictors of sarcopenia and severe sarcopenia were the same regardless of the choice of biomarkers of selenium status. The equations including β ± SD for the significant predictors are displayed in Table 4.3, whilst the full output can be found in Appendix Table 4.3. There were no associations between any of the biomarkers of selenium status and sarcopenia in Model 1 or Model 2. The regressions were repeated using tertiles of the biomarkers of selenium status; in these analyses, none of the biomarkers of selenium status were predictors of HGS or sarcopenia. However, those with high (tertile 3) serum selenium (≥ 62 μg/L), compared to those with low (tertile 1) serum selenium (≤ 46.7 μg/L) were associated to have better TUG performance (β -0.02 ± 0.01 , P = 0.015, data not shown).

Table 4.3: Equations derived from regressions of the relationships between the biomarkers of selenium status and musculoskeletal function outcomes using the significant predictors and intercept values, β(SE).

MSK Function	Selenium Status Biomarker	Equation = intercept, significant predictors, β(SE)
HGS	Serum Selenium	=8.57(2.61)-8.00(0.59)Sex+1.66(0.30)PA+0.17(0.03)FFM+0.12(0.06)SMMSE+0.001(0.001)Kcal-0.94(0.26)SRH-4.30(0.80)Arthritis
	GPx3 Activity	=9.82(2.61)-7.92(0.59)Sex+1.64(0.30)PA+0.17(0.03)FFM+0.13(0.06)SMMSE+0.001(0.001)Kcal-0.97(0.26)SRH-4.24(0.80)Arthritis
	Selenoprotein P	=9.61(2.56) -7.90(0.59)Sex+1.67(0.30)PA+0.17(0.03)FFM+0.13(0.06)SMMSE+0.001(0.001)Kcal-0.96(0.26)SRH-4.27(0.80)Arthritis
Log10 TUG	Serum Selenium	=1.58(0.09)-0.09(0.01)PA-0.01(0.002)SMMSE-0.01(0.002)Education+0.04(0.01)SRH+0.25(0.02)Walking Aid
	GPx3 Activity	=1.54(0.09)-0.09(0.01)PA-0.01(0.002)SMMSE-0.01(0.002)Education+0.04(0.01)SRH+0.25(0.02)Walking Aid
	Selenoprotein P	=1.56(0.09)-0.09(0.01)PA-0.01(0.002)SMMSE-0.01(0.002)Education+0.04(0.01)SRH+0.25(0.02)Walking Aid
Sarcopenia	Serum Selenium	=20.89(2.53)-5.71(0.64)Sex-0.44(0.05)FFM+0.77(0.39)Walking Aid
	GPx3 Activity	=21.15(2.53)-5.67(0.64)Sex-0.45(0.05)FFM+0.77(0.39)Walking Aid
	Selenoprotein P	=21.17(2.52)-5.69(0.64)Sex-0.45(0.05)FFM+0.77(0.39)Walking Aid
Severe Sarcopenia	Serum Selenium	=16.84(2.66)-4.33(0.64)Sex-0.36(0.05)FFM+0.95(0.43)Walking Aid
	GPx3 Activity	=17.01(2.65)-4.39(0.65)Sex-0.37(0.05)FFM+0.97(0.42)Walking Aid
	Selenoprotein P	=17.12(2.63)-4.35(0.64)Sex-0.37(0.05)FFM+0.99(0.43)Walking Aid

MSK: musculoskeletal; HGS: hand grip strength; Log10 TUG: Timed-Up-and-Go with log10 transformation; PA: physical activity; FFM: fat free mass; SMMSE: standardised mini mental state examination; Kcal: total energy intake in kcal; SRH: self-rated health; Arthritis: presence of hand arthritis; Walking Aid: use of walking appliances

4.4.4 Descriptives of MSK Measures over Time

MSK measures at baseline and follow-up (1.5, 3 and 5 years) were categorised by tertiles of serum selenium and are summarised in Appendix Table 4.4. There were no significant differences in HGS over the 5 years between the tertiles of serum selenium. In contrast, there was a significant difference in TUG performance between the tertiles of serum selenium; low (tertile 1) selenium concentrations were associated with worse TUG performance (15 s) compared to those with medium (tertile 2) (14.9 s) and high (tertile 3) concentrations (14.2 s) in the first follow-up at 1.5 years (P = 0.005). However, the differences in performance were inconsistent during the remaining follow-ups, where medium (tertile 2) selenium concentrations were associated with worse TUG performance. The use of walking aids was significantly lower in those in with high (tertile 3) selenium concentrations compared to those with low (tertile 1) selenium at the 1.5 year and 3-year follow-up. For example, 1.5 years after baseline, there were approximately 10 % more participants with low (tertile 1) selenium using walking aids compared to those with high (tertile 3) selenium concentrations (P = 0.037). As with HGS, there were no significant differences in sarcopenia prevalence between serum selenium tertiles, however, severe sarcopenia prevalence was more likely in those with low (tertile 1) selenium concentrations compared to those with medium (tertile 2) selenium concentrations (P = 0.013).

4.4.5 Relationship between Biomarkers of Selenium Status and the Change in MSK Function Over Time

Prospective investigations of the relationships between the biomarkers of selenium status at baseline, and rate of change in MSK function over 5 years are shown in Table 4.4, including the unadjusted model (Model 1) and fully adjusted model (Model 2). The graphical outputs from these results for HGS and TUG are displayed in Figure 4.2, and for sarcopenia and severe sarcopenia, in Figure 4.3. In the results below, I will first present the findings of

HGS, followed by TUG performance and sarcopenia prevalence and within each MSK function measure I will first present the continuous format of the biomarkers of selenium status, in the unadjusted and adjusted model, followed by the tertiles of biomarkers of selenium status.

In the unadjusted model (Model 1), when using continuous biomarkers of selenium status, serum selenium was associated with an improvement in HGS (β 0.04 ± 0.01, P = 0.003), whereas there were no significant associations between serum selenium and HGS in the fully adjusted model (Model 2) when using the continuous or tertile format. In the unadjusted model, there was an interaction between SePP and time which was associated with a greater rate of decline in HGS (β -0.04 ± 0.07, P = 0.046) and in the fully adjusted model, time was associated with a significant decline in HGS. In the unadjusted model, when using tertiles of the biomarkers of selenium status, low (tertile 1) serum selenium concentrations were more likely to be associated with lower HGS than high (tertile 3) serum selenium (β -1.83 ± 0.067, P = 0.007); however this was not maintained in the fully adjusted model.

In the unadjusted model (Model 1), when using continuous biomarkers of selenium status, serum selenium (β -2.53^{E-3} ± 6.12^{E-4}, P < 0.001) and SePP (β −0.02 ± 0.01, P = 0.006) were associated with greater rates of change in TUG performance. However, these were not maintained in the fully adjusted model. In the fully adjusted model, time was associated with a greater rate of change in TUG performance (β 0.06 ± 0.01, P < 0.001). There was an interaction between serum selenium and sex which was also associated with a greater rate of change in TUG performance (β 1.51^{E-3} ± 4.97^{E-4}, P = 0.002). There was no association between GPx3 activity and the rate of change in TUG performance over time. In the unadjusted model, when using tertiles of the biomarkers of selenium status, those with low (tertile 1) concentrations were associated with a greater rate of change in TUG performance than those with high (tertile 3) concentrations (serum selenium β 0.13 ± 0.03, P < 0.001; GPx3 activity β 0.06 ± 0.03 P = 0.050; SePP β 0.09 ± 0.03, P = 0.002) and this was also seen

between medium (tertile 2) GPx3 activity and TUG performance (β 0.06 ± 0.03, P = 0.032). In the fully adjusted model, the association remained for serum selenium; those with low (tertile 1) selenium concentrations had a greater rate of change in TUG performance (1.14 s increase) than those with high (tertile 3) selenium concentrations (β 0.06 ± 0.02, P = 0.013).

In the unadjusted model (Model 1), when using continuous biomarkers of selenium status, there was no association between the biomarkers of selenium status with the change in prevalence of sarcopenia or severe sarcopenia. In fully adjusted model, GPx3 activity was associated with a greater change in prevalence of sarcopenia (β 8.44^{E-4} ± 3.88^{E-4}, P = 0.030), as was the interaction between GPx3 activity and time (β -6.67^{E-4} ± 2.62^{E-4}, P = 0.011) (Table 4.4). Time was not associated with a change in prevalence of sarcopenia or severe sarcopenia in Model 2 (Table 4.4). In the unadjusted model, when using tertiles of the biomarkers of selenium status, low (tertile 1) selenium concentrations compared to high (tertile 3) were associated with a greater change in the prevalence of sarcopenia (β -0.12 ± 0.06, P = 0.035) and, in the fully adjusted model, medium (tertile 2) selenium concentrations compared to high (tertile 3) were associated with a lower change in the prevalence of severe sarcopenia (β -0.16 ± 0.07, P = 0.020). Additionally, the interaction between low (tertile 1) and medium (tertile 2) concentrations of each biomarker of selenium status, and sex, were associated with greater rates of change in each MSK function measure (see Appendix Table 4.5).

Table 4.4. Hand grip strength (kg), Timed-Up-and-Go (log_{10}-s), sarcopenia and severe sarcopenia estimates from an unadjusted (Model 1) and fully adjusted model (Model 2) including all covariates and each biomarker of selenium status.

Outcome	Variable	Selenium Model		GPx3 Activity Model		SePP Model	
		β (SE)	p	β (SE)	p	β (SE)	p
HGS (kg) Model 1	Intercept	12.57 (0.83)	< 0.001	14.45 (0.88)	< 0.001	14.69 (0.67)	< 0.001
	Biomarker	0.04 (0.01)	0.003*	2.59^{E-3} (5.62^{E-3})	0.645	0.06 (0.19)	0.772
Model 2	Intercept	-0.16 (1.81)	0.929	0.64 (1.84)	0.727	-0.19 (1.72)	0.914
	Biomarker	1.38^{E-3} (0.01)	0.926	5.91^{E-3} (0.01)	0.296	-0.07 (0.19)	0.724
	Time	-1.24 (0.36)	< 0.001	-1.63 (0.34)	< 0.001	-1.29 (0.26)	< 0.001
	Biomarker × Time	1.15^{E-3} (0.01)	0.846	3.13^{E-3} (2.21^{E-3})	0.157	-0.04 (0.07)	0.046*
	Biomarker x Sex	0.01 (0.01)	0.300	1.42^{E-3} (4.94^{E-3})	0.774	-0.34 (0.17)	0.054
TUG (log_{10}-s) Model 1	Intercept	1.33 (0.04)	< 0.001	1.25 (0.04)	< 0.001	1.26 (0.03)	< 0.001
	Biomarker	-2.53^{E-3} (6.12^{E-4})	< 0.001*	4.58^{E-4} (2.40^{E-4})	0.057	-0.02 (0.01)	0.006*
Model 2	Intercept	1.52 (0.07)	< 0.001	1.49 (0.09)	< 0.001	1.53 (0.06)	<0.001
	Biomarker	-4.99^{E-4} (5.59^{E-4})	0.346	2.00^{E-5} (9.94^{E-5})	0.841	-0.01 (0.01)	0.052
	Time	0.06 (0.01)	< 0.001	0.05 (0.09)	0.519	0.05 (0.01)	< 0.001
	Biomarker × Time	-2.60^{E-4} (2.14^{E-4})	0.225	-6.03^{E-5} (3.91^{E-5})	0.123	-2.28^{E-5} (2.72^{E-3})	0.800
	Biomarker x Sex	1.51^{E-3} (4.97^{E-4})	0.002	-4.11^{E-4} (8.76^{E-5})	< 0.001	0.03 (0.01)	<0.001
Sarcopenia Model 1	Intercept	0.22 (0.10)	0.027	0.12 (0.10)	0.222	0.15 (0.07)	0.037
	Biomarker	3.38^{E-4} (1.66^{E3})	0.838	5.57^{E-4} 6.21^{E4}	0.370	0.02 (0.02)	0.470
Model 2	Intercept	1.37 (0.20)	< 0.001	1.29 (0.22)	< 0.001	1.34 (0.18)	< 0.001
	Biomarker	1.12^{E-3} (1.99^{E-3})	0.573	8.44^{E-4} (3.88^{E-4})	0.030*	0.02 (0.03)	0.323
	Time	0.03 (0.08)	0.714	0.13 (0.20)	0.521	0.06 (0.06)	0.283
	Biomarker × Time	-7.77^{E-5} (1.38^{E-3})	0.955	-6.67^{E-4} (2.62^{E-4})	0.011*	-0.01 (0.02)	0.477
	Biomarker x Sex	-8.22^{E-5} (1.41^{E-3})	0.953	-4.68^{E-4} (2.58^{E-4})	0.070	-0.02 (0.02)	0.260
Severe Sarcopenia	Intercept	0.01 (0.08)	0.943	-4.74^{E-3} (0.08)	0.951	0.06 (0.06)	0.283
Model 1	Biomarker	1.68^{E-3} (0.05)	0.200	7.19^{E-4} (4.89^{E-4})	0.141	0.01 (0.02)	0.454
Model 2	Intercept	0.88 (0.16)	< 0.001	0.89 (0.16)	< 0.001	1.01 (0.14)	< 0.001
	Biomarker	2.78^{E-3} (1.58^{E-3})	0.078	9.25^{E-4} (5.88^{E-3})	0.116	0.01 (0.02)	0.548
	Time	0.11 (0.07)	0.092	0.12 (0.06)	0.052	0.04 (0.05)	0.383
	Biomarker × Time	1.50^{E-3} (1.09^{E-3})	0.169	6.51^{E-4} (3.96^{E-4})	0.101	5.10^{E-3} (0.01)	0.703
	Biomarker x Sex	1.94^{E-4} (1.11^{E-3})	0.862	9.17^{E-5} (3.91^{E-4})	0.815	0.02 (0.01)	0.195

SE: standard error; Se: serum selenium; GPx3: glutathione peroxidase 3; SePP: selenoprotein P. Model 1 included: the biomarker of selenium status; time; sex; interaction between time and the biomarker; and interaction between sex and the biomarker. Model 2 was further adjusted for: presence of hand arthritis for HGS, or use of walking aids in TUG, sarcopenia and severe sarcopenia; National Statistics Socio-Economic Classification (NS-SEC); self-rated health; energy intake; protein intake; medication use; fat-free mass (FFM); physical activity; SMMSE: standardised mini mental state examination; alcohol intake; and smoking status.

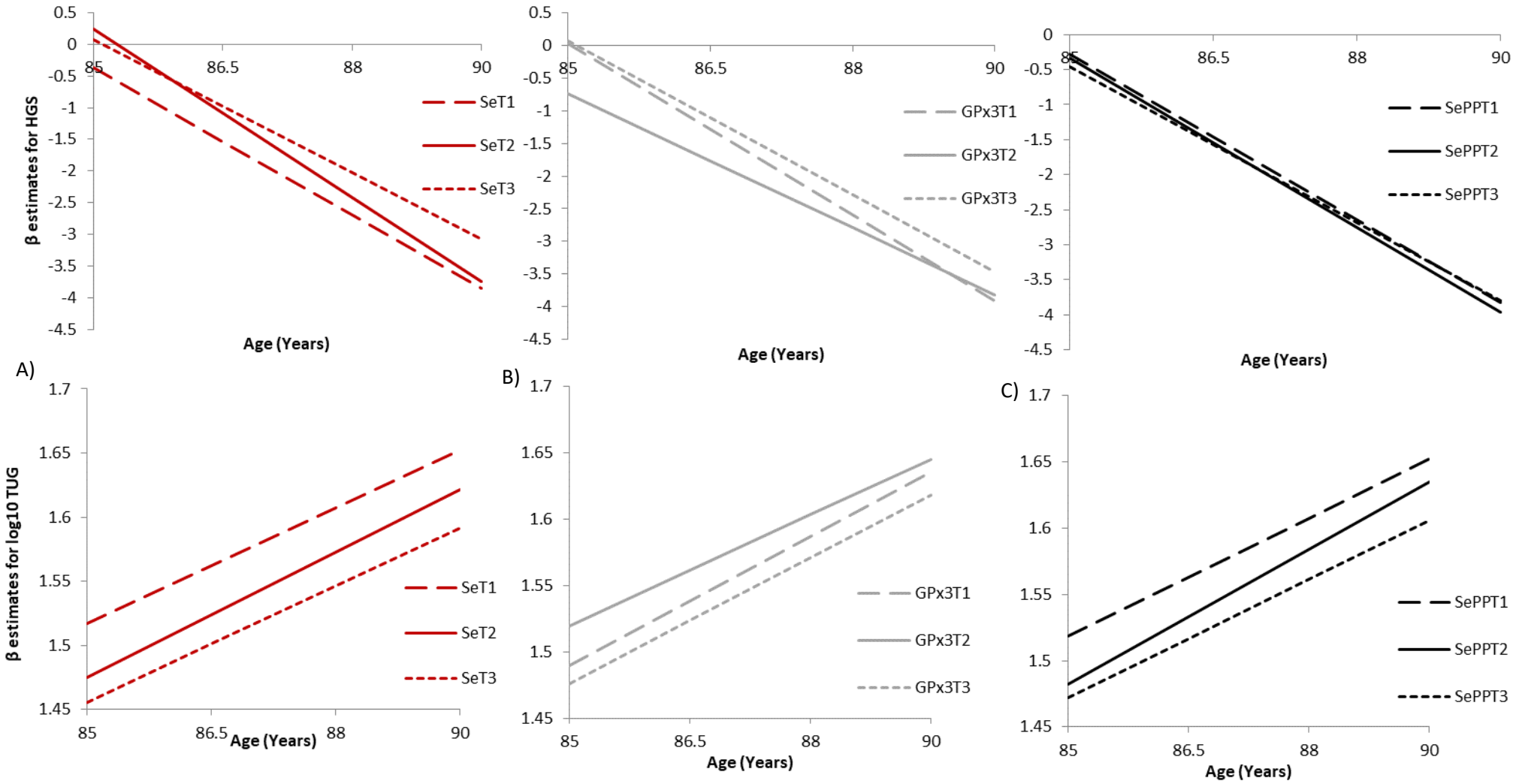


Figure 4.2. Linear slopes for hand grip strength (HGS) and Timed-Up-and-Go (TUG) A) Serum selenium tertiles; B) Glutathione peroxidase 3 activity tertiles; C) Selenoprotein P tertiles.

T1: tertile 1 low, T2: tertile 2 medium and T3: tertile 3 high concentrations of the biomarker of selenium status.

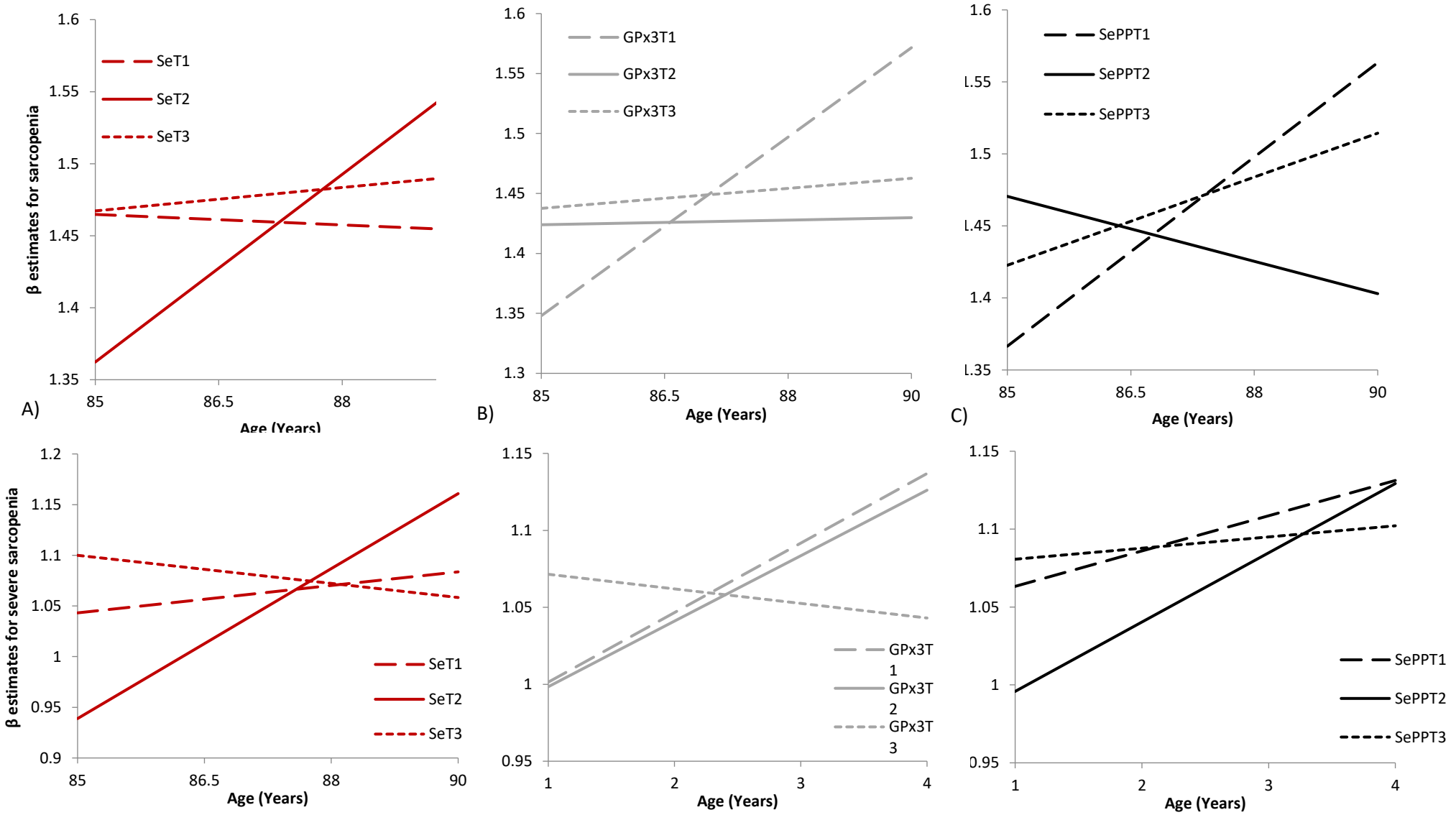


Figure 4.3. Linear slopes for sarcopenia and severe sarcopenia A) Serum selenium tertiles; B) Glutathione peroxidase 3 activity tertiles; C) Selenoprotein P tertiles.

T1: tertile 1 low, T2: tertile 2 medium and T3: tertile 3 high concentrations of the biomarker of selenium status.

4.5 Discussion

At baseline, in adjusted models, there was no association between the biomarkers of selenium status and MSK outcomes. Over time, in adjusted models, when using the continuous biomarkers of selenium status, the longitudinal analyses revealed that GPx3 activity was associated with a greater change in the prevalence of sarcopenia and, when using tertiles, low (tertile 1) selenium concentration was associated with a greater rate of decline in TUG performance and, medium (tertile 2) selenium concentration was associated with a greater change in the prevalence of severe sarcopenia. Each aspect of this summary will be reviewed below in more detail.

Relationship between Biomarkers of Selenium Status and MSK Function at Baseline

At baseline, the significant predictors of HGS were sex, physical activity, FFM, SMMSE, energy intake, self-rated health and hand arthritis. These findings are common predictors of muscle strength in other studies (García-Esquinas *et al.*, 2021; Beck *et al.*, 2007). Unlike the findings from Beck *et al.*, (2007), serum selenium in my analyses was not a predictor of HGS in the fully adjusted model. The lack of association in my analyses may be due to the suboptimal concentrations of biomarkers of selenium status in this population, when applying the concentrations from studies that derived the selenium DRVs (as detailed in Chapter 1, Section 1.6 and Chapter 3, Section 3.2). In my analyses the predictors of HGS, as noted above, similarly predicted TUG, in addition to education duration and walking aids. Likewise, none of the continuous biomarkers of selenium status were predictors of TUG performance, although, when using tertiles of the biomarkers of selenium status, low (tertile 1) selenium concentrations were associated with worse TUG performance. This may be explained by the fact that TUG requires muscle power as well as neural inputs (Savva *et al.*, 2013), and it is known that selenium has a positive effect on cognition (Berr *et al.*, 2000; Arnaud *et al.*, 2007; Gray *et al.*, 2003; Shahar *et al.*, 2010). Furthermore, recent research has

also shown bi-directional temporal relationships between cognitive domains and TUG, where sustained attention and processing speed had the greatest influence on TUG as compared to global cognition, executive function and verbal fluency (Hartley *et al.,* 2022). Another important factor that may play a role in TUG performance is motor function, which is also known to decline with age (Voelcker-Rehage, 2010; Hunter *et al.*, 2016). There was an overlap in some of the predictors for both HGS and TUG; these were sex, FMM and walking aids, again, some of which are common predictors in other reports (Cruz-Jentoft *et al.*, 2019). In the fully adjusted regressions, the findings suggest that biomarkers of selenium status (as continuous variables) were not significant predictors of the chosen MSK outcomes at baseline; however, when using statistically derived tertiles, low (tertile 1) selenium concentrations were associated with worse TUG performance.

Previous studies have found biomarkers of selenium status to be associated with different MSK outcomes. Serum selenium was positively associated with BMD in postmenopausal women (Hoeg *et al.*, 2012). In another study, there was an inverse association between serum selenium and HGS up to, and beyond, whole-blood selenium concentrations of 140 µg/L in older cohorts (≥ 60-65 years from NHANES and Seniors-ENRICA-2) (García-Esquinas *et al.*, 2021). Similarly, in the HORTEGA study (≥ 50 years), there were positive associations between plasma selenium < 100 µg/L and BMD, although concentrations above this were positively associated with femur fractures, suggesting a dose-response curve where optimal levels are beneficial. Using NHANES data (≥ 40 years), where participants had baseline serum selenium of 131 µg/L, there was a positive association between serum selenium and bone health (Wu *et al.*, 2021); in this study, a higher selenium status was associated with increased total femur BMD, lower incidence of previous fractures and reduced FRAX scores. This may suggest the need to explore different MSK outcomes, as well as using populations with higher baseline selenium status.

Relationship between Biomarkers of Selenium Status and Change in MSK Function Over Time

When using the continous biomarkers of selenium status, there was an association between GPx3 activity and the change in prevalence of sarcopenia over time. Similarly, in another study, despite serum selenium deficiency, GPx3 activity remained unaffected (Bunker *et al.*, 1988) and likewise, in my analyses, GPx3 activity was the only biomarker of selenium status to be considered optimal according to the selected cut-offs. However, this contrasts other data that suggests in times of selenium deficiency, GPx3 is the first enzyme to be affected (Fairweather-Tait *et al.*, 2010) and is depedent on SePP (Schweizer *et al.*, 2005; Renko *et al.,* 2008). Time was a signficant predictor of HGS in all models including each biomarker and, of TUG performance in the models including serum selenium and SePP, but not GPx3 activity, nor in any of the sarcopenia models. It would be expected that MSK function would ineveitably decline over time, as evidenced in various studies and reviews (McCormick and Vasilaki, 2018; Wilkinson, Piasecki and Atherton, 2018; Cruz-Jentoft *et al.*, 2019; Larsson *et al.*, 2018). Similar to the cross-sectional analyses, serum selenium or SePP concentrations (continous format) were not associated with rates of change in MSK function over time. This could relate to the same issue of a supoptimal selenium status reducing the effectiveness or contribution of selenoprotein activity towards MSK fucntion. Skeletal muscle can hold between 30-45 % of the total selenium pool and it is known that this store is mobilised during supoptimal selenium status (Rayman, 2012; Zachara *et al.*, 2001; Oster, Schmiedel and Prellwitz, 1988). When serum selenium concentrations are supoptimal, these skeletal muscle stores will be depleted to shuttle selenium to priortiy organs such as the brain and thus will limit the function of selenoproteins in regard to muscle function (Burk and Hill, 2009; Burk *et al.*, 2014). When using tertiles, low (tertile 1) selenium concentrations were associated with a greater rate of change in TUG performance and medium (tertile 2) selenium concentrations were associated with a greater change in the prevalence of severe sarcopenia. The interaction between a low (tertile 1) and medium (tertile 2) biomarker concentration, and sex, was a signifcant predictor of the rate of change in each of the MSK

function measures. This finding could suggest that the biomarker concentrations differ between men and women and MSK outcomes. This corroborates previous literature that shows differences in MSK function between men and women. Older women generally have poorer MSK function, usually related to lower baseline muscle mass, strength and power; this can lead to lower values over time. Other sex differences are lower intakes of calories and nutrients (i.e. protein) associated with MSK function and a higher disease burden in women, including poorer thyroid function and a reduction in sex steroids following menopause. More recently, research has suggested female muscle function is largely influenced by muscle disuse, and less so by inflammation when compared to males and, in this population, males were significantly more active than females (48.1 % males highly active versus 27.1 % females) (Weitoft *et al.*, 2005; Anderson, Liu and Garcia, 2017; Rosa-Caldwell and Greene, 2019).

Details on the strengths and limitations of the study design are detailed in Chapter 7, Section 7.2. To date, this is the only study using large-scale, observational data to explore the associations between biomarkers of selenium status and MSK function in very old adults. This study focused on an important area of ageing, MSK function, which is crucial for longevity and independence in older age. In addition to this, a single-birth cohort of very old adults was examined which helps bridge the knowledge gap between selenium status and MSK function in very old adults, as research in this area was previously lacking.

4.6 Conclusion

The detailed analysis of these results presented in this chapter show that GPx3 activity was associated with a greater change in the prevalence of sarcopenia and, when using tertiles, low (tertile 1) and medium (tertile 2) selenium concentrations were associated with greater rates of change in TUG performance and severe sarcopenia, respectively. To further develop these analyses in this population, I will next explore the associations between the biomarkers of selenium status and disability scores. The findings from this chapter suggest

that selenium may play a role in MSK function in association with mental capacity and cognition as evidenced by the baseline associations between SePP and TUG, and longitudinal associations between serum selenium and TUG performance. One way to explore this would be by using disability scores which would provide a greater insight into MSK ageing in very old adults and provide a meaningful measure that incorporates both muscle function and its connection with cognition.

4.7 Appendix

Table 4.1: Correlations between tertiles of biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) and hand grip strength, Timed-Up-and-Go, sarcopenia and severe sarcopenia.

		HGS kg	TUG s	Sarcopenia	Severe Sarcopenia
Serum Selenium Tertile µg/L	Pearson Correlation	-0.013	-0.085	0.021	0.056
	p	0.732	0.025*	0.593	0.149
	N	732	700	675	675
GPx3 Activity Tertile U/L	Pearson Correlation	-0.054	-0.031	-0.045	-0.001
	p	0.148	0.411	0.238	0.974
	N	730	698	673	673
SePP Tertile mg/L	Pearson Correlation	-0.033	-0.093	-0.025	0.004
	p	0.366	0.014*	0.521	0.927
	N	732	700	675	675

SePP: selenoprotein P; GPx3: glutathione peroxidase 3; HGS: hand grip strength; TUG: Timed-Up-and-Go

Table 4.2: Predictors of musculoskeletal function with each biomarker of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P), derived from linear regressions.

Outcome	Variable	Serum Selenium		GPx3 Activity		Selenoprotein P	
		β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
HGS (kg)	Intercept	16.18 (0.89)	< 0.001	19.05 (0.87)	< 0.001	18.72 (0.67)	< 0.001
	Biomarker	0.03 (0.12)	0.045*	-0.01 (0.01)	0.146	-0.28 (0.20)	0.156
	Intercept	8.57 (2.61)	<0.001	9.82 (2.61)	< 0.001	9.61 (2.56)	< 0.001
	Biomarker	0.01 (0.01)	0.312	-0.003 (0.004)	0.410	-0.14 (0.13)	0.288
	Sex	-8.00 (0.59)	<0.001	-7.92 (0.59)	< 0.001	-7.90 (0.59)	< 0.001
	Physical Activity	1.66 (0.30)	< 0.001	1.64 (0.30)	< 0.001	1.67 (0.30)	< 0.001
	Fat Free Mass (kg)	0.17 (0.03)	< 0.001	0.17 (0.03)	< 0.001	0.17 (0.03)	< 0.001
	SMMSE	0.12 (0.06)	0.040	0.13 (0.06)	0.045	0.13 (0.06)	0.042
	Energy Intake (Kcal)	0.001 (0.001)	0.044	0.001 (0.001)	0.046	0.001 (0.001)	0.056
	Protein Intake (g)	-0.003 (0.01)	0.834	0.000 (0.01)	0.989	0.000 (0.01)	0.922
	NS-SEC	-0.23 (0.23)	0.328	-0.25 (0.23)	0.274	-0.24 (0.23)	0.300
	Education	0.02 (0.30)	0.948	0.02 (0.30)	0.938	0.004 (0.30)	0.990
	Living Status	-1.49 (1.15)	0.196	-1.66 (1.15)	0.149	-1.66 (1.15)	0.149
	Self-rated Health	-0.94 (0.26)	< 0.001	-0.97 (0.26)	< 0.001	-0.96 (0.26)	< 0.001
	Total Medications	-0.41 (0.26)	0.111	-0.46 (0.26)	0.071	-0.44 (0.26)	0.082
	Smoking Status	-0.02 (0.21)	0.912	-0.03 (0.21)	0.882	-0.02 (0.21)	0.934
	Alcohol Intake	-0.42 (0.42)	0.314	-0.42 (0.42)	0.321	-0.42 (0.42)	0.320
	Hand Arthritis	-4.30 (0.80)	< 0.001	-4.24 (0.80)	< 0.001	-4.27 (0.80)	< 0.001
Log10 TUG (log10 s)	Intercept	1.30 (0.03)	< 0.001	1.21 (0.03)	< 0.001	1.22 (0.02)	< 0.001
	Biomarker	-0.002 (0.00)	< 0.001*	0.00 (0.00)	0.421	-0.01 (0.01)	0.156
	Intercept	1.58 (0.09)	< 0.001	1.54 (0.09)	< 0.001	1.56 (0.09)	< 0.001
	Biomarker	-0.001 (0.00)	0.096	-1.08^{E-5} (0.00)	0.934	-0.006 (0.00)	0.181
	Sex	0.04 (0.02)	0.060	0.04 (0.02)	0.074	0.04 (0.02)	0.062
	Physical Activity	-0.09 (0.01)	< 0.001	-0.09 (0.01)	< 0.001	-0.09 (0.01)	< 0.001
	Fat Free Mass (kg)	0.001 (0.001)	0.555	0.001 (0.001)	0.498	0.001 (0.001)	0.509
	SMMSE	-0.01 (0.002)	< 0.001	-0.01 (0.002)	< 0.001	-0.01 (0.002)	< 0.001
	Energy Intake (Kcal)	-3.51^{E-5} (0.00)	0.072	-3.37^{E-5} (0.00)	0.085	-3.45^{E-5} (0.00)	0.076
	Protein Intake (g)	0.00 (0.00)	0.785	4.42^{E-5} (0.00)	0.920	0.00 (0.00)	0.794
	NS-SEC	0.01 (0.01)	0.113	0.01 (0.01)	0.109	0.01 (0.01)	0.129
	Education	-0.02 (0.01)	0.022	-0.02 (0.01)	0.021	-0.02 (0.01)	0.017
	Living Status	-0.05 (0.04)	0.251	-0.04 (0.04)	0.317	-0.05 (0.04)	0.271
	Self-rated Health	0.04 (0.01)	< 0.001	0.04 (0.01)	< 0.001	0.04 (0.01)	< 0.001
	Total Medications	0.01 (0.01)	0.314	0.01 (0.01)	0.202	0.01 (0.01)	0.204
	Smoking Status	0.002 (0.007)	0.831	0.001 (0.007)	0.864	0.002 (0.007)	0.801
	Alcohol Intake	-0.02 (0.01)	0.230	-0.02 (0.01)	0.216	-0.02 (0.01)	0.177
	Waking Aid	0.25 (0.02)	< 0.001	0.25 (0.02)	< 0.001	0.25 (0.02)	< 0.001

SE: standard error; BMI: body mass index; SMMSE: standardised mini mental state examination; NS-SEC: National Statistics Socio-economic classification.

Table 4.3: Predictors of sarcopenia and severe sarcopenia with each biomarker of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P), derived from logistic regressions.

Outcome	Variable	Serum Selenium		GPx3 Activity		Selenoprotein P	
Sarcopenia		**B (SE)**	***p***	**B (SE)**	***p***	**B (SE)**	***p***
	Intercept	-1.39 (0.30)	< 0.001	-1.11 (0.29)	< 0.001	-1.26 (0.22)	< 0.001
	Biomarker	0.001 (0.01)	0.785	-0.001 (0.002)	0.451	-0.02 (0.07)	0.770
	Intercept	20.89 (2.53)	< 0.001	21.15 (2.53)	< 0.001	21.17 (2.52)	< 0.001
	Biomarker	0.004 (0.007)	0.573	0.00 (0.002)	0.887	-0.01 (0.09)	0.995
	Sex	-5.71 (0.64)	< 0.001	-5.67 (0.64)	< 0.001	-5.69 (0.64)	< 0.001
	Physical Activity (1)	-0.41 (0.40)	0.313	-0.40 (0.40)	0.322	-0.39 (0.40)	0.325
	Physical Activity (2)	-0.64 (0.44)	0.144	-0.62 (0.44)	0.159	-0.63 (0.44)	0.151
	Fat Free Mass (kg)	-0.44 (0.05)	< 0.001	-0.45 (0.05)	< 0.001	-0.45 (0.05)	< 0.001
	SMMSE	0.03 (0.04)	0.536	0.03 (0.04)	0.540	0.03 (0.04)	0.544
	Energy Intake (Kcal)	0.00 (0.00)	0.961	0.00 (0.00)	0.903	0.00 (0.00)	0.915
	Protein Intake (g)	-0.01 (0.01)	0.513	-0.01 (0.01)	0.596	-0.01 (0.01)	0.591
	NS-SEC (1)	-0.48 (0.42)	0.251	-0.46 (0.42)	0.269	-0.46 (0.42)	0.272
	NS-SEC (2)	0.35 (0.31)	0.272	0.35 (0.31)	0.264	0.35 (0.31)	0.270
	Education (1)	0.22 (0.31)	0.492	0.21 (0.31)	0.509	0.21 (0.31)	0.501
	Education (2)	-0.67 (0.47)	0.157	-0.70 (0.48)	0.145	-0.69 (0.48)	0.148
	Living Status	-0.18 (0.76)	0.809	-0.22 (0.77)	0.777	-0.22 (0.77)	0.770
	Self-rated Health (1)	0.13 (0.29)	0.664	0.14 (0.30)	0.646	0.12 (0.29)	0.672
	Self-rated Health (2)	-0.48 (0.37)	0.195	-0.49 (0.37)	0.184	-0.49 (0.37)	0.182
	Total Medications (1)	-0.06 (0.38)	0.877	-0.05 (0.38)	0.890	-0.06 (0.38)	0.871
	Total Medications (2)	0.05 (0.36)	0.896	0.03 (0.36)	0.942	0.03 (0.36)	0.938
	Smoking Status (1)	0.30 (0.50)	0.541	0.28 (0.50)	0.577	0.28 (0.50)	0.568
	Smoking Status (2)	-0.26 (0.28)	0.771	-0.26 (0.28)	0.363	-0.26 (0.28)	0.362
	Alcohol Intake	0.03 (0.28)	0.919	0.05 (0.28)	0.849	0.04 (0.28)	0.877
	Walking Aid	0.77 (0.39)	0.048	0.77 (0.39)	0.049	0.77 (0.39)	0.049
Severe Sarcopenia	Intercept	-2.20 (0.39)	< 0.001	-2.25 (0.38)	< 0.001	-2.29 (0.30)	< 0.001
	Biomarker	0.003 (0.007)	0.777	0.001 (0.002)	0.666	0.06 (0.08)	0.459
	Intercept	16.84 (2.66)	< 0.001	17.01 (2.65)	< 0.001	17.12 (2.63)	< 0.001
	Biomarker	0.01 (0.008)	0.171	0.01 (0.003)	0.129	0.16 (0.11)	0.145
	Sex	-4.33 (0.64)	< 0.001	-4.39 (0.65)	< 0.001	-4.35 (0.64)	< 0.001
	Physical Activity (1)	-0.46 (0.44)	0.304	-0.43 (0.45)	0.340	-0.41 (0.44)	0.353
	Physical Activity (2)	-0.91 (0.52)	0.078	-0.85 (0.51)	0.097	-0.88 (0.51)	0.086
	Fat Free Mass (kg)	-0.36 (0.05)	< 0.001	-0.37 (0.05)	< 0.001	-0.37 (0.05)	< 0.001
	SMMSE	-0.06 (0.05)	0.199	-0.06 (0.05)	0.233	-0.06 (0.05)	0.203
	Energy Intake (Kcal)	0.00 (0.00)	0.582	0.00 (0.00)	0.667	0.00 (0.00)	0.637
	Protein Intake (g)	-0.02 (0.01)	0.140	-0.02 (0.01)	0.157	-0.02 (0.01)	0.158
	NS-SEC (1)	-0.20 (0.55)	0.711	-0.20 (0.55)	0.715	-0.19 (0.55)	0.733
	NS-SEC (2)	0.49 (0.42)	0.243	0.52 (0.41)	0.213	0.50 (0.42)	0.225
	Education (1)	0.19 (0.41)	0.642	0.19 (0.41)	0.635	0.20 (0.41)	0.625
	Education (2)	-0.22 (0.64)	0.735	-0.22 (0.64)	0.736	-0.18 (0.64)	0.783
	Living Status	-1.02 (0.92)	0.270	-1.03 (0.92)	0.266	-1.00 (0.93)	0.284
	Self-rated Health (1)	0.18 (0.37)	0.625	0.20 (0.37)	0.590	0.21 (0.37)	0.572
	Self-rated Health (2)	-0.06 (0.43)	0.889	-0.07 (0.43)	0.863	-0.09 (0.43)	0.830
	Total Medications (1)	-0.07 (0.55)	0.903	-0.03 (0.54)	0.949	-0.01 (0.55)	0.990
	Total Medications (2)	0.87 (0.51)	0.086	0.81 (0.50)	0.068	0.77 (0.50)	0.122
	Smoking Status (1)	-0.29 (0.66)	0.660	-0.26 (0.66)	0.700	-0.30 (0.66)	0.647
	Smoking Status (2)	-0.10 (0.36)	0.780	-0.04 (0.36)	0.919	-0.10 (0.36)	0.784
	Alcohol Intake	0.35 (0.35)	0.323	0.36 (0.35)	0.302	0.39 (0.35)	0.261
	Walking Aid	0.95 (0.43)	0.026	0.97 (0.42)	0.022	0.99 (0.43)	0.020

SE: standard error; BMI: body mass index; SMMSE: standardised mini mental state examination; NS-SEC: National Statistics Socio-economic classification.

Table 4.4: Untransformed musculoskeletal measures (hand grip strength, timed up-and-go, sarcopenia, disability scores, IADL, BADL, falls and fractures) represented by tertiles of serum selenium, at baseline, and follow-up.

MSK measures	All Participants	Low Selenium ≤ 46.7 µg/L (Tertile 1)	Medium Selenium 46.73-62.0 µg/L (Tertile 2)	High Selenium ≥ 62.0 µg/L (Tertile 3)	p
Hand Grip Strength kg (M, SD)					
Baseline	19.4, 8.3	20.2, 7.5	18.8, 8.2	19.3, 7.2	0.030
Hand arthritis % (n) yes	6.9 (51)	8.2 (20)	5.3 (13)	7.2 (18)	0.440
Follow-up at 1.5 years	19.5, 8.7	19.1, 8.2	17.9, 7.5	19.0, 7.3	0.139
Follow-up at 3 years	18.5, 8.1	18.1, 7.1	17.6, 7.1	17.8, 7.2	0.924
Follow-up at 5 years	17.1, 7.9	16.5, 7.6	14.9, 6.7	16.1, 6.8	0.455
Timed Up-and-Go s (Median, IQR)					
Baseline	12.5, 5.0	11.5, 5.5	13.4, 6.0	11.5, 5.5	< 0.001
Use of walking aids % (n) yes	17.4 (122)	24.1 (53)	14.4 (34)	14.3 (35)	0.007
Follow-up at 1.5 years	13.5, 5.5	15.0, 9.7	14.9, 7.6	14.2, 6.6	0.005
Use of walking aids % (n) yes	16.1 (83)	20.4 (30)	18.2 (32)	10.8 (21)	0.037
Follow-up at 3 years	13.8, 6.9	14.5, 11.1	15.3, 10.1	14.6, 7.3	0.030
Use of walking aids % (n) yes	17.8 (68)	23.2 (22)	19.5 (26)	13.1 (20)	0.107
Follow-up at 5 years	13.0, 6.3	15.2, 14.9	18.7, 12.6	14.8, 9.8	0.009
Use of walking aids % (n) yes	25.0 (65)	30.5 (18)	29.2 (96)	18.1 (19)	0.105
Sarcopenia % (n) yes					
Baseline	21.2 (143)	24.5 (52)	17.2 (39)	22.0 (52)	0.157
Follow-up at 3 years	20.0 (73)	19.4 (18)	19.5 (25)	20.8 (30)	0.949
Severe Sarcopenia Baseline	11.0 (74)	14.6 (31)	6.2 (14)	12.3 (29)	0.013
Severe Sarcopenia Follow-up at 3 years	10.1 (37)	11.8 (11)	10.2 (13)	9.0 (13)	0.784

Table 4.5. Hand grip strength (kg), Timed-Up-and-Go (log_{10}-s), sarcopenia and severe sarcopenia estimates from a fully adjusted model including all covariates and tertiles of each biomarker of selenium status.

Outcome	Variable	Selenium Model		GPx3 Activity Model		SePP Model	
		β (SE)	p	β (SE)	p	β (SE)	p
HGS (kg) Model 1	Intercept	15.61 (0.46)	< 0.001	14.46 (0.47)	< 0.001	15.14 (0.48)	< 0.001
	Low compared to High Concentration	-1.83 (0.67)	0.007*	-0.91 (0.70)	0.194	-0.47 (0.69)	0.495
	Medium compared to High Concentration	-0.40 (0.68)	0.552	-1.06 (0.67)	0.116	-0.34 (0.69)	0.624
Model 2	Intercept	0.07 (1.67)	0.966	0.07 (1.67)	0.968	-0.46 (1.68)	0.783
	Low compared to High Concentration	-0.45 (0.69)	0.517	-0.04 (0.69)	0.950	0.19 (0.67)	0.781
	Medium compared to High Concentration	0.17 (0.67)	0.799	-0.81 (0.67)	0.230	0.13 (0.68)	0.850
	Time	-1.05 (0.18)	< 0.001	-1.18 (0.18)	< 0.001	-1.11 (1.57)	0.479
	Low × Time	-0.11 (0.287)	0.686	-0.13 (0.27)	0.617	-0.07 (0.26)	0.784
	Medium × Time	-0.28 (0.26)	0.277	0.15 (0.26)	0.573	-0.10 (0.26)	0.716
	Low x Sex	7.48 (0.51)	< 0.001	7.50 (0.51)	< 0.001	7.75 (0.51)	< 0.001
	Medium x Sex	6.90 (0.52)	< 0.001	7.40 (0.52)	< 0.001	7.14 (0.51)	< 0.001
TUG (log_{10}-s) Model 1	Intercept	1.13 (0.02)	< 0.001	1.15 (0.02)	< 0.001	1.14 (0.02)	< 0.001
	Low compared to High Concentration	0.13 (0.03)	< 0.001*	0.06 (0.03)	0.050*	0.09 (0.03)	0.002*
	Medium compared to High Concentration	0.05 (0.03)	0.112	0.06 (0.03)	0.032*	0.03 (0.03)	0.241
Model 2	Intercept	1.46 (0.06)	< 0.001	1.48 (0.06)	< 0.001	1.47 (0.06)	< 0.001
	Low compared to High Concentration	0.06 (0.02)	0.013*	0.01 (0.02)	0.579	0.05 (0.02)	0.052
	Medium compared to High Concentration	0.02 (0.02)	0.422	0.04 (0.02)	0.071	0.01 (0.02)	0.668
	Time	0.05 (0.01)	< 0.001	0.05 (0.01)	< 0.001	0.04 (0.01)	< 0.001
	Low × Time	2.75^{E-4} (0.01)	0.978	1.07^{E-3} (0.01)	0.911	2.89^{E-4} (0.01)	0.976
	Medium × Time	3.60^{E-3} (0.01)	0.694	-5.52^{E-3} (0.01)	0.561	6.08^{E-3} (0.01)	0.519
	Low x Sex	-0.10 (0.02)	< 0.001	-0.08 (0.02)	< 0.001	-0.11 (0.02)	< 0.001
	Medium x Sex	-0.04 (0.02)	0.027	-0.08 (0.02)	< 0.001	-0.06 (0.02)	0.002
Sarcopenia Model 1	Intercept	0.20 (0.05)	< 0.001	0.23 (0.05)	< 0.001	0.21 (0.05)	< 0.001
	Low compared to High Concentration	0.04 (0.08)	0.615	-0.08 (0.08)	0.291	-0.03 (0.08)	0.734
	Medium compared to High Concentration	-0.04 (0.07)	0.593	-3.92^{E-3} (0.07)	0.958	-1.86^{E-3} (0.07)	0.980
Model 2	Intercept	1.47 (0.17)	< 0.001	1.44 (0.17)	< 0.001	1.42 (0.17)	< 0.001
	Low compared to High Concentration	-2.44^{E-3} (0.09)	0.979	-0.09 (0.09)	0.318	-0.06 (0.09)	0.526
	Medium compared to High Concentration	-0.10 (0.09)	0.228	-0.01 (0.09)	0.878	0.05 (0.09)	0.594
	Time	0.01 (0.04)	0.831	0.01 (0.04)	0.840	0.03 (0.04)	0.461
	Low × Time	-0.01 (0.06)	0.850	0.07 (0.06)	0.270	0.04 (0.06)	0.556
	Medium × Time	0.06 (0.06)	0.324	-0.01 (0.06)	0.914	-0.05 (0.06)	0.377
	Low x Sex	0.40 (0.05)	< 0.001	0.48 (0.05)	< 0.001	0.43 (0.05)	< 0.001
	Medium x Sex	0.45 (0.05)	< 0.001	0.38 (0.05)	< 0.001	0.42 (0.05)	< 0.001
Severe Sarcopenia Model 1	Intercept	0.15 (0.04)	< 0.001	0.14 (0.04)	< 0.001	0.14 (0.04)	< 0.001
	Low compared to High Concentration	-0.01 (0.06)	0.815	-0.06 (0.06)	0.318	-0.01 (0.06)	0.853
	Medium compared to High Concentration	-0.12 (0.06)	0.035*	-0.05 (0.06)	0.385	-0.11 (0.06)	0.061
Model 2	Intercept	1.10 (0.14)	< 0.001	1.07 (1.47)	0.467	1.08 (0.14)	< 0.001
	Low compared to High Concentration	-0.06 (0.07)	0.434	-0.07 (0.07)	0.328	-0.02 (0.07)	0.804
	Medium compared to High Concentration	-0.16 (0.07)	0.020*	-0.07 (0.07)	0.302	-0.08 (0.07)	0.234
	Time	-0.01 (0.03)	0.663	-0.01 (0.03)	0.774	0.01 (0.03)	0.828
	Low × Time	0.03 (0.05)	0.577	0.05 (0.05)	0.254	0.02 (0.05)	0.742
	Medium × Time	0.09 (0.05)	0.056	0.05 (0.05)	0.273	0.04 (0.05)	0.433
	Low x Sex	0.22 (0.04)	< 0.001	0.24 (0.04)	< 0.001	0.18 (0.04)	< 0.001
	Medium x Sex	0.24 (0.04)	< 0.001	0.21 (0.04)	< 0.001	0.26 (0.04)	< 0.001

Tertiles: Low, medium and high: Low Selenium ≤ 46.7 µg/L, GPx3 activity ≤ 120.3 U/L; SePP ≤ 2.32 mg/L; Medium Selenium 46.7-62.0 µg/L; GPx3 120.3-166.1 U/L; SePP 2.33-3.57 mg/L; High Selenium ≥ 62 µg/L; GPx3 activity ≥ 166.1 U/L; SePP ≥ 3.58 mg/L. SE, standard error, Se: serum selenium; GPx3: glutathione peroxidase 3; SePP: selenoprotein P. The high concentration of each biomarker was used a reference. Model 1 included: the biomarker of selenium status; time; sex; interaction between time and the biomarker; and interaction between sex and the biomarker. Model 2 was adjusted for: presence of hand arthritis for HGS, or use of walking aids in TUG, sarcopenia and severe sarcopenia; selected biomarker; sex; National Statistics Socio-Economic Classification (NS-SEC); self-rated health; energy intake; protein intake; medication use; fat-free mass (FFM); physical activity; SMMSE: standardised mini mental state examination; alcohol intake; and smoking status.

4.8 Sensitivity Analyses

Table 4.1: Musculoskeletal measures at baseline for participants separated by serum selenium cut-offs.

Characteristic	Participants	Suboptimal Selenium < 70 µg/L n = 619	Optimal Selenium ≥ 70 µg/L n = 138	P
HGS Phase 1 (M, SD) n = 700	19.4, 8.3	19.2, 7.9	21.8, 9.5	0.732
TUG Phase 1 (M, IQR)	12.5, 5.0	11.5, 4.7	8.7, 5.3	0.013
Phase 1 Arthritis in hands % (n) n = 739				0.545
Yes	6.9 (51)	6.6 (40)	8.1 (11)	
No	93.1 (688)	93.4 (563)	91.9 (125)	
Phase 1 Walking Aids % (n) n = 700				0.117
Yes	17.4 (122)	18.5 (105)	12.8 (17)	
No	82.6 (578)	81.5 (462)	87.2 (116)	
Sarcopenia n = 675				0.592
Yes	21.2 (143)	20.8 (113)	22.9 (30)	
No	78.8 (532)	79.2 (431)	77.1 (101)	
Severe Sarcopenia n = 675				0.148
Yes	11.0 (74)	10.1 (55)	14.5 (19)	
No	89.0 (601)	89.9 (489)	85.5 (112)	

HGS: hand grip strength; TUG: Timed-Up-and-Go

Table 4.2: Baseline correlations between cut-offs of biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) and hand grip strength and Timed-Up-and-Go.

		HGS kg	TUG s	Sarcopenia	Severe Sarcopenia
Serum Selenium 70 µg/L	Pearson Correlation	-0.013	-0.085	0.021	0.056
	p	0.732	0.025*	0.593	0.149
	N	732	700	675	675
GPx3 Activity 115 U/L	Pearson Correlation	-0.054	-0.031	-0.045	-0.001
	p	0.148	0.411	0.238	0.974
	N	730	698	673	673
SePP 4.5 mg/L	Pearson Correlation	-0.033	-0.093	-0.025	0.004
	p	0.366	0.014*	0.521	0.927
	N	732	700	675	675

SePP: selenoprotein P; GPx3: glutathione peroxidase 3; HGS: hand grip strength; TUG: Timed-Up-and-Go

Table 4.3. Untransformed musculoskeletal measures (hand grip strength, timed up-and-go, sarcopenia, disability scores, IADL, BADL, falls and fractures) represented by serum selenium cut-offs, at baseline and follow-up.

MSK measures	All Participants	Low Selenium < 70 µg/L	Optimal Selenium ≥ 70 µg/L	p
Hand Grip Strength kg (M, SD)				
Baseline	19.4, 8.3	19.2, 7.9	21.8, 9.5	0.995
Hand arthritis % (n) yes	6.9 (51)	6.6 (40)	8.1 (11)	0.545
Follow-up at 1.5 years	19.5, 8.7	18.8, 8.1	21.8, 10.5	0.964
Follow-up at 3 years	18.5, 8.1	18.0, 7.4	20.5, 10.2	0.485
Follow-up at 5 years	17.1, 7.9	16.5, 7.1	18.9, 10.1	0.972
Timed Up-and-Go s (Median, IQR)				
Baseline	12.5, 5.0	11.4, 4.7	8.7, 5.3	0.013
Use of walking aids % (n) yes	17.4 (122)	18.5 (105)	12.8 (17)	0.117
Follow-up at 1.5 years	13.5, 5.5	13.9, 5.6	12.1, 3.9	0.029
Use of walking aids % (n) yes	16.1 (83)	17.5 (72)	10.5 (11)	0.081
Follow-up at 3 years	13.8, 6.9	13.7, 7.4	14.0, 7.8	0.002
Use of walking aids % (n) yes	17.8 (68)	20.3 (61)	8.8 (7)	0.017
Follow-up at 5 years	13.0, 6.3	12.8, 6.2	13.6, 7.5	0.012
Use of walking aids % (n) yes	25.0 (65)	28.0 (56)	15.0 (9)	0.041
Sarcopenia % (n) yes				
Baseline	21.2 (143)	20.8 (113)	22.9 (30)	0.592
Follow-up at 3 years	20.0 (73)	19.9 (58)	20.3 (15)	0.948
Severe Sarcopenia Baseline	11.0 (74)	10.1 (55)	14.5 (19)	0.148
Severe Sarcopenia Follow-up at 3 years	10.1 (37)	10.7 (31)	8.1 (6)	0.517

Table 4.4. Hand grip strength (kg), Timed-Up-and-Go (log_{10}-s), sarcopenia and severe sarcopenia estimates from an unadjusted model (Model 1) and fully adjusted model (Model 2) including all covariates and each biomarker of selenium status, represented by a cut-off for optimal status.

Outcome	Variable	Selenium Model		GPx3 Activity Model		SePP Model	
		β (SE)	p	β (SE)	p	β (SE)	p
HGS (kg)	Intercept	15.49 (0.61)	< 0.001	14.95 (0.33)	< 0.001	15.03 (0.65)	< 0.001
Model 1	Suboptimal compared to Optimal	-0.79 (0.69)	0.253	-0.44 (0.63)	0.490	-0.20 (0.72)	0.782
Model 2	Intercept	-0.61 (2.52)	0.808	-0.35 (1.63)	0.829	-5.38 (1.69)	0.958
	Biomarker Low	0.62 (0.34)	0.066	0.47 (0.63)	0.487	-0.08 (0.34)	0.822
	Time	-0.93 (2.38)	0.697	-1.09 (0.13)	< 0.001	-1.04 (2.38)	1.000
	Biomarker Low × Time	-0.31 (0.13)	0.017	-0.28 (0.24)	0.246	-0.16 (0.14)	0.235
	Biomarker Low x Sex	7.05 (0.20)	< 0.001	7.61 (0.53)	< 0.001	7.25 (0.20)	< 0.001
TUG (log_{10}-s)	Intercept	1.13 (0.03)	< 0.001	1.17 (0.01)	< 0.001	1.12 (0.03)	< 0.001
Model 1	Suboptimal compared to Optimal	0.07 (0.03)	0.011*	0.05 (0.03)	0.066	0.08 (0.03)	0.008*
Model 2	Intercept	1.49 (0.06)	< 0.001	1.49 (0.06)	< 0.001	1.43 (3.08)	0.643
	Biomarker Low	8.85^{E-4} (0.02)	0.971	3.64^{E-5} (0.02)	0.999	0.06 (0.03)	0.020
	Time	0.04 (0.09)	0.697	0.04 (0.004)	< 0.001	0.05 (41.8)	1.000
	Biomarker Low × Time	0.01 (0.01)	0.171	4.54^{E-3} (0.01)	0.606	-7.95^{E-3} (0.01)	0.431
	Biomarker Low x Sex	-0.06 (0.02)	< 0.001	-0.09 (0.02)	< 0.001	-0.07 (0.01)	< 0.001
Sarcopenia	Intercept	0.19 (0.07)	0.004	0.23 (0.04)	< 0.001	0.17 (0.07)	0.017
Model 1	Suboptimal compared to Optimal	0.01 (0.08)	0.901	-0.10 (0.07)	0.166	0.04 (0.08)	0.642
Model 2	Intercept	1.47 (0.18)	< 0.001	1.44 (0.16)	< 0.001	1.42 (0.18)	< 0.001
	Biomarker Low	-0.06 (0.09)	0.518	-0.09 (0.08)	0.272	0.01 (0.10)	0.941
	Time	-0.01 (0.05)	0.904	1.12^{E-3} (0.03)	0.970	0.06 (0.06)	0.322
	Biomarker Low × Time	0.04 (0.06)	0.506	0.10 (0.05)	0.084	-0.04 (0.06)	0.533
	Biomarker Low x Sex	0.43 (0.04)	< 0.001	0.45 (0.05)	< 0.001	0.44 (0.04)	< 0.001
Severe Sarcopenia	Intercept	0.17 (0.05)	0.001	0.11 (0.03)	< 0.001	0.14 (0.06)	0.012
Model 1	Suboptimal compared to Optimal	-0.09 (0.06)	0.137	-0.03 (0.05)	0.558	-0.05 (0.06)	0.435
Model 2	Intercept	1.14 (0.14)	< 0.001	1.03 (0.13)	< 0.001	1.07 (0.15)	< 0.001
	Biomarker Low	-0.14 (0.07)	0.049*	-0.02 (0.06)	0.784	-0.04 (0.08)	0.617
	Time	-0.05 (0.04)	0.299	0.02 (0.02)	0.446	0.02 (0.05)	0.734
	Biomarker Low × Time	0.09 (0.05)	0.070	0.02 (0.04)	0.571	0.01 (0.05)	0.838
	Biomarker Low x Sex	0.23 (0.03)	< 0.001	0.20 (0.04)	< 0.001	0.23 (0.03)	< 0.001

SE, standard error; Se: serum selenium; GPx3: glutathione peroxidase 3; SePP: selenoprotein P. Model 1 included: the biomarker of selenium status; time; sex; interaction between time and the biomarker; and interaction between sex and the biomarker. Model 2 was further adjusted for: presence of hand arthritis for HGS, or use of walking aids in TUG, sarcopenia and severe sarcopenia; National Statistics Socio-Economic Classification (NS-SEC); self-rated health; energy intake; protein intake; medication use; fat-free mass (FFM); physical activity; SMMSE: standardised mini mental state examination; alcohol intake; and smoking status.

Chapter 5. Selenium Status and Disability in Very Old Adults: The Newcastle 85+ Study

5.1 Abstract

Background: Disability is defined as a difficulty in performing common daily activities of life related to basic functioning, care, and independence. Selenium may influence disability through its putative roles in maintaining muscle and brain health.

Objectives: To explore the relationships between biomarkers of selenium status and disability scores among participants in The Newcastle 85+ Study at baseline and prospectively.

Methods: Biomarkers of selenium status (serum selenium, GPx3 activity and SePP) at baseline were measured using standard laboratory techniques in 757 participants from The Newcastle 85+ Study. Disability was measured using a self-reported questionnaire asking questions regarding one's ability to perform 17 ADLs, including basic ADL (washing hands, dressing oneself) and instrumental ADL (managing finances, grocery shopping); difficulty in performance in each activity was summed. The relationships between the biomarkers of selenium status and disability scores were analysed at baseline and at 5 years using linear mixed models that adjusted for appropriate covariates.

Results: At baseline, in fully adjusted models, there was a strong, negative association between the biomarkers of selenium status and baseline disability score; serum selenium (β -0.014 ± 0.06, P = 0.019); and SePP (β -0.15 ± 0.07 P = 0.038). Over time, in fully adjusted models, participants with low (tertile 1) SePP concentrations had a greater change in prevalence of disability compared with those with high (tertile 3) SePP concentrations (β 0.56 ± 0.22, P = 0.012).

Conclusion: In cross-sectional analyses, selenium and SePP concentrations were negatively associated with lower disability scores in very old adults. Over time, low (tertile 1) SePP concentrations were associated with a greater prevalence of disability.

5.2 Introduction

In this penultimate experimental chapter, I explored the associations between biomarkers of selenium status and MSK function, focussing on the clinically important endpoint, disability. Disability is defined as a difficulty in performing basic activities of daily life including, but not limited to, dressing oneself, getting out of bed and washing hands. These BADL, in addition to more complex activities, such as managing finances, shopping and housework, IADL, are components that determine the disability status of an individual or population (Gobbens and van Assen, 2014). Therefore, disability is a complex construct of activities of daily living encompassing both physical and motor function and cognition. There is a high prevalence of disability in very old adults (≥ 85 years) (Yu *et al.*, 2016) with 40 % of those over 85 years estimated to be affected (Gobbens and van Assen, 2014). Disability is an important MSK-related outcome because it is associated with adverse health outcomes including increased risk of hospitalisation, greater need for 24 h care, higher socioeconomics costs (Millán-Calenti *et al.*, 2010) and greater likelihood of mortality (Majer *et al.*, 2011). Disability scores combine both physical and cognitive function, and are therefore strongly correlated with performance in TUG (Jagger *et al.*, 2011). Since serum selenium and SePP concentrations were associated with TUG performance among participants in The Newcastle 85+ Study (reported in Chapter 4), I hypothesised that selenium status may also be associated with disability. Moreover, previous publications from this population have reported associations between protein intake (Mendonça *et al.*, 2019) and vitamin D status (Hakeem *et al.*, 2020) and disability trajectories.

Further rationale for the link between selenium and diability is provided by evidence that selenium has a positive effect on cognitive function (Berr *et al.*, 2000; Akbaraly *et al.*, 2007; Gray *et al.*, 2003; Shahar *et al.*, 2010). As for age-related loss of MSK function, cognitive impairment is associated with excessive oxidative stress and inflammation (Baierle *et al.*, 2015; Schweizer *et al.*, 2004), which can lead to increased protein oxidation, advanced glycation end products and lipid peroxidation, all of which ehance neurodegeneration (Popa-Wagner *et al.*, 2013). As mentioned in Chapter 1, Sections 1.4 and 1.8, higher selenium concentratons and SeMet supplementation have been associated with lower concentrations of inflammation probably through selenium's incorporation into

selenoproteins acting as antioxidants (Tseng *et al.*, 2013; Walston *et al.*, 2006; Cheng *et al.*, 2011). In mouse models, SePP knockout led to synaptic dsyfunction in the hippocampus and decreased spatial learning, similar to that seen in Alzheimer's Disease (Peters *et al.*, 2006). In another SePP knockout study, mice exposed to selenium-deficient diets experienced neurological seizures and movement disorders, suggesting the role of selenoproteins, especially SePP, in cognition (Schomburg *et al.*, 2003; Schweizer *et al.*, 2004). Furthermore, SePP transports selenium to brain by binding to the ApoER2 surface receptor and this is maintained during times of selenium defiency to prioritise delivery of selenium to the brain (Rayman, 2012; Burk *et al.*, 2014). As described in Chapter 1, Section 1.5, there is a hierarchy amongst organs in the body that determines how selenium is priortised during selenium deficiency. In addition, there is an hierarchy among selenoproteins where SePP is often preserved over other selenoproteins, such as GPx3 (Schomburg and Schweizer, 2009). These key considerations suggest that selenium status, especially SePP, may play an important role in determining the risk of disabilty, a more complex measure of MSK function, combining muscle power, motor function and cogntion (Savva *et al.*, 2013), in addition to the role of selenium in measures of MSK function such as TUG performance (see Chapter 4).

To my knowledge, only one study has explored the associations between selenium status (whole-blood and serum selenium) and MSK function, IADL and fraility in older people using NHANES (≥ 60 years) and Seniors-ENRICA-2 cohorts (≥ 65 years) (García-Esquinas *et al.*, 2021). However, many of the participants were relatively young and, in both cohorts, participants had higher baseline serum selenium than the participants from The Newcastle 85+ Study (129.7 and 113.4 µg/L versus 53.6 µg/L, respectively). The findings revealed that higher whole-blood selenium concentration was associated with better MSK function and a lower risk of mobility disability (García-Esquinas *et al.*, 2021). Thus, to date, no epidemiological studies have investigated the association between multiple biomarkers of selenium status and disability in very old adults.

I hypothesised that participants in The Newcastle 85+ Study with optimal selenium status will have lower disability scores than participants with suboptimal selenium status. The aims of this study were: 1) to explore the associations between biomarkers of selenium status (serum selenium, GPx3 activity, and SePP) and disability scores in very old adults at baseline; 2) to identify the determinants of disability including biomarkers of selenium status, socioeconomics, health and lifestyle factors; 3) to determine the relationships between biomarkers of selenium status and disability scores at baseline and the change in prevalence of disability over 5 years.

5.3 Methods

5.3.1. Study Population

Data and samples were obtained from The Newcastle 85+ Study, a longitudinal, population-based study of a single-year birth cohort in the Northeast of England that explored health outcomes and trajectories in adults aged 85 years and over. The study was initiated in 2006 recruiting 1042 participants born in 1921, for full details, see Chapter 2, Section 2.2.1.

5.3.2 Socioeconomic, Lifestyle and Other Covariates

Assessments included questionnaires, functional tests, fasting blood samples, medical record reviews, dietary intakes and body weight measurements which were taken at the initial health assessment (2006/2007) and three other visits (1.5, 3, 5 years), details of which are provided in Chapter 2, Sections 2.2.1.1 and 2.2.1.2. These covariates used in these analyses were: sex; use of walking aids; occupational status; education; self-rated health; SMMSE; energy intake; protein intake; medication use; FFM; smoking; and alcohol intake. These covariates were selected based on previous research investigating the effects of vitamin D and protein on disability trajectories in the same cohort (Mendonça *et al.*, 2019; Hakeem *et al.*, 2020).

5.3.3 Assessment of Disability

Full details of disability scores are provided in Chapter 2, Section 2.2.1.4. In brief, disability scores were created by using a self-reported questionnaire that summed the scores of 17 activities. The activities included: BADL; IADL; mobility issues; lower limb mobility; chair rises; stair climbing; grocery shopping; and walking 370 m. A score of 1 indicated difficulty in

performance, or unable to perform without an aid/appliance or carer, and a score of 0 indicated no difficulty. Scores for each activity were summed so that a total score of 17 indicated greatest disability, whilst a score of 0 indicated an absence of disability.

5.3.4. Biomarkers of Selenium Status

Baseline blood samples from 2006/2007 (n = 757) that had been stored at -80 °C were analysed for biomarkers of selenium status. Serum selenium was measured using TXRF, GPx3 activity was measured using a coupled-enzyme reaction measuring NADPH consumption and SePP was measured using a commercial ELISA. For full details, see Chapter 2, Section 2.2.1.5.

5.3.5. Statistical Analyses

IBM statistical software package version 27.0 (SPSS) was used to perform the exploratory and statistical analyses; $p < 0.05$ was considered statistically significant. To determine normality of distributions of the variables, the Shapiro-Wilk test and quantile–quantile (QQ) plots were used. Full participant characteristics are described in detail in Chapter 3, Section 3.4.1. Biomarkers of selenium status were used as continuous independent variables in the initial analyses and subsequently categorised into statistically derived tertiles as described in Chapter 4, Section 4.3.5. The tertiles 1, 2, and 3 of selenium status, hereafter, low, medium and high, were as follows: serum selenium concentration (μg/L): ≤ 46.7, 46.7-62.0, ≥ 62.0; GPx3 activity (U/L): ≤ 120.3, 120.3-166.1, ≥ 166.1; serum SePP concentration (mg/L): ≤ 2.32, 2.33-3.57, ≥ 3.58. Differences in disability scores according to serum selenium status (tertiles) were assessed Chi-square test (categorical) and Kruskal–Wallis (ordered and non-normally distributed).

5.3.5.1 Relationships between Biomarkers of Selenium Status and Disability at Baseline

Relationships between each biomarker of selenium status (serum selenium, GPx3 activity, SePP) and disability score were investigated by linear regression. The biomarkers of selenium status (in continuous and tertile format) were first added into an unadjusted model (Model 1), followed by the additional covariates. These were: sex (men/women, binary); use of walking aids; NS-SEC (routine/manual, intermediate, managerial/professional

occupations, categorical); education (0-9, 10-11, ≥ 12 years, categorical); self-rated health (excellent/very good, good, fair/poor, ordinal); SMMSE (continuous); energy intake (continuous); protein intake (continuous); medication use (continuous); FFM (continuous); smoking (current/former/never); and alcohol intake (binary).

5.3.5.2 Relationships between Biomarkers of Selenium Status and the Change in Disability Over Time

Linear mixed models were used to determine the relationships between each biomarker of selenium status and disability score at baseline, and the rate of change in disability scores over 5 years. For each time point (baseline, 1.5, 3 and 5 years), time was treated as a categorical variable. The random effects were time and the intercept. Fixed effects were the selected variables of interest including the biomarkers of selenium status (continuous and tertile format) and the associated covariates (listed above in 5.3.5.1). I used two different models: (Model 1) time, sex, biomarker, time x biomarker, sex x biomarker interactions; (Model 2) adjustments made for presence of hand arthritis or use of walking aids (binary), sex, NS-SEC, education, self-rated health, energy intake, protein intake, medication use, FFM, SMMSE, smoking and alcohol intake. Restricted maximum likelihood (RML) and unstructured or heterogeneous first-order autoregressive covariance matrixes were applied to derive parameter estimates (β). Negative β estimates indicated lower disability scores, and thus reduced disability. Graphical outputs were created in Microsoft Excel 2010 using the equation: Intercept value + Time × (Time-beta + Time × selenium-beta interaction term) + selenium-beta.

5.4 Results

5.4.1. Participant Characteristics

Baseline disability scores of participants according to the statistically derived tertiles of serum selenium concentration are summarised in Table 5.1 (see Chapter 3 for full characteristics of participants at baseline). A higher proportion of participants with low (tertile 1) serum selenium reported high levels of disability (15.3 %) compared with those with high (tertile 3) serum selenium (4 %) ($P < 0.001$). Similarly, fewer participants reported no disability (16.5 %) among those with low (tertile 1) serum selenium compared to high

(tertile 3) serum selenium concentration (28.4 %) (P < 0.001). Similar trends were observed for other measures of disability including BADL, IADL and mobility scores (P < 0.001).

Table 5.1: Disability scores and associated measures at baseline for participants represented by tertiles of serum selenium.

Characteristic	Participants N = 748	Low Selenium ≤ 46.7 µg/L (Tertile 1)	Medium Selenium 46.7-62.0 µg/L (Tertile 2)	High Selenium ≥ 62.0 µg/L (Tertile 3)	P
Disability Score % (n)					
No Disability	21.1 (158)	16.5 (41)	18.4 (46)	28.4 (71)	< 0.001
Low (score 1–6)	51.6 (386)	46.8 (116)	55.6 (139)	52.4 (131)	
Moderate (score 7–12)	18.9 (141)	21.4 (53)	20.0 (50)	15.2 (38)	
High (score 13–17)	8.4 (63)	15.3 (38)	6.0 (15)	4.0 (10)	
BADL Score % (n)					
0	31.4 (235)	25.4 (63)	31.6 (79)	37.2 (93)	< 0.001
1	30.7 (230)	29.8 (74)	30.0 (75)	32.4 (81)	
2	15.2 (114)	13.3 (33)	16.0 (40)	16.4 (41)	
3	7.9 (59)	7.3 (18)	9.6 (24)	6.8 (17)	
4	3.3 (25)	5.2 (13)	1.2 (3)	3.6 (9)	
5	2.9 (22)	4.8 (12)	3.6 (9)	0.4 (1)	
6	4.3 (32)	7.3 (18)	4.0 (10)	1.6 (4)	
7	1.3 (10)	2.4 (6)	0.8 (2)	0.8 (2)	
8	2.8 (21)	4.4 (11)	3.2 (8)	0.8 (2)	
IADL Score % (n)					
0	39.7 (297)	32.3 (80)	37.2 (93)	49.6 (124)	< 0.001
1	17.8 (133)	16.1 (40)	19.6 (49)	17.6 (44)	
2	14.8 (111)	14.9 (37)	15.6 (39)	14.0 (35)	
3	7.9 (59)	6.9 (17)	10.8 (27)	6.0 (15)	
4	6.1 (46)	6.0 (15)	6.8 (17)	5.6 (14)	
5	5.2 (39)	8.9 (22)	4.0 (10)	2.8 (7)	
6	8.4 (63)	14.9 (37)	6.0 (15)	4.4 (11)	
Mobility Score % (n)					
0	45.1 (337)	35.5 (88)	44.0 (110)	55.6 (139)	< 0.001
1	16.0 (120)	16.1 (40)	16.0 (40)	16.0 (40)	
2	19.1 (143)	19.4 (48)	22.0 (55)	16.0 (40)	
3	19.8 (148)	29.0 (72)	18.0 (45)	12.4 (31)	

BADL: basic activities of daily living; IADL: instrumental activities of daily living. Higher scores indicate poor performance

5.4.2 Relationship between Biomarkers of Selenium Status and Disability at Baseline

Relationships between the three biomarkers of selenium status and disability scores are presented in Table 5.2. At baseline, when using the continuous biomarkers of selenium status, there was a strong, negative association between serum selenium and disability score ($r(748) = -0.256$, $P < 0.001$). Likewise, there was a strong, negative association between GPx3 activity and disability score ($r(746) = -0.090$, $P = 0.014$) and between SePP and disability score ($r(748) = -0.092$, $P = 0.012$). When using tertiles of the biomarkers of selenium status, the strong, negative association between serum selenium and disability scores

((r(748)= -0.222, P < 0.001) and between SePP and disability scores (r(748)= -0.111, P = 0.002) remained highly significant. However, there were no associations between GPx3 activity tertiles and disability score (r(746)= -0.068, P = 0.065).

Table 5.2: Correlations between biomarkers of selenium status (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) and disability scores at baseline.

		Disability Score
Serum Selenium µg/L	Pearson Correlation	-0.256*
	p	< 0.001
	N	748
GPx3 Activity U/L	Pearson Correlation	-0.090*
	p	0.014
	N	746
SePP mg/L	Pearson Correlation	-0.092*
	p	0.012
	N	748

SePP: selenoprotein P; GPx3: glutathione peroxidase 3 * denotes significance

5.4.3 Predictors of Disability Score at Baseline

At baseline, when using the continuous format of the biomarkers of selenium status, the significant predictors of disability score were the same regardless of the choice of biomarker of selenium status (serum selenium, SePP, GPx3 activity). The equations including β ± SD for the significant predictors are displayed in Table 5.3, whilst the full output can be found in Appendix Table 5.1. These predictors were: physical activity; SMMSE; self-rated health; medication use; walking aids; and living status. In the unadjusted model (Model 1), a 1 µg/L increase in serum selenium was associated with a decrease in disability score (β -0.06 ± 0.01, P < 0.001) as was a 1 U/L increase in GPx3 activity (β -0.01 ± 0.003, P = 0.014), and a 1 mg/L increase in SePP (β -0.30 ± 0.12, P = 0.012). These associations were maintained in the fully adjusted model (Model 2) of associations between serum selenium (β -0.01 ± 0.01, P = 0.019) and SePP (β -0.15 ± 0.07, P = 0.038) and disability score. In the unadjusted model, when using tertiles of the biomarkers of selenium status, both low (tertile 1) and medium (tertile 2) concentrations of each biomarker (serum selenium, GPx3 activity and SePP) were significant predictors of disability with low (tertile 1) concentrations being associated with a higher disability score: low (tertile 1) serum selenium (β 2.66 ± 0.55, P < 0.001) and medium (tetile 2) serum selenium (β 1.80 ± 0.55, P < 0.001); low (tertile 1) GPx3 activity (β 1.64 ±

0.57, P = 0.004) and medium (tertile 2) GPx3 activity (β 1.10 ± 0.55, P = 0.046); low (tertile 1) SePP concentrations (β 1.74 ± 0.56, P = 0.002) and medium (tertile 2) SePP concentrations (β 1.26 ± 0.56, P = 0.024). However, in the fully adjusted model (Model 2), these associations were not maintained (Appendix Table 5.2).

Table 5.3: Equations derived from regressions of the relationships between the biomarkers of selenium status and disability scores using the significant predictors and intercept values, β(SE).

Outcome	Selenium Status Biomarker	Equation =intercept, significant predictors, β(SE)
Disability Score	Serum Selenium	=11.53(1.50)-0.01(0.01)Selenium-2.06(0.18)PA -0.17(0.04)SMMSE+0.58(0.15)SRH+0.35(0.15)Medication+3.04(0.31)Walking Aid
	GPx3 Activity	=11.29(1.50) -2.08(0.18)PA -0.18(0.04)SMMSE+1.29(0.67)Living Status+0.59(0.15)SRH+0.39(0.15)Medication+3.05(0.31)Walking Aid
	Selenoprotein P	=11.20(1.50)-0.15(0.07)SePP-2.08(0.18)PA -0.18(0.04)SMMSE+1.32(0.67)Living Status+0.61(0.15)SRH+0.41(0.15)Medication+3.01(0.31)Walking Aid

PA: physical activity; SMMSE: standardised mini mental state examination; SRH: self-rated health; Walking Aid: use of walking appliances; GPx3: glutathione peroxidase 3; SePP: selenoprotein P

5.4.4 Prevalence of Disability over Time

Disability scores and associated measures at baseline and follow-up (1.5, 3 and 5 years) were categorised by tertiles of serum selenium and are summarised in Appendix Table 5.3. As expected, the prevalence of disability increased over the 5 years of follow-up. Participants with low (tertile 1) serum selenium were more likely to have higher disability scores (greatest disability) compared to those with high (tertile 3) serum selenium (1.5-year follow-up; 21.1 % low (tertile 1) serum selenium versus 10.0 % in high (tertile 3) serum selenium, P = 0.003, and 3-year follow-up; 25.8 % versus 10.9 %, P = 0.001). The same trend, where participants with low (tertile 1) serum selenium had higher mobility and BADL scores than those with high (tertile 3) serum selenium, at 1.5-year and 3-year follow-ups. Likewise, similar trends were seen for IADL scores in addition to those with low (tertile 1) serum selenium having significantly worse performance at 5-year follow-ups compared to those with high (tertile 3) serum selenium.

5.4.5 Relationship between Biomarkers of Selenium Status and Change in Prevalence of Disability Over Time

Prospective investigations of relationships between the biomarkers of selenium status at baseline and the change in prevalence of disability over 5 years are shown in Table 5.4,

including the unadjusted model (Model 1) and fully adjusted model (Model 2). The graphical outputs from these results are displayed in Figure 5.1. In the unadjusted model (Model 1), when using continuous biomarkers of selenium status, each biomarker of selenium status was associated with an improvement in disability score, where SePP was associated with the lowest change in prevalence of disability (serum selenium β −0.06 ± 0.01, P < 0.001; GPx3 activity β −0.01 ± 0.005, P = 0.003; SePP β −0.43 ± 0.16, P = 0.006). However, in the fully adjusted model, none of the biomarkers of selenium status were associated with a change in prevalence of disability.

In the unadjusted model (Model 1), when using tertiles of the biomarkers of selenium status, participants with low (tertile 1) and medium (tertile 2) concentrations of each biomarkers were more likely to have a greater change in prevalence of disability, than those with high (tertile 3) concentrations (low serum selenium β 2.66 ± 0.55, P < 0.001, medium serum selenium β 1.80 ± 0.55, P = 0.001; low GPx3 activity β 2.64 ± 0.57, P = 0.004, medium GPx3 activity β 1.10 ± 0.57, P = 0.046; low SePP concentration β 1.74 ± 0.56, P = 0.002, medium SePP concentration β 1.26 ± 0.56, P = 0.024). The associations with a change in prevalence of disability were strongest for serum selenium. In the fully adjusted model, those with low (tertile 1) SePP concentrations were more likely to be associated with a greater change in the prevalence of disability than those with high (tertile 3) SePP concentrations (β 0.56 ± 0.22, P = 0.012). There were no associations between the remaining biomarkers of selenium status and change in prevalence of disability. In the fully adjusted model, the change in prevalence of disability over 5 years was predicted by the interactions between low (tertile 1), and medium (tertile 3) biomarker concentrations, and sex (except for medium (tertile 2) serum selenium concentrations) (Appendix Table 5.4).

Table 5.4. Disability score estimates from an unadjusted (Model 1) and fully adjusted model (Model 2) including all covariates and each biomarker of selenium status.

Outcome	Variable	Selenium Model		GPx3 Activity Model		SePP Model	
		β (SE)	p	β (SE)	p	β (SE)	p
Disability	Intercept	7.44 (0.67)	< 0.001	5.93 (0.72)	< 0.001	5.22 (0.05)	< 0.001
Model 1	Biomarker	-0.06 (0.01)	< 0.001*	-0.01 (0.005)	0.003*	-0.43 (0.16)	0.006*
Model 2	Intercept	10.40 (1.19)	< 0.001	10.97 (1.21)	< 0.001	10.93 (1.13)	< 0.001
	Biomarker	4.44^{E-4} (0.01)	0.964	-0.002 (0.004)	0.516	-0.13 (0.13)	0.320
	Time	1.72 (0.23)	< 0.001	1.21 (0.23)	< 0.001	1.34 (0.17)	< 0.001
	Biomarker × Time	-0.02 (0.004)	0.165	0.002 (0.003)	0.343	0.02 (0.05)	0.636
	Biomarker x Sex	0.005 (0.01)	0.605	-1.28 (0.54)	0.561	0.19 (0.11)	0.097

SE: standard error; Se: serum selenium; GPx3: glutathione peroxidase 3; SePP: selenoprotein P. Model 1 included: the biomarker of selenium status; time; sex; interaction between time and the biomarker; and interaction between sex and the biomarker. Model 2 was further adjusted for: presence of hand arthritis for HGS, or use of walking aids in TUG, sarcopenia and severe sarcopenia; National Statistics Socio-Economic Classification (NS-SEC); self-rated health; energy intake; protein intake; medication use; fat-free mass (FFM); physical activity; SMMSE: standardised mini mental state examination; alcohol intake; and smoking status.

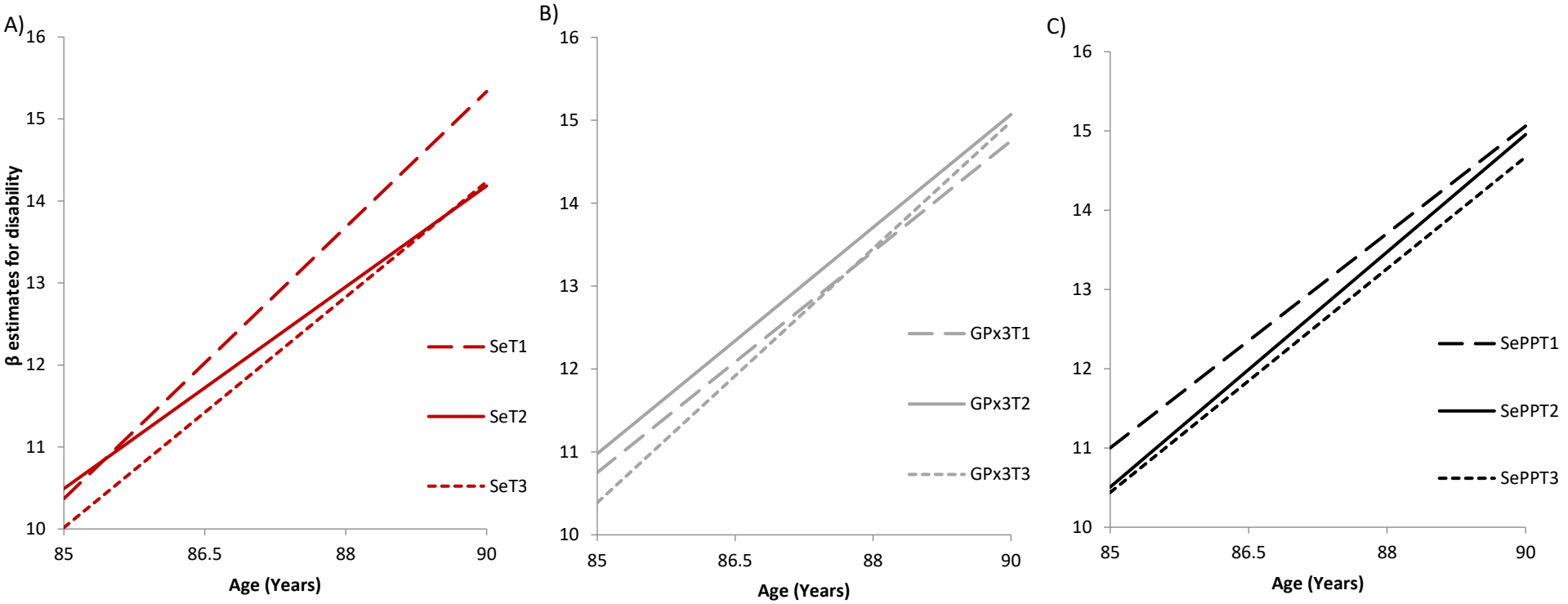


Figure 5.1: Linear relationships between disability scores and biomarkers of selenium status in fully adjusted models. A) Serum selenium tertiles; B) Glutathione peroxidase 3 activity tertiles; C) Selenoprotein P tertiles. T1: tertile 1 low, T2: tertile 2 medium, T3: tertile 3 high concentrations of the biomarker of selenium status.

5.5 Discussion

At baseline, in adjusted models, there was a strong, negative association between serum selenium and disability score, and a weaker, negative association between SePP and disability score. Predictors of disability remained the same between the biomarkers of selenium status, where serum selenium and SePP predicted lower disability. Over time, in adjusted models, when using tertiles of the biomarkers of selenium status, low (tertile 1) SePP concentrations, compared to high (tertile 3), were associated with a greater change in prevalence of disability. Each aspect of this summary will be reviewed below in more detail.

Relationships between Biomarkers of Selenium Status and Disability at Baseline

At baseline, the significant predictors of disability were physical activity; SMMSE; self-rated health; medication use; walking aids and living status. These findings are common predictors of disability in other studies (Gobbens and van Assen, 2014; Oliveira *et al.*, 2017; Wu *et al.*, 2022). Among the biomarkers of selenium status, when using continuous variables, serum selenium and SePP were associated with lower disability scores. The significance of serum selenium and SePP predicting disability scores is not surprising as these biomarkers have been associated with brain health, where higher concentrations of selenium are associated with enhanced cognition and therefore, potentially improved performance in activities of daily living (Popa-Wagner *et al.*, 2013; Peters *et al.*, 2006; Schweizer *et al.*, 2004).

Relationships between Biomarkers of Selenium Status and Change in Prevalence of Disability Over Time

When using tertiles of the biomarkers of selenium status, participants with low (tertile 1) SePP concentrations were more likely to have greater increase in prevalence of disability than those with high (tertile 3) SePP concentrations. Since disability scores combine MSK function and motor function (through BADL) and cognition (through IADL), it seems plausible that low (tertile 1) SePP would be associated with increased disability (Berr *et al.*, 2000; Gray *et al.*, 2003; Shahar *et al.*, 2010; Akbaraly *et al.*, 2007). SePP is a known selenium transporter (Saito and Takahashi, 2002); it delivers selenium to different tissues, one of

which is the brain by binding to the surface receptor, ApoER2. During selenium deficiency, transport of selenium to the brain is prioritised (Burk and Hill, 2009; Burk *et al.*, 2014). In addition to this, there is selenoprotein hierarchy, where SePP is preferentially maintained over other selenoproteins, such as GPx3 (Behne *et al.*, 1988), and this could explain why SePP was a predictor of disability score, and the change in prevalence of disability despite the low levels of SePP in this population. Furthermore, this contrasts the lack of association between the biomarkers of selenium status and HGS as seen in Chapter 4; during selenium deficiency, selenium is preserved in the brain and major organs whilst selenium stores in the muscle, albeit one of the largest stores, are also one of the first to be compromised (Schomburg and Schweizer, 2009; Combs and Combs, 1986).

In the fully adjusted model, the change in prevalence of disability over 5 years was predicted by the interactions between low (tertile 1), and medium (tertile 2) biomarker concentrations, and sex (except for medium serum selenium concentrations). This finding suggests that the biomarkers of selenium status and prevalence of disability are different between men and women. Previous research has suggested sex-differences in biomarkers of selenium status, although there is some controversy (see Chapter 3, Section 3.2 and 3.5). Furthermore, in this population and other studies, disability is known to affect women more than men (Gill *et al.*, 2013; Millán-Calenti *et al.*, 2010) and other studies have found fat mass, rather than muscle mass to be a stronger predictor of disability (Sternfeld *et al.*, 2002; Koster *et al.*, 2011). This could be one explanation why disability is greater in women, especially in this population, as post-menopausal women are more likely to have a greater distribution of visceral fat compared to older men (Kodoth, Scaccia and Aggarwal, 2022).

To date, this is the only large-scale study assessing the association between three biomarkers of selenium status and disability scores, and the change in prevalence of disability over time, in very old adults with suboptimal selenium status. This study focused on an important area of ageing, disability, which is crucial for longevity and independence in older age. However, these results should be interpreted with caution as this was an observational study. Please see the general limitations to The Newcastle 85+ Study design in Chapter 7, Section 7.2.

5.6 Conclusion

The additional analyses of this population presented in this penultimate experimental chapter reveal that biomarkers of selenium status, specifically serum selenium and SePP are associated with disability scores in older adults, cross-sectionally and prospectively. It was found that serum selenium and SePP predicted disability at baseline, and over time, low (tertile 1) SePP concentrations were associated with an increased change in the prevalence of disability.

5.7 Appendix

Table 5.1. Correlations between tertiles of biomarkers of selenium (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) and disability score.

		Disability Score
Serum Selenium Tertile µg/L	Pearson Correlation	-0.222
	p	< 0.001*
	N	748
GPx3 Activity Tertile U/L	Pearson Correlation	-0.068
	p	0.065
	N	746
SePP Tertile mg/L	Pearson Correlation	-0.111
	p	0.002*
	N	748

SePP: selenoprotein P; GPx3: glutathione peroxidase 3

Table 5.2: Predictors of disability with each selenium status biomarker (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) determined using linear regression.

Outcome	Variable	Serum Selenium		GPx3 Activity		Selenoprotein P	
		B (SE)	p	B (SE)	p	B (SE)	p
Disability	Intercept	8.01 (0.50)	< 0.001	5.77 (0.51)	< 0.001	5.47 (0.40)	< 0.001
Model 1	Biomarker	-0.06 (0.01)	< 0.001*	-0.01 (0.003)	0.014*	-0.30 (0.12)	0.012*
Model 2	Intercept	11.53 (1.50)	< 0.001	11.29 (1.51)	< 0.001	11.20 (1.48)	< 0.001
	Biomarker	-0.01 (0.01)	0.019*	-0.004 (0.002)	0.089	-0.15 (0.07)	0.038*
	Sex	0.53 (0.34)	0.117	0.53 (0.34)	0.118	0.53 (0.34)	0.119
	Physical Activity	-2.06 (0.18)	< 0.001	-2.08 (0.18)	< 0.001	-2.08 (0.18)	< 0.001
	Fat Free Mass (kg)	-0.003 (0.02)	0.865	-0.002 (0.02)	0.915	-0.001 (0.02)	0.938
	SMMSE	-0.17 (0.04)	< 0.001	-0.18 (0.04)	< 0.001	-0.18 (0.04)	< 0.001
	Energy Intake (Kcal)	0.00 (0.00)	0.673	0.00 (0.00)	0.603	0.00 (0.00)	0.658
	Protein Intake (g)	-0.001 (0.01)	0.856	-0.002 (0.01)	0.781	-0.001 (0.01)	0.866
	NS-SEC	0.12 (0.13)	0.364	0.11 (0.13)	0.411	0.11 (0.13)	0.424
	Education	-0.23 (0.17)	0.178	-0.24 (0.17)	0.159	-0.25 (0.17)	0.135
	Living Status	1.29 (0.67)	0.056	1.41 (0.67)	0.037	1.32 (0.67)	0.050
	Self-rated Health	0.58 (0.15)	< 0.001	0.59 (0.15)	< 0.001	0.61 (0.15)	< 0.001
	Total Medications	0.35 (0.15)	0.016	0.39 (0.15)	0.008	0.41 (0.15)	0.005
	Smoking Status	0.04 (0.12)	0.738	0.03 (0.12)	0.794	0.05 (0.12)	0.696
	Alcohol Intake	-0.10 (0.24)	0.682	-0.11 (0.24)	0.649	-0.15 (0.24)	0.523
	Walking Aid	3.04 (0.31)	< 0.001	3.05 (0.31)	< 0.001	3.01 (0.31)	< 0.001

SE: standard error; BMI: body mass index; MMSE: standardised mini mental state examination; NS-SEC: National Statistics Socio-economic classification.

Table 5.3: Predictors of disability with each selenium status biomarker as tertiles (serum selenium, glutathione peroxidase 3 activity, selenoprotein P) determined using linear regression.

Outcome	Variable	Serum Selenium		GPx3 Activity		Selenoprotein P	
		B (SE)	p	B (SE)	p	B (SE)	p
Disability	Intercept	7.07 (0.44)	< 0.001	5.34 (0.45)	< 0.001	5.81 (0.44)	< 0.001
Model 1	Biomarker	-1.25 (0.20)	< 0.001*	-0.38 (0.21)	0.065	-0.63 (0.21)	0.002*
Model 2	Intercept	11.17 (1.48)	< 0.001	11.10 (1.51)	< 0.001	11.22 (1.48)	< 0.001
	Biomarker	-0.26 (0.14)	0.053	-0.16 (0.13)	0.225	-0.26 (0.13)	0.051
	Sex	0.52 (0.34)	0.127	0.52 (0.34)	0.128	0.51 (0.34)	0.136
	Physical Activity	-2.07 (0.18)	< 0.001	-2.07 (0.18)	< 0.001	-2.08 (0.18)	< 0.001
	Fat Free Mass (kg)	-0.001 (0.02)	0.937	-0.001 (0.02)	0.940	-0.002 (0.02)	0.951
	SMMSE	-0.17 (0.04)	< 0.001	-0.18 (0.04)	< 0.001	-0.18 (0.04)	< 0.001
	Energy Intake (Kcal)	0.00 (0.00)	0.678	0.00 (0.00)	0.598	0.00 (0.00)	0.645
	Protein Intake (g)	-0.002 (0.01)	0.819	-0.002 (0.01)	0.742	-0.002 (0.01)	0.828
	NS-SEC	0.11 (0.13)	0.384	0.11 (0.13)	0.398	0.11 (0.13)	0.402
	Education	-0.22 (0.17)	0.188	-0.24 (0.17)	0.158	-0.25 (0.17)	0.135
	Living Status	1.38 (0.67)	0.040	1.39 (0.68)	0.042	1.34 (0.67)	0.047
	Self-rated Health	0.60 (0.15)	< 0.001	0.60 (0.15)	< 0.001	0.62 (0.15)	< 0.001
	Total Medications	0.36 (0.15)	0.015	0.40 (0.15)	0.007	0.40 (0.15)	0.007
	Smoking Status	0.04 (0.12)	0.760	0.03 (0.12)	0.777	0.04 (0.12)	0.745
	Alcohol Intake	-0.11 (0.24)	0.738	-0.13 (0.24)	0.577	-0.15 (0.24)	0.531
	Walking Aid	3.03 (0.31)	< 0.001	3.03 (0.31)	< 0.001	3.01 (0.31)	< 0.001

SE: standard error; BMI: body mass index; SMMSE: standardised mini mental state examination; NS-SEC: National Statistics Socio-economic classification.

Table 5.4. Untransformed disability measures (disability score, mobility score, instrumental activities of daily living and basic activities of daily living) separated by tertiles of serum selenium.

Disability measures	All Participants	Low Selenium ≤ 46.7 µg/L (Tertile 1)	Medium Selenium 46.73-62.0 µg/L (Tertile 2)	High Selenium ≥ 62.0 µg/L (Tertile 3)	p
		Disability Score % (n)			
Baseline No Disability	21.1 (158)	16.5 (41)	18.4 (46)	28.4 (71)	< 0.001
Low (score 1–6)	51.6 (386)	46.8 (116)	55.6 (139)	52.4 (131)	
Moderate (score 7–12)	18.9 (141)	21.4 (53)	20.0 (50)	15.2 (38)	
High (score 13–17)	8.4 (63)	15.3 (38)	6.0 (15)	4.0 (10)	
Follow-up at 1.5 years No Disability	10.5 (61)	8.0 (14)	8.2 (16)	14.7 (31)	0.003
Low (score 1–6)	49.1 (286)	41.1 (72)	52.0 (102)	53.1 (112)	
Moderate (score 7–12)	25.9 (151)	29.7 (52)	26.5 (52)	22.3 (47)	
High (score 13–17)	14.4 (84)	21.1 (37)	13.3 (26)	10.0 (21)	
Follow-up at 3 years No Disability	6.7 (30)	3.1 (4)	6.3 (10)	9.7 (16)	0.001
Low (score 1–6)	47.5 (214)	38.3 (49)	45.6 (72)	56.4 (93)	
Moderate (score 7–12)	28.2 (127)	32.8 (42)	29.7 (47)	23.0 (38)	
High (score 13–17)	17.7 (80)	25.8 (33)	18.4 (29)	10.9 (18)	
Follow-up at 5 years No Disability	7.4 (24)	3.4 (3)	6.8 (8)	10.7 (13)	0.097
Low (score 1–6)	42.2 (137)	36.8 (32)	40.2 (47)	47.9 (58)	
Moderate (score 7–12)	32.3 (105)	34.5 (30)	34.2 (40)	28.9 (35)	
High (score 13–17)	18.2 (59)	25.3 (22)	18.8 (22)	12.4 (15)	
		Mobility Score % (n)			
Baseline No Issues	45.1 (337)	35.5 (88)	44.0 (110)	55.6 (139)	< 0.001
Low	16.0 (120)	16.1 (40)	16.0 (40)	16.0 (40)	
Moderate	19.1 (143)	19.4 (48)	22.0 (55)	16.0 (40)	
High	19.8 (148)	29.0 (72)	18.0 (45)	12.4 (31)	
Follow-up at 1.5 years No Issues	32.0 (186)	28.0 (49)	29.1 (57)	37.9 (80)	0.001
Low	16.5 (96)	10.9 (19)	16.8 (33)	20.9 (44)	
Moderate	25.3 (147)	25.1 (44)	28.1 (55)	22.7 (48)	
High	26.3 (153)	36.0 (63)	26.0 (51)	18.5 (39)	
Follow-up at 3 years No Issues	23.5 (106)	15.6 (20)	22.8 (36)	30.3 (50)	
Low	15.7 (71)	18.0 (23)	10.8 (17)	18.8 (31)	0.002
Moderate	30.2 (136)	27.3 (35)	31.6 (50)	30.9 (51)	
High	30.6 (138)	39.1 (50)	34.8 (55)	20.0 (33)	
Follow-up at 5 years No Issues	24.6 (80)	23.0 (20)	18.8 (22)	31.4 (38)	0.059
Low	14.2 (46)	13.8 (12)	13.7 (16)	14.9 (18)	
Moderate	24.0 (78)	16.1 (14)	29.9 (35)	24.0 (29)	
High	37.2 (121)	47.1 (41)	37.6 (44)	29.8 (36)	

			IADL % (n)		
Baseline 0	39.7 (297)	32.3 (80)	37.2 (93)	49.6 (124)	< 0.001
1	17.8 (133)	16.1 (40)	19.6 (49)	17.6 (44)	
2	14.8 (111)	14.9 (37)	15.6 (39)	14.0 (35)	
3	7.9 (59)	6.9 (17)	10.8 (27)	6.0 (15)	
4	6.1 (46)	6.0 (15)	6.8 (17)	5.6 (14)	
5	5.2 (39)	8.9 (22)	4.0 (10)	2.8 (7)	
6	8.4 (63)	14.9 (37)	6.0 (15)	4.4 (11)	
Follow-up at 1.5 years 0	22.5 (131)	20.6 (36)	20.4 (40)	26.1 (55)	0.025
1	17.5 (102)	11.4 (20)	16.8 (33)	23.2 (49)	
2	18.7 (109)	16.0 (28)	21.9 (43)	18.0 (38)	
3	13.2 (77)	15.4 (27)	15.3 (30)	9.5 (20)	
4	6.9 (40)	8.0 (14)	7.7 (15)	5.2 (11)	
5	8.8 (51)	10.9 (19)	7.7 (15)	8.1 (17)	
6	12.4 (72)	17.7 (31)	10.2 (20)	10.0 (21)	
Follow-up at 3 years 0	18.0 (81)	7.8 (10)	19.0 (30)	24.8 (41)	< 0.001
1	12.2 (55)	12.5 (16)	13.3 (21)	10.9 (18)	
2	20.6 (93)	20.3 (26)	16.5 (26)	24.8 (41)	
3	15.5 (70)	12.5 (16)	19.0 (30)	14.5 (24)	
4	9.3 (42)	8.6 (11)	11.4 (18)	7.9 (13)	
5	6.7 (30)	9.4 (12)	6.3 (10)	4.8 (8)	
6	17.7 (80)	28.9 (37)	14.6 (23)	12.1 (20)	
Follow-up at 5 years 0	17.2 (56)	9.2 (8)	17.9 (21)	22.3 (27)	0.029
1	13.2 (43)	14.9 (13)	12.0 (14)	13.2 (16)	
2	23.1 (75)	17.2 (15)	19.7 (23)	30.6 (37)	
3	10.5 (34)	12.6 (11)	12.8 (15)	6.6 (8)	
4	7.4 (24)	4.6 (4)	9.4 (11)	7.4 (9)	
5	8.9 (29)	12.6 (11)	8.5 (10)	6.6 (8)	
6	19.7 (64)	28.7 (25)	19.7 (23)	13.2 (16)	
			BADL % (n)		
Baseline 0	31.4 (235)	25.4 (63)	31.6 (79)	37.2 (93)	< 0.001
1	30.7 (230)	29.8 (74)	30.0 (75)	32.4 (81)	
2	15.2 (114)	13.3 (33)	16.0 (40)	16.4 (41)	
3	7.9 (59)	7.3 (18)	9.6 (24)	6.8 (17)	
4	3.3 (25)	5.2 (13)	1.2 (3)	3.6 (9)	
5	2.9 (22)	4.8 (12)	3.6 (9)	0.4 (1)	
6	4.3 (32)	7.3 (18)	4.0 (10)	1.6 (4)	
7	1.3 (10)	2.4 (6)	0.8 (2)	0.8 (2)	
8	2.8 (21)	4.4 (11)	3.2 (8)	0.8 (2)	
Follow-up at 1.5 years 0	19.1 (111)	15.4 (27)	18.4 (36)	22.7 (48)	0.057
1	32.8 (191)	26.9 (47)	33.7 (66)	37.0 (78)	
2	16.3 (95)	18.9 (33)	15.8 (31)	14.7 (31)	
3	8.2 (48)	9.7 (17)	7.1 (14)	8.1 (17)	
4	6.5 (38)	5.7 (10)	8.2 (16)	5.7 (12)	
5	4.8 (28)	5.7 (10)	5.1 (10)	3.8 (8)	
6	5.5 (32)	8.0 (14)	6.6 (13)	24 (5)	
7	3.3 (19)	2.9 (5)	2.6 (5)	4.3 (9)	
8	3.4 (20)	6.9 (12)	2.6 (5)	1.4 (3)	0.007
Follow-up at 3 years 0	15.3 (69)	11.7 (15)	13.3 (21)	20.0 (33)	
1	30.6 (138)	19.5 (25)	29.1 (46)	40.6 (57)	
2	19.1 (86)	24.2 (31)	20.9 (33)	13.3 (22)	
3	8.9 (40)	11.7 (15)	8.2 (13)	7.3 (12)	
4	8.2 (37)	7.8 (10)	10.1 (16)	6.7 (11)	
5	3.5 (16)	3.1 (4)	3.2 (5)	4.2 (7)	
6	7.8 (35)	10.9 (14)	8.2 (13)	4.8 (8)	
7	2.2 (10)	3.9 (5)	1.9 (3)	1.2 (2)	
8	4.4 (20)	7.0 (9)	5.1 (8)	1.8 (3)	
Follow-up at 5 years 0	14.2 (46)	5.7 (5)	14.5 (17)	19.8 (24)	0.123
1	25.2 (82)	27.6 (24)	21.4 (25)	27.3 (33)	
2	23.7 (77)	25.2 (22)	25.6 (30)	20.7 (25)	
3	10.2 (33)	6.9 (6)	12.0 (14)	10.7 (13)	
4	5.8 (19)	4.6 (4)	6.0 (7)	6.6 (8)	
5	4.6 (15)	4.6 (4)	3.4 (4)	5.8 (7)	
6	7.7 (25)	10.3 (9)	7.7 (9)	5.8 (7)	
7	2.8 (9)	3.4 (3)	4.3 (5)	0.8 (1)	
8	5.8 (19)	11.5 (10)	5.1 (6)	2.5 (3)	

IADL: instrumental activities of daily living; BADL: basic activities of daily living. Higher scores indicate poor performance

Table 5.5. Disability estimates from a fully adjusted model including all covariates and tertiles of each biomarker of selenium status.

Outcome	Variable	Selenium Model		GPx3 Activity Model		SePP Model	
		β (SE)	**p**	**β (SE)**	**p**	**β (SE)**	**p**
Disability	Intercept	2.41 (0.38)	< 0.001	3.02 (0.38)	< 0.001	2.89 (0.39)	< 0.001
Model 1	Low compared to High Concentration	2.66 (0.55)	< 0.001*	1.64 (0.57)	0.004*	1.74 (0.56)	0.002*
	Medium compared to High Concentration	1.80 (0.55)	0.001*	1.10 (0.55)	0.046*	1.26 (0.56)	0.024*
Model 2	Intercept	10.02 (2.53)	< 0.001	10.39 (1.09)	< 0.001	10.44 (1.71)	< 0.001
	Low compared to High Concentration	0.35 (0.46)	0.447	0.36 (0.46)	0.434	0.56 (0.22)	0.012*
	Medium compared to High Concentration	0.47 (0.45)	0.290	0.59 (0.45)	0.193	0.07 (0.23)	0.765
	Time	1.41 (9.87)	1.000	1.53 (0.12)	< 0.001	1.41 (1.62)	1.000
	Low × Time	0.25 (0.18)	0.162	-0.20 (0.18)	0.257	-0.06 (0.09)	0.502
	Medium × Time	-0.18 (0.17)	0.301	-0.17 (0.17)	0.481	0.07 (0.09)	0.403
	Low x Sex	-1.54 (0.34)	< 0.001	-0.83 (0.34)	0.015	-1.36 (0.17)	< 0.001
	Medium x Sex	-0.61 (0.36)	0.088	-1.79 (0.35)	< 0.001	-1.29 (0.17)	< 0.001

Tertiles: Low, medium and high: Low Selenium ≤ 46.7 µg/L, GPx3 activity ≤ 120.3 U/L; SePP ≤ 2.32 mg/L; Medium Selenium 46.7-62.0 µg/L; GPx3 120.3-166.1 U/L; SePP 2.33-3.57 mg/L; High Selenium ≥ 62 µg/L; GPx3 activity ≥ 166.1 U/L; SePP ≥ 3.58 mg/L. SE, standard error, Se: serum selenium; GPx3: glutathione peroxidase 3; SePP: selenoprotein P. The high concentration of each biomarker was used a reference. Model 1 included: the biomarker of selenium status; time; sex; interaction between time and the biomarker; and interaction between sex and the biomarker. Model 2 was adjusted for: presence of hand arthritis for HGS, or use of walking aids in TUG, sarcopenia and severe sarcopenia; selected biomarker; sex; National Statistics Socio-Economic Classification (NS-SEC); self-rated health; energy intake; protein intake; medication use; fat-free mass (FFM); physical activity; SMMSE: standardised mini mental state examination; alcohol intake; and smoking status.

Chapter 6. The Effect of Selenium Supplementation on Biomarkers of Bone Turnover

6.1 Abstract

Background: Optimal selenium status has been associated with lower BTMs in epidemiological studies. However, the long-term impact of selenium supplementation on BTMs has not been studied.

Objectives: To investigate the effects of selenium supplementation on BTMs including OC, PINP, CTX and BALP in the short-term (6 months) and long-term (5 years).

Methods: A total of 481 Danish men and women (60-74 years) were randomised to receive a daily tablet containing placebo-yeast versus 100, 200 or 300 µg Se-enriched yeast, for 5 years. Plasma selenium concentration was measured using ICP-MS and BTMs were measured in non-fasted samples at baseline, 6 months, and 5 years. Data were analysed by ANCOVA to investigate the shape of the dose-response relationships. Covariates included age, BMI, baseline selenium status, baseline BTM, smoking, alcohol, supplement use and medication.

Results: Plasma selenium concentration (mean 86.5 µg/d) increased significantly with increasing selenium supplementation to 152.6, 209.1 and 253.7 µg/L after 6 months and remained elevated at 5 years (158.4, 222.4 and 275.9 µg/L, for 100, 200 and 300 µg/d respectively ($P < 0.001$)). There was no change in the plasma selenium concentration in the placebo-treated group over the duration of the intervention study. There was no significant effect of selenium supplementation on OC (6 months $P = 0.37$; 5 years $P = 0.63$), PINP (6 months $P = 0.37$; 5 years $P = 0.79$), CTX (6 months $P = 0.91$; 5 years $P = 0.58$) or BALP (6 months $P = 0.17$; 5 years $P = 0.53$).

Conclusion: The relatively optimal baseline selenium status in the study participants may explain this lack of effect. Testing in populations with suboptimal status may provide further insights into the impact of selenium supplementation on bone health.

6.2 Introduction

This final experimental chapter of my PhD thesis will utilise data from a RCT to determine the effect of selenium supplementation on BTMs. As discussed in detail throughout this thesis, suboptimal selenium status impairs expression of the consortium of selenoproteins (Combs, 2001). As discussed, selenium intakes vary greatly (Combs, 2001) and among Europeans, selenium intakes have declined from 60-63 μg/d to 29-39 μg/d from 1970 to 2000 (Rayman, 2002; MoA, 1997). For example, in Denmark, selenium intake fell from 51 to 42 μg/d during 1990-1995 (Bro and Heydorn, 1990) and plasma selenium concentration dropped from 42 to 37 μg/L between 1995-2000 and 2002 (Andersen *et al.*, 1996; Lyhne *et al.*, 2005). However, among Danish adults (65-75 years), selenium intakes have increased by approximately 25 μg/d between 1995-2001 and 2011-2013 (Bro and Heydorn, 1990).

When incorporated into selenoproteins, selenium is important for MSK function. Most selenoproteins are involved in redox reactions that reduce concentrations of ROS, such as hydroperoxides (Steinbrenner and Sies, 2009; Hariharan and Dharmaraj, 2020). Multiple studies have shown an inverse relationship between selenium status and inflammatory molecules, such as IL-6 and tumour-necrosis factor α (TNF-α) (Tseng *et al.*, 2013). Both ROS and pro-inflammatory molecules (i.e. IL-6, TNF-α) can initiate bone resorption (Harmer, Falank and Reagen, 2018; Kitaura *et al.*, 2020). Since selenoproteins are expressed within osteoblasts (bone formation) and osteoclasts (bone resorption) (Zhang, Zhang and Xiao, 2014) they may regulate bone resorption by moderating oxidative stress through ROS reduction (Cao, Gregoire and Zeng, 2012; Beukhof *et al.*, 2016; Cervellati *et al.*) and concentrations of IL-6 are higher in osteoporotic individuals (Scheidt-Nave *et al.*, 2001; Ferrari *et al.*, 2003; Manolagas, 2010). Higher ROS concentration increases bone loss through the RANKL pathway (Manolagas, 2010). In animal models, selenium-deficient mice had higher concentrations of inflammatory markers and bone resorption markers with poorer bone microarchitecture compared to Se-supplemented mice (Yang *et al.*, 1993). Similarly, abnormal skeletal growth and poor bone health was observed in selenium-deficient rats (Hurt, Cary and Visek, 1971; Ewan, 1976; Thompson, Haibach and Sunde, 1995) whereas selenium supplementation improved bone microarchitecture (Cao, Gregoire and Zeng, 2012). In humans, those with lower selenium status have higher concentrations of

IL-6 (Tseng *et al.*, 2013; Prystupa *et al.*, 2017) and supplementation with 200 µg/d selenium for 12 weeks reduced IL-6 concentration (Salehi *et al.*, 2013). In addition, higher selenium concentrations are associated with higher BMD and lower BTMs (Hoeg *et al.*, 2012) and NHANES data suggested that higher serum selenium concentrations(mean 131 µg/l), especially in postmenopausal women, were positively associated with femur BMD (Wu *et al.*, 2021).

Consequently, improving selenium status could be an effective and inexpensive approach to reducing the rate of age-related decline in bone health. However, a recent RCT in 120 postmenopausal women showed that supplementation with sodium selenite for 6 months did not affect BTMs or BMD (Walsh *et al.*, 2021). I hypothesised that participants in The PRECISE Study receiving higher doses of selenium supplementation would have better bone health (i.e. improved concentrations of BTMs). The aims of this secondary analysis were: 1) to extend the findings from the investigation by Walsh *et al.*, (2021) by using both men and women, a larger sample size, and a longer study duration; 2) to explore the associations in older adults between plasma selenium and BTMs at baseline; 3) to determine the effect of selenium supplementation on BTMs over 6 months and 5 years.

6.3 Materials and Methods

6.3.1. Study Population

Participants were from The PRECISE Study, a RCT exploring the effects of Se-yeast supplementation on CVD risk in 491 Danish adult aged 60-74 years. For details on recruitment, inclusion criteria and ethics, see Chapter 2, Section 2.2.2.

6.3.2. Intervention

Se-yeast in tablet form was provided to participants in 100, 200, 300 µg/d, in addition to a placebo. The placebo was identical to the supplementation tablets and consisted of inactive spray-dried baker's yeast. The intervention was undertaken for 5 years. For full details please see Chapter 2, Section 2.2.2.2.

6.3.3 Socioeconomic, Lifestyle and Other Covariates

Assessments included questionnaires, blood samples, medical record reviews, body weight measurements, tablet count, records of side effects and the provision of new tablets, as previously described (Cold *et al.*, 2015). These were taken at the initial health assessment at baseline, 6 months and 5 years, details of which are provided in Chapter 2, Section 2.2.2.3. Other covariates included in the analyses were: BMI; education; living status; smoking status; alcohol intake; medication and supplementation use. Covariates were selected based on previous literature showing their effect on bone health (Aspray and Hill, 2019).

6.3.4. Plasma Selenium

Plasma selenium was measured by ICP-MS in non-fasted samples, collected at baseline, 6 months and 5 years. Plasma was prepared and stored at -80 °C. For full details see Chapter 2, Section 2.2.2.4.

6.3.5 Biomarkers of Bone Turnover

The BTMs included OC, PINP, CTX and BALP, and were measured by IDS-iSYS automated immunoassays in non-fasted samples collected at baseline, 6 months and 5 years. For full details see Chapter 2, Section 2.2.2.5. The BMTs were selected based on previous research and recommendations (Table 2.5).

6.3.6. Statistical Analyses

Data were analysed using the IBM statistical software package version 24.0 (SPSS). A p value < 0.05 was considered statistically significant. One participant out of the 482 with BTMs was removed from the analyses because this participant's BTM concentrations for OC, PINP, CTX and BALP were 6, 5, 7, and 2-fold higher than the population mean concentrations. The removal of this participant had no significant effect on the main findings or baseline descriptives (data not shown). To determine the normality of the variables, quantile–

quantile (QQ) plots were used. Log 10 transformation was applied to all BTM measurements to normalise the data. Participant baseline characteristics are presented according to the supplementation dose. Differences in characteristics across supplementation doses were assessed using Chi-square test (categorical) or Kruskal-Wallis (ordered and non-normally distributed). Data were analysed using intention-to-treat.

A linear regression was used to determine predictors of baseline BTM. The effects of selenium supplementation on BTMs were investigated using four models; Model 1 included baseline plasma selenium; Model 2: Model 1 plus BMI, sex, smoking and alcohol intake; Model 3: Model 2 plus thyroid, AR, HRT, inhaled and systemic GC medication and finally Model 4: Model 3 plus multivitamins, calcium and vitamin D supplementation. For the main analyses, the shape of the dose-response relationships between selenium supplementation and each of the BTM (OC, PINP, CTX, BALP) at each time (6 months and 5 years, respectively) was investigated separately using an ANCOVA with orthogonal polynomials. Outcomes are reported as estimated marginal means with upper and lower 95 % confidence intervals after back transformation.

6.3.7 Sample Size

As this was a secondary analysis, sample size was not determined for this study. The initial pilot study of this RCT proposed a sample size of 500 participants (Cold *et al.*, 2015). Furthermore, the selenium supplemental trial of the effects on BTMs in 120 post-menopausal women conducted by Walsh *et al.*, (2021) was estimated to have 90 % power to be able to detect a 20 % between-group difference in urine N–terminal cross-linking telopeptide of type I collagen:creatinine ratio. Since my study had similar outcome measures, in 482 participants, I am confident that there was sufficient power to detect changes in BTMs concentrations following selenium supplementation. In addition, a retrospective power analysis was performed using SAS version 9.4. Least significant changes (i.e. the smallest change between BTMs which is associated with a clinical significance) were set to 20 % for OC, 21 % for PINP, 30 % for CTX and BALP (Bergmann *et al.*, 2009; Tsujimoto *et al.*, 2011; Schousboe and Bauer 2012). These calculations showed that there was over 90

% power to detect least significant changes in OC, PINP and BALP at 6 months and 5 years, but only 5 % and 16 % for CTX at 6 months and 5 years, respectively.

6.3.8 Sensitivity Analyses

Further analyses were undertaken after excluding those participants receiving systemic GC (n = 4) because of their potential to influence BTMs (Barahona *et al.*, 2009; Burch *et al.*, 2014; Devogelaer *et al.*, 2017; Eastell *et al.*, 2018). A second analysis was run excluding systemic GC, inhaled GC, antiresorptive and thyroid medication users (n = 53). Whilst some research suggests inhaled GC have minimal effects on bone (Loke *et al.*, 2015), a recent study suggested inhaled GC increased the risk of osteoporosis (Chiu, Lee and Chen, 2021). Likewise, thyroid medication has been shown to have a detrimental effect on bone health (Turner *et al.*, 2011; Karimifar *et al.*, 2014). Another sensitivity analysis was run after exclusion of those using HRT (n = 75) and dietary supplement users (n = 215) because intakes of calcium, vitamin D and multivitamins can influence bone metabolism. A final sensitivity analysis removed those participants using AR at baseline, 6 months and 5 years, as well as those who had fractures, as a proxy to estimate those with osteoporosis (n = 14). The analyses were also repeated after categorising baseline plasma selenium concentration into a binary variable, above and below 70 µg/L, based on evidence that this concentration is required to optimise glutathione peroxidase 3 (GPx) activity (Nève, 1995). These results are reported in the Sensitivity Analyses in Table 6.1-6.8, respectively.

6.4 Results

6.4.1. Participant Characteristics

Of the 491 participants randomised into the trial, 481 participants had BTM measurements at baseline (Figure 2.5). The mean age of participants was 66.2 ± 4.1 years and there was an almost equal split of male and female participants (52.0 versus 48.0 %, P = 0.476) (Table 6.1). There were significant differences at baseline between the supplementation groups for living status (P = 0.047), calcium supplementation (P = 0.028) and vitamin D supplementation (P = 0.020), otherwise, the supplementation groups were well matched (Table 6.1). Overall mean plasma selenium concentration at baseline was 86.5 ± 16.2 µg/L

and did not differ between groups (P = 0.190, Table 6.2). Across all supplementation groups, 12 % of participants had evidence of suboptimal selenium concentrations (plasma concentration < 70 μg/L). Mean ± (SD) baseline concentrations of OC, PINP, CTX and BALP were 18.7 ± 8.5, 42.7 ± 18.1, 0.20 ± 0.22 and 15.7 ± 5.7 μg/L, respectively (Table 6.3). Over the 5 years of study, 127 participants were lost to follow-up, leaving 354 for the full study duration (Figure 2.5). There were no differences between supplementation groups in loss to follow-up (P = 0.847) or reasons for dropout (P = 0.816). However, participants who dropped out were more likely to have suboptimal plasma selenium at 6 months (P = 0.009), but not at baseline. There were no other significant differences in baseline characteristics between participants who dropped out and those who did not (Sensitivity Analyses Table 6.7). BTM concentrations did not differ significantly between those who dropped out and those who remained in the study (Sensitivity Analyses Table 6.7).

6.4.2 Predictors of Bone Turnover Markers at Baseline

Linear regression revealed that plasma selenium concentration was not associated with baseline concentrations of BTMs (Appendix Table 6.1 Model 1 and 4). Those who were male had a lower concentration of OC (0.045 ± 0.021, P = 0.037). Those who used inhaled GC were more likely to have higher PINP (0.106 ± 0.047, P = 0.025) and CTX concentrations (0.152 ± 0.077, P = 0.050).

6.4.3 Effects of increasing doses of supplemental selenium on plasma selenium concentration

Over the 5 years of the study, mean (± SD) plasma selenium concentration in the placebo group remained unchanged (85.9 ± 15.3, 85.2 ± 14.3 and 87.5 ± 24.1 μg/L) at baseline, 6 months and 5 years, respectively (P = 0.190). In contrast, at 6 months plasma selenium concentration increased significantly in a dose-dependent manner with increasing supplemental selenium to reach 152.6, 209.1 and 253.7 μg/L for selenium doses 100-300 μg/d, respectively, and remained elevated at 5 years (Table 6.2).

6.4.4. Effects of increasing dose of supplemental selenium on concentrations of bone turnover markers

Concentrations of each BTM in serum at 6 months and, at 5 years were similar to those at baseline and there was no evidence that selenium supplementation altered any of the BTMs at either time-point (Table 6.4). These findings remained robust in sensitivity analyses after excluding i) users of systemic GC, ii) combined users of systemic GC, inhaled GC, antiresorptives and thyroid medication, iii) users of HRT, iv) users of nutritional supplements, v) users of antiresorptives at baseline, 6 months and 5 years, and those having fractures (Sensitivity Analyses Table 6.1-6.5). When analyses were limited to participants with plasma selenium concentrations below 70 µg/L, supplementation had a significant effect on CTX concentrations at 5 years leading to an overall decrease in CTX, with lowest concentrations at 200 µg/d supplementation (Sensitivity Analyses Section 6.3.6 and Table 6.6).

Table 6.1: Baseline characteristics of participants with bone turnover markers measurements, randomised to selenium supplementation (0-300 µg/d).

Characteristic	All Participants n = 481	Selenium Dosage (µg/d)				P
		0 n = 124	100 n = 122	200 n = 118	300 n = 117	
Male n (%)	250 (52.0)	59 (23.6)	69 (27.6)	64 (25.6)	58 (23.3)	0.476
Female n (%)	231 (48.0)	65 (28.1)	53 (22.9)	54 (23.4)	59 (25.5)	
Age years, Mean (SD)	66.16 (4.10)	65.42 (3.8)	66.49 (4.2)	66.32 (4.3)	66.45 (4.1)	0.155
BMI, kg/m^2 (SD)	26.83 (4.02)	26.51 (4.1)	27.01 (3.8)	27.24 (4.3)	26.51 (4.0)	0.320
Height m, Mean (SD)	1.69 (0.09)	1.68 (0.09)	1.69 (0.08)	1.70 (0.09)	1.69 (0.08)	0.538
Weight kg, Mean (SD)	76.87 (13.5)	75.41 (12.6)	76.84 (11.6)	79.21 (15.4)	75.83 (14.3)	0.255
Alcohol units per week, Mean (SD)	7.30 (7.5)	7.75 (8.4)	7.75 (8.0)	7.25 (7.3)	6.37 (6.3)	0.741
Smokers, n (%)						
Never	158 (32.8)	36 (22.8)	40 (25.3)	39 (24.7)	43 (27.2)	0.590
Previous	178 (37.0)	45 (25.3)	47 (26.4)	49 (27.5)	37 (20.8)	
Present	145 (30.1)	43 (29.7)	35 (24.1)	30 (20.7)	37 (25.5)	
Education, n (%)						
No further education	134 (28.7)	38 (28.4)	41 (30.6)	30 (22.4)	25 (18.7)	0.267
1-3 y	76 (16.3)	13 (17.1)	19 (25.0)	21 (27.6)	23 (30.3)	
3-4 y	212 (45.4)	57 (26.9)	44 (20.8)	53 (25.0)	58 (27.4)	
> 4 y	45 (9.6)	12 (26.7)	14 (31.1)	10 (22.2)	9 (20.0)	
Live Alone, n (%)						
No	400 (85.7)	94 (23.5)	107 (26.8)	99 (24.8)	100 (25.2)	0.047
Yes	67 (14.3)	26 (38.8)	11 (16.4)	15 (22.4)	15 (22.4)	
Thyroid Medication, n (%)						
None	467 (97.1)	122 (26.1)	118 (25.3)	113 (24.2)	114 (24.4)	0.659
LT4	11 (2.3)	2 (18.2)	3 (27.3)	3 (27.3)	3 (27.3)	
ATD	3 (0.6)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	
Inhaled GC, n (%)						
No	450 (96.4)	118 (26.2)	111 (24.7)	110 (24.4)	111 (24.7)	0.374
Yes	17 (3.6)	2 (11.8)	7 (41.2)	4 (23.5)	4 (23.5)	
Systemic GC, n (%)						
No	475 (99.2)	122 (25.7)	121 (25.5)	117 (24.6)	115 (24.2)	0.595
Yes	4 (0.8)	2 (50.0)	1 (25.0)	0 (0.0)	1 (25.0)	
Antiresorptives, n (%)						
No	461 (98.7)	118 (25.6)	117 (25.4)	113 (24.5)	113 (24.7)	0.884
Yes	6 (1.3)	2 (33.3)	1 (16.7)	1 (16.7)	2 (33.3)	
HRT, n (%)						
No	392 (83.9)	98 (25.0)	98 (25.0)	99 (25.3)	97 (24.7)	0.740
Yes	75 (16.1)	22 (29.3)	20 (26.7)	15 (20.0)	18 (24.0)	
Calcium, n (%)						
No	419 (89.7)	110 (26.3)	112 (26.7)	95 (22.7)	102 (24.3)	0.028
Yes	48 (10.3)	10 (20.8)	6 (12.5)	19 (39.6)	13 (27.1)	
Vitamin D, n (%)						
No	442 (94.6)	115 (26.0)	117 (26.5)	103 (23.3)	107 (24.2)	0.020
Yes	25 (5.4)	5 (20.0)	1 (4.0)	11 (44.0)	8 (32.0)	
Multivitamin, n (%)						
No	325 (69.6)	90 (27.7)	86 (26.5)	70 (21.5)	79 (24.3)	0.116
Yes	142 (30.4)	30 (21.1)	32 (22.5)	44 (31.0)	36 (25.4)	
Dropout, n (%)						
6 months	23 (18.1)	10 (43.5)	2 (8.7)	5 (21.7)	6 (26.1)	0.133
5 years	104 (81.9)	26 (25.0)	30 (28.8)	26 (25.0)	22 (21.2)	

SD: standard deviation; inhaled GC: inhaled glucocorticoid; systemic GC: systemic glucocorticoid; LT4: levothyroxine; ATD: antithyroid drugs; HRT: hormone replacement therapy. Age, alcohol n = 481; height, weight, BMI n = 478; Smoking, sex, thyroid medication n = 480; BMI, weight, height n = 478; systemic GC n = 479; education, live alone, inhaled GC, antiresorptives, HRT and supplement users n = 467.

Table 6.2: Plasma selenium concentration at baseline, 6 months and 5 years measurements for participants with bone turnover marker measurements randomised to selenium supplementation (0-300 µg/d).

Plasma Selenium (µg/L) Mean (SD)	All Participants	Selenium Dosage (µg/d)				P
		0	100	200	300	
Baseline (n = 479)	86.5 (16.2)	85.9 (15.3)	87.8 (16.2)	88.3 (16.4)	84.0 (16.9)	0.190
6 months (n = 426)	174.1 (72.4)	85.2 (14.3)	152.6 (23.7)	209.1 (42.2)	253.7 (53.7)	< 0.001
5 years (n = 349)	185.6 (85.4)	87.5 (24.1)	158.4 (28.4)	222.4 (41.1)	275.9 (78.9)	< 0.001

Baseline selenium status n = 479: 124, 122, 117, 116; 6 months n = 426: 106, 112, 106, 102; 5 years n = 349: 88, 88, 86, 87 for 0-300 µg/d selenium, respectively.

Table 6.3: Plasma concentration of bone turnover markers at baseline for participants randomised to selenium supplementation (0-300 µg/d).

Bone Turnover (µg/L) Mean (SD)	All Participants (n = 481)	Selenium Dosage (µg/d)				P
		0	100	200	300	
OC	18.7 (8.5)	19.1 (8.2)	18.3 (8.3)	18.0 (8.8)	19.3 (8.5)	0.321
PINP	42.7 (18.1)	43.4 (19.3)	43.0 (16.4)	41.6 (19.8)	42.7 (16.5)	0.629
CTX	0.20 (0.22)	0.21 (0.13)	0.18 (0.11)	0.22 (0.40)	0.21 (0.14)	0.167
BALP	15.7 (5.7)	15.3 (5.5)	15.8 (5.7)	15.6 (5.4)	16.0 (6.2)	0.901

SD: standard deviation; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. OC and PINP n = 481: 124, 122, 118, 117; CTX, n = 459: 118, 117, 110, 114; BALP n = 479: 124, 121, 117, 117 for 0-300 µg/d selenium, respectively.

Table 6.4: Estimated marginal means from ANCOVA of bone turnover markers by supplementation group at 6 months and 5 years. Upper and lower 95 % confidence intervals are displayed in parentheses.

Bone Turnover (µg/L) Mean (CI)	Selenium dosage (µg/d)				P
	0	100	200	300	
OC 6 months	17.1 (16.3-17.9)	16.7 (16.0-17.5)	17.5 (16.6-18.3)	16.5 (15.7-17.3)	0.373
OC 5 years	16.9 (15.6-18.2)	17.1 (15.8-18.6)	17.1 (15.7-18.6)	16.0 (14.8-17.3)	0.630
PINP 6 months	38.7 (36.8-40.8)	36.6 (34.7-38.5)	38.4 (36.4-40.5)	38.6 (36.6-40.8)	0.370
PINP 5 years	39.5 (36.1-43.3)	40.0 (36.5-43.8)	39.4 (35.8-43.3)	37.6 (34.3-41.1)	0.793
CTX 6 months	0.16 (0.14-0.17)	0.16 (0.14-0.17)	0.16 (0.15-0.18)	0.15 (0.14-0.17)	0.910
CTX 5 years	0.16 (0.14-0.18)	0.17 (0.15-0.19)	0.16 (0.14-0.18)	0.15 (0.14-0.19)	0.582
BALP 6 months	14.4 (13.8-14.9)	13.7 (13.2-14.3)	13.7 (13.2-14.3)	14.3 (13.7-14.9)	0.170
BALP 5 years	14.0 (13.2-14.8)	14.8 (14.0-15.7)	14.5 (13.6-15.4)	14.5 (13.7-15.3)	0.525

CI: confidence intervals; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. Covariates in the ANCOVA included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives, hormone replacement therapy (HRT)). OC 6 months n = 404: 107, 105, 98, 94; OC 5 years n = 328: 84, 84, 76, 84; PINP 6 months n = 403: 106, 105, 98, 94; PINP 5 years n = 328: 84, 84, 76, 84; CTX 6 months n = 378: 99, 98, 93, 88; CTX 5 years n = 299: 74, 77, 73, 75; BALP 6 months n = 402: 106, 104, 98, 94; BALP 5 years n = 328: 84, 83, 77, 84 participants for 0-300 µg/d selenium, respectively.

6.3.5 Adverse Events

Adverse events for the full study have been previously reported and consisted of grooved nails, hair loss and skin reactions (Cold *et al.*, 2015; Rayman *et al.*, 2018). During the 5 years, 22 (4.6 %) participants with BTM measurements died and 57 (11.9 %) withdrew due to non-fatal adverse events and reactions with no significant differences between supplementation groups (P = 0.727).

6.3.6 Sensitivity Analyses

After removal of users of systemic GC (n = 4); systemic or inhaled GC, antiresorptive or thyroid medication (n = 53); supplements (n = 215); or AR at baseline, 6 months and 5 years or with fractures (n = 14), selenium supplementation did not have a significant effect on any of the BTMs at either time point (Sensitivity Analyses Table 6.1, 6.2, 6.4, 6.5). After removal of HRT users (n = 75), 200 µg supplementation had a significant effect on BALP at 6 months (P = 0.041, η2 = 0.013), but not overall dosage (Sensitivity Analyses Table 6.3). A final sensitivity analysis was performed that was limited to participants with a baseline plasma selenium concentration below 70 µg/L (12 % of the population). Concentrations of CTX at 5 years significantly differed in a linear trend (P = 0.011) between dosages in those participants with a baseline selenium concentration below 70 µg/L (P = 0.045, η2 = 0.036) where 200 and 300 µg dosages led to a linear reduction in CTX compared to the placebo (P = 0.011, η2 = 0.282 and P = 0.020, η2 = 0.243, for each dose, respectively) (Sensitivity Analyses Table 6.6).

6.5 Discussion

In this RCT of selenium supplementation in older Danish adults, supplementation with up to 300 µg/d selenium did not have a significant effect on BTMs in a trial lasting 5 years. These results are consistent with findings from a RCT of selenium supplementation for 6 months in older women in the UK (Walsh *et al.*, 2021). That study recruited 120 osteoporotic and osteopenic post-menopausal women (55-83 years) with a baseline plasma selenium concentration (79.4 µg/L) similar to the present findings (86.5 µg/L) and found no effect of selenium supplementation on any of the measured BTMs.

The plasma selenium concentration for optimal bone health is not known with certainty. However, if it is assumed that the optimal concentration for wider aspects of health (≥ 70 μg/L) (Rasmussen *et al.*, 2009; Xia *et al.*, 2005; Nève, 1995; Combs, 2001) is also optimal for bone, then it is likely that only a small minority of PRECISE Study participants (12.1 %) had the potential to benefit from selenium supplementation. Studies that have observed responses to selenium, such as cancer prevalence or BMD, are usually in populations with suboptimal intakes or status, whilst populations with optimal status are often non-responsive (Duffield-Lillico *et al.*, 2003; Lippman *et al.*, 2009; Galvez-Fernandez *et al.*, 2021). The relatively optimal baseline selenium concentration (86.5 μg/L) in my study may explain the lack of response of BTMs to selenium supplementation. Further research could have explored unsupervised hierarchical clustering to identify potential associations of subgroups of this population.

Despite the historical low intakes in European countries, similar research carried out in Denmark reported that healthy participants have adequate selenium status (Suadicani, Hein and Gyntelberg, 2012; Pedersen, 2015) with mean serum selenium concentration of 94 μg/L (Suadicani, Hein and Gyntelberg, 1992) and 107-116 μg/L (Tarp, Thorling and Hansen, 1990). These studies, in accordance with the findings from this chapter, suggest Danish populations have baseline levels > 70 μg/L allowing for GPx3 activity optimisation (Combs, 2001; Nève, 1995; Rasmussen *et al.*, 2009). Chapter 1, Section 1.6 explained how soil quality is an important contributor to selenium concentrations in food. The soils of Scandinavian countries are affected by glacial erosion which influences their quality (Tolonen, 1990). However, Danish populations generally do not consume locally grown food therefore, it is unlikely that soil quality played a major role in influencing selenium intakes (Rasmussen *et al.*, 2009). Another contributor to plasma selenium concentration could be the sociodemographic background of the study population. Socioeconomic status and education in many populations, including Denmark, have been associated with enhanced dietary patterns and reduced comorbidities (Groth *et al.*, 2014; Groth, Fagt and Brondsted, 2001; Groth *et al.*, 2009; Diderichsen *et al.*, 2012; Mackenbach *et al.*, 2008; Koch, Davidsen and Juel, 2012; Osler *et al.*, 2001). The study took place in Funen in Southern Denmark; Funen holds about 9 % of the Danish population and is thought to be representative of the overall Danish population with a sufficient economic status (Henriksen *et al.*, 2015; European

Parliament, 2002; Nielsen *et al.*, 2006). This suggests that this population may have been predisposed to a more optimal baseline selenium status.

This RCT included a large sample of healthy, older Danish adult men and women and explored the responses of four different BTMs to supplementation with selenium (0-300 µg/d) for up to 5 years. BTMs are recognised as a useful surrogate marker of bone health (Bonjour *et al.*, 2014) and the BTMs measured in my analyses (OC, PINP and BALP) correlate well with bone formation (Hlaing and Compston, 2014; Delmas *et al.*, 1985; Kuo and Chen, 2017) and fracture risk (Sornay-Rendu *et al.*, 2005; Vilaca, Gossiel and Eastell, 2017; Shigdel *et al.*, 2015; Szulc and Delmas, 2008; Tromp *et al.*, 2000). As well as using the BTMs suggested by the IOF, using a range of BTMs overcame some of the individual limitations of each BTM (Burch *et al.*, 2014; Vasikaran *et al.*, 2011). However, BTMs can be sensitive to the post-prandial state (Clowes *et al.*, 2002) and circadian rhythm where concentrations are generally higher in the morning, especially in resorption markers (Christgau, 2000; Schlemmer and Hassager, 1999; Scott *et al.*, 2012; Hannon and Eastell, 2000). Consequently, the use of non-fasted samples in this study may have increased BTM variance, but this was likely to be similar for all supplementation groups and thereby would not alter responses between groups. Furthermore, fasting for 24 hours had no effect on BTMs (CTX and PINP) when compared to control participants (Clayton *et al.,* 2020). It may be that significant reductions in BTM would have occurred earlier than the first testing at the 6-month follow-up, such as 1 month following supplementation, as seen with pharmaceutical treatment (Takada *et al.*, 2020; Miyauchi *et al.*, 2019). Although response times in other studies have been inconsistent; 6 months was not long enough to observe changes in CTX (Astorino, Harness and Witzke, 2013), whilst 1-3 months of teriparatide treatment was sufficient in other studies (Glover *et al.*, 2009; Eastell *et al.*, 2011). However, for selenium supplementation to be effective, any changes in bone turnover markers would need to be sustained in the long term, and this was not seen in the findings from this chapter.

BMI is a well-known risk factor for osteoporosis, where lower values are associated with poor bone health (Bolland *et al.*, 2015; Armstrong *et al.*, 2012). BMI was only available at baseline, however, mean values were similar for all supplemented groups, so this was an unlikely confounder. It is well known that there are key nutrients and minerals required for

optimal bone health. In this study, there was no information on the dietary intakes of selenium or other beneficial nutrients for bone health, such as calcium and vitamin D (Garnero *et al.*, 1996; Theiler *et al.*, 2000), however, supplementation was accounted for in these analyses; the effects of nutrients will be discussed further in Chapter 7, Section 7.2.

It is difficult to generalise to other populations such as the USA or UK due to differences in baseline selenium status. Historically, like the UK, Nordic countries (Denmark, Finland, Sweden, Norway) have lower selenium intakes (Rayman, 2000) at around 42 µg/d and 33 µg/d for men and women, respectively (Lyhne *et al.*, 2005), although supplementation use can increase intakes by 25 µg/d (Ravn-Haren *et al.*, 2008). Furthermore, generalisation to older populations (> 74 years) where osteoporosis is more likely is not possible with this study (Aspray and Hill, 2019; Johansen *et al.*, 1997; De Laet *et al.*, 1997). Data on osteoporosis status or years since menopause were unavailable, therefore, antiresorptive usage was used as a proxy to estimate osteoporosis prevalence. In this study, there were a total of 9 antiresorptive users at 5 years, which was a small percentage (1.8 %) of the study population. If these were the only osteoporotic participants, then the healthy status of the remaining participants may not have benefited from selenium supplementation. It could be helpful to repeat the study in those with osteoporosis or osteopenia and suboptimal selenium status. Recommendations for future studies will be discussed in Chapter 7, Section 7.4.

6.6 Conclusion

This was the first, long-term (5 years), large-scale, RCT exploring the effects of selenium supplementation on BTMs in older men and women. Supplementation resulted in a large dose-dependent increase in plasma selenium concentration which was apparent at 6 months, and that was maintained at 5 years. In contrast, the plasma selenium concentration remained similar to baseline in the placebo group. Selenium supplementation did not have any significant effect on the BTMs. However, this does not rule out the potential of selenium supplementation to improve bone health in people with suboptimal selenium status and/or poor bone health at baseline.

6.7 Appendix

Table 6.1: BTM estimates from an unadjusted (Model 1) and fully adjusted model (Model 2) including all covariates.

Model	Covariates	OC		PINP		CTX		BALP	
		Model 1: R^2 = 0.122		Model 1: R^2 = 0.122		Model 1: R^2 = 0.122		Model 1: R^2 = 0.122	
		Model 4: R^2 = 0.643		Model 4: R^2 = 0.643		Model 4: R^2 = 0.643		Model 4: R^2 = 0.643	
		β ± SE	*p*	β ± SE	*p*	β ± SE	*p*	β ± SE	*p*
Model 1	(Constant)	1.179 ± 0.048	< 0.001	1.511 ± 0.048	< 0.001	-0.832 ± 0.075	< 0.001	1.126 ± 0.039	< 0.001
	Baseline Selenium	0.001 ± 0.001	0.264	0.001 ± 0.001	0.081	0.001 ± 0.001	0.552	0.000 ± 0.000	0.271
Model 4	(Constant)	1.171 ± 0.086	< 0.001	1.562 ± 0.086	< 0.001	-0.944 ± 0.135	< 0.001	1.165 ± 0.070	< 0.001
	Baseline Selenium	0.001 ± 0.001	0.113	0.001 ± 0.001	0.133	0.001 ± 0.001	0.206	0.000 ± 0.000	0.386
	Sex	-0.045 ± 0.021	0.037	-0.037 ± 0.021	0.085	-0.036 ± 0.034	0.285	-0.026 ± 0.017	0.128
	BMI	0.000 ± -0.002	0.919	-0.001 ± -0.002	0.779	0.003 ± -0.004	0.398	-0.001 ± -0.002	0.695
	Smoking	0.014 ± 0.011	0.230	0.009 ± 0.011	0.457	0.000 ± 0.018	0.980	0.001 ± 0.009	0.917
	Alcohol	0.000 ± 0.001	0.761	-0.002 ± 0.001	0.055	0.000 ± 0.002	0.899	-0.001 ± 0.001	0.599
	Thyroid Medication	0.001 ± 0.054	0.988	-0.033 ± 0.054	0.540	0.038 ± 0.083	0.652	-0.014 ± 0.044	0.749
	Inhaled GC	0.055 ± 0.047	0.241	0.106 ± 0.047	0.025	0.152 ± 0.077	0.050	0.048 ± 0.038	0.210
	Systemic GC	-0.117 ± 0.097	0.232	0.015 ± 0.098	0.876	0.086 ± 0.150	0.569	0.005 ± 0.079	0.946
	HRT	-0.007 ± 0.027	0.786	-0.030 ± 0.027	0.271	-0.002 ± 0.043	0.972	-0.005 ± 0.022	0.838
	AR	-0.032 ± 0.083	0.705	-0.030 ± 0.083	0.717	-0.251 ± 0.129	0.052	0.026 ± 0.067	0.701
	Multivitamins	-0.010 ± 0.020	0.615	0.003 ± 0.020	0.897	-0.031 ± 0.032	0.322	0.000 ± 0.016	0.986
	Calcium	0.018 ± 0.040	0.646	0.021 ± 0.040	0.601	-0.014 ± 0.063	0.820	0.033 ± 0.033	0.307
	Vitamin D	-0.037 ± 0.054	0.499	-0.047 ± 0.054	0.390	-0.047 ± 0.087	0.587	-0.052 ± 0.044	0.233

Dependent Variable: Baseline bone turnover marker

Model 1 accounted for baseline selenium status

Model 4 accounted for baseline selenium status, BMI, smoking, alcohol, medication (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives (AR), hormone replacement therapy (HRT)) and supplement use (multivitamins, calcium, vitamin D).

6.8 Sensitivity Analyses

Table 6.1: Estimated marginal means from ANCOVA of bone turnover markers by supplementation group at 6 months and 5 years after the removal of systemic glucocorticoid users. Upper and lower 95 % confidence intervals are displayed in parentheses.

Bone Turnover (µg/L), Mean (CI)	Selenium dosage (µg/d)				P
	0	100	200	300	
OC 6 months	17.1 (16.3-17.9)	16.7 (16.0-17.5)	17.5 (16.7-18.4)	16.5 (15.7-17.4)	0.381
OC 5 years	17.0 (15.6-18.4)	17.2 (15.8-18.7)	17.2 (15.8-18.7)	16.1 (14.9-17.5)	0.642
PINP 6 months	38.7 (36.8-40.8)	36.6 (34.8-38.5)	38.4 (36.4-40.6)	38.7 (36.6-40.9)	0.394
PINP 5 years	39.7 (36.2-43.5)	40.0 (36.5-43.9)	39.4 (35.9-43.5)	37.7 (34.4-41.2)	0.801
CTX 6 months	0.16 (0.14-0.17)	0.16 (0.14-0.17)	0.16 (0.15-0.18)	0.15 (0.14-0.17)	0.916
CTX 5 years	0.16 (0.14-0.18)	0.17 (0.15-0.19)	0.16 (0.14-0.18)	0.15 (0.14-0.17)	0.621
BALP 6 months	14.4 (13.8-14.9)	13.8 (13.3-14.3)	13.7 (13.2-14.3)	14.3 (13.8-14.9)	0.192
BALP 5 years	14.0 (13.2-14.8)	14.9 (14.0-15.8)	14.5 (13.7-15.4)	14.6 (13.7-15.4)	0.526

CI: confidence intervals; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. Covariates in the ANCOVA included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives, hormone replacement therapy (HRT)). OC 6 months n = 400: 105, 104, 98, 93; OC 5 years n = 325: 82, 83, 76, 84; PINP 6 months n = 399: 104, 104, 98, 93; PINP 5 years n = 325: 82, 83, 76, 84; CTX 6 months n = 374: 97, 97, 93, 87; CTX 5 years n = 296: 72, 76, 73, 75; BALP 6 months n = 398: 104, 103, 98, 93; BALP 5 years n = 325: 82, 82, 77, 84 participants for 0-300 µg/d selenium, respectively.

Table 6.2: Estimated marginal means from ANCOVA of bone turnover markers by supplementation group at 6 months and 5 years after the removal of systemic glucocorticoid, inhaled glucocorticoid, thyroid medication and antiresorptive users. Upper and lower 95 % confidence intervals are displayed in parentheses.

Bone Turnover (µg/L), Mean (CI)	Selenium dosage (µg/d)				P
	0	100	200	300	
OC 6 months	17.1 (16.3-17.9)	16.6 (15.6-17.4)	17.4 (16.6-18.3)	16.3 (15.5-17.1)	0.244
OC 5 years	16.9 (15.5-18.4)	16.9 (15.5-18.5)	17.1 (15.6-18.7)	15.9 (14.6-17.4)	0.689
PINP 6 months	38.9 (36.9-41.0)	36.4 (34.4-38.5)	38.2 (36.1-40.4)	38.4 (36.3-40.6)	0.346
PINP 5 years	39.0 (35.4-43.0)	39.9 (36.1-44.1)	39.4 (35.5-43.6)	37.5 (34.0-41.4)	0.847
CTX 6 months	0.16 (0.14-0.17)	0.15 (0.14-0.16)	0.16 (0.15-0.18)	0.15 (0.14-0.17)	0.715
CTX 5 years	0.17 (0.15-0.19)	0.17 (0.15-0.19)	0.16 (0.14-0.18)	0.16 (0.14-0.18)	0.815
BALP 6 months	14.4 (13.9-15.0)	13.7 (13.2-14.2)	13.7 (1.32-14.3)	14.2 (13.6-14.7)	0.174
BALP 5 years	14.0 (13.2-14.8)	14.7 (13.8-15.7)	14.5 (13.6-15.5)	14.4 (13.5-15.3)	0.673

CI: confidence intervals; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. Covariates in the ANCOVA included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives, hormone replacement therapy (HRT)). OC 6 months n = 371: 101, 92, 90, 88; OC 5 years n = 296: 78, 73, 69, 76; PINP 6 months n = 370: 100, 92, 90, 88; PINP 5 years n = 296: 78, 73, 69, 76; CTX 6 months n = 347: 94, 86, 85, 82; CTX 5 years n = 269: 69, 67, 66, 67; BALP 6 months n = 369: 100, 91, 90, 88; BALP 5 years n = 296: 78, 72, 70, 76 participants for 0-300 µg/d selenium, respectively.

Table 6.3: Estimated marginal means from ANCOVA of bone turnover markers by supplementation group at 6 months and 5 years after the removal of hormone replacement therapy users. Upper and lower 95 % confidence intervals are displayed in parentheses.

Bone Turnover	Selenium dosage (µg/d)				P
(µg/L), Mean (CI)	0	100	200	300	
OC 6 months	17.4 (16.6-18.2)	17.3 (16.5-18.2)	17.5 (16.6-18.4)	16.7 (16.0-17.6)	0.649
OC 5 years	17.4 (15.9-19.0)	17.3 (15.8-18.9)	17.5 (16.0-18.9)	16.1 (14.7-17.5)	0.505
PINP 6 months	38.7 (36.6-40.9)	37.9 (35.9-40.2)	39.4 (37.2-41.6)	40.1 (37.8-42.5)	0.605
PINP 5 years	40.9 (36.9-45.3)	41.1 (37.1-45.5)	40.9 (36.9-45.3)	38.1 (34.4-42.1)	0.684
CTX 6 months	0.16 (0.14-0.17)	0.16 (0.14-0.18)	0.16 (0.15-0.18)	0.16 (0.14-0.17)	0.846
CTX 5 years	0.16 (0.14-0.18)	0.17 (0.15-0.20)	0.16 (0.14-0.19)	0.15 (0.13-0.17)	0.523
BALP 6 months	14.6 (14.0-15.2)	14.0 (13.2-14.6)	13.7 (13.2-14.3)	14.5 (13.9-15.1)	0.149
BALP 5 years	14.4 (13.5-15.3)	15.1 (14.1-16.1)	14.9 (14.0-16.0)	14.6 (13.7-15.6)	0.737

CI: confidence intervals; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. Covariates in the ANCOVA included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives, hormone replacement therapy (HRT)). OC 6 months n = 341: 87, 87, 86, 81; OC 5 years n = 296: 78, 73, 69, 76; PINP 6 months n = 340: 86, 87, 86, 81; PINP 5 years n = 268: 66, 67, 66, 69; CTX 6 months n = 321: 81, 82, 81, 77; CTX 5 years n = 244: 59, 61, 63, 61; BALP 6 months n = 339: 86, 86, 86, 81; BALP 5 years n = 269: 66, 67, 67, 69 participants for 0-300 µg/d selenium, respectively.

Table 6.4: Estimated marginal means from ANCOVA of bone turnover markers by supplementation group at 6 months and 5 years after the removal of calcium, vitamin D and multivitamin users. Upper and lower 95 % confidence intervals are displayed in parentheses.

Bone Turnover	Selenium dosage (µg/d)				P
(µg/L), Mean (CI)	0	100	200	300	
OC 6 months	17.1 (16.1-18.0)	16.8 (15.8-17.8)	17.6 (16.4-18.7)	17.1 (16.0-18.2)	0.785
OC 5 years	16.7 (15.1-18.5)	17.9 (16.2-19.9)	16.7 (14.7-18.9)	16.4 (14.7-18.4)	0.671
PINP 6 months	38.3 (35.9-40.8)	35.8 (33.6-38.2)	38.0 (35.3-40.9)	39.5 (36.9-42.5)	0.226
PINP 5 years	38.6 (34.4-43.4)	41.2 (36.6-46.2)	37.5 (32.6-43.2)	38.2 (33.7-43.4)	0.743
CTX 6 months	0.17 (0.15-0.19)	0.16 (0.14-0.17)	0.16 (0.14-0.18)	0.16 (0.14-0.18)	0.879
CTX 5 years	0.18 (0.15-0.21)	0.18 (0.16-0.21)	0.16 (0.14-0.19)	0.17 (0.14-0.19)	0.589
BALP 6 months	14.1 (13.6-14.7)	13.6 (13.1-14.2)	13.8 (13.2-14.4)	14.5 (13.9-15.2)	0.138
BALP 5 years	14.1 (13.1-15.2)	15.2 (14.1-16.4)	14.2 (13.0-15.5)	14.3 (13.2-15.4)	0.488

CI: confidence intervals; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. Covariates in the ANCOVA included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives, hormone replacement therapy (HRT)). OC 6 months n = 246: 70, 69, 50, 57; OC 5 years n = 199: 57, 57, 37, 48; PINP 6 months n = 245: 69, 69, 50, 57; PINP 5 years n = 200: 57, 57, 38, 48; CTX 6 months n = 232: 67, 64, 47, 54; CTX 5 years n = 179: 50, 52, 35, 42; BALP 6 months n = 244: 69, 68, 50, 57; BALP 5 years n = 199: 57, 56, 38, 48 participants for 0-300 µg/d selenium, respectively.

Table 6.5: Estimated marginal means from ANCOVA of bone turnover markers by supplementation group at 6 months and 5 years after the removal of antiresorptive users and fractures. Upper and lower 95 % confidence intervals are displayed in parentheses.

Bone Turnover (µg/L), Mean (CI)	Selenium dosage (µg/d)				P
	0	100	200	300	
OC 6 months	17.1 (16.3-17.9)	16.7 (15.9-17.5)	17.4 (16.6-18.3)	16.5 (15.7-17.3)	0.405
OC 5 years	16.8 (15.5-18.2)	17.3 (15.9-18.8)	17.1 (15.7-18.6)	16.0 (14.8-17.4)	0.602
PINP 6 months	38.9 (36.9-41.0)	36.6 (34.7-38.5)	38.4 (36.3-40.6)	38.5 (36.5-40.8)	0.373
PINP 5 years	39.4 (36.0-43.3)	40.6 (36.9-44.6)	39.4 (35.7-43.4)	37.3 (34.0-40.9)	0.658
CTX 6 months	0.16 (0.14-0.17)	0.16 (0.15-0.18)	0.16 (0.15-0.18)	0.16 (0.14-0.17)	0.914
CTX 5 years	0.16 (0.14-0.18)	0.18 (0.16-0.20)	0.16 (0.14-0.18)	0.15 (0.14-0.17)	0.447
BALP 6 months	14.4 (13.8-14.9)	13.8 (13.2-14.3)	13.7 (13.2-14.2)	14.3 (13.7-14.9)	0.183
BALP 5 years	13.9 (13.1-14.7)	13.1 (14.2-15.9)	14.5 (13.6-15.3)	14.4 (13.6-14.4)	0.316

CI: confidence intervals; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. Covariates in the ANCOVA included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives, hormone replacement therapy (HRT)). OC 6 months n = 392: 104, 100, 97, 91; OC 5 years n = 316: 81, 79, 75, 81; PINP 6 months n = 391: 103, 100, 97, 91; PINP 5 years n = 316: 81, 79, 75, 81; CTX 6 months n = 366: 96, 93, 92, 85; CTX 5 years n = 289: 71, 73, 72, 73; BALP 6 months n = 390: 103, 99, 97, 91; BALP 5 years n = 316: 81, 78, 76, 81 participants for 0-300 µg/d selenium, respectively.

Table 6.6: Sensivity analyses including participants with plasma selenium concentration below 70 µg/L at baseline. Upper and lower 95 % confidence intervals are displayed in parentheses.

Bone Turnover (µg/L), Mean (CI)	Selenium dosage (µg/d)				P
	0	100	200	300	
OC 6 months	16.4 (14.3-18.7)	16.8 (14.7-19.3)	18.8 (16.4-21.5)	15.7 (14.2-17.3)	0.231
OC 5 years	25.6 (18.3-35.7)	14.5 (10.2-20.6)	16.1 (10.5-24.7)	18.7 (14.8-23.5)	0.139
PINP 6 months	37.3 (30.5-45.6)	37.6 (30.5-46.3)	40.6 (33.3-49.4)	38.8 (33.5-44.9)	0.930
PINP 5 years	56.5 (40.4-78.9)	37.1 (26.2-52.4)	37.1 (24.2-56.6)	43.6 (34.8-54.6)	0.318
CTX 6 months	0.17 (0.12-0.24)	0.15 (0.10-0.22)	0.15 (0.10-0.21)	0.15 (0.11-0.19)	0.931
CTX 5 years	0.27 (0.19-0.38)	0.16 (0.11-0.25)	0.12 (0.10-0.19)	0.16 (0.12-0.20)	0.045
BALP 6 months	13.5 (11.8-15.6)	12.9 (11.3-14.7)	13.5 (11.8-15.3)	14.4 (13.1-15.8)	0.580
BALP 5 years	16.5 (12.4-21.9)	13.5 (10.0-18.1)	13.2 (9.9-18.7)	14.9 (12.3-17.9)	0.728

CI: confidence intervals; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. Covariates in the ANCOVA included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid (GC), antiresorptives, hormone replacement therapy (HRT)). OC 6 months n = 49: 10, 9, 11, 19; OC 5 years n = 38: 8, 7, 6, 17; PINP 6 months n = 49: 10, 9, 11, 19; PINP 5 years n = 38: 8, 7, 6, 17; CTX 6 months n = 44: 9, 7, 10, 18; CTX 5 years n = 35: 8, 6, 6, 15; BALP 6 months n = 48:9, 9, 11, 19; BALP 5 years n = 38: 8, 7, 6, 17 participants for 0-300 µg/d selenium, respectively.

Table 6.7: Baseline characteristics of participants with bone turnover markers represented by dropout status.

Characteristic	All Participants	Dropout		P
		No	Yes	
Sex, n (%) Male	250 (52.0)	177 (50.0)	73 (57.5)	0.148
Sex, n (%) Female	231 (48.0)	177 (50.0)	54 (42.5)	
Age years, Mean (SD)	66.16 (4.1)	66.10 (4.1)	66.34 (4.2)	0.571
Plasma Selenium µg/L, Mean (SD)	86.54 (16.2)	86.71 (16.4)	85.89 (15.8)	0.742
BMI kg/m^2, Mean (SD)	26.82 (4.03)	26.81 (4.0)	26.88 (4.0)	0.672
Height m, Mean (SD)	1.69 (0.09)	1.69 (0.09)	1.69 (0.09)	0.719
Weight kg, Mean (SD)	76.81 (13.6)	76.76 (13.6)	77.17 (13.2)	0.720
Alcohol units per week, Mean (SD)	7.29 (7.5)	7.27 (7.6)	7.39 (7.4)	0.865
Smokers, n (%)				0.220
Never	158 (32.8)	120 (33.9)	38 (29.9)	
Previous	178 (37.0)	135 (38.1)	43 (33.9)	
Present	145 (30.1)	99 (28.0)	46 (36.2)	
Education, n (%)				0.397
None	134 (28.7)	103 (29.7)	31 (25.8)	
1-3 y	76 (16.3)	53 (15.3)	23 (19.2)	
3-4 y	212 (5.4)	161 (6.4)	51 (42.5)	
> 4 y	45 (9.6)	30 (8.6)	15 (12.5)	
Live Alone, n (%)				0.713
No	400 (85.7)	296 (85.3)	104 (86.7)	
Yes	67 (14.3)	51 (14.7)	16 (13.3)	
Thyroid Medication, n (%)				0.961
None	468 (97.1)	344 (97.2)	123 (96.9)	
LT4	11 (2.3)	8 (2.3)	3 (2.4)	
ATD	3 (0.6)	2 (0.6)	1 (0.8)	
Inhaled GC, n (%)				0.721
No	451 (96.4)	335 (96.5)	115 (95.8)	
Yes	17 (3.6)	12 (3.5)	5 (4.2)	
Systemic GC, n (%)				0.274
No	475 (99.2)	352 (99.4)	123 (98.4)	
Yes	4 (0.8)	2 (0.6)	2 (1.6)	
Antiresorptives, n (%)				0.667
No	461 (98.7)	343 (98.8)	118 (98.3)	
Yes	6 (1.3)	4 (1.2)	2 (1.7)	
HRT, n (%)				0.128
No	392 (83.9)	286 (82.4)	106 (88.3)	
Yes	75 (16.1)	61 (17.6)	14 (11.7)	
Calcium, n (%)				0.642
No	419(89.7)	310 (89.3)	109 (90.8)	
Yes	48 (10.3)	37 (10.7)	11 (9.2)	
Vitamin D, n (%)				0.254
No	442 (94.6)	326 (93.9)	116 (96.7)	
Yes	25 (5.4)	21 (6.1)	4 (3.3)	
Multivitamin, n (%)				0.911
No	325 (69.6)	241 (69.5)	84 (70.0)	
Yes	142 (30.4)	106 (30.5)	36 (30.0)	
Dosage µg/d, n (%)				0.847
0	124 (25.8)	88 (24.9)	36 (28.3)	
100	122 (25.3)	90 (25.4)	32 (25.2)	
200	118 (24.5)	87 (24.6)	31 (24.4)	
300	117 (24.3)	89 (25.1)	28 (22.0)	

SD: standard deviation; inhaled GC: inhaled glucocorticoid; systemic GC: systemic glucocorticoid; LT4: levothyroxine; ATD: antithyroid drugs; HRT: hormone replacement therapy. Age, alcohol, smokers n = 482; weight, height, BMI n = 479; plasma selenium baseline n = 479.

Table 6.8: Bone turnover markers at baseline of participants represented by dropout status.

Bone Turnover (µg/L), Mean (SD)	All participants	Dropout		P
		No	Yes	
OC	18.67 (8.46)	18.60 (8.23)	18.86 (9.09)	0.883
PINP	42.68 (18.05)	42.14 (17.23)	44.17 (20.16)	0.520
CTX	0.20 (0.22)	0.21 (0.25)	0.20 (0.13)	0.876
BALP	15.66 (5.68)	15.60 (5.83)	15.84 (5.24)	0.279

SD: standard deviation; OC: osteocalcin; PINP: procollagen type 1 N-terminal propeptide; CTX: collagen type 1 cross-linked C-telopeptide; BALP: bone alkaline phosphatase. OC and PINP n = 482; CTX n = 460; BALP n = 480. OC and PINP n = 354 remained, 127 dropped out; CTX n = 336 remained, 123 dropped out; BALP n = 354 remained, 125 dropped out.

Chapter 7. General Discussion

7.1 Overview

Chapter 7 is the final chapter of this thesis and will critically discuss the main findings from the four experimental chapters. In this chapter I will discuss the strengths and limitations of the experimental approaches used in this thesis including The Newcastle 85+ Study (Chapters 3-5), and The PRECISE Study (Chapter 6). Finally, this chapter will explore some of the potential public health implications arising out of the PhD findings and provide recommendations for future research.

Selenium is a nutrient for optimal functioning of the human body, one such function being its antioxidant role through Sec-containing selenoproteins. Historically, much of the research on selenium and health has focused on cardiovascular disease, cancer, diabetes, cognition, immunity and fertility, with limited research exploring selenium's role in MSK function (Rayman, 2012). It is well known that selenoproteins such as SePP are found in bone and muscle and evidence from animal models shows that selenium supplementation, or selenium deficiency, has an impact on MSK function; for example selenium-supplemented mice have better bone microarchitecture than selenium-deficient mice (Cao, Gregoire and Zeng, 2012) and in selenium-deficient rats, skeletal growth was impaired (Moreno-Reyes *et al.*, 2001). Despite this, there is a dearth of related data on the role of selenium in MSK function in humans. The aim of this PhD thesis was to fill some of these knowledge gaps by elucidating the role of selenium in MSK ageing using different study designs. This aim was achieved successfully within the experimental chapters of this thesis using epidemiological and RCT approaches and by examining MSK outcomes including muscle function, sarcopenia, disability and BTMs.

Overall, the findings from The Newcastle 85+ Study reveal that, when compared with selenium DRVs, serum selenium and SePP concentrations in these very old adults were suboptimal (82 % below 70 µg/L serum selenium and 83 % below 4.5 mg/L SePP). On the other hand, and rather surprisingly, GPx3 activity appeared optimal (70 % at, or above, 115 U/L). There were strong linear relationships between serum selenium and GPx3 activity and serum selenium and SePP concentration that suggested that neither outcome was

maximised within the range of serum selenium concentrations observed in these analyses. To fulfil the aim of elucidating the role of selenium in MSK function, relationships between the biomarkers of selenium status and HGS, TUG and sarcopenia were explored, and these analyses were taken further by using a clinical endpoint of MSK function, disability. There were some inconsistencies in the associations between the biomarkers of selenium status and MSK function between cross-sectional and prospective analyses. For example, in unadjusted, cross-sectional models, when used in the continuous format, biomarkers of selenium status were associated with HGS, TUG, and disability, but not sarcopenia. Furthermore, when using tertiles of the biomarkers of selenium status, those with low (tertile 1) serum selenium concentration had a greater rate of change in TUG performance over 5 years in comparison to those with high (tertile 3) serum selenium. Additionally, those with medium (tertile 2) selenium concentrations had a greater prevalence of severe sarcopenia over 3 years in comparison to those with high (tertile 3) serum selenium. Finally, those with low (tertile 1) SePP concentrations had a greater change in the prevalence of disability over 5 years in comparison to those with high (tertile 3) SePP concentrations.

The second study design was a secondary analysis of data from The PRECISE Study, a RCT that explored the effects of selenium supplementation on biomarkers of bone turnover in middle-aged and older adults in Denmark. In contrast with The Newcastle 85+ Study, participants in The PRECISE Study were younger and had higher plasma selenium at baseline. Supplementation with Se-yeast increased plasma selenium concentration in a dose-dependent manner. These effects were observed at 6 months and were maintained after 5 years of selenium supplementation. However, there was no evidence that selenium supplementation improved BTMs.

Therefore, the findings from this PhD project have offered new insights into the role of selenium status, and of the effects of selenium supplementation on MSK ageing. The potential implications of these findings will be discussed in subsequent sections within this chapter. I will now critically discuss the strengths and limitations of the study designs used in this PhD thesis.

7.2 Strengths and Limitations

Strengths of The Newcastle 85+ Study

The major strength of this prospective cohort study was the large sample size (n = 757 for biomarkers of selenium status) with inclusion of all participants regardless of living status, providing a sociodemographically representative sample of very old adults in the UK. The Newcastle 85+ Study was the first UK based study to assess three biomarkers of selenium status in very old adults. The availability of three commonly used biomarkers of selenium status in this cohort is a major strength particularly because comparable studies involving a large sample size, such as the EPIC-Oxford study (20-97 years), or older adults, such as the NDNS (19-64 years) have only assessed one biomarker of selenium status i.e., plasma selenium concentration (Hughes *et al.*, 2015; Roberts *et al.*, 2018). Another study has assessed the same three biomarkers of selenium status that were used in The Newcastle 85+ Study but the participants in the American study were younger (40-79 years) and had a higher baseline selenium concentration (mean 117.6 µg/L) (Hargreaves *et al.*, 2014). However, an unavoidable phenomenon of using very old populations was that attrition and mortality were high which could lead to a healthy-survivor bias (Davies *et al.*, 2014); over the 5 year follow-up 15 % withdrew, and 38 % died.

The Use of Biomarkers of Selenium Status and their Potential Limitations

A strength of my analyses was the use of nutritional biomarkers to help overcome an array of issues with dietary intake assessments, as discussed in Chapter 1, Section 1.6. My PhD thesis was a direct follow on from my MRes project which explored the relationship between dietary selenium intake and MSK function (Perri *et al.*, 2020). Nevertheless, as with all nutritional biomarkers, there are limitations to each and difficulty in deciphering their effect since nutrients are not consumed in isolation. The biomarkers of selenium status measured in serum from The Newcastle 85+ participants were selected based on their suitability to assess selenium status in a very old population, who were expected to have suboptimal selenium status, as suggested by their low selenium intakes. As this was a secondary analysis, the study did not initially set out to explore selenium status in this population. The blood samples were only available as serum so this refined the selection of biomarkers, for example, whole-blood selenium could not be assessed, nor could the

selenite-exchangeable pool, thus the biomarkers of selenium status selected were serum selenium, GPx3 activity and SePP. These biomarkers were appropriate for my population as they are all sensitive to a range of selenium intakes (Hurst *et al.*, 2013; Burk *et al.*, 2006; Ashton *et al.*, 2009). Serum selenium is a good biomarker since this is commonly used in other studies, so allowed for comparison and it also houses the selenoproteins, GPx3 and SePP. However, the selenite-exchangeable pool has been suggested to be more reliable in determining selenium status, compared to serum or plasma (Broome *et al.*, 2004) or erythrocyte selenium, as this is not affected by inflammation (Oakes *et al.*, 2008). GPx3 activity may be less sensitive to higher selenium intakes, since it plateaus at lower concentrations of serum selenium, although, this was not an issue in my population due to their suboptimal serum selenium concentration of 53.6 µg/L. However, one potential limitation to the GPx3 activity measurements in my analyses was that the comparability across studies of GPx3 activity is lower since it reflects enzymatic processes rather than a concentration of a biomarker. The use of SePP was appropriate due to its greater sensitivity to selenium intake than GPx3 activity as evidenced by its requirement for higher serum selenium concentrations to achieve a plateau (Hurst *et al.*, 2010; Xia *et al.*, 2010; Schomburg *et al.*, 2019). Additionally, according to the selenoprotein hierarchy, SePP is thought to be reserved in times of selenium deficiency.

Selenium biomarkers are diverse and reflect many processes related to the biological roles of selenium. For example, GPx3 is synthesised in the kidney, whilst SePP is synthesised in the liver, yet both are distributed throughout the body, and furthermore, rodent studies have suggested differences in selenoprotein synthesis between the liver and kidney, despite similar serum levels (Riese *et al.*, 2006). This suggest differences in liver metabolism (Schomburg *et al.*, 2007) which could be further enhanced due to the increased heterogeneity in older populations whereby there is disparity in health states including daily functioning, disease burden and mortality (Nguyen *et al.*, 2021). For example, in US adults aged over 85 years, almost one third reported to have good health and over 50 % reported to have no health limitations in their ability to perform daily tasks (Lowsky *et al.*, 2014). This adds to the complexity of deriving nutritional recommendations in very old adults as the requirements will vary on an individual basis. For example, Kidney Care UK reported kidney

problems to double in prevalence in adults aged over 80 years, further complicating the DRV for selenium in older adults (Kidney Care UK, 2022).

Strengths of The PRECISE Study

In Chapter 6, I performed secondary analyses of data from The PRECISE Study, a RCT exploring the effects of selenium supplementation on BTMs in both men and women; this was the first RCT to explore these effects in older, healthy adults over 5 years. In summary, Se-yeast supplementation (100-300 µg/d) led to a dose-dependent increase in plasma selenium concentration which was sustained for the entire study duration. However, there was no effect of supplementation on the blood-derived biomarkers of bone formation, or bone resorption, at either 6 months or 5 years. Prior to my analyses, data on the biological effects of long-term selenium supplementation in humans was scarce (Behne, Alber and Kyriakopoulos, 2010). A recent UK-based RCT reported the effects of selenium supplementation, in the form of sodium selenite (50, 200 µg/d), on BTMs and BMD (Walsh *et al.*, 2021). This RCT was carried out for a shorter period (6 months) than The PRECISE Study and was restricted to post-menopausal women with osteoporosis or osteopenia but like The PRECISE Study, there was no effect of selenium supplementation on BTMs. My analyses had the advantage of recruiting healthy men and women, and continuing supplementation for 5 years. In the RCT conducted by Walsh *et al.,* (2021) sodium selenite was used, The Selenium and Vitamin E Cancer Prevention Trial (SELECT) (Klein *et al.*, 2011) used L-SeMet, whilst in my analyses and the NPC trial (Clark *et al.*, 1996), Se-yeast was used. Studies suggest that the bioavailability of SeMet is over 90 %, whilst it is around 50 % for the inorganic forms, selenite and selenate (Thomson, 1986). Overall, studies suggest Se-yeast is effective in increasing selenoenzyme activity and can be stored, which can be beneficial during deficiency (Schrauzer *et al.*, 2000; Alfthan *et al.*, 1991).

Potential Limitations to The PRECISE Study

In this secondary analysis, the blood samples were taken in a non-fasted state. Research has shown that the post-prandial state and circadian rhythm has been associated with a decrease in BTMs (Hannon *et al.*, 2000). Consequently, the use of non-fasted samples may

have increased the variance in BTM measurements, but this was likely to be similar for all supplementation groups. Another potential limitation was lack of available data on nutritional intakes; selenium absorption is not homeostatically regulated, and it can be affected by other dietary factors. Furthermore, vitamin C from a varied diet can improve selenium bioavailability (Fairweather-Tait, 1997), and vitamin A, E and antioxidants have been seen to improve selenium bioavailability in animals (Combs, 1988). Likewise, protein intake can promote selenium absorption, likely due to the competition between SeMet and methionine for incorporation into proteins. On the other hand, heavy metals, high dietary sulphur (Combs, 1988) and guar gum can reduce selenium absorption by binding of selenium to these compounds and thus reducing selenium's bioavailability (Fairweather-Tait, 1997). Other factors that may affect selenium status are sex differences (Combs *et al.*, 2012), although these differences are not always obvious in plasma selenium (Monget *et al.*, 1996; Imai *et al.*, 1990) and there were no significant differences in serum selenium between sex in my Chapter 6 analyses. Nonetheless, studies have suggested differences in the responses to selenium supplementation between sexes (Schomburg and Schweizer, 2009) and SePP and GPx4 expression are known to differ depending on sex (Méplan *et al.*, 2007). In addition to these sex differences, other genetic differences, such as SNPs that are common in Caucasian populations (Méplan *et al.*, 2007) could also play a role in modulating the biological response to selenium supplementation. For example, women with the with GPx1 679 T/T genotype had higher levels of urinary selenium excretion after selenium supplementation than women with the C/C genotype (Combs *et al.*, 2012). Finally, it may have been useful to have other biomarkers of selenium status in this study, however, supplementation (0-200 μg/d) with SeMet in US, non-deficient adults for 12 months did not lead to differences in GPx activity or SEPP concentrations (Combs *et al.*, 2012), suggesting that selenoprotein biomarkers may have not added further value due to the fact that The PRECISE Study population had optimal selenium concentrations (86.5 μg/L).

It is important to acknowledge that secondary analysis of data has limitations. The initial data collection may not catch information on key variables since it is not set out to answer the retrospective research question. As I experienced, due to not being involved in the data collection, there can be difficulties in understanding study-specific nuances which can

increase the time required to clean and interpret the data, as well as not having access to files or data due to geographical or temporal limitations.

Impact of Ageing on Selenium Status

As discussed in Chapter 1, and throughout this thesis, a disproportionate amount of oxidative stress is associated with chronic inflammation, increased disease risk and the ageing process (Chrousos, 2009). The suppressive effect of inflammation on serum selenium likely occurred in The Newcastle 85+ Study due to the older age, and furthermore a quarter of the population had three or more diseases. Higher levels of inflammation have been associated with decreased selenoprotein synthesis (Dreher *et al.*, 1997) which can alter serum and erythrocyte concentrations (Miller *et al.*, 1983; MacDonell *et al.*, 2018), although another study reported that erythrocyte selenium was unaffected by inflammation (Oakes *et al.*, 2008). Inflammation can lead to a cytokine-mediated redistribution of selenium from the plasma to the liver, causing apparent plasma selenium deficiencies leading to an overestimation of selenium deficiency (MacDonell *et al.*, 2018; Oakes *et al.*, 2008). Excessive oxidative stress can inactivate GPx activity (Pigeolet *et al.*, 1990) and conversely, inactivation of GPx can induce oxidative stress (Miyamoto *et al.*, 2003; Lubos, Loscalzo and Hardy, 2011). This increase in oxidative stress is most likely since GPx detoxifies lipid hydroperoxides and reduces the negative effects of ROS (Martínez, García and Galarza, 1982). Excessive ROS, if left unchecked, can increase DNA damage and protein degradation (Lubos, Loscalzo and Hardy, 2011). The increase in oxidative stress and inflammation that occurs with the ageing process, in addition to the decline in GPx activity as seen in older women (> 65 years) with disability (Espinoza *et al.*, 2008) may further increase the requirements for these antioxidant compounds, such as GPx (Lubos, Loscalzo and Hardy, 2011) in order to synthesise selenoproteins to keep up with the increase in demand.

7.3 Public Health Implications

Concern regarding inadequate selenium intakes in the elderly arose in the 1980s (Bates *et al.*, 2002). It is estimated that 15 % of the world's population have deficient intakes of selenium (Fordyce, 2013; White, Broadley and Gregory, 2012; Tan *et al.* 2016). This PhD thesis has shown that very old adults in the UK have suboptimal selenium status as

indicated by suboptimal concentrations of serum selenium (mean 53.6 μg/L) and SePP (mean 2.9 mg/L). Other studies across the globe have shown that older adults have suboptimal selenium concentrations, ranging from 50.0-67.1 μg/L (MacDonell *et al.*, 2021; Alehagen *et al.*, 2016; Koç *et al.*, 2015; Rita Cardoso *et al.*, 2016) with some studies reporting lower serum selenium in adults aged > 81 years compared to younger adults (Lymbury *et al.*, 2008). Therefore, it would be expected that very old adults (≥ 85 years) have a further reduced selenium status due to the increased prevalence of disease and its associated inflammation, in addition to an overall reduction in micronutrient intakes (Chrousos, 2009; Gonzalez *et al.* 2006). However, an interesting finding from my analyses of very old adults was that, despite the suboptimal serum selenium and SePP concentrations, GPx3 activity was considered optimal (over 70 % with GPx3 activity above 115 U/L). The results of Chapters 3 and 4 bring into focus the aptness of the current DRVs for selenium in very old adults and emphasise the need for age specific DRVs beyond 65 years and as seen in Table 7.1, the current DRVs estimate suboptimal selenium status using a population of very old adults. However, this inference from my analyses is based upon the apparent relationships between serum selenium and GPx3 activity and SePP in very old adults without the use of selenium supplementation as used in the studies that derived the current selenium DRVs.

Table 7.1: Estimated serum selenium concentrations (µg/L), stratified by sex, from participants of The Newcastle 85+ Study derived using various selenium dietary recommendation values.

Organisation	Recommendation		Type	The Newcastle 85+ Study Estimated Serum Selenium µg/L		Reference
	Men	Women		Men	Women	
Committee of IOM National Academy of Sciences Food and Nutrition Board 2000 (USA and Canada)	55	55	RDA	53.58	57.64	(Yang, 1987; Duffield et al., 1999)
COMA/Department of Health 1991 (UK)	75	60	RNI	54.78	58.14	(Yang, 1987)
	40	40	LRNI	52.68	56.14	
FAO/WHO 2002/2004	33	25	NR	52.26	54.64	(Walzel, 1988)
NHMRC Australia and New Zealand	70	60	RDI	54.58	58.14	(Duffield et al., 1999)
	60	50	EAR	53.88	57.14	(Xia et al., 2005)
NNR 2014 (Nordic)	60	50	RNI	53.88	57.14	(Xia et al., 2010)
	35	30	AR	52.38	55.14	
	70	60	RI	54.58	58.14	
	20	20	LI	51.48	54.14	
Scientific Committee for Food 1992	40	40	AR	52.68	56.14	(Yang, 1987)
	55	55	PRI	53.58	57.64	
	20	20	LI	51.48	54.14	
D-A-CH Germany (D), Austria (A) and Switzerland (CH) 2015 -Revised	70	60	RNI	54.58	58.14	(Xia et al., 2010)

IOM: Institute of Medicine; RDA: Recommended Daily Allowance; EAR: Estimated Average Requirement; COMA: The Committee on Medical Aspects of Food and Nutrition Policy; RNI: Reference Nutrient Intake; LRNI: Lower Reference Nutrient Intake; FOA/WHO: World Health Organisation/ Food and Agriculture Organisation; NHMRC: National Health and Medical Research Council; NR: normative requirement; NNR: Nordic Nutrition Recommendations; AR: Average Requirement; RI: Recommended Intake; LI: Lower Intake; D-A-CH: Germany (D), Austria (A) and Switzerland (CH)

These analyses from The Newcastle 85+ Study have taken a step forward in revealing the importance of understanding the selenium requirements of very old adults and, possibly deriving new recommendations to ensure very old adults optimise their health through full functioning of selenoproteins. This is important as it could prevent, or delay, MSK dysfunction, helping to retain autonomy and independence, a key determinant of quality of life in older years. In order to do this, greater efforts would be required to standardise the DRVs across countries by using the same study protocols to ensure consistency. However, before this is achieved, there needs to be an improved understanding of the changes in selenium requirements in very old adults; for example, studies have implied that selenium requirements may be lower due to nutrient retention in older age (Bunker *et al.*, 1988) or increased selenoprotein synthesis (Schomburg *et al.*, 2007). On the other hand, requirements may be higher, as seen in Table 7.1, due to selenium malabsorption, altered gut microbiomes, whereby intestinal bacteria can reduce selenium concentrations in the gut which has been associated with further disease (Ferreira *et al.*, 2021), or increased inflammation, as discussed earlier in this chapter, where antioxidant selenoproteins are

required to help reduce ROS (Dreher *et al.*, 1997; Lubos, Loscalzo and Hardy, 2011). Care must also be taken with those living in institutions, or who are receiving parenteral nutrition, where selenium status is often suboptimal (van Rij *et al.*, 1979; Brown *et al.*, 1986). In addition to these altered requirements; considerations should be made for very old adults in their ability to achieve these requirements, for example, lower dentition can make it difficult to consume meat, a major source of selenium, and likewise, low income can restrict purchasing these forms of selenium-rich foods, alongside the difficulties posed in preparing these foods (Clegg and Williams, 2018). It is also important to consider vegetarians and vegans, one study found lower selenium concentrations in non-meat eaters compared with meat-eaters (Hoeflich *et al.*, 2010), however, another study reported vegetarians to have optimal selenium status despite lower selenium intakes (Akesson and Ockerman, 1985). Furthermore, culinary tastes in various societies may lead to a reduction in consumption of selenium-rich foods like meat and offal and an increase in cereal consumption, which now forms a large proportion of daily selenium intakes (Xie *et al.*, 2021). Thus, alternatives for those who cannot, or do not consume selenium-rich animal produce should be provided to help those adults or inform their carers to meet the required nutritional recommendations (Winkel *et al.*, 2015; Jones *et al.*, 2017; Clegg and Williams, 2018).

Raising Selenium Intakes and Status Among Older Adults

One way to enhance selenium intakes in those unable to consume animal produce could be the mandatory fortification of certain food produce, for example, cereals and cereal products, or, alternatively, the use of selenium-fertilisers, which has increased selenium concentrations in a range of foods (Combs, 2001) and proven successful in increasing selenium status since 1984 in Finnish populations (Alfthan *et al.*, 2015). These solutions would also meet the EAT-Lancet Commission which focuses on increasing the consumption of healthy foods via vegetables, whole grains, legumes, and reducing the consumption of meats, sugar and refined grains (Willett *et al.*, 2019). If biofortification or selenium-fortified fertilisers were implemented, extensive research would inevitably be required to ensure the end products did not exceed the safe upper limit for selenium of 255 µg/d (ESFA, 2023) (since updated from 400 µg/d (IoM, 2000)). In the meantime, whilst research was

undertaken, nutritional education programmes could be provided to improve the understanding of nutritional requirements in older adults and reiterate the importance of nutrition in health to aid behaviour change. Healthy food choices would form the basis of the recommendations, followed by supplementation in special cases, where these may be required. However, before supplementation was commenced, individuals would require a blood test to determine their baseline status of selenium because, as seen in Chapter 6, supplementing with selenium when baseline concentrations are already optimal may provide no further benefits and excessive intakes can be detrimental (Roman, Jitaru and Barbante, 2014); for instance there is a U-shaped curve between selenium status and diabetes and cancer risk (Rayman, 2012; Roman, Jitaru and Barbante, 2014; Vinceti *et al.*, 2013; Solovyev Nikolay, Vanhaecke and Michalke, 2019). If supplementation was required, as evidenced from Chapter 6 of this thesis, Se-yeast would be an appropriate candidate due to its efficient absorption and safety regarding toxicity. Notwithstanding the importance of studying the role of selenium in MSK ageing, it would be pertinent that once knowledge was improved in older adults, including the mechanisms of selenium on MSK function and disability, this would be used to inform and implement guidelines to help prevent these issues earlier in adult life before substantial decline set in.

7.4 Recommendations for Future Research

Future studies should explore the basis for selenium requirements in very old adults; the quantification of the relationship between serum selenium and selenoproteins in this thesis was limited to cross-sectional data, as opposed to the published DRVs that are based on experimental studies. Future studies could overcome this issue by supplementing older adults (≥ 85 years) with Se-yeast to quantify the relationship between serum selenium and selenoproteins to update the selenium DRVs for very old adults. Furthermore, additional future studies could repeat the analyses undertaken in Chapter 4 and 5 of this thesis in very old populations, considering those with suboptimal and with optimal selenium status, and exploring a range of MSK outcomes, such as BMD, BTM, falls and fractures, as animal models have shown the effect of selenium on bone health. Previous research on these outcomes in The Newcastle 85+ Study reported 12 % of participants to have osteoporosis, 38 % to have experienced a fall in 12 months and 35 % required osteoporosis treatment (Duncan *et al.*, 2015); this emphasises the high prevalence of fracture risk in very old adults.

In addition to the biomarkers of selenium status measured in this thesis, whole-blood selenium could also provide insight to explore whether selenium retention is greater in older adults (Thomson, 2003) and it correlates well with serum selenium (Wang *et al.*, 2019; Longnecker *et al.*, 1996; Clausen and Nielsen, 1988). To strengthen the causal inferences from this observational study, Mendelian randomisation (MR) analyses could be incorporated to determine modifiable risk factors derived from genetics (Smith and Ebrahim, 2003). For example, the use of MR has been used in the SELECT trial which explored the associations between baseline selenium and prostate cancer risk (Yarmolinsky *et al.*, 2018).

Following these observational studies, the ultimate study to understanding these associations between selenium and MSK function would be using a RCT. To make the findings generalisable, it would be important to ensure there was adequate ethnic diversity and gender distribution. These factors are important as they may alter the interpretation of the results since disease risks alter across ethnicities, such as MSK pain and disease (Allison *et al.*, 2002; Jordan *et al.*, 2002), and gender, such as disability (Alexandre Tda *et al.*, 2012), osteoarthritis (Felson *et al.*, 1987) and osteoporosis (Wade *et al.*, 2014; Alswat, 2017). The intervention would consist of selenium supplementation in the form of Se-yeast which, as seen from Chapter 6, is a suitable form of selenium and the slower turnover rate of SeMet in the Se-yeast will allow plasma selenium to increase over a ranges of intakes in the study population (Burk *et al.*, 2006). In this study, a variety of MSK outcomes could be measured including clinical outcomes, such as sarcopenia and disability; biomarkers, such as urinary creatinine; and BTMs. Having a range of MSK outcomes could help provide representative findings of MSK function as the heterogeneity of older adults leads to floor and ceiling limits; and these can prevent successful assessment in frail or highly-able participants (Francis *et al.*, 2017). Assessing other selenoproteins, such as SELENOW and SELENON would also provide a better understanding of their involvement in MSK function. It would also be valuable to measure SNPs that can alter selenoproteins and their responses (Combs, 2015; Sempértegui *et al.*, 2003; Huang, Rose and Hoffmann, 2012). A genome-wide association study in European adults (40-80-year-olds) found that SNPs in NIMA-related kinase 6 (NEK6) and dimethylglycine dehydrogenase (DMGDH)/ betaine-homocysteine S-methyltransferase (BHMT) regions influenced responses to Se-yeast supplementation, where 2 alleles of SNPs

from DMGDH/BHMT were associated with a greater increase in plasma selenium and higher basal selenium concentrations (Batai *et al.*, 2021) and, in a small UK study, the C variant of rs713041 in the GPx4 gene was associated with an increased risk of colorectal cancer (Hesketh, 2008). Furthermore, genetic polymorphisms could also be explored in MSK function measures, for example, a meta-analysis of genome-wide linkage found associations between OP and the chromosome position where GPx1 is located (Lee *et al.*, 2006; Huang *et al.*, 2008) and lower activity was seen in those with OP (Sontakke and Tare, 2002; Sánchez-Rodríguez *et al.*, 2007). Other studies have also identified associations between GPx1 and OP in mice (Muthusami *et al.*, 2005) and postmenopausal women (Ozgocmen *et al.*, 2007). Combining these suggestions in future studies would continue to shed further light onto elucidating the role of selenium in MSK ageing.

8. References

Adamson, A. J., Collerton, J., Davies, K., Foster, E., Jagger, C., Stamp, E., Mathers, J. C. and Kirkwood, T. (2009) 'Nutrition in advanced age: dietary assessment in the Newcastle 85+ study', *Eur J Clin Nutr,* 63(Suppl 1), pp. S6-18.

Age UK, (2019) 'Briefing: Health and care of older people in England 2019'. https://www.ageuk.org.uk/globalassets/age-uk/documents/reports-and-publications/reports-and-briefings/health--wellbeing/age_uk_briefing_state_of_health_and_care_of_older_people_july2019.pdf Accessed on 5/11/22

Ahmed, T., and Haboubi, N. (2010) 'Assessment and management of nutrition in older people and its importance to health', *Clinical Interventions in Aging,* 5, pp. 207-216.

Ahn, S. H., Park, S. Y., Yoo, J. I., Chung, Y.-J., Jeon, Y. K., Yoon, B.-H., Kim, H. Y., Lee, S. H., Lee, J., and Hong, S. (2019) 'Use of Bone Turnover Markers in Clinical Practice for the Management of Osteoporosis in Korea: From the Survey on the Prescription Pattern of Bone Turnover Markers.', *Journal of Bone Metabolism*, 26(4), pp. 271-277.

Akbaraly, N. T., Arnaud, J., Hininger-Favier, I., Gourlet, V., Roussel, A. M. and Berr, C. (2005) 'Selenium and mortality in the elderly: results from the EVA study', *Clinica Chemica Acta,* 51(11), pp. 2117-23.

Akbaraly, N. T., Hininger-Favier, I., Carrière, I., Arnaud, J., Gourlet, V., Roussel, A. M., and Berr, C. (2007) 'Plasma selenium over time and cognitive decline in the elderly', *Epidemiology*, 18(1), pp. 52-58.

Akesson, B. and Ockerman, P. A. (1985) 'Selenium status in vegans and lactovegetarians', *The British Journal of Nutrition,* 53(2), pp. 199-205.

Al-E-Ahmad, A., Parsian, H., Fathi, M., Faghihzadeh, S., Hosseini, S. R., Nooreddini, H. G., and Mosapour, A. (2018) 'ALOX12 gene polymorphisms and serum selenium status in elderly osteoporotic patients', *Advances in Clinical and Experimental Biology*, 27(12), pp. 1717-1722.

Alehagen, U., Johansson, P., Björnstedt, M., Rosén, A., Post, C. and Aaseth, J. (2016) 'Relatively high mortality risk in elderly Swedish subjects with low selenium status', *European Journal of Clinical Nutrition,* 70(1), pp. 91-96.

Alexandre Tda, S., Corona, L. P., Nunes, D. P., Santos, J. L., Duarte, Y. A. and Lebrão, M. L. (2012) 'Gender differences in incidence and determinants of disability in activities of daily living among elderly individuals: SABE study', *Arch Gerontol Geriatr,* 55(2), pp. 431-7.

Alfthan, G. and Neve, J. (1996) 'Reference Values for Serum Selenium in Various AreasEvaluated According to the TRACY Protocol', *Journal of Trace Elements in Medicine and Biology,* 10(2), pp. 77-87.

Alfthan, G., Aro, A., Arvilommi, H., and Huttunen, J. K. (1991) 'Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite, and selenate', *Am J Clin Nutr,* 53(1), pp. 120-5.

Alfthan, G., Eurola, M., Ekholm, P., Venäläinen, E. R., Root, T., Korkalainen, K., Hartikainen, H., Salminen, P., Hietaniemi, V., Aspila, P., and Aro, A. (2015) 'Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population', *J Trace Elem Med Biol,* 31, pp. 142-7.

Allison, T. R., Symmons, D. P. M., Brammah, T., Haynes, P., Rogers, A., Roxby, M. and Urwin, M. (2002) 'Musculoskeletal pain is more generalised among people from ethnic minorities than among white people in Greater Manchester', *Annals of the Rheumatic Diseases,* 61(2), pp. 151.

Al-Mubarak, A. A., Grote Beverborg, N., Anker, S. D., Samani, N. J., Dickstein, K., Filippatos, G., van Veldhuisen, D. J., Voors, A. A., Bomer, N. and van der Meer, P. (2020) 'A Clinical Tool to Predict Low Serum Selenium in Patients with Worsening Heart Failure', *Nutrients,* 12(9), pp. 2541.

Alswat, K. A. (2017) 'Gender Disparities in Osteoporosis', *Journal of Clinical Medicine Research,* 9(5), pp. 382-387.

Andersen, N., Fagt, S., Groth, M., Hartkopp, H., Moeller, A., Ovesen, L. and Warming, D. (1996) '[Food habits in Denmark 1995: Main results].[Danish]', *Publikation. LST. Levnedsmiddelstyrelsen (Denmark). no. 235.*

Anderson, L. J., Liu, H. and Garcia, J. M. (2017) 'Sex Differences in Muscle Wasting', *Adv Exp Med Biol,* 1043, pp. 153-197.

Arbogast, S., Beuvin, M., Fraysse, B., Zhou, H., Muntoni, F. and Ferreiro, A. (2009) 'Oxidative stress inSEPN1-related myopathy: From pathophysiology to treatment', *Annals of Neurology,* 65(6), pp. 677-686.

Arikan, D. C., Coskun, Ayhan, Ozer, Ali, Kilinc, Metin, Atalay, Filiz, Arikan, Tugba (2011) 'Plasma selenium, zinc, copper and lipid levels in postmenopausal Turkish women and their relation with osteoporosis', *Biol Trace Elem Res,* 144(1-3), pp. 407-417.

Armstrong, M. E., Cairns, B. J., Banks, E., Green, J., Reeves, G. K., Beral, V. and Million Women Study, C. (2012) 'Different effects of age, adiposity and physical activity on the risk of ankle, wrist and hip fractures in postmenopausal women', *Bone,* 50(6), pp. 1394-400.

Arnaud, J. 'Stability of serum copper, selenium and zinc ', *Unpublished, on behalf the French Trace Element External Quality Assessment Scheme and Thematic Network Organizers of external quality assessment*.

Arnaud, J., Akbaraly, N. T., Hininger, I., Roussel, A. M., and Berr, C (2007) 'Factors associated with longitudinal plasma selenium decline in the elderly: The EVA Study', *The Journal of Nutritional Biochemistry,* 18(7), pp. 482-487.

Arnaud, J., Bertrais, S., Roussel, A. M., Arnault, N., Ruffieux, D., Favier, A., Berthelin, S., Estaquio, C., Galan, P., Czernichow, S. and Hercberg, S. (2006) 'Serum selenium determinants in French adults: the SU.VI.M.AX study', *The British Journal of Nutrition,* 95(2), pp. 313-320.

Ashton, K., Hooper, L., Harvey, L. J., Hurst, R., Casgrain, A. and Fairweather-Tait, S. J. (2009) 'Methods of assessment of selenium status in humans: a systematic review', *Am J Clin Nutr,* 89(6), pp. 2025S-2039S.

Aspray, T. J. and Hill, T. R. (2019) 'Osteoporosis and the Ageing Skeleton', *Subcell Biochem,* 91, pp. 453-476.

Astorino, T. A., Harness, E. T. and Witzke, K. A. (2013) 'Effect of chronic activity-based therapy on bone mineral density and bone turnover in persons with spinal cord injury', *Eur J Appl Physiol,* 113(12), pp. 3027-37.

Baierle, M., Nascimento, S. N., Moro, A. M., Brucker, N., Freitas, F., Gauer, B., Durgante, J., Bordignon, S., Zibetti, M., Trentini, C. M., Duarte, M. M. M. F., Grune, T., Breusing, N. and Garcia, S. C. (2015) 'Relationship between inflammation and oxidative stress and cognitive decline in the institutionalized elderly', *Oxidative Medicine and Cellular Longevity,* 2015, pp. 804198-804198.

Bakker, A. D. and Jaspers, R. T. (2015) 'IL-6 and IGF-1 Signaling Within and Between Muscle and Bone: How Important is the mTOR Pathway for Bone Metabolism?', *Current Osteoporosis Reports,* 13(3), pp. 131-139.

Barahona, M. J., Sucunza, N., Resmini, E., Fernandez-Real, J. M., Ricart, W., Moreno-Navarrete, J. M., Puig, T., Wagner, A. M., Rodriguez-Espinosa, J., Farrerons, J. and Webb, S. M. (2009) 'Deleterious effects of glucocorticoid replacement on bone in women after long-term remission of Cushing's syndrome', *J Bone Miner Res,* 24(11), pp. 1841-6.

Bartali, B., Semba, R. D., Frongillo, E. A., Varadhan, R., Ricks, M. O., Blaum, C. S., Ferrucci, L., Guralnik, J. M. and Fried, L. P. (2006) 'Low micronutrient levels as a predictor of incident disability in older women', *Arch Intern Med,* 166(21), pp. 2335-40.

Batai, K., Trejo, M. J., Chen, Y., Kohler, L. N., Lance, P., Ellis, N. A., Cornelis, M. C., Chow, H. H. S., Hsu, C.-H. and Jacobs, E. T. (2021) 'Genome-Wide Association Study of Response to Selenium Supplementation and Circulating Selenium Concentrations in Adults of European Descent', *The Journal of Nutrition,* 151(2), pp. 293-302.

Bates, C. J., Thane, C. W., Prentice, A. and Delves, H. T. (2002) 'Selenium status and its correlates in a British national diet and nutrition survey: people aged 65 years and over', *J Trace Elem Med Biol,* 16(1), pp. 1-8.

Bauer, D., Krege, J., Lane, N., Leary, E., Libanati, C., Miller, P., Myers, G. et al. (2012) 'National Bone Health Alliance Bone Turnover Marker Project: Current practices and the need for US harmonization, standardization, and common reference ranges', *Osteoporosis International*, 23(10), pp. 2425-2433.

Baumann, C. W., Kwak, D., Liu, H. M. and Thompson, L. V. (2016) 'Age-induced oxidative stress: how does it influence skeletal muscle quantity and quality?', *Journal of Applied Physiology,* 121(5), pp. 1047-1052.

Beaudart, C., Rolland, Y., Cruz-Jentoft, A. J., Bauer, J. M., Sieber, C., Cooper, C., et al. (2019) 'Assessment of Muscle Function and Physical Performance in Daily Clinical Practice : A position paper endorsed by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO)', *Calcif Tissue Int,* 105(1), pp. 1-14.

Beck M. A., Levander, O., and Handy, J. (2003) 'Selenium Deficiency and Viral Infection', *The Journal of Nutrition,* 133(5 Suppl 1). 1463S–7S

Beck, B. R., Daly, R. M., Singh, M. A. and Taaffe, D. R. (2017) 'Exercise and Sports Science Australia (ESSA) position statement on exercise prescription for the prevention and management of osteoporosis', *J Sci Med Sport,* 20(5), pp. 438-445.

Beck, J., Ferrucci, L., Sun, K., Walston, J., Fried, L. P., Varadhan, R., Guralnik, J. M. and Semba, R. D. (2007) 'Low serum selenium concentrations are associated with poor grip strength among older women living in the community', *BioFactors,* 29(1), pp. 37-44.

Becker, N.P., Martitz, J., Renko, K., Stoedter, M., Hybsier, S., Cramer, T. and Schomburg, L. (2014) 'Hypoxia reduces and redirects selenoprotein biosynthesis', *Metallomics,* 6(5), pp. 1079-1086.

Behne, D., Alber, D., and Kyriakopoulos, A. (2010) 'Long-term selenium supplementation of humans: Selenium status and relationships between selenium concentrations in skeletal muscle and indicator materials'*, J Trace Elem Med Biol*, 24(2), pp. 99-105.

Behne, D., Hilmert, H., Scheid, S., Gessner, H., and Elger, W. (1988) 'Evidence for specific selenium target tissues and new biologically important selenoproteins', *Biochimica et Biophysica Acta,* 966(1), pp. 12-21.

Bellinger, F. P., Raman, A. V., Reeves, M. A. and Berry, M. J. (2009) 'Regulation and function of selenoproteins in human disease', *The Biochemical Journal,* 422(1), pp. 11-22.

Berchtold, M. W., Brinkmeier, H. and Müntener, M. (2000) 'Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease', *Physiol Rev,* 80(3), pp. 1215-65.

Bergmann, P., Body, J. J., Boonen, S., Boutsen, Y., Devogelaer, J. P., Goemaere, S., Kaufman, J. M., Reginster, J. Y., and Gangji, V. (2009) 'Evidence-based guidelines for the use of biochemical markers of bone turnover in the selection and monitoring of bisphosphonate treatment in osteoporosis: A consensus document of the Belgian Bone Club', *Int J Clin Pract*, 63(1), pp. 19-26.

Bermano, G., Nicol, F., Dyer, J. A., Sunde, R. A., Beckett, G. J., Arthur, J. R. and Hesketh, J. E. (1995) 'Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats', *The Biochemical Journal,* 311(Pt 2), pp. 425-430.

Berr, C., Balansard, B., Arnaud, J., Roussel, A. M., and Alpérovitch, A. (2000) 'Cognitive decline is associated with systemic oxidative stress: The EVA study', *J Am Geriatr Soc*, 48(10), pp. 1285-1291.

Beukhof, C. M., Medici, M., van den Beld, A. W., Hollenbach, B., Hoeg, A., Visser, W. E., de Herder, W. W., Visser, T. J., Schomburg, L. and Peeters, R. P. (2016) 'Selenium Status Is Positively Associated with Bone Mineral Density in Healthy Aging European Men', *PLoS One,* 11(4), pp. e0152748.

Bian, A. L., Hu, H. Y., Rong, Y. D., Wang, J., Wang, J. X. and Zhou, X. Z. (2017) 'A study on relationship between elderly sarcopenia and inflammatory factors IL-6 and TNF-α', *Eur J Med Res,* 22(1), pp. 25.

Bleys, J., Navas-Acien, A., and Guallar, E. (2008) 'Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults', *Archives of internal medicine*, *168*(4), pp. 404–410.

Bodnár, D., Ruzsnavszky, O., Oláh, T., Dienes, B., Balatoni, I., Ungvári, É., Benkő, I., Babka, B., Prokisch, J., Csernoch, L. and Szentesi, P. (2016) 'Dietary selenium augments sarcoplasmic calcium release and mechanical performance in mice', *Nutrition and Metabolism,* 13, pp. 76-76.

Bodnar, M., Szczyglowska, M., Konieczka, P. and Namiesnik, J. (2016) 'Methods of Selenium Supplementation: Bioavailability and Determination of Selenium Compounds', *Critical Reviews in Food Science and Nutrition,* 56(1), pp. 36-55.

Bolland, M. J., Leung, W., Tai, V., Bastin, S., Gamble, G. D., Grey, A., and Reid, I. R. (2015) 'Calcium intake and risk of fracture: systematic review', *BMJ,* 351, pp. h4580.

Bonjour, J. P., Kohrt, W., Levasseur, R., Warren, M., Whiting, S., and Kraenzlin, M. (2014) 'Biochemical markers for assessment of calcium economy and bone metabolism: application in clinical trials from pharmaceutical agents to nutritional products', *Nutr Res Rev,* 27(2), pp. 252-67.

Borawska, M. H., Witkowska, A. M., Hukałowicz, K., and Markiewicz, R. (2004) 'Influence of Dietary Habits on Serum Selenium Concentration', *Annals of Nutrition and Metabolism,* 48(3), pp. 134-140.

Bortoli, A., Fazzin, G., Marchiori, M., Mello, F., Brugiolo, R., and Martelli, F. (1991). 'Selenium status and effect of selenium supplementation in a group of elderly women'. *Journal of Trace Elements and Electrolytes in Health and Disease*, 5(1), pp. 19-21 .

Bos, S. D., Kloppenburg, M., Suchiman, E., van Beelen, E., Slagboom, P. E., and Meulenbelt, I. (2009) 'The role of plasma cytokine levels, CRP and Selenoprotein S gene variation in OA', *Osteoarthritis Cartilage,* 17(5), pp. 621-6.

Bratakos, M. S., Zafiropoulos, T. F., Siskos, P. A., and Ioannou, P. V. (1988) 'Selenium losses on cooking Greek foods', *International Journal of Food Science & Technology,* 23(6), pp. 585-590.

Brigelius-Flohé, R. (1999) 'Tissue-specific functions of individual glutathione peroxidases', *Free Radic Biol Med*, 27(9-10), pp. 951-965.

Brigelius-Flohé, R., and Maiorino, M. (2013) 'Glutathione peroxidases', *Biochim* Biophys *Acta* 1830(5), pp. 3289-3303.

Bro, S. S., B., and Heydorn, K. (1990) 'Intake of essential and toxic trace elements in a random sample of Danish men as determined by the duplicate portion sampling technique', *J Trace Elem Electrolytes Health Dis,* 4(3), pp. 147-55.

roadley, M. R., White, P. J., Bryson, R. J., Meacham, M. C., Bowen, H. C., Johnson, S. E., Hawkesford, M. J., McGrath, S. P., Zhao, F. J., Breward, N., Harriman, M., & Tucker, M. (2006) 'Biofortification of UK food crops with selenium', *The Proceedings of the Nutrition Society*, 65(2), pp. 169-181.

Brodin, O., Hackler, J., Misra, S., Wendt, S., Sun, Q., Laaf, E., Stoppe, C., Björnstedt, M., and Schomburg, L. (2020) 'Selenoprotein P as biomarker of selenium status in clinical trials with therapeutic dosages of selenite', *Nutrients*, *12*(4), pp. 1067.

Broome, C. S., McArdle, F., Kyle, J. A., Andrews, F., Lowe, N. M., Hart, C. A., Arthur, J. R., and Jackson, M. J. (2004) 'An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status', *Am J Clin Nutr*, 80(1), pp. 154-162.

Brown, K. M., Pickard, K., Nicol, F., Beckett, G. J., Duthie, G. G., and Arthur, J. R. (2000) 'Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphoctyes, granulocytes, platelets and erythrocytes', *Clinical Science*, 98(5), pp. 593-599.

Brown, M. R., Cohen, H. J., Lyons, J. M., Curtis, T. W., Thunberg, B., Cochran, W. J. and Klish, W. J. (1986) 'Proximal muscle weakness and selenium deficiency associated with long term parenteral nutrition', *The American Journal of Clinical Nutrition,* 43(4), pp. 549-554.

Buchner, D. M., Larson, E. B., Wagner, E. H., Koepsell, T. D. and de Lateur, B. J. (1996) 'Evidence for a non-linear relationship between leg strength and gait speed', *Age Ageing,* 25(5), pp. 386-91.

Bunker, V. W., Lawson, M. S., Stansfield, M. F. and Clayton, B. E. (1988) 'Selenium balance studies in apparently healthy and housebound elderly people eating self-selected diets', *British Journal of Nutrition,* 59(2), pp. 171-180.

Burch, J., Rice, S., Yang, H., Neilson, A., Stirk, L., Francis, R., Holloway, P., Selby, P. and Craig, D. (2014) 'Systematic review of the use of bone turnover markers for monitoring the response to osteoporosis treatment: the secondary prevention of fractures, and primary prevention of fractures in high-risk groups', *Health Technol Assess,* 18(11), pp. 1-180.

Bureau, I., Anderson, R. A., Arnaud, J., Raysiguier, Y., Favier, A. E., and Roussel, A. M. (2002) 'Trace mineral status in post-menopausal women: Impact of hormonal replacement therapy', *Journal of Trace Elements in Medicine and Biology*, 16(1), pp. 9-13.

Burk, R. F., and Hill, K. E. (2015) 'Regulation of Selenium Metabolism and Transport', *Annu Rev Nutr*, 35, pp. 109-134.

Burk, R. F., Hill, K. E., and Motley, A. K. (2001) 'Plasma selenium in specific and non-specific forms', *Biofactors*, 14(1-4), pp. 107-114.

Burk, R. F., Hill, K. E., Motley, A. K., Winfrey, V. P., Kurokawa, S., Mitchell, S. L. and Zhang, W. (2014) 'Selenoprotein P and apolipoprotein E receptor-2 interact at the blood-brain barrier and also within the brain to maintain an essential selenium pool that protects against neurodegeneration', *Faseb j,* 28(8), pp. 3579-88.

Burk, R. F., Hill, K. E., Read, R., and Bellew, T. (1991) 'Response of rat selenoprotein P to selenium administration and fate of its selenium', *The American Journal of Physiology*, 261, pp. E26-E30.

Burk, R. F., and Hill, K. E. (2009) 'Selenoprotein P-Expression, functions, and roles in mammals', *Biochim Biophys Acta*, 1790(11), pp. 1441-1447.

Burk, R. F., Norsworthy, B. K., Hill, K. E., Motley, A. K. and Byrne, D. W. (2006) 'Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial', *Cancer Epidemiol Biomarkers Prev,* 15(4), pp. 804-10.

Cabral, M., Kuxhaus, O., Eichelmann, F., Kopp, J. F., Alker, W., Hackler, J., Kipp, A. P., Schwerdtle, T., Haase, H., Schomburg, L., and Schulze, M. B. (2021) 'Trace element profile and incidence of type 2 diabetes, cardiovascular disease and colorectal cancer: results from the EPIC-Potsdam cohort study', *European Journal of Nutrition*, *60*(6), pp. 3267–3278.

Campbell, D., Bunker, V. W., Thomas, A. J., and Clayton, B. E. (1989) 'Selenium and vitamin E status of healthy and institutionalized elderly subjects: analysis of plasma, erythrocytes and platelets', *Br J Nutr,* 62(1), pp. 221-7.

Cao, J. J., Gregoire, B. R., and Zeng, H. (2012) 'Selenium deficiency decreases antioxidative capacity and is detrimental to bone microarchitecture in mice', *J Nutr,* 142(8), pp. 1526-31.

Castets, P., Maugenre, S., Gartioux, C., Rederstorff, M., Krol, A., Lescure, A., Tajbakhsh, S., Allamand, V. and Guicheney, P. (2009) 'Selenoprotein N is dynamically expressed during mouse development and detected early in muscle precursors', *BMC Developmental Biology*, 9, pp. 46.

Cawthon, P. M., Fullman, R. L., Marshall, L., Mackey, D. C., Fink, H. A., Cauley, J. A., Cummings, S. R., Orwoll, E. S., Ensrud, K. E. and Osteoporotic Fractures in Men Research, G. (2008) 'Physical performance and risk of hip fractures in older men', *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research,* 23(7), pp. 1037-1044.

Cervellati, C., Bonaccorsi, G., Cremonini, E., Romani, A., Fila, E., Castaldini, M. C., Ferrazzini, S., Giganti, M. and Massari, L. 'Oxidative stress and bone resorption interplay as a possible trigger for postmenopausal osteoporosis', *Biomed Res Int*, (2314-6141 (Electronic)).

Cesari, M., Kritchevsky, S. B., Newman, A. B., Simonsick, E. M., Harris, T. B., Penninx, B. W., Brach, J. S., Tylavsky, F. A., Satterfield, S. and Bauer, D. C. (2009) 'Added value of physical performance measures in predicting adverse health-related events: results from the Health, Aging and Body Composition Study', *Journal of the American Geriatrics Society,* 57(2), pp. 251-259.

Cesari, M., Penninx, B. W., Pahor, M., Lauretani, F., Corsi, A. M., Rhys Williams, G., Guralnik, J. M. and Ferrucci, L. (2004) 'Inflammatory markers and physical performance in older persons: the InCHIANTI study', *J Gerontol A Biol Sci Med Sci,* 59(3), pp. 242-8.

Chan, R., Woo, J., Lau, W., Leung, J., Xu, L., Zhao, X., Yu, W., Lau, E. and Pocock, N. (2009) 'Effects of Lifestyle and Diet on Bone Health in Young Adult Chinese Women Living in Hong Kong and Beijing', *Food and Nutrition Bulletin,* 30(4), pp. 370-378.

Chandola, T., and Jenkinson, C. (2000) 'The new UK national statistics socio-economic classification (NS-SEC); investigating social class differences in self-reported health status', *Journal of Public Health,* 22(2), pp. 182-190.

Chaput, J. P., Lord, C., Cloutier, M., Aubertin Leheudre, M., Goulet, E. D., Rousseau, S., Khalil, A., and Dionne, I. J. (2007) 'Relationship Between Antioxidant Intakes and Class I Sarcopenia in Elderly Men and Women', *The Journal of Nutrition, Health & Aging,* 11(4), pp. 363-369.

Chariot, P. and Bignani, O. (2003) 'Skeletal muscle disorders associated with selenium deficiency in humans', *Muscle & Nerve,* 27(6), pp. 662-668.

Chen, X., Lei, L., Tian, L., Zhu, G. and Jin, T. (2012) 'Bone mineral density and polymorphisms in metallothionein 1A and 2A in a Chinese population exposed to cadmium', *Sci Total Environ,* 423, pp. 12-7.

Chen, Y. L. Yang, K. C., Chang, H. H., Lee, L. T,. Lu, C. W., Huang, K. C., (2014) 'Low Serum Selenium Level Is Associated With Low Muscle Mass inthe Community-Dwelling Elderly', 15(11), pp. 807-811.

Cheng, A. W. M., Stabler, T. V., Bolognesi, M. and Kraus, V. B. (2011) 'Selenomethionine inhibits IL-1b2; inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) expression in primary human chondrocytes', *Osteoarthritis and Cartilage,* 19(1), pp. 118-125.

Chiu, K.L., Lee, C.C. and Chen, C.Y. (2021) 'Evaluating the association of osteoporosis with inhaled corticosteroid use in chronic obstructive pulmonary disease in Taiwan', *Scientific Reports,* 11(1), pp. 724.

Christensen, M. J., Nartey, E. T., Hada, A. L., Legg, R. L., and Barzee, B. R. (2007) 'High selenium reduces NF-κB-regulated gene expression in uninduced human prostate cancer cells', *Nutrition and Cancer*, 58(2), pp. 197-204.

Christgau, S. (2000) 'Circadian variation in serum CrossLaps concentration is reduced in fasting individuals', *Clin Chem,* 46(3), pp. 431.

Chrousos, G. P. (2009) 'Stress and disorders of the stress system', *Nat Rev Endocrinol,* 5(7), pp. 374-81.

Chung, Y. W., Kim, T. S., Lee, S. Y., Lee, S. H., Choi, Y., Kim, N., Min, B. M., Jeong, D. W., and Kim, I. Y. (2006) 'Selenite-induced apoptosis of osteoclasts mediated by the mitochondrial pathway', 160(2), pp. 143-150.

Chung, S. S., Kim, M., Youn, B. S., Lee, N. S., Park, J. W., Lee, I. K., Lee, Y. S., Kim, J. B., Cho, Y. M., Lee, H. K., and Park, K. S. (2009). 'Glutathione peroxidase 3 mediates the antioxidant effect of peroxisome proliferator-activated receptor gamma in human skeletal muscle cells', *Molecular and cellular biology*, *29*(1), pp. 20–30.

Churchward-Venne, T. A., Breen, L. and Phillips, S. M. (2014) 'Alterations in human muscle protein metabolism with aging: Protein and exercise as countermeasures to offset sarcopenia', *Biofactors,* 40(2), pp. 199-205.

Clark, L. C., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Chalker, D. K., Chow, J., Davis, L. S., Glover, R. A., Graham, G. F., Gross, E. G., Krongrad, A., Lesher, J. L., Jr., Park, H. K., Sanders, B. B., Jr., Smith, C. L. and Taylor, J. R. (1996) 'Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group', *JAMA,* 276(24), pp. 1957-63.

Clausen, J. and Nielsen, S. A. (1988) 'Comparison of whole-blood selenium values and erythrocyte glutathione peroxidase activities of normal individuals on supplementation with selenate, selenite, L-selenomethionine, and high selenium yeast', *Biol Trace Elem Res,* 15, pp. 125-38.

Clayton, D. J., James, L. J., Sale, C., Templeman, I., Betts, J. A., and Varley, I. (2020) 'Severely restricting energy intake for 24 h does not affect markers of bone metabolism at rest or in response to re-feeding', *European journal of nutrition*, *59*(8), pp. 3527–3535.

Clegg, M. E. and Williams, E. A. (2018) 'Optimizing nutrition in older people', *Maturitas,* 112, pp. 34-38.

Clinical Practice Research Datalink (2006-2015). https://cprd.com/ Accessed 12/11/22

Clowes, J. A., Hannon, R. A., Yap, T. S., Hoyle, N. R., Blumsohn, A. and Eastell, R. (2002) 'Effect of feeding on bone turnover markers and its impact on biological variability of measurements', *Bone,* 30(6), pp. 886-90.

Cohen, S., Nathan, J. A. and Goldberg, A. L. (2015) 'Muscle wasting in disease: molecular mechanisms and promising therapies', *Nature Reviews Drug Discovery,* 14(1), pp. 58-74.

Cold, F., Winther, K. H., Pastor-Barriuso, R., Rayman, M. P., Guallar, E., Nybo, M., Griffin, B. A., Stranges, S. and Cold, S. (2015) 'Randomised controlled trial of the effect of long-term selenium supplementation on plasma cholesterol in an elderly Danish population', *Br J Nutr,* 114(11), pp. 1807-18.

Collerton, J., Barrass, K., Bond, J., Eccles, M., Jagger, C., James, O., Martin-Ruiz, C., Robinson, L., von Zglinicki, T. and Kirkwood, T. (2007) 'The Newcastle 85+ study: biological, clinical and psychosocial factors associated with healthy ageing: study protocol', *BMC Geriatr,* 7, pp. 14.

Collerton, J., Davies, K., Jagger, C., Kingston, A., Bond, J., Eccles, M. P., Robinson, L. A., Martin-Ruiz, C., von Zglinicki, T., James, O. F. W. and Kirkwood, T. B. L. (2009) 'Health and disease in 85 year olds: baseline findings from the Newcastle 85+ cohort study', *BMJ (Clinical research ed.),* 339, pp. b4904-b4904.

Combs, G. F. (1988) 'Selenium in Foods', Selenium in foods. *Advances in food research*, 32, pp. 85-113.

Combs, G. F. (2001) 'Selenium in global food systems', *Br J Nutr,* 85(5), pp. 517-47.

Combs, G. F. (2015) 'Biomarkers of selenium status', *Nutrients*, 7(4), pp. 2209-2236.

Combs, G. F., Jr, Jackson, M. I., Watts, J. C., Johnson, L. K., Zeng, H., Idso, J., Schomburg, L., Hoeg, A., Hoefig, C. S., Chiang, E. C., Waters, D. J., Davis, C. D., and Milner, J. A. (2012)

'Differential responses to selenomethionine supplementation by sex and genotype in healthy adults.', *The British journal of nutrition*, 107(10), 1514–1525.

Combs, G., and Combs, S. B. (1986) 'The Role of Selenium in Nutrition'. Academic Press, New York. ISBN: 9780323146531.

Cosman, F., de Beur, S. J., LeBoff, M. S., Lewiecki, E. M., Tanner, B., Randall, S., Lindsay, R. and National Osteoporosis, F. (2014) 'Clinician's Guide to Prevention and Treatment of Osteoporosis', *Osteoporosis international*, 25(10), pp. 2359-2381.

Cruz-Jentoft, A. J., Baeyens, J. P., Bauer, J. M., Boirie, Y., Cederholm, T., Landi, F., Martin, F. C., Michel, J.P., Rolland, Y. and Schneider, S. M. (2010) 'Sarcopenia: European consensus on definition and diagnosis Report of the European Working Group on Sarcopenia in Older PeopleA. J. Cruz-Gentoft et al', *Age and ageing,* 39(4), pp. 412-423.

Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A. A., Schneider, S. M., Sieber, C. C., Topinkova, E., Vandewoude, M., Visser, M., Zamboni, M., Writing Group for the European Working Group on Sarcopenia in Older, P. and the Extended Group for European Working Group on Sarcopenia in Older People 2. (2019) 'Sarcopenia: revised European consensus on definition and diagnosis', *Age and ageing,* 48(1), pp. 16-31.

Cusack, S. and Cashman, K. D. (2003) 'Impact of genetic variation on metabolic response of bone to diet', *Proceedings of the Nutrition Society,* 62(4), pp. 901-912.

Daniels, L. A. (2004) 'Selenium: does selenium status have health outcomes beyond overt deficiency?', *Med J Aust,* 180(8), pp. 373-4.

Davies, K., Kingston, A., Robinson, L., Hughes, J., Hunt, J. M., Barker, S. A. H., Edwards, J., Collerton, J., Jagger, C. and Kirkwood, T. B. L. (2014) 'Improving retention of very old participants in longitudinal research: experiences from the Newcastle 85+ study', *PloS one,* 9(10), pp. e108370-e108370.

de Jong, N., Gibson, R. S., Thomson, C. D., Ferguson, E. L., McKenzie, J. E., Green, T. J. and Horwath, C. C. (2001) 'Selenium and Zinc Status Are Suboptimal in a Sample of Older New Zealand Women in a Community-Based Study', *The Journal of Nutrition,* 131(10), pp. 2677-2684.

De Laet, C. E. D. H., Van Hout, Ben A., Burger, Huibert, Hofman, Albert, Pols, and Huibert A.P (1997) 'Bone density and risk of hip fracture in men and women: Cross sectional analysis', *BMJ*, 315(7102), pp. 221-225.

Deagen, J. T., Butler, J. A., Zachara, B. A., and Whanger, P. D (1993) 'Determination of the distribution of selenium between glutathione peroxidase, selenoprotein P, and albumin in plasma', *Analytical biochemistry,* 208(1), pp. 176-181.

Delesalle, C., de Bruijn, M., Wilmink, S., Vandendriessche, H., Mol, G., Boshuizen, B., Plancke, L. and Grinwis, G. (2017) 'White muscle disease in foals: focus on selenium soil content. A case series', *BMC Veterinary Research,* 13(1), pp. 121.

Delmas, P. D., Malaval, L., Arlot, M. E., and Meunier, P. J. (1985) 'Serum bone gal-protein compared to bone histomorphometry in endocrine diseases', *Bone*, 6(5), pp. 329-41.

Delmas, P. D., Stenner, D., Wahner, H. W., Mann, K. G., and Riggs, B. L. (1983) 'Serum bone gla-protein increases with ageing in normal women: implications for the mechanism of age-related bone loss', *The Journal of Clinical Investigation*, 71(5), pp. 1316-21.

Demircan, K., Bengtsson, Y., Sun, Q., Brange, A., Vallon-Christersson, J., Rijntjes, E., Malmberg, M., Saal, L. H., Rydén, L., Borg, Å., Manjer, J. and Schomburg, L. (2021) 'Serum selenium, selenoprotein P and glutathione peroxidase 3 as predictors of mortality and recurrence following breast cancer diagnosis: A multicentre cohort study', *Redox Biology,* 47, pp. 102145.

Deng, H. W., Xu, F. H., Huang, Q. Y., Shen, H., Deng, H., Conway, T., Liu, Y. J., Liu, Y. Z., Li, J. L., Zhang, H. T., Davies, K. M. and Recker, R. R. (2002) 'A whole-genome linkage scan suggests several genomic regions potentially containing quantitative trait loci for osteoporosis', *Journal of Clinical Endocrinology and Metabolism,* 87(11), pp. 5151-5159.

Department of Health (1991) 'Dietary Reference Values for Food and Energy and Nutrients in the UK, Report on Health and Social Subjects ', *41. London: HMSO.*

Devogelaer, J.P., Durnez, A., Gruson, D., and Manicourt, D. H., (2017) 'Bone Turnover Markers and Glucocorticoid Treatments' in Biomarkers in Bone Disease, pp. 905-932, ISBN : 978-94-007-7692-0.

Devoto, M., Shimoya, K., Caminis, J., Ott, J., Tenenhouse, A., Whyte, M. P., Sereda, L., Hall, S., Considine, E., Williams, C. J., Tromp, G., Kuivaniemi, H., Ala-Kokko, L., Prockop, D. J. and Spotila, L. D. (1998) 'First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p and 4q', *European Journal of Human Genetics,* 6(2), pp. 151-157.

Diderichsen, F., Manuel, I. A. C., Andersen, AN., Bach, E., and Baadsgaard, M. (2012) 'Health Inequality - determinants and policies', 40, pp. 12-105.

Di Micco, R., Krizhanovsky, V., Baker, D. and d'Adda di Fagagna, F. (2021) 'Cellular senescence in ageing: from mechanisms to therapeutic opportunities', *Nature reviews. Molecular cell biology,* 22(2), pp. 75-95.

Dikiy, A., Novoselov, S. V., Fomenko, D. E., Sengupta, A., Carlson, B. A., Cerny, R. L., Ginalski, K., Grishin, N. V., Hatfield, D. L. and Gladyshev, V. N. (2007) 'SelT, SELENOW, SelH, and Rdx12: genomics and molecular insights into the functions of selenoproteins of a novel thioredoxin-like family', *Biochemistry,* 46(23), pp. 6871-82.

Dodds, R. M., Granic, A., Davies, K., Kirkwood, T. B. L., Jagger, C. and Sayer, A. A. (2017) 'Prevalence and incidence of sarcopenia in the very old: findings from the Newcastle 85+ Study', *Journal of Cachexia, Sarcopenia and Muscle,* 8(2), pp. 229-237.

Downey, C. M., Horton, C. R., Carlson, B. A., Parsons, T. E., Hatfield, D. L., Hallgrímsson, B. and Jirik, F. R. (2009) 'Osteo-Chondroprogenitor–Specific Deletion of the Selenocysteine tRNA Gene, Trsp, Leads to Chondronecrosis and Abnormal Skeletal Development: A Putative Model for Kashin-Beck Disease', *PLOS Genetics,* 5(8), pp. e1000616.

Dreher, I., Schmutzler, C., Jakob, F. and Köhrle, J. (1997) 'Expression of selenoproteins in various rat and human tissues and cell lines', *J Trace Elem Med Biol,* 11(2), pp. 83-91.

Dreher, I., Schütze, N., Baur, A., Hesse, K., Schneider, D., Köhrle, J., and Jakob, F. (1998) 'Selenoproteins are expressed in fetal human osteoblast-like cells', *Biochemical and biophysical research communications,* 245(1), pp. 101-107.

Drutel, A., Archambeaud, F. and Caron, P. (2013) 'Selenium and the thyroid gland: more good news for clinicians', *Clinical Endocrinology,* 78(2), pp. 155-164.

Dubois, F., Teby, A., Belleville, F., Nabet, P., Paysant, P. (1990) 'Valeurs usuelles du sélénium et de la glutathion peroxydase dans une population belge.', 48, pp. 28-32.

Duffield, A. J., Thomson, C. D., Hill, K. E. and Williams, S. (1999) 'An estimation of selenium requirements for New Zealanders', *Am J Clin Nutr,* 70(5), pp. 896-903.

Duffield-Lillico, A. J., Dalkin, B. L., Reid, M. E., Turnbull, B. W., Slate, E. H., Jacobs, E. T., Marshall, J. R., Clark, L. C. and Nutritional Prevention of Cancer Study, G. (2003) 'Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial', *BJU Int,* 91(7), pp. 608-12.

Dumont, E., Vanhaecke, F. and Cornelis, R. (2006) 'Selenium speciation from food source to metabolites: a critical review', *Anal Bioanal Chem,* 385(7), pp. 1304-23.

Duncan, R., Francis, R. M., Jagger, C., Kingston, A., McCloskey, E., Collerton, J., Robinson, L., Kirkwood, T. B. L. and Birrell, F. (2015) 'Magnitude of fragility fracture risk in the very old—are we meeting their needs? The Newcastle 85+ Study', *Osteoporosis International,* 26(1), pp. 123-130.

Dzhagaryan, A., Milenkovic, A., Jovanov, E. and Milosevic, M. (2015) Smart Button: A Wearable System for Assessing Mobility in Elderly. *17th International Conference on E-health Networking, Application & Services (HealthCom),* 416-421.

Eastell, R., Christiansen, C., Grauer, A., Kutilek, S., Libanati, C., McClung, M. R., Reid, I. R., Resch, H., Siris, E., Uebelhart, D., Wang, A., Weryha, G., and Cummings, S. R. (2011) 'Effects of denosumab on bone turnover markers in postmenopausal osteoporosis', 26(3), pp. 530-537.

Eastell, R., Pigott, T., Gossiel, F., Naylor, K. E., Walsh, J. S. and Peel, N. F. A. (2018) 'Diagnosis of Endocrine Disease: Bone turnover markers: are they clinically useful?', *European Journal of Endocrinology,* 178(1), pp. R19-R31.

Ebert, R., Ulmer, M., Zeck, S., Meissner-Weigl, J., Schneider, D., Stopper, H., Schupp, N., Kassem, M., and Jakob, F. (2006) 'Selenium Supplementation Restores the Antioxidative Capacity and Prevents Cell Damage in Bone Marrow Stromal Cells In Vitro', *Stem Cells*, 24(5), pp. 1226-1235.

EFSA (2014) 'Scientific Opinion on Dietary Reference Values for selenium', *European Food Safety Authority Journal,* 12(10):3846

EFSA (2023) 'Scientific opinion on the tolerable upper intake level for selenium', *European Food Safety Authority Journal*, 21(1):7704.

ELSA (2018) 'The English Longitudinal Study of Ageing (ELSA)'. https://www.elsa-project.ac.uk/

Elsom, R., Sanderson, P., Hesketh, J. E., Jackson, M. J., Fairweather-Tait, S. J., Akesson, B., Handy, J., and Arthur, J. R. (2006) 'Functional markers of selenium status: UK Food Standards Agency workshop report', 96(5), pp. 980-984.

EPIC (2015) 'The EPIC-Oxford Study: European Prospective Investigation of Cancer'. https://www.ceu.ox.ac.uk/research/epic-oxford-1 Accessed on: 10/11/22

Espinoza, S. E., Guo, H., Fedarko, N., DeZern, A., Fried, L. P., Xue, Q.-L., Leng, S., Beamer, B. and Walston, J. D. (2008) 'Glutathione peroxidase enzyme activity in aging', *The Journals of Gerontology,* 63(5), pp. 505-509.

European Parliament (2002) 'Danish Economy', *European Parliament.* https://www.europarl.europa.eu/RegData/etudes/note/join/2002/290605/DG-4-ECON_NT(2002)290605_EN.pdf Accessed on: 05/01/22

Evans, D. M., and Ralston, S. H. (1996) 'Nitric oxide and bone', *J Bone Miner Res,* 11(3), pp. 300-5.

Ewan, R. C. (1976) 'Effect of selenium on rat growth, growth hormone and diet utilization', *J Nutr,* 106(5), pp. 702-9.

Fordyce, F. M., Hughes, J., Reay, G., Thomas, L., Walker, A., Luo, W., and Lewis, J. (2009) 'The Selenium Content of Scottish Soil and Food Products', *COMA. FSA Scotland*.

Fairweather-Tait, S. J. (1997) 'Bioavailability of selenium', *Eur J Clin Nutr,* 51(Suppl 1), pp. S20-3.

Fairweather-Tait, S. J., Bao, Y., Broadley, M. R., Collings, R., Ford, D., Hesketh, J. E., and Hurst, R. (2010) 'Selenium in Human Health and Disease', *Antioxidants & Redox Signaling,* 14(7), pp. 1337-1383.

Fairweather-Tait, S. J., Collings, R., and Hurst, R. (2010) 'Selenium bioavailability: current knowledge and future research requirements', *The American journal of clinical nutrition*, *91*(5), pp. 1484S-1491S.

Faulkner, J. A., Larkin, L. M., Claflin, D. R., and Brooks, S. V. (2007) 'Age-related changes in the structure and function of skeletal muscles', *Clinical and experimental pharmacology & physiology*, *34*(11), 1091–1096.

Fedacko, J., Pella, D., Fedackova, P. Hänninen, O., Tuomainen, P., Jarcuska, P., Lopuchovsky, T., Jedlickova, L., Merkovska, L., and Littarru, G. P. (2013) 'Coenzyme Q(10) and selenium in statin-associated myopathy treatment', *Can J Physiol Pharmacol,* 91(2), pp. 165-70.

Felson, D. T., Naimark, A., Anderson, J., Kazis, L., Castelli, W. and Meenan, R. F. (1987) 'The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study', *Arthritis Rheum,* 30(8), pp. 914-8.

Ferrari, S. L., Ahn-Luong, L., Garnero, P., Humphries, S. E., and Greenspan, S. L. (2003) 'Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women', *J Clin Endocrinol Metab,* 88(1), pp. 255-9.

Ferreira, R. L. U., Sena-Evangelista, K. C. M., de Azevedo, E. P., Pinheiro, F. I., Cobucci, R. N. and Pedrosa, L. F. C. (2021) 'Selenium in Human Health and Gut Microflora: Bioavailability of Selenocompounds and Relationship With Diseases', *Frontiers in Nutrition,* 8, 685317.

Finley, J. W. (2006) 'Bioavailability of selenium from foods'*, Nutr Rev*, 64, pp. 146-151.

Fischer, J., Bosse, A. and Pallauf, J. (2008) 'Effect of selenium deficiency on the antioxidative status and muscle damage in growing turkeys', *Arch Anim Nutr,* 62(6), pp. 485-97.

Flynn, A., Hirvonen, T., Mensink, G. B., Ocké, M. C., Serra-Majem, L., Stos, K., Szponar, L., Tetens, I., Turrini, A., Fletcher, R. and Wildemann, T. (2009) 'Intake of selected nutrients from foods, from fortification and from supplements in various European countries', *Food Nutr Res,* 12, pp. 53.

Fodor, J., Al-Gaadi, D., Czirják, T., Oláh, T., Dienes, B., Csernoch, L., and Szentesi, P. (2020). 'Improved Calcium Homeostasis and Force by Selenium Treatment and Training in Aged Mouse Skeletal Muscle', *Scientific reports*, *10*(1), 1707.

Fontana, L., Partridge, L. and Longo, V. D. (2010) 'Extending Healthy Life Span—From Yeast to Humans', *Science,* 328(5976), pp. 321-326.

Forceville, X., Vitoux, D., Gauzit, R., Combes, A., Lahilaire, P. and Chappuis, P. (1998) 'Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients', *Crit Care Med,* 26(9), pp. 1536-44.

Fordyce, F. (2007) 'Selenium geochemistry and health', *Ambio,* 36(1), pp. 94-7.

Fordyce, F. (2013) 'Selenium deficiency and toxicity in the environment'. In: Selinus, O. (eds) Essentials of Medical Geology. Springer, Dordrecht, pp. 375–416.

Francis, P., Lyons, M., Piasecki, M., Mc Phee, J., Hind, K. and Jakeman, P. (2017) 'Measurement of muscle health in aging', *Biogerontology,* 18(6), pp. 901-911.

Freiberger, E., Sieber, C., and Pfeifer, K. (2011) 'Physical activity, exercise, and sarcopenia - Future challenges', *Wiener medizinische Wochenschrift,* 161(17-18), pp. 416-425.

Galvez-Fernandez, M., Grau-Perez, M., Garcia-Barrera, T., Ramirez-Acosta, S., Gomez-Ariza, J. L., Perez-Gomez, B., Galan-Labaca, I., Navas-Acien, A., Redon, J. and Briongos-Figuero, L. S. (2021) 'Arsenic, cadmium, and selenium exposures and bone mineral density-related endpoints: The HORTEGA study', *Free Radical Biology and Medicine,* 162, pp. 392-400.

Gámez, C., Ruiz-López, D., Artacho, R., Navarro, M., Puerta, A. and López, C. (1997) 'Serum selenium in institutionalized elderly subjects and relation to other nutritional markers', *Clinical chemistry,* 43(4), pp. 693-694.

Ganapathy, A., and Nieves, J. W. (2020). 'Nutrition and Sarcopenia-What Do We Know?', *Nutrients*, *12*(6), 1755.

Gao, J., Nie, W., Wang, F. and Guo, Y. (2018) 'Maternal Selenium Supplementation Enhanced Skeletal Muscle Development Through Increasing Protein Synthesis and SELENOW mRNA Levels of their Offspring', *Biol Trace Elem Res,* 186(1), pp. 238-248.

García-Esquinas, E., Carrasco-Rios, M., Ortolá, R., Sotos Prieto, M., Pérez-Gómez, B., Gutiérrez-González, E., Banegas, J. R., Queipo, R., Olmedo, P., Gil, F., Tellez-Plaza, M., Navas-Acien, A., Pastor-Barriuso, R. and Rodríguez-Artalejo, F. (2021) 'Selenium and impaired physical function in US and Spanish older adults', *Redox Biology,* 38, pp. 101819.

Garnero, P., Sornay-Rendu, E., Chapuy, M.C., and Delmas, P.D. (1996) 'Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis', *Journal of bone and mineral research,* 11(3), pp. 337-349.

Garrett, I. R., Boyce, B. F., Oreffo, R. O.C., Bonewald, L., Poser, J., Mundy, G. R. (1990) 'Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo', *Journal of Clinical Investigation,* 85(3), pp. 632-639.

Ghayour-Mobarhan, M., Taylor, A., New, S. A., Lamb, D. J. and Ferns, G. A. (2005) 'Determinants of serum copper, zinc and selenium in healthy subjects', *Ann Clin Biochem,* 42(Pt 5), pp. 364-75.

Gill, T. M., Gahbauer, E. A., Lin, H., Han, L. and Allore, H. G. (2013) 'Comparisons between older men and women in the trajectory and burden of disability over the course of nearly 14 years', *J Am Med Dir Assoc,* 14(4), pp. 280-6.

Gimble, J. M., Zvonic, S., Floyd, Z. E., Kassem, M. and Nuttall, M. E. (2006) 'Playing with bone and fat', *J Cell Biochem,* 98(2), pp. 251-66.

Giorgi, C., Marchi, S., Simoes, I. C. M., Ren, Z., Morciano, G., Perrone, M., et al. (2018) 'Mitochondria and Reactive Oxygen Species in Aging and Age-Related Diseases', *International review of cell and molecular biology,* 340, pp. 209-344.

Giovannini, S., Onder, G., Lattanzio, F., Bustacchini, S., Di Stefano, G., Moresi, R., Russo, A., Bernabei, R., and Landi, F. (2018) 'Selenium Concentrations and Mortality among Community-Dwelling Older Adults: Results from IlSIRENTE Study', *The journal of nutrition, health & aging*, *22*(5), pp. 608–612.

Gladyshev, V. N., Arnér, E. S., Berry, M. J., Brigelius-Flohé, R., Bruford, E. A., Burk, R. F., et al. (2016) 'Selenoprotein Gene Nomenclature', *The Journal of biological chemistry*, 291(46), pp. 24036–24040.

Glass, T. A. (1998) 'Conjugating the "tenses" of function: Discordance among hypothetical, experimental, and enacted function in older adults', *The Gerontologist,* 38(1), pp. 101-112.

Glover, S. J., Eastell, R., McCloskey, E. V., Rogers, A., Garnero, P., Lowery, J., Belleli, R., Wright, T. M., and John, M. R. (2009) 'Rapid and robust response of biochemical markers of bone formation to teriparatide therapy', 45(6), pp. 1053-1058.

Gobbens, R. J. and van Assen, M. A. (2014) 'The Prediction of ADL and IADL Disability Using Six Physical Indicators of Frailty: A Longitudinal Study in the Netherlands', *Curr Gerontol Geriatr Res,* 2014, pp. 358137.

Goenawan, H., Daba, M., Murniati Tarawan, V., Lesmana, R., Mardanarian Rosdianto, A., and Nur Fatimah, S. (2022) 'Selenium Supplementation Alters IL-1β and IL-6 Protein Levels in Contusion Model Rats', *Pak J Biol Sci,* 25(3), pp. 226-233.

Goldberg, G. R., Black, A. E., Jebb, S. A., Cole, T. J., Murgatroyd, P. R., Coward, W. A. and Prentice, A. M. (1991) 'Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording', *Eur J Clin Nutr,* 45(12), pp. 569-81.

González, S., Huerta, José M., Fernández, Serafina, Patterson, Ángeles M., Lasheras, Cristina (2006) 'Food intake and serum selenium concentration in elderly people', *Annals of nutrition & metabolism,* 50(2), pp. 126-131.

Granic, A., Mendonça, N., Sayer, A. A., Hill, T. R., Davies, K., Adamson, A., Siervo, M., Mathers, J. C. and Jagger, C. (2018) 'Low protein intake, muscle strength and physical performance in the very old: The Newcastle 85+ Study', *Clinical nutrition (Edinburgh, Scotland),* 37(6 Pt A), pp. 2260-2270.

Gray, S. L., Hanlon, J. T., Landerman, L. R., Artz, M., Schmader, K. E. and Fillenbaum, G. G. (2003) 'Is antioxidant use protective of cognitive function in the community-dwelling elderly?', *Am J Geriatr Pharmacother,* 1(1), pp. 3-10.

Greene, B. R., Doheny, E. P., O'Halloran, A. and Anne Kenny, R. (2013) 'Frailty status can be accurately assessed using inertial sensors and the TUG test', *Age and Ageing,* 43(3), pp. 406-411.

Grosch, M., Fuchs, J., Bösl, M., Winterpacht, A. and Tagariello, A. (2013) 'Selenoprotein M is expressed during bone development', *EXCLI journal,* 12, pp. 967-979.

Groth, M. V., Fagt, S. and Brondsted, L. (2001) 'Social determinants of dietary habits in Denmark', *Eur J Clin Nutr,* 55(11), pp. 959-66.

Groth, M. V., Fagt, S., Stockmarr, A., Matthiessen, J. and Biltoft-Jensen, A. (2009) 'Dimensions of socioeconomic position related to body mass index and obesity among Danish women and men', *Scandinavian journal of public health,* 37(4), pp. 418-426.

Groth, M. V., Sorensen, M. R., Matthiessen, J., Fagt, S., Landvad, N. and Knudsen, V. K. (2014) 'Disparities in dietary habits and physical activity in Denmark and trends from 1995 to 2008', *Scand J Public Health,* 42(7), pp. 611-20.

Grumolato, L., Ghzili, H., Montero-Hadjadje, M., Gasman, S., Lesage, J., Tanguy, Y., Galas, L., Ait-Ali, D., Leprince, J., Guérineau, N. C., Elkahloun, A. G., Fournier, A., Vieau, D., Vaudry, H. and Anouar, Y. (2008) 'Selenoprotein T is a PACAP-regulated gene involved in intracellular Ca2+ mobilization and neuroendocrine secretion', *Faseb j,* 22(6), pp. 1756-68.

Guo, X., Ma, W. J., Zhang, F., Ren, F. L., Qu, C. J., and Lammi, M. J. (2014) 'Recent advances in the research of an endemic osteochondropathy in China: Kashin-Beck disease', *Osteoarthritis and cartilage,* 22(11), pp. 1774-1783.

Guralnik, J. M., Ferrucci, L., Pieper, C. F., Leveille, S. G., Markides, K. S., Ostir, G. V., Studenski, S., Berkman, L. F. and Wallace, R. B. (2000) 'Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery', *J Gerontol A Biol Sci Med Sci,* 55(4), pp. M221-31.

Guralnik, J. M., Simonsick, E. M., Ferrucci, L., Glynn, R. J., Berkman, L. F., Blazer, D. G., Scherr, P. A. and Wallace, R. B. (1994) 'A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission', *J Gerontol,* 49(2), pp. M85-94.

Ha, E. J. and Smith, A. M. (2003) 'Plasma selenium and plasma and erythrocyte glutathione peroxidase activity increase with estrogen during the menstrual cycle', *J Am Coll Nutr,* 22(1), pp. 43-51.

Hakeem, S., Mendonca, N., Aspray, T., Kingston, A., Ruiz-Martin, C., Jagger, C., Mathers, J. C., Duncan, R. and Hill, T. R. (2020) 'The Association between 25-Hydroxyvitamin D Concentration and Disability Trajectories in Very Old Adults: The Newcastle 85+ Study', *Nutrients,* 12(9).

Hannon, R. and Eastell, R. (2000) 'Preanalytical variability of biochemical markers of bone turnover', *Osteoporos Int,* 11 Suppl 6, pp. S30-44.

Hargreaves, M. K., Liu, J., Buchowski, M. S., Patel, K. A., Larson, C. O., Schlundt, D. G., Kenerson, D. M., Hill, K. E., Burk, R. F. and Blot, W. J. (2014) 'Plasma selenium biomarkers in low income black and white americans from the southeastern United States', *PLoS One,* 9(1), pp. e84972.

Hariharan, S., and Dharmaraj, S. (2020) 'Selenium and selenoproteins: it's role in regulation of inflammation', *Inflammopharmacology,* 28(3), pp. 667-695.

Harmer, D., Falank, C., and Reagan, M. R (2018) 'Interleukin-6 Interweaves the Bone Marrow Microenvironment, Bone Loss, and Multiple Myeloma', *Front Endocrinol (Lausanne),* 9, pp. 788.

Hartley, P., Monaghan, A., Donoghue, O. A., Kenny, R. A., and Romero-Ortuno, R. (2022). 'Exploring bi-directional temporal associations between timed-up-and-go and cognitive domains in the Irish longitudinal study on ageing (TILDA)', *Archives of gerontology and geriatrics*, *99*, pp.104611.

Hauffe, R., Stein, V., Chudoba, C., Flore, T., Rath, M., Ritter, K., Schell, M., Wardelmann, K., Deubel, S., Kopp, J. F., Schwarz, M., Kappert, K., Blüher, M., Schwerdtle, T., Kipp, A. P., and Kleinridders, A. (2020). 'GPx3 dysregulation impacts adipose tissue insulin receptor expression and sensitivity', *JCI insight*, *5*(11), pp. e136283.

Haug, A., Graham, R. D., Christophersen, O. A., and Lyons, G. H. (2007) 'How to use the world's scarce selenium resources efficiently to increase the selenium concentration in food', 19(4), pp. 209-228.

Heffernan, S. M., Horner, K., De Vito, G. and Conway, G. E. (2019) 'The Role of Mineral and Trace Element Supplementation in Exercise and Athletic Performance: A Systematic Review', *Nutrients,* 11(3), pp. 696.

Helmersson, J., Arnlöv, J., Vessby, B., Larsson, A., Alfthan, G. and Basu, S. (2005) 'Serum selenium predicts levels of F2-isoprostanes and prostaglandin F2alpha in a 27 year follow-up study of Swedish men', *Free Radic Res,* 39(7), pp. 763-70.

Hendrickson, N. R., Pickhardt, P. J., Del Rio, A. M., Rosas, H. G. and Anderson, P. A. (2018) 'Bone Mineral Density T-Scores Derived from CT Attenuation Numbers (Hounsfield Units): Clinical Utility and Correlation with Dual-energy X-ray Absorptiometry', *The Iowa orthopaedic journal,* 38, pp. 25-31.

Henriksen, D. P., Rasmussen, L., Hansen, M. R., Hallas, J. and Pottegård, A. (2015) 'Comparison of the Five Danish Regions Regarding Demographic Characteristics, Healthcare Utilization, and Medication Use—A Descriptive Cross-Sectional Study', *PLOS ONE,* 10(10), pp. e0140197.

Hernlund, E., Svedbom, A., Ivergård, M., Compston, J., Cooper, C., Stenmark, J., McCloskey, E. V., Jönsson, B. and Kanis, J. A. (2013) 'Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA)', *Arch Osteoporos,* 8(1), pp. 136.

Hesketh, J. (2008) 'Nutrigenomics and selenium: gene expression patterns, physiological targets, and genetics', *Annu Rev Nutr,* 28, pp. 157-77.

Hesketh, J. and Méplan, C. (2011) 'Transcriptomics and functional genetic polymorphisms as biomarkers of micronutrient function: focus on selenium as an exemplar', *Proc Nutr Soc*, 70(3), pp. 365-373.

Higgs, D. J., Morris, V. C., and Levander, O. A. (1972) 'Effect of Cooking on Selenium Content of Foods', *Journal of agricultural and food chemistry*, 20(3), pp. 678-680.

Hill, K. E., Motley, A. K., Li, X., May, J. M., Burk, R. F. (2001) 'Combined selenium and vitamin E deficiency causes fatal myopathy in guinea pigs', *J Nutr,* 131(6), pp. 1798-802.

Hill, K. E., Xia, Y., Akesson, B., Boeglin, M. E. and Burk, R. F. (1996) 'Selenoprotein P concentration in plasma is an index of selenium status in selenium-deficient and selenium-supplemented Chinese subjects', *J Nutr,* 126(1), pp. 138-45.

Hlaing, T. T., and Compston, J. E. (2014) 'Biochemical markers of bone turnover - uses and limitations', *Annals of Clinical Biochemistry*, 51(Pt 2), pp. 189-202.

Hoeflich, J., Hollenbach, B., Behrends, T., Hoeg, A., Stosnach, H., and Schomburg, L. (2010) 'The choice of biomarkers determines the selenium status in young German vegans and vegetarians', *British Journal of Nutrition*, 104(11), pp. 1601-1604.

Hoeg, A., Gogakos, A., Murphy, E., Mueller, S., Köhrle, J., Reid, D. M., Glüer, C. C., Felsenberg, D., Roux, C., Eastell, R., Schomburg, L., and Williams, G. R. (2012) 'Bone turnover and bone mineral density are independently related to selenium status in healthy euthyroid postmenopausal women', *The Journal of clinical endocrinology and metabolism,* 97(11), pp. 4061-4070.

Hollenbach, B., Morgenthaler, N. G., Struck, J., Alonso, C., Bergmann, A., Köhrle, J., and Schomburg, L. (2008) 'New assay for the measurement of selenoprotein P as a sepsis biomarker from serum', *Journal of trace elements in medicine and biology,* 22(1), pp. 24-32.

Hong Du, X., Xia Dai, X., Xia Song, R., Zhen Zou, X., Yan Sun, W., Yan Mo, X., Lu Bai, G. and Min Xiong, Y. (2012) 'SNP and mRNA expression for glutathione peroxidase 4 in Kashin-Beck disease', *British Journal of Nutrition,* 107(2), pp. 164-169.

Hu, Y. J., and Diamond, A. M (2003) 'Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium', *Cancer Res,* 63(12), pp. 3347-51.

Huang, J. Q., Ren, F. Z., Jiang, Y. Y., Xiao, C. and Lei, X. G. (2015) 'Selenoproteins protect against avian nutritional muscular dystrophy by metabolizing peroxides and regulating redox/apoptotic signaling', *Free Radic Biol Med,* 83, pp. 129-38.

Huang, J.Q., Li, D.-L., Zhao, H., Sun, L.-H., Xia, X.-J., Wang, K.-N., Luo, X. and Lei, X. G. (2011) 'The selenium deficiency disease exudative diathesis in chicks is associated with downregulation of seven common selenoprotein genes in liver and muscle', *The Journal of nutrition,* 141(9), pp. 1605-1610.

Huang, Q. Y., Li, G. H. Y., Cheung, W. M. W., Song, Y. Q. and Kung, A. W. C. (2008) 'Prediction of osteoporosis candidate genes by computational disease-gene identification strategy', *J Hum Genet,* 53(7), pp. 644-655.

Huang, Z., Rose, A. H. and Hoffmann, P. R. (2012) 'The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities', *Antioxid Redox Signal,* 16(7), pp. 705-43.

Hughes, D. J., Fedirko, V., Jenab, M., Schomburg, L., Méplan, C., Freisling, H., et al. (2015). 'Selenium status is associated with colorectal cancer risk in the European prospective investigation of cancer and nutrition cohort', *International journal of cancer*, *136*(5), pp. 1149–1161.

Hunter, S. K., Pereira, H. M., and Keenan, K. G. (2016). 'The aging neuromuscular system and motor performance', *Journal of applied physiology (Bethesda, Md. : 1985)*, *121*(4), pp. 982–995.

Hurst, R., Armah, C. N., Dainty, J. R., Hart, D. J., Teucher, B., Goldson, A. J., Broadley, M. R., Motley, A. K. and Fairweather-Tait, S. J. (2010) 'Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial', *Am J Clin Nutr,* 91(4), pp. 923-31.

Hurst, R., Collings, R., Harvey, L. J., King, M., Hooper, L., Bouwman, J., Gurinovic, M. and Fairweather-Tait, S. J. (2013) 'EURRECA-Estimating selenium requirements for deriving dietary reference values', *Crit Rev Food Sci Nutr,* 53(10), pp. 1077-96.

Hurt, H. D., Cary, E. E. and Visek, W. J. (1971) 'Growth, reproduction, and tissue concentrations of selenium in the selenium-depleted rat', *J Nutr,* 101(6), pp. 761-6.

Hybsier, S., Schulz, T., Wu, Z., Demuth, I., Minich, W. B., Renko, K., Rijntjes, E., Köhrle, J., Strasburger, C. J., Steinhagen-Thiessen, E., and Schomburg, L. (2017) 'Sex-specific and inter-individual differences in biomarkers of selenium status identified by a calibrated ELISA for selenoprotein P', *Redox Biol*, 11, pp. 403-414.

Ichikawa, S., Koller, D. L., Johnson, M. L., Lai, D., Xuei, X., Edenberg, H. J., Klein, R. F., Orwoll, E. S., Hui, S. L., Foroud, T. M., Peacock, M. and Econs, M. J. (2006) 'Human ALOX12, but Not ALOX15, Is Associated With BMD in White Men and Women', *Journal of Bone and Mineral Research,* 21(4), pp. 556-564.

Ilich, J. Z., Cvijetic, S., Baric, I. C., Cecic, I., Saric, M., Crncevic-Orlic, Z., Blanusa, M. and Korsic, M. (2009) 'Nutrition and lifestyle in relation to bone health and body weight in Croatian postmenopausal women', *Int J Food Sci Nutr,* 60(4), pp. 319-32.

Imai, H., Suzuki, T., Kashiwazaki, H., Takemoto, T.-I., Izumi, T. and Moji, K. (1990) 'Dietary habit and selenium concentrations in erythrocyte and serum in a group of middle-aged and elderly japanese', *Nutrition Research,* 10(11), pp. 1205-1214.

Innerd, P., Catt, M., Collerton, J., Davies, K., Trenell, M., Kirkwood, T. B. L. and Jagger, C. (2015) 'A comparison of subjective and objective measures of physical activity from the Newcastle 85+ study', *Age and ageing,* 44(4), pp. 691-694.

IoM (2000) 'Institute of Medicine Panel on Dietary, Antioxidants Related, Compounds', *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington (DC): National Academies Press (US) Copyright 2000 by the National Academy of Sciences. All rights reserved.

Islam, S., Carter, K., Mhurchu, C. N. and Anderson, C. S. (2007) 'Selenium and zinc in hair and toenails in relation to the physical and mental health status of older adults: The Freemasons Health study in New Zealand', *Australasian Journal on Ageing,* 26(4), pp. 180-186.

Jagger, C., Collerton, J. C., Davies, K., Kingston, A., Robinson, L. A., Eccles, M. P., von Zglinicki, T., Martin-Ruiz, C., James, O. F. W., Kirkwood, T. B. L. and Bond, J. (2011) 'Capability and dependency in the Newcastle 85+ cohort study. Projections of future care needs', *BMC geriatrics,* 11, pp. 21-21.

Jakob, F., Becker, K., Paar, E., Ebert-Duemig, R., and Schütze, N. (2002). 'Expression and regulation of thioredoxin reductases and other selenoproteins in bone', *Methods in enzymology*, *347*, 168–179.

Janssen, I., Heymsfield, S. B. and Ross, R. (2002) 'Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability', *J Am Geriatr Soc,* 50(5), pp. 889-96.

Janssen, I., Heymsfield, S. B., Baumgartner, R. N. and Ross, R. (2000) 'Estimation of skeletal muscle mass by bioelectrical impedance analysis', *Journal of Applied Physiology,* 89(2), pp. 465-471.

Johansen, A., Evans, R. J., Stone, M. D., Richmond, P. W., Lo, S. V. and Woodhouse, K. W. (1997) 'Fracture incidence in England and Wales: a study based on the population of Cardiff', *Injury,* 28(9-10), pp. 655-60.

Jones, G. D., Droz, B., Greve, P., Gottschalk, P., Poffet, D., McGrath, S. P., Seneviratne, S. I., Smith, P., and Winkel, L. H. (2017) 'Selenium deficiency risk predicted to increase under future climate change', *PNAS*, 114(11), pp. 2848-2853.

Jordan, J. M., Lawrence, R., Kington, R., Fraser, P., Karlson, E., Lorig, K. and Liang, M. H. (2002) 'Ethnic health disparities in arthritis and musculoskeletal diseases: Report of a scientific conference', *Arthritis & Rheumatism,* 46(9), pp. 2280-2286.

Jurynec, M. J., Xia, R., Mackrill, J. J., Gunther, D., Crawford, T., Flanigan, K. M., Abramson, J. J., Howard, M. T. and Grunwald, D. J. (2008) 'Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle', *Proc Natl Acad Sci U S A,* 105(34), pp. 12485-90.

Kafai, M. R. and Ganji, V. (2003) 'Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third National Health and Nutrition Examination Survey, 1988-1994', *J Trace Elem Med Biol,* 17(1), pp. 13-8.

Kanis, J. A., McCloskey, E. V., Johansson, H., Strom, O., Borgstrom, F., Odén, A., Group, N.O. (2008). 'Case finding for the management of osteoporosis with FRAX®—assessment and intervention thresholds for the UK'. *Osteoporosis International*, 19(10), pp. 1395-1408.

Kanis, J. A., Norton, N., Harvey, N. C., Jacobson, T., Johansson, H., Lorentzon, M., McCloskey, E. V., Willers, C. and Borgström, F. (2021) 'SCOPE 2021: a new scorecard for osteoporosis in Europe', *Arch Osteoporos,* 16(1), pp. 82.

Karimifar, M., Esmaili, F., Salari, A., Kachuei, A., Faragzadegan, Z. and Karimifar, M. (2014) 'Effects of Levothyroxine and thyroid stimulating hormone on bone loss in patients with primary hypothyroidism', *Journal of research in pharmacy practice,* 3(3), pp. 83-87.

Karita, K., Yamanouchi, Y., Takano, T., Oku, J., Kisaki, T. and Yano, E. (2008) 'Associations of blood selenium and serum lipid levels in Japanese premenopausal and postmenopausal women', *Menopause,* 15(1), pp. 119-24.

Kawaguchi, H., Akune, T., Yamaguchi, M., Ohba, S., Ogata, N., Chung, U.-I., Kubota, N., Terauchi, Y., Kadowaki, T. and Nakamura, K. (2005) 'Distinct effects of PPARγ insufficiency on bone marrow cells, osteoblasts, and osteoclastic cells', *Journal of Bone and Mineral Metabolism,* 23(4), pp. 275-279.

Keaney, J. F., Jr., Larson, M. G., Vasan, R. S., Wilson, P. W., Lipinska, I., Corey, D., Massaro, J. M., Sutherland, P., Vita, J. A. and Benjamin, E. J. (2003) 'Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study', *Arterioscler Thromb Vasc Biol,* 23(3), pp. 434-9.

Keck, A. S., and Finley, J. W. (2006) 'Database values do not reflect selenium contents of grain, cereals, and other foods grown or purchased in the upper Midwest of the United States', *Nutrition Research,* 26(1), pp. 17-22.

Kempen, G. I., Miedema, I., Ormel, J. and Molenaar, W. (1996) 'The assessment of disability with the Groningen Activity Restriction Scale. Conceptual framework and psychometric properties', *Soc Sci Med,* 43(11), pp. 1601-10.

Kenkre, J. S., and Bassett, J. (2018) 'The bone remodelling cycle', *Annals of clinical biochemistry*, 55(3), pp. 308-327.

Key, L. L., Wolf, W. C., Gundberg, C. M., and Ries, W. L. (1994) 'Superoxide and bone resorption', *Bone,* 15(4), pp. 431-436.

Khanam, A. and Platel, K. (2016) 'Influence of domestic processing on the bioaccessibility of selenium from selected food grains and composite meals', *J Food Sci Technol,* 53(3), pp. 1634-9.

Kidney Care UK (2022) 'Chronic Kidney Disease'. https://www.kidneycareuk.org/about-kidney-health/conditions/ckd/ Accessed on: 25/11/22

Kieliszek, M., and Błazejak, S., (2013) 'Selenium: Significance, and outlook for supplementation', *Nutrition*, 29(5), pp. 713-718.

Kieliszek, M., and Błażejak, S., (2016) 'Current Knowledge on the Importance of Selenium in Food for Living Organisms: A Review', *Molecules*, 21,(5) pp. 609.

Kim, B. J., Lee, S. H., Kwak, M. K., Isales, C. M., Koh, J. M., and Hamrick, M. W. (2018) 'Inverse relationship between serum hsCRP concentration and hand grip strength in older adults: a nationwide population-based study', *Aging,* 10(8), pp. 2051-2061.

Kim, H., Lee, K., Kim, J. M., Kim, M. Y., Kim, J.-R., Lee, H.-W., Chung, Y. W., Shin, H.-I., Kim, T., Park, E.-S., Rho, J., Lee, S. H., Kim, N., Lee, S. Y., Choi, Y. and Jeong, D. (2021) 'Selenoprotein W ensures physiological bone remodeling by preventing hyperactivity of osteoclasts', *Nature Communications,* 12(1), pp. 2258.

Kim, K. W., Cho, M. L., Lee, S. H., Oh, H. J., Kang, C. M., Ju, J. H., Min, S. Y., Cho, Y. G., Park, S. H., and Kim, H. Y. (2007) 'Human rheumatoid synovial fibroblasts promote osteoclastogenic

activity by activating RANKL via TLR-2 and TLR-4 activation', *Immunology letters*, 110(1), pp. 54-64.

Kingston, A., Collerton, J., Davies, K., Bond, J., Robinson, L.., Jagger, C. (2012) 'Losing the ability in activities of daily living in the oldest old: a hierarchic disability scale from the Newcastle 85+ study', *PloS one,* 7(2), pp. e31665-e31665.

Kipp, A. P., Strohm, D., Brigelius-Flohe, R., Schomburg, L., Bechthold, A., Leschik-Bonnet, E., Heseker, H. and German Nutrition, S. (2015) 'Revised reference values for selenium intake', *J Trace Elem Med Biol,* 32, pp. 195-9.

Kitaura, H., Marahleh, A., Ohori, F., Noguchi, T., Shen, W. R., Qi, J., Nara, Y., Pramusita, A., Kinjo, R. and Mizoguchi, I. (2020) 'Osteocyte-Related Cytokines Regulate Osteoclast Formation and Bone Resorption', *Int J Mol Sci,* 21(14), pp. 5169.

Klein, E. A., Thompson, I. M., Jr., Tangen, C. M., Crowley, J. J., Lucia, M. S., Goodman, P. J., et al. (2011) 'Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT)', *JAMA,* 306(14), pp. 1549-56.

Kobayashi, R., Hasegawa, M., Kawaguchi, C., Ishikawa, N., Tomiwa, K., Shima, M. and Nogami, K. (2021) 'Thyroid function in patients with selenium deficiency exhibits high free T4 to T3 ratio', *Clin Pediatr Endocrinol,* 30(1), pp. 19-26.

Koç, E. R., Ilhan, A., Zübeyde, A., Acar, B., Gürler, M., Altuntaş, A., Karapirli, M. and Bodur, A. S. (2015) 'A comparison of hair and serum trace elements in patients with Alzheimer disease and healthy participants', *Turk J Med Sci,* 45(5), pp. 1034-9.

Koch, M. B., Davidsen, M. and Juel, K. (2012) 'Social ulighed i sundhed, sygelighed og trivsel 2010 og udviklingen siden 1987'.

Kodoth, V., Scaccia, S. and Aggarwal, B. (2022) 'Adverse Changes in Body Composition During the Menopausal Transition and Relation to Cardiovascular Risk: A Contemporary Review', *Women's health reports (New Rochelle, N.Y.),* 3(1), pp. 573-581.

Koh, J. M., Khang, Y. H., Jung, C. H., Bae, S., Kim, D. J., Chung, Y. E., and Kim, G. S. (2005) 'Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: Evidence for a link between systemic inflammation and osteoporosis', *Osteoporosis International*, 16(10), pp. 1263-1271.

Köhrle, J. (2000) 'The deiodinase family: selenoenzymes regulating thyroid hormone availability and action', *Cell Mol Life Sci,* 57(13-14), pp. 1853-63.

Köhrle, J., Jakob, F., Contempré, B., Dumont, J. E. (2005) 'Selenium, the thyroid, and the endocrine system', *Endocrine reviews,* 26, pp. 944-984.

Koster, A., Ding, Jingzhong, Stenholm, Sari, Caserotti, Paolo, Houston, Denise K., Nicklas, Barbara J. et al (2011) 'Does the amount of fat mass predict age-related loss of lean mass, muscle strength, and muscle quality in older adults?', *The Journals of Gerontology.* 66 A, pp. 888-895.

Koyama, H., Watanabe, C., Satoh, H., Hosokai, H. and Tamura, S. (1995) 'Consistent relationship between selenium and apolipoprotein A-II concentrations in the sera of fasting middle-aged male abstainers and regular consumers of alcohol', *Biological Trace Element Research,* 50(1), pp. 33-42.

Kryukov, G. V., Castellano, Sergi, Novoselov, Sergey V., Lobanov, Alexey V., Zehtab, Omid, Guigó, Roderic, Gladyshev, Vadim N (2003) 'Characterization of mammalian selenoproteomes', *Science*, 300(5624), pp. 1439-1443.

Kuhnle, G. G. C. (2012) 'Nutritional biomarkers for objective dietary assessment', *Journal of the Science of Food and Agriculture,* 92(6), pp. 1145-1149.

Kuo, T. R., and Chen, C. H. (2017) 'Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives', *Biomark Res,* 5(2050-7771 (Print)), pp. 18.

Labunskyy, V. M., Hatfield, D. L. and Gladyshev, V. N. (2014) 'Selenoproteins: molecular pathways and physiological roles', *Physiol Rev,* 94(3), pp. 739-77.

Laclaustra, M., Stranges, S., Navas-Acien, A., Ordovas, J. M., and Guallar, E. (2010) 'Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004', *Atherosclerosis* 210(2), pp. 643-648.

Larsen, E. H., Hansen, M., Paulin, H., Moesgaard, S., Reid, M. and Rayman, M. (2004) 'Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies', *Journal of AOAC Int,* 87(1), pp. 225-32.

Larsson, L., Degens, H., Li, M., Salviati, L., Lee, Y. i., Thompson, W., Kirkland, J. L. and Sandri, M. (2018) 'Sarcopenia: Aging-Related Loss of Muscle Mass and Function', *Physiological Reviews,* 99(1), pp. 427-511.

Lauretani, F., Russo, C. R., Bandinelli, S., Bartali, B., Cavazzini, C., Di Iorio, A., Corsi, A. M., Rantanen, T., Guralnik, J. M. and Ferrucci, L. (2003) 'Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia', *J Appl Physiol (1985),* 95(5), pp. 1851-60.

Lauretani, F., Semba, R. D., Bandinelli, S., Ray, A. L., Guralnik, J. M. and Ferrucci, L. (2007) 'Association of low plasma selenium concentrations with poor muscle strength in older community-dwelling adults: the InCHIANTI Study', *The American journal of clinical nutrition,* 86(2), pp. 347-352.

Lean, J. M., Jagger, C. J., Kirstein, B., Fuller, K., and Chambers, T. J. (2005) 'Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoclast formation', *Endocrinology*, 146(2), pp. 728-735.

Lecka-Czernik, B., Moerman, E. J., Grant, D. F., Lehmann, J. M., Manolagas, S. C. and Jilka, R. L. (2002) 'Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation', *Endocrinology,* 143(6), pp. 2376-84.

Lee, S. Y., Tung, H. H., Liu, C. Y., and Chen, L. K. (2018) 'Physical Activity and Sarcopenia in the Geriatric Population: A Systematic Review', *Journal of the American Medical Directors Association*, 19(5), pp. 378-383.

Lee, Y. H., Rho, Y. H., Choi, S. J., Ji, J. D. and Song, G. G. (2006) 'Meta-analysis of genome-wide linkage studies for bone mineral density', *Journal of Human Genetics,* 51(5), pp. 480-486.

Lescure, A., Rederstorff, M., Krol, A., Guicheney, P. and Allamand, V. (2009) 'Selenoprotein function and muscle disease', *Biochim Biophys Acta,* 1790(11), pp. 1569-74.

Letavayová, L., Vlčková, V., and Brozmanová, J. (2006) 'Selenium: From cancer prevention to DNA damage', *Toxicology*, 227(1-2), pp. 1-14.

Letsiou, S., Nomikos, T., Panagiotakos, D. B., Pergantis, S. A., Fragopoulou, E., Pitsavos, C. et al. (2014). 'Gender-specific distribution of selenium to serum selenoproteins: associations with total selenium levels, age, smoking, body mass index, and physical activity', *BioFactors (Oxford, England)*, *40*(5), pp. 524–535.

Levander, O. A. (1982) 'Clinical consequences of low selenium intake and its relationship to vitamin E', *Ann N Y Acad Sci,* 393, pp. 70-82.

Levander, O. A., and Burk, R. F. (2006) 'Update of human dietary standards for selenium', in Hatfield, D.L., Berry, M.J. and Gladyshev, V.N. (eds.) *Selenium: Its Molecular Biology and Role in Human Health*. Boston, MA: Springer US, pp. 399-410.

Levander, O. A., Alfthan, G., Arvilommi, H. Gref, C. G., Huttunen, J. K., Kataja, M., Koivistoinen, P., Pikkarainen, J. (1983) 'Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters', *The American Journal of Clinical Nutrition*, 37(6), pp. 887-897.

Lippman, S. M., Klein, E. A., Goodman, P. J., Lucia, M. S., Thompson, I. M., Ford, L. G., et al. (2009) 'Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT)', *JAMA,* 301(1), pp. 39-51.

Liu, C., Fu, J., Lin, S., Wang, X. and Li, S. (2014) 'Effects of Dietary Selenium Deficiency on mRNA Levels of Twenty-One Selenoprotein Genes in the Liver of Layer Chicken', *Biological Trace Element Research,* 159(1-3), pp. 192-198.

Liu, H., Bian, Weixia, Liu, Songxiu, Huang, Kaixun (2012) 'Selenium protects bone marrow stromal cells against hydrogen peroxide-induced inhibition of osteoblastic differentiation by suppressing oxidative stress and ERK signaling pathway', *Biological Trace Element Research*, 150, pp. 441-450.

Liu, S. Z., Yan, H., Xu, P., Li, J., et al. (2009) 'Correlation analysis between bone mineral density and serum element contents of postmenopausal women in Xi'an urban area', *Biological Trace Element Research*, 131, pp. 205-214.

Lloyd, B., Lloyd, R. S., and Clayton, B. E. (1983) 'Effect of smoking, alcohol, and other factors on the selenium status of a healthy population', *Journal of Epidemiology and Community Health,* 37(3), pp. 213-217.

Lobanov, A. V., Hatfield, D. L. and Gladyshev, V. N. (2009) 'Eukaryotic selenoproteins and selenoproteomes', *Biochim Biophys Acta,* 1790(11), pp. 1424-8.

Loeschner, K., Hadrup, N., Hansen, M., Pereira, S. A., Gammelgaard, B., Møller, L. H., Mortensen, A., Lam, H. R., and Larsen, E. H. (2014) 'Absorption, distribution, metabolism and excretion of selenium following oral administration of elemental selenium nanoparticles or selenite in rats', *Metallomics : Integrated Biometal Science*, 6(2), pp. 330-337.

Loke, Y. K., Gilbert, D., Thavarajah, M., Blanco, P., and Wilson, A. M. (2015) 'Bone mineral density and fracture risk with long-term use of inhaled corticosteroids in patients with asthma: systematic review and meta-analysis', *BMJ Open*, 5, pp. e008554.

Longnecker, M. P., Stram, D. O., Taylor, P. R., Levander, O. A., Howe, M., Veillon, C., McAdam, P. A., Patterson, K. Y., Holden, J. M., Morris, J. S., Swanson, C. A., Willett, W. C. (1996) 'Use of selenium concentration in whole-blood, serum, toenails, or urine as a surrogate measure of selenium intake', *Epidemiology*, 7(4), pp. 384-390.

Lopes, G., Fabrício, A. W. and Luiz, R. G. (2017) 'Comportamento de selênio no solo e sua implicação para a saúde humana', *Ciência e Agrotecnologia*, 41(6), pp. 605-615.

Lopes, P. A., Santos, M. C., Vicente, L., Rodrigues, M. O., Pavão, M. L., Nève, J., and Viegas-Crespo, A. M. (2004) 'Trace element status (Se, Cu, Zn) in healthy Portuguese subjects of Lisbon population: A reference study', *Biological Trace Element Research*, 101(1), pp. 1-17.

Low, S. C., Grundner-Culemann, E., Harney, J. W. and Berry, M. J. (2000) 'SECIS-SBP2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy', *The EMBO journal,* 19(24), pp. 6882-6890.

Löwik, M. R., van den Berg, H., Schrijver, J., Odink, J., Wedel, M. and van Houten, P. (1992) 'Marginal nutritional status among institutionalized elderly women as compared to those living more independently (Dutch Nutrition Surveillance System)', *J Am Coll Nutr,* 11(6), pp. 673-81.

Lowsky, D. J., Olshansky, S. J., Bhattacharya, J. and Goldman, D. P. (2014) 'Heterogeneity in healthy aging', *The journals of gerontology. Series A, Biological Sciences and Medical Sciences,* 69(6), pp. 640-649.

Lu, X., He, Z., Lin, Z., Zhu, Y., Yuan, L., Liu, Y. and Yin, X. (2018) 'Effects of Chinese Cooking Methods on the Content and Speciation of Selenium in Selenium Bio-Fortified Cereals and Soybeans', *Nutrients,* 10(3), pp. 317.

Lubos, E., Loscalzo, J., and Handy, D.E. (2011) 'Glutathione peroxidase-1 in health and disease: From molecular mechanisms to therapeutic opportunities', *Antioxidants and Redox Signalling*, 15(7), pp. 1957-1997.

Luo, X., Wei, H., Yang, C., Xing, J., Liu, X., Qiao., C. H., Feng, Y. M., Liu, J., Liu, Y. X., and Wu, Q. (1985) 'Bioavailability of selenium to residents in a low-selenium area of China', *American Journal of Clinical Nutrition*, 42(3), pp. 439-448.

Lyhne, N., Christensen, T., Groth, M., Fagt, S., Biltoft-Jensen, A., Hartkopp, H., Hinsch, H., Matthiessen, J., Møller, A. and Saxholt, E. (2005) 'Danskernes kostvaner 2000–2002', *Hovedresultater (DFVF Publikation nr. 11. Danish Institute for Food and Veterinary Research.(Dietary habits in Denmark 2000–2002. Main results: in Danish)*.

Lymbury, R., Tinggi, U., Griffiths, L., Rosenfeldt, F. and Perkins, A. V. (2008) 'Selenium status of the Australian population: effect of age, gender and cardiovascular disease', *Biol Trace Elem Res,* 126 Suppl 1, pp. S1-10.

Lyons, G. H., Judson, G. J., Stangoulis, J. C., Palmer, L. T., Jones, J. A., and Graham, R. D. (2004) 'Trends in selenium status of South Australians', *The Medical journal of Australia*, 180(8), 383–386.

Ma, P., Hu, Z., Li, L., Li, D. and Tang, R. (2021) 'Dietary selenium promotes the growth performance through growth hormone-insulin-like growth factor and hypothalamic-pituitary-thyroid axes in grass carp (Ctenopharyngodon idella)', *Fish Physiol Biochem,* 47(4), pp. 1313-1327.

MacDonell, S. O., Miller, J. C., Harper, M. J., Reid, M. R., Haszard, J. J., Gibson, R. S. and Houghton, L. A. (2018) 'A comparison of methods for adjusting biomarkers of iron, zinc, and selenium status for the effect of inflammation in an older population: a case for interleukin 6', *Am J Clin Nutr,* 107(6), pp. 932-940.

MacDonell, S. O., Miller, J. C., Harper, M. J., Reid, M. R., Haszard, J. J., Gibson, R. S. and Houghton, L. A. (2021) 'Multiple Micronutrients, Including Zinc, Selenium and Iron, Are Positively Associated with Anemia in New Zealand Aged Care Residents', *Nutrients,* 13(4), pp. 1072.

Macfarlane, D. J., Chou, K. L., Cheng, Y. H. and Chi, I. (2006) 'Validity and normative data for thirty-second chair stand test in elderly community-dwelling Hong Kong Chinese', *Am J Hum Biol,* 18(3), pp. 418-21.

Mackenbach, J. P., Stirbu, I., Roskam, A. J., Schaap, M. M., Menvielle, G., Leinsalu, M., Kunst, A. E., and European Union Working Group on Socioeconomic Inequalities in, H. (2008) 'Socioeconomic inequalities in health in 22 European countries', *N Engl J Med,* 358(23), pp. 2468-81.

Maggio, M., Ceda, G. P., Lauretani, F., Bandinelli, S., Dall'Aglio, E., Guralnik, J. M., Paolisso, G., Semba, R. D., Nouvenne, A., Borghi, L., Ceresini, G., Ablondi, F., Benatti, M. and Ferrucci, L. (2010) 'Association of plasma selenium concentrations with total IGF-1 among older community-dwelling adults: the InCHIANTI study', *Clin Nutr,* 29(5), pp. 674-7.

Majer, I. M., Nusselder, W. J., Mackenbach, J. P., Klijs, B. and van Baal, P. H. (2011) 'Mortality risk associated with disability: a population-based record linkage study', *Am J Public Health,* 101(12), pp. e9-15.

Manolagas, S. C. (2010) 'From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis', *Endocr Rev,* 31(3), pp. 266-300.

Marchaluk, E., Persson-Moschos, M., and Thorling, E. B. (1995) 'Variation in selenoprotein P concentration in serum from different European regions', 49(1), pp. 42-48.

Margaritis, I., Tessier, F., Prou, E., Marconnet, P. and Marini, J. F. (1997) 'Effects of endurance training on skeletal muscle oxidative capacities with and without selenium supplementation', *J Trace Elem Med Biol,* 11(1), pp. 37-43.

Martin, H., Aihie Sayer, A., Jameson, K., Syddall, H., Dennison, E. M., Cooper, C. and Robinson, S. (2011) 'Does diet influence physical performance in community-dwelling older people? Findings from the Hertfordshire Cohort Study', *Age and Ageing,* 40(2), pp. 181-186.

Martínez, J. I. R., García, R. D. and Galarza, A. M. (1982) 'The kinetic mechanism of glutathione peroxidase from human platelets', *Thrombosis Research,* 27(2), pp. 197-203.

Martin-Ruiz, C., Jagger, C., Kingston, A., Collerton, J., Catt, M., Davies, K., Dunn, M., Hilkens, C., Keavney, B., Pearce, S. H., den Elzen, W. P., Talbot, D., Wiley, L., Bond, J., Mathers, J. C., Eccles, M. P., Robinson, L., James, O., Kirkwood, T. B. and von Zglinicki, T. (2011) 'Assessment of a large panel of candidate biomarkers of ageing in the Newcastle 85+ study', *Mech Ageing Dev,* 132(10), pp. 496-502.

Marzani, B., Balage, M., Vénien, A., Astruc, T., Papet, I., Dardevet, D. and Mosoni, L. (2008) 'Antioxidant supplementation restores defective leucine stimulation of protein synthesis in skeletal muscle from old rats', *J Nutr,* 138(11), pp. 2205-11.

Mathias, S., Nayak, U. S. and Isaacs, B. (1986) 'Balance in elderly patients: the "get-up and go" test', *Arch Phys Med Rehabil,* 67(6), pp. 387-9.

McCambridge, J., Witton, J. and Elbourne, D. R. (2014) 'Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects', *Journal of Clinical Epidemiology,* 67(3), pp. 267-277.

McCance, R. A. (2002) 'McCance and Widdowson's The Composition of Foods, 6th Summary Edition', *Food Standards Agency*, Print ISBN 978-0-85404-428-3

McCormick, R. and Vasilaki, A. (2018) 'Age-related changes in skeletal muscle: changes to lifestyle as a therapy', *Biogerontology,* 19(6), pp. 519-536.

Mendonça, N., Granic, A., Hill, T. R., Siervo, M., Mathers, J. C., Kingston, A. and Jagger, C. (2019) 'Protein Intake and Disability Trajectories in Very Old Adults: The Newcastle 85+ Study', *Journal of the American Geriatrics Society,* 67(1), pp. 50-56.

Mendonça, N., Hill, T. R., Granic, A., Davies, K., Collerton, J., Mathers, J. C., Siervo, M., Wrieden, W. L., Seal, C. J., Kirkwood, T. B. L., Jagger, C. and Adamson, A. J. (2016a) 'Macronutrient intake and food sources in the very old: analysis of the Newcastle 85+ Study', *British Journal of Nutrition,* 115(12), pp. 2170-2180.

Mendonça, N., Hill, T. R., Granic, A., Davies, K., Collerton, J., Mathers, J. C., Siervo, M., Wrieden, W. L., Seal, C. J., Kirkwood, T. B. L., Jagger, C. and Adamson, A. J. (2016b) 'Micronutrient intake and food sources in the very old: analysis of the Newcastle 85+ Study', *British Journal of Nutrition,* 116(4), pp. 751-761.

Méplan, C., Crosley, L. K., Nicol, F., Beckett, G. J., Howie, A. F., Hill, K. E., Horgan, G., Mathers, J. C., Arthur, J. R. and Hesketh, J. E. (2007) 'Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study)', *Faseb j,* 21(12), pp. 3063-74.

Michelsen, J., Wallaschofski, H., Friedrich, N., Spielhagen, C., Rettig, R., Ittermann, T., Nauck, M. and Hannemann, A. (2013) 'Reference intervals for serum concentrations of three bone turnover markers for men and women', *Bone,* 57(2), pp. 399-404.

Michelucci, A., Liang, C., Protasi, F. and Dirksen, R. T. (2021) 'Altered Ca(2+) Handling and Oxidative Stress Underlie Mitochondrial Damage and Skeletal Muscle Dysfunction in Aging and Disease', *Metabolites,* 11(7), pp. 424.

Mijnarends, D. M., Meijers, J. M. M., Halfens, R. J. G., ter Borg, S., Luiking, Y. C., Verlaan, S., Schoberer, D., Cruz Jentoft, A. J., van Loon, L. J. C. and Schols, J. M. G. A. (2013) 'Validity and Reliability of Tools to Measure Muscle Mass, Strength, and Physical Performance in Community-Dwelling Older People: A Systematic Review', *Journal of the American Medical Directors Association,* 14(3), pp. 170-178.

Millán-Calenti, J. C., Tubío, J., Pita-Fernández, S., González-Abraldes, I., Lorenzo, T., Fernández-Arruty, T. and Maseda, A. (2010) 'Prevalence of functional disability in activities of daily living (ADL), instrumental activities of daily living (IADL) and associated factors, as predictors of morbidity and mortality', *Arch Gerontol Geriatr,* 50(3), pp. 306-10.

Miller, L., Mills, B. J., Blotcky, A. J. and Lindeman, R. D. (1983) 'Red blood cell and serum selenium concentrations as influenced by age and selected diseases', *J Am Coll Nutr,* 2(4), pp. 331-41.

Min, Z., Zhao, W., Zhong, N., Guo, Y., Sun, M., Wang, Q., Zhang, R., Yan, J., Tian, L., Zhang, F., Han, Y., Ning, Q., Meng, L., Sun, J. and Lu, S. (2015) 'Abnormality of epiphyseal plate induced by selenium deficiency diet in two generation DA rats', *APMIS,* 123(8), pp. 697-705.

Ministry of Agriculture, Fisheries and Food (1997) 'Dietary intake of selenium food surveillance information sheet no. 126', *Ministry of Agriculture, Fisheries and Food, Joint Food Safety and Standards Group, London*.

Misu, H., Takayama, H., Saito, Y., Mita, Y., Kikuchi, A., Ishii, K. A., et al., (2017). 'Deficiency of the hepatokine selenoprotein P increases responsiveness to exercise in mice through upregulation of reactive oxygen species and AMP-activated protein kinase in muscle', *Nature medicine*, *23*(4), pp. 508–516.

Miyamoto, Y., Koh, Y. H., Park, Y. S., Fujiwara, N., Sakiyama, H., Misonou, Y., Ookawara, T., Suzuki, K., Honke, K. and Taniguchi, N. (2003) 'Oxidative stress caused by inactivation of glutathione peroxidase and adaptive responses', *Biol Chem,* 384(4), pp. 567-74.

Miyauchi, A., Dinavahi, Rajani V., Crittenden, Daria B., Yang, Wenjing, Maddox, Judy C., Hamaya, Etsuro, Nakamura, Yoichi, Libanati, Cesar, Grauer, Andreas, Shimauchi, Junichiro (2019) 'Increased bone mineral density for 1 year of romosozumab, vs placebo, followed by 2 years of denosumab in the Japanese subgroup of the pivotal FRAME trial and extension', *Archives of Osteoporosis*, 14(1), 59.

Mlakar, S. J., Osredkar, J., Prezelj, J. and Marc, J. (2010) 'The Antioxidant Enzyme GPX1 Gene Polymorphisms Are Associated with Low BMD and Increased Bone Turnover Markers', *Disease Markers,* 29(2), pp. 71-80.

Mody, N., Parhami, Farhad, Sarafian, Theodore A., Demer, Linda L. (2001) 'Oxidative stress modulates osteoblastic differentiation of vascular and bone cells', 31(4), pp. 509-519.

Moghadaszadeh, B., Petit, N., Jaillard, C., Brockington, M., Quijano Roy, S., Merlini, L., Romero, N., Estournet, B., Desguerre, I., Chaigne, D., Muntoni, F., Topaloglu, H. and Guicheney, P. (2001) 'Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome', *Nat Genet,* 29(1), pp. 17-8.

Monget, A. L., Galan, P., Preziosi, P., Keller, H., Bourgeois, C., Arnaud, J., Favier, A. and Hercberg, S. (1996) 'Micronutrient status in elderly people. Geriatrie/Min. Vit. Aux Network', *Int J Vitam Nutr Res,* 66(1), pp. 71-6.

Monsen, E. R. (2000) 'Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids', *Journal of the Academy of Nutrition and Dietetics,* 100(6), pp. 637.

Moreno, P., Quijano, M. A., Gutiérrez, A. M., Pérez-Conde, M. C. and Cámara, C. (2002) 'Stability of total selenium and selenium species in lyophilised oysters and in their enzymatic extracts', *Anal Bioanal Chem,* 374(3), pp. 466-76.

Moreno-Reyes, R., Egrise, D., Nève, J., Pasteels, J. L., and Schoutens, A. (2001) 'Selenium deficiency-induced growth retardation is associated with an impaired bone metabolism and osteopenia', *Journal of Bone and Mineral Research*, 16(8), pp. 1556-1563.

Morovat, A., Catchpole, A., Meurisse, A., Carlisi, A., Bekaert, A. C., Rousselle, O., Paddon, M., James, T. and Cavalier, E. (2013) 'IDS iSYS automated intact procollagen-1-N-terminus pro-peptide assay: method evaluation and reference intervals in adults and children', *Clin Chem Lab Med,* 51(10), pp. 2009-18.

Morris, H. A., Eastell, R., Jorgensen, N. R., Cavalier, E., Vasikaran, S., Chubb, S. A. P., Kanis, J. A., Cooper, C. and Makris, K. (2017) 'Clinical usefulness of bone turnover marker concentrations in osteoporosis', *Clinica Chimica Acta,* 467, pp. 34-41.

Morris, S., Cater, J. D., Green, M. A., Johnstone, A. M., Brunstrom, J. M., Stevenson, E. J., Williams, E. A. and Corfe, B. M. (2020) 'Inadequacy of Protein Intake in Older UK Adults', *Geriatrics*, 5(1) pp. 6.

Moylan, J. S. and Reid, M. B. (2007) 'Oxidative stress, chronic disease, and muscle wasting', *Muscle & Nerve,* 35(4), pp. 411-429.

Muñoz Olivas, R., Quevauviller, P. and Donard, O. F. X. (1998) 'Long term stability of organic selenium species in aqueous solutions', *Fresenius' Journal of Analytical Chemistry,* 360(5), pp. 512-519.

Murray, C. J., Barber, R. M., Foreman, K. J., Abbasoglu Ozgoren, A., Abd-Allah, F., Abera, S. F. et al. (2015) 'Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition', *Lancet,* 386(10009), pp. 2145-91.

Muthusami, S., Ramachandran, I., Muthusamy, B., Vasudevan, G., Prabhu, V., Subramaniam, V., Jagadeesan, A., Narasimhan, S. (2005) 'Ovariectomy induces oxidative stress and impairs bone antioxidant system in adult rats' *Clinica Chimica Acta*, 360(1-2), pp. 81-86.

Naska, A., Lagiou, A. and Lagiou, P. (2017) 'Dietary assessment methods in epidemiological research: current state of the art and future prospects', *F1000Research,* 6, pp. 926-926.

National Research Council. (1980) 'Mineral Tolerance of Domestic Animals, National Research Council Subcommittee on the Tenth Edition of the Recommended Dietary, Allowances', Washington, DC: The National Academies Press, pp. 1-577.

Navarro-Alarcon., M., and Cabrera-Vique, C. (2008) 'Selenium in food and the human body: A review', T*he Science of the Total Environment*, 400(1-3), pp. 115-141.

Nelson, M. (1997) 'A Photographic Atlas of Food Portion Sizes' Ministry of Agriculture Fisheries and Food, MAFF Publications, pp. 1-78

Nève, J. (1991) 'Methods in determination of selenium states', *Journal of Trace Elements and Electrolytes in Health and Disease*, 5(1), pp. 1-17.

Nève, J. (1995) 'Human Selenium Supplementation as Assessed by Changes in Blood Selenium Concentration and Glutathione Peroxidase Activity', *Journal of Trace Elements in Medicine and Biology*, 9(2), pp. 65-73.

Nève, J., Vertongen, F. and Molle, L. (1985) 'Selenium deficiency', *Clin Endocrinol Metab,* 14(3), pp. 629-56.

Nguyen, Q. D., Moodie, E. M., Forget, M.-F., Desmarais, P., Keezer, M. R. and Wolfson, C. (2021) 'Health Heterogeneity in Older Adults: Exploration in the Canadian Longitudinal Study on Aging', *Journal of the American Geriatrics Society,* 69(3), pp. 678-687.

NICE (2017) 'Osteoporosis: assessing the risk of fragility fracture'. https://www.nice.org.uk/Guidance/CG146 Accessed on 15/08/2021.

Nichol, C., Herdman, J., Sattar, N., O'Dwyer, P. J., O'Reilky, D., S.J., Littlejohn, D. Fell, G. (1998) 'Changes in the concentrations of plasma selenium and selenoproteins after minor elective surgery: Further evidence for a negative acute phase response?', *Clinical Chemistry*, 44, pp. 1764-1766.

Nielsen, T. L., Wraae, K., Brixen, K., Hermann, A. P., Andersen, M. and Hagen, C. (2006) 'Prevalence of overweight, obesity and physical inactivity in 20- to 29-year-old, Danish men. Relation to sociodemography, physical dysfunction and low socioeconomic status: the Odense Androgen Study', *Int J Obes (Lond),* 30(5), pp. 805-15.

Niskar, A. S., Paschal, D. C., Kieszak, S. M., Flegal, K. M., Bowman, B., Gunter, E. W., Pirkle, J. L., Rubin, C., Sampson, E. J., and McGeehin, M. (2003) 'Serum selenium levels in the US population: third National Health and Nutrition Examination Survey, 1988–1994', *Biological Trace Element Research,* 91(1), pp. 1-10.

Nizet, A., Cavalier, E., Stenvinkel, P., Haarhaus, M. and Magnusson, P. (2020) 'Bone alkaline phosphatase: An important biomarker in chronic kidney disease - mineral and bone disorder', *Clin Chim Acta,* 501, pp. 198-206.

Oakes, E. J. C., Lyon, T. D. B., Duncan, A., Gray, A., Talwar, D. and O'Reilly, D. S. J. (2008) 'Acute inflammatory response does not affect erythrocyte concentrations of copper, zinc and selenium', *Clinical Nutrition,* 27(1), pp. 115-120.

Odabasi, E., Turan, M., Aydin, A., Akay, C. and Kutlu, M. (2008) 'Magnesium, zinc, copper, manganese, and selenium levels in postmenopausal women with osteoporosis. Can magnesium play a key role in osteoporosis?', *Ann Acad Med Singap,* 37(7), pp. 564-7.

Office for National Statistics (2018) 'Living longer: how our population is changing and why it matters' https://www.ons.gov.uk/releases/livinglongerhowourpopulationischangingandwhyitmatters Accessed on: 15/11/22

Office for National Statistics (2022a) 'National population projections: 2020-based interim'. https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationprojections/bulletins/nationalpopulationprojections/2020basedinterim Accessed on: 15/11/22

Office for National Statistics (2022b) 'Principal Projection - UK population in age groups'. https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationprojections/datasets/tablea21principalprojectionukpopulationinagegroups Accessed on: 15/11/22

Oliveira, S. R., Simão, A. N. C., Alfieri, D. F., Flauzino, T., Kallaur, A. P., Mezzaroba, L., Lozovoy, M. A. B., Sabino, B. S., Ferreira, K. P. Z., Pereira, W., Kaimen-Maciel, D. R., Dichi, I. and Reiche, E. M. V. (2017) 'Vitamin D deficiency is associated with disability and disease progression in multiple sclerosis patients independently of oxidative and nitrosative stress', *J Neurol Sci,* 381, pp. 213-219.

Olivieri, D. G., M Azzini, AM Stanzila, C Russo, M Ferroni, R Corrocher (1995) 'Low selenium status in the elderly influences thyroid hormones', *Clinical Science*, 89(6), pp. 637-642.

Olivieri, O., Stanzial, A. M., Girelli, D., Trevisan, M. T., Guarini, P., Terzi, M., Caffi, S., Fontana, F., Casaril, M., and Ferrari, S. (1994) 'Selenium status, fatty acids, vitamins A and E, and aging: The Nove study', *The American Journal of Clinical Nutrition*, 60(4), pp. 510-517.

Osler, M., Jorgensen, T., Davidsen, M., Gronbaek, M., Bronnum-Hansen, H., Madsen, M., Gerdes, U. and Schroll, M. (2001) 'Socioeconomic status and trends in alcohol drinking in the Danish MONICA population, 1982-92', *Scand J Public Health,* 29(1), pp. 40-3.

Oster, O., Schmiedel, G., Prellwitz, W. (1988) 'The organ distribution of selenium in German adults', *Biological Trace Element Research*, 15, pp. 23-45.

Outzen, M., Tjønneland, A., Larsen, E. H., Andersen, K. K., Christensen, J., Overvad, K. and Olsen, A. (2015) 'The effect on selenium concentrations of a randomized intervention with fish and mussels in a population with relatively low habitual dietary selenium intake', *Nutrients,* 7(1), pp. 608-24.

Ozgocmen, S., Kaya, H., Fadillioglu, E. and Yilmaz, Z. (2007) 'Effects of Calcitonin, Risedronate, and Raloxifene on Erythrocyte Antioxidant Enzyme Activity, Lipid Peroxidation, and Nitric Oxide in Postmenopausal Osteoporosis', *Archives of Medical Research,* 38(2), pp. 196-205.

Papp, L. V., Lu, J., Holmgren, A., and Khanna, K. K. (2007) 'From selenium to selenoproteins: Synthesis, identity, and their role in human health', *Antioxidants and Redox Signalling*, 9(7), pp. 775-80.

Pappa, E. C., Pappas, A. C. and Surai, P. F. (2006) 'Selenium content in selected foods from the Greek market and estimation of the daily intake', *Sci Total Environ,* 372(1), pp. 100-8.

Park, K. C., Kwon, Y., Lee, Y., Kim, D. K., Jang, Y., Lee, S. (2020) 'Low selenium levels are associated with decreased bone mineral densities', *Journal of Trace Elements on Medicine and Biology*, 61, pp. 126534.

Pedersen, A., Christensen, T, Matthiessen, J, Knudsen, VK, Rosenlund-Sørensen, M, Biltoft-Jensen, A (2015) 'Dietary habits in Denmark 2011–2013 [In Danish]'. DTU Fødevareinstituttet, Søborg, pp. 1-208.

Pedrera-Zamorano, J. D., Calderon-García, J. F., Roncero-Martin, R., Mañas-Nuñez, P., Moran, J. M., Lavado-Garcia, J. M. (2012) 'The protective effect of calcium on bone mass in postmenopausal women with high selenium intake', *The Journal of Nutrition, Health and Aging*, 16(9), pp. 743-748.

Perri, G., Hill, T. R., Mathers, J. C., Walsh, J. S., Gossiel, F., Winther, K., Frölich, J., Folkestad, L., Cold, S. and Eastell, R. (2022) 'Long-Term Selenium-Yeast Supplementation Does Not Affect Bone Turnover Markers: A Randomized Placebo-Controlled Trial', *J Bone Miner Res,* 37(11), pp. 2165-2173.

Perri, G., Mendonça, N., Jagger, C., Walsh, J., Eastell, R., Mathers, J. C., and Hill, T. R. (2020) 'Dietary selenium intakes and musculoskeletal function in very old adults: Analysis of the newcastle 85+ study', *Nutrients,* 12(7), pp. 1-22.

Persson -Moschos, M., Huang, W., Srikumar, T. S., Akesson, B., and Lindeberg, S. (1995) 'Selenoprotein P in serum as a biochemical marker of selenium status', The Analyst, 120(3), pp. 833-836.

Persson-Moschos, M., Alfthan, G., and Åkesson, B (1998) 'Plasma selenoprotein P levels of healthy males in different selenium status after oral supplementation with different forms of selenium', *European Journal of Clinical Nutrition*, 52(5), pp. 363-367.

Persson-Moschos, M., Stavenow, L., Åkesson, B., Lindgärde, F. (2000) 'Selenoprotein P in plasma in relation to cancer morbidity in middle-aged Swedish men', *Nutrition and Cancer*, 36(1), pp. 19-26.

Persson-Moschos, M. P. (2000) 'Selenoprotein P', Cell Mol Life Sci, 57(13-14), pp. 1836-45.

Peters, M. M., Hill, K. E., Burk, R. F., and Weeber, E. J. (2006) 'Altered hippocampus synaptic function in selenoprotein P deficient mice', *Mol Neurodegener,* 1, pp. 1-12.

Petit, N., Lescure, A., Rederstorff, M., Krol, A., Moghadaszadeh, B., Wewer, U. M. and Guicheney, P. (2003) 'Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern', *Hum Mol Genet,* 12(9), pp. 1045-53.

Pietschmann, N., Rijntjes, E., Hoeg, A., Stoedter, M., Schweizer, U., Seemann, P., and Schomburg, L. (2014) 'Selenoprotein P is the essential selenium transporter for bones', *Metallomics: Integrated Biometal Science* 6(5), pp. 1043-1049.

Pigeolet, E., Corbisier, P., Houbion, A., Lambert, D., Michiels, C., Raes, M., Zachary, M. D. and Remacle, J. (1990) 'Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals', *Mech Ageing Dev,* 51(3), pp. 283-97.

Podsiadlo, D., Richardson, S. (1991) 'The timed "Up & Go": a test of basic functional mobility for frail elderly persons', *J Am Geriatr Soc,* 39(2), pp. 142-8.

Poljsak, B., Šuput, D. and Milisav, I. (2013) 'Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants', *Oxid Med Cell Longev,* 2013, pp. 956792.

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E. and Buga, A. M. (2013) 'ROS and brain diseases: the good, the bad, and the ugly', *Oxid Med Cell Longev,* 2013, pp. 963520.

Powers, S. K., Ji, L. L., Kavazis, A. N. and Jackson, M. J. (2011) 'Reactive oxygen species: impact on skeletal muscle', *Comprehensive Physiology,* 1(2), pp. 941-969.

Powers, S. K., and Howley, E., T. (2012) 'Exercise physiology: theory and application to fitness and performance', McGraw-Hill ISBN: 0071316264, 9780071316262

Powers, S. K., Radak, Z. and Ji, L. L. (2016) 'Exercise-induced oxidative stress: past, present and future', *J Physiol,* 594(18), pp. 5081-92.

Prabhu, K. S., Zamamiri-Davis, F., Stewart, J. B., Thompson, J. T., Sordillo, L. M., and Reddy, C. C. (2002) 'Selenium deficiency increases the expression of inducible nitric oxide synthase in RAW 264.7 macrophages: Role of nuclear factor-κB in up-regulation', *Biochemical Journal*, 366(Pt 1), pp. 203-209.

Prystupa, A., Kiciński, P., Luchowska-Kocot, D., Błażewicz, A., Niedziałek, J., Mizerski, G., Jojczuk, M., Ochal, A., Sak, J. J. and Załuska, W. (2017) 'Association between Serum Selenium Concentrations and Levels of Proinflammatory and Profibrotic Cytokines—Interleukin-6 and Growth Differentiation Factor-15, in Patients with Alcoholic Liver Cirrhosis', *International Journal of Environmental Research and Public Health,* 14(4), pp. 437.

Public Health England (2021) 'McCance and Widdowson's The Composition of Foods Integrated Dataset User guide' Public Health England https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/971021/McCance_and_Widdowsons_Composition_of_Foods_integrated_dataset_2021.pdf Accessed: 10/11/22

Qu, Z., Yang, F., Yan, Y., Hong, J., Wang, W., Li, S., Jiang, G. and Yan, S. (2021) 'Relationship between Serum Nutritional Factors and Bone Mineral Density: A Mendelian Randomization Study', *The Journal of Clinical Endocrinology & Metabolism,* 106(6), pp. e2434-e2443.

Quevauviller, P., de la Calle-Guntiñas, M. B., Maier, E. A. and Cámara, C. (1995) 'A survey on stability of chemical species in solution during storage: the BCR experience', *Microchimica Acta,* 118(1), pp. 131-141.

Rammell, C. G., Pearson, A. B. and Bentley, G. R. (1988) 'Vitamin E, selenium and polyunsaturated fatty acids in clinically normal grower (9-16 weeks old) pigs and their feed: their relationship to the vitamin E/selenium deficiency ("VESD") syndrome', *N Z Vet J,* 36(3), pp. 133-7.

Rannem, T., Ladefoged, K., Hylander, E., Christiansen, J., Laursen, H., Kristensen, J. H. et al. (1995) 'The effect of selenium supplementation on skeletal and cardiac muscle in selenium-depleted patients', *Journal of Parenteral and Enteral Nutritio*n, 19(5), pp. 351-355.

Rasmussen, L. B., Hollenbach, B., Laurberg, P., Carle, A., Hog, A., Jorgensen, T., Vejbjerg, P., Ovesen, L. and Schomburg, L. (2009) 'Serum selenium and selenoprotein P status in adult Danes - 8-year followup', *J Trace Elem Med Biol,* 23(4), pp. 265-71.

Ravn-Haren, G., Krath, B. N., Overvad, K., Cold, S., Moesgaard, S., Larsen, E. H., and Dragsted, L. O. (2008) 'Effect of long-term selenium yeast intervention on activity and gene expression of antioxidant and xenobiotic metabolising enzymes in healthy elderly volunteers from the Danish Prevention of Cancer by Intervention by Selenium (PRECISE) Pilot Study', *The British Journal of Nutrition*, 99, pp. 1190-1198.

Rayman, M. (2002) 'The argument for increasing selenium intake', *Proceedings of the Nutrition Society*, 61(2), pp. 203-215.

Rayman, M. P. (1997) 'Dietary selenium: Time to act', *BMJ*, 314(7078), pp. 387-388.

Rayman, M. P. (2000) 'The importance of selenium to human health', *Lancet,* 356(9225), pp. 233-41.

Rayman, M. P. (2004) 'The use of high-selenium yeast to raise selenium status: how does it measure up?', *The British Journal of Nutrition,* 92(4), pp. 557-573.

Rayman, M. P. (2005) 'Selenium in cancer prevention: a review of the evidence and mechanism of action', *The British Journal of Nutrition*, 64(4), pp. 527-542.

Rayman, M. P. (2008a) 'Food-chain selenium and human health: emphasis on intake', *The British Journal of Nutrition*, 100(2), pp. 254-68.

Rayman, M. P., Infante, H. G., and Sargent, M.(2008b) 'Food-chain selenium and human health: Spotlight on speciation', *The British Journal of Nutrition*, 100, pp. 238-253.

Rayman, M. P., Thompson, A. J., Bekaert, B., Catterick, J., Galassini, R., Hall, E., Warren-Perry, M., and Beckett, G. J. (2008c) 'Randomized controlled trial of the effect of selenium supplementation on thyroid function in the elderly in the United Kingdom', *The American Journal of Clinical Nutrition*, 87(2), pp. 370-378.

Rayman, M. P. (2012) 'Selenium and human health', *Lancet*, 379(9822), pp. 1256-1268.

Rayman, M. P., Stranges, S., Griffin, B. A., Pastor-Barriuso, R., and Guallar, E. (2011) 'Effect of supplementation with high-selenium yeast on plasma lipids: A randomized trial', *Annals of Internal Medicine,* 154(10), pp. 656-665.

Rayman, M. P., Winther, K. H., Pastor-Barriuso, R., Cold, F., Thvilum, M., Stranges, S., Guallar, E., and Cold, S. (2018) 'Effect of long-term selenium supplementation on mortality: Results from a multiple-dose, randomised controlled trial', *Free Radicl Biology and Medicine*, 127, pp. 46-54.

Raymond, A., Bazeer, N., Barclay, C., Krelle, H., Idriss, O., Tallack, C., and Kelly, E. (2021) 'Our ageing population: how ageing affects health and care need in England. https://doi.org/10.37829/HF-2021-RC16'. Accessed on: 10/11/22.

Rea, H. M., Thomson C. D., Campbell, D. R., and Robinson, M. F. (1979) 'Relation between erythrocyte selenium concentrations and glutathione peroxidase (EC 1.11.1.9) activities of New Zealand residents and visitors to New Zealand', *The British Journal of Nutrition*, 42(2), pp. 201-208.

Rederstorff, M., Krol, A., and Lescure, A. (2006) 'Understanding the importance of selenium and selenoproteins in muscle function', *Cellular and Molecular Life Sciences*, 63(1), pp. 52-59.

Reeder, R. J., Schoonen, M. A. A. and Lanzirotti, A. (2006) 'Metal Speciation and Its Role in Bioaccessibility and Bioavailability', *Reviews in Mineralogy and Geochemistry,* 64(1), pp. 59-113.

Reeves, M. A. and Hoffmann, P. R. (2009) 'The human selenoproteome: recent insights into functions and regulation', *Cell Mol Life Sci,* 66(15), pp. 2457-78.

Reeves, M. A., Bellinger, F. P. and Berry, M. J. (2010) 'The neuroprotective functions of selenoprotein M and its role in cytosolic calcium regulation', *Antioxidants and Redox Signaling,* 12(7), pp. 809-818.

Reinhard, P., Schweinsberg, F., Wernet, D. and Kötter, I. (1998) 'Selenium status in fibromyalgia', *Toxicol Lett,* 96-97, pp. 177-80.

Ren, F. L., Guo, X., Zhang, R. J., Wang, Sh J., Zuo, H., Zhang, Z. T., Geng, D., Yu, Y., and Su, M. (2007) 'Effects of selenium and iodine deficiency on bone, cartilage growth plate and chondrocyte differentiation in two generations of rats', *Osteoarthritis and cartilage,* 15(10), pp. 1171-1177.

Renko, K., Werner, M., Renner-Müller, I., Cooper, T. G., Yeung, C. H., Hollenbach, B., Scharpf, M., Köhrle, J., Schomburg, L. and Schweizer, U. (2008) 'Hepatic selenoprotein P (SePP) expression restores selenium transport and prevents infertility and motor-incoordination in Sepp-knockout mice', *Biochem J,* 409(3), pp. 741-9.

Reszka, E., Jablonska, E., Gromadzinska, J. and Wasowicz, W. (2012) 'Relevance of selenoprotein transcripts for selenium status in humans', *Genes & Nutrition,* 7(2), pp. 127-137.

Riese, C., Michaelis, M., Mentrup, B., Götz, F., Köhrle, J., Schweizer, U., and Schomburg, L. (2006) 'Selenium-dependent pre- and posttranscriptional mechanisms are responsible for sexual dimorphic expression of selenoproteins in murine tissues', *Endocrinology*, 147(12), pp. 5883-5892.

Rikli, R. E. and Jones, C. J. (1999) 'Development and validation of a functional fitness test for community-residing older adults', *Journal of Aging and Physical Activity,* 7(2), pp. 129-161.

Rita Cardoso, B., Apolinário, D., da Silva Bandeira, V., Busse, A. L., Magaldi, R. M., Jacob-Filho, W. and Cozzolino, S. M. (2016) 'Effects of Brazil nut consumption on selenium status and

cognitive performance in older adults with mild cognitive impairment: a randomized controlled pilot trial', *Eur J Nutr,* 55(1), pp. 107-16.

Rivas, A., Romero, A., Mariscal-Arcas, M., Monteagudo, C., López, G., Lorenzo, M. L., Ocaña-Peinado, F. M., and Olea-Serrano, F. (2012) 'Association between dietary antioxidant quality score (DAQs) and bone mineral density in Spanish women', *Nutr Hosp,* 27(6), pp. 1886-93.

Rizzoli, R., Bruyere, O., Cannata-Andia, J. B., Devogelaer, J., Lyritis, G., Ringe, J. D., Vellas, B. and Reginster, J. (2009) 'Management of osteoporosis in the elderly', *Curr Med Res Opin,* 25(10), pp. 2373-87.

Robberecht, H. J., and Deelstra, H. A. (1984) 'Selenium in human urine: concentration levels and medical implications', *Clinica Chemica Acta*, 136(2-3), pp. 107-120.

Robberecht, H., De Bruyne, T., Davioud-Charvet, E., Mackrill, J. and Hermans, N. (2019) 'Selenium Status in Elderly People: Longevity and Age-Related Diseases', *Curr Pharm Des,* 25(15), pp. 1694-1706.

Roberts C., Steer T., Maplethorpe, N., Cox, L., Meadows, S., Nicholson, S., Page, P., and Swan, G. (2018) 'Results from Years 7 and 8 (combined) of the Rolling Programme (2014/2015 to 2015/2016)', Public Health England, National Diet and Nutrition Survey (NDNS).

Roberts, H. C., Denison, H. J., Martin, H. J., Patel, H. P., Syddall, H., Cooper, C. and Sayer, A. A. (2011) 'A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach', *Age and Ageing,* 40(4), pp. 423-429.

Robinson M. F., Stewart, R. D., Rea, H. M., Thomson, C. D., Snow, P. G., Squires, I. H. (1981) 'Effect of Daily Supplements of Selenium on Patients With Muscular Complaints in Otago and Canterbury', *The New Zealand Medical Journal,* 93(683), pp. 289-292.

Robinson, J. R., Robinson, M. F., Levander, O. A., and Thomson, C. D (1985) 'Urinary excretion of selenium by New Zealand and North American human subjects on differing intakes', *The American Journal of Clinical Nutrition*, 41(5), pp. 1023-1031.

Robinson, M. F., Rea, H. M., Friend, G. M., Stewart, R. D., Snow, P. C. and Thomson, C. D. (1978) 'On supplementing the selenium intake of New Zealanders. 2. Prolonged metabolic experiments with daily supplements of selenomethionine, selenite and fish',*The British Journal of Nutrition,* 39(3), pp. 589-600.

Robinson, S. M., Jameson, K. A., Batelaan, S. F., Martin, H. J., Syddall, H. E., Dennison, E. M., Cooper, C., Sayer, A. A., and Hertfordshire Cohort Study Group (2008) 'Diet and its relationship with grip strength in community-dwelling older men and women: The Hertfordshire cohort study', *Journal of American Geriatrics Society,* 56(1), pp. 84-90.

Robinson, S., Cooper, C. and Aihie Sayer, A. (2012) 'Nutrition and Sarcopenia: A Review of the Evidence and Implications for Preventive Strategies', *Journal of Aging Research,* 2012, pp. 510801.

Roca-Perez, L., Gil, C., Cervera, M. L., Gonzálvez, A., Ramos-Miras, J., Pons, V., Bech, J., and Boluda, R. (2010) 'Selenium and heavy metals content in some Mediterranean soils', *Journal of Geochemical Exploration*, 107(2), pp. 110-116.

Roman, M., Jitaru, P., and Barbante, C. (2014) 'Selenium biochemistry and its role for human health', *Metallomics: Integrated Biometal Science,* 6(1), pp. 25-54.

Rosa-Caldwell, M. E. and Greene, N. P. (2019) 'Muscle metabolism and atrophy: let's talk about sex', *Biology of Sex Differences, Biology of Sex Differences*, 10(1), pp. 43.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G. and Hoekstra, W. G. (1973) 'Selenium: biochemical role as a component of glutathione peroxidase', *Science,* 179(4073), pp. 588-90.

Rucci, N. (2008) 'Molecular biology of bone remodelling', *Clinical cases in mineral and bone metabolism : the official journal of the Italian Society of Osteoporosis, Mineral Metabolism, and Skeletal Diseases,* 5(1), pp. 49-56.

Rusip G., and Suhartini S., M. (2020). ' Effects of moderate intentsity exercse on glutathione peroxidase activity and VO_2max in elderly women', *Journal of Medical Sciences*, 25(8), 230-233.

Russell, R. M., Rasmussen, H., and Fada. (1999) 'The Impact of Nutritional Needs of Older Adults on Recommended Food Intakes', *Nutrition in Clinical Care,* 2(3), pp. 164-176.

Sabé, R., Rubio, R. and García-Beltrán, L. (2003) 'Selenium measurement in human plasma with Zeeman effect electrothermal atomic absorption spectrometry: sample stability and calibration method', *Journal of Trace Elements in Medicine and Biology,* 17(2), pp. 73-77.

Saito, Y., Sato, N., Hirashima, M., Takebe, G., Nagasawa, S., and Takahashi, K. (2004) 'Domain structure of bi-functional selenoprotein P', *Biochemical Journal*, 381(Pt 3), pp. 841-846.

Saito, Y., and Takahashi, K. (2002) 'Characterization of selenoprotein P as a selenium supply protein', *Eur J Biochem,* 269(22), pp. 5746-51.

Salehi, M., Sohrabi, Z., Ekramzadeh, M., Fallahzadeh, M. K., Ayatollahi, M., Geramizadeh, B., Hassanzadeh, J., and Sagheb, M. M. (2013) 'Selenium supplementation improves the nutritional status of hemodialysis patients: a randomized, double-blind, placebo-controlled trial', *Nephrology, Dialysis, Transplantation,* 28(3), pp. 716-723.

Sánchez-Rodríguez, M. A., Ruiz-Ramos, M., Correa-Muñoz, E. and Mendoza-Núñez, V. M. (2007) 'Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes', *BMC Musculoskeletal Disorders,* 8(1), pp. 124.

Sasaki, S., Iwata, H., Ishiguro, N., Habuchi, O., and Miura, T. (1994) 'Low-selenium Diet, Bone, and Articular Cartilage in Rats', *Nutrition,* 10(6) pp. 538-543.

Savory, L. A., Kerr, C. J., Whiting, P., Finer, N., McEneny, J. and Ashton, T. (2012) 'Selenium supplementation and exercise: effect on oxidant stress in overweight adults', *Obesity,* 20(4), pp. 794-801.

Savva, G. M., Donoghue, O. A., Horgan, F., O'Regan, C., Cronin, H. and Kenny, R. A. (2013) 'Using timed up-and-go to identify frail members of the older population', *J Gerontol A Biol Sci Med Sci,* 68(4), pp. 441-6.

Schaap, L. A., Pluijm, S. M., Deeg, D. J. and Visser, M. (2006) 'Inflammatory markers and loss of muscle mass (sarcopenia) and strength', *Am J Med,* 119(6), pp. 526.e9-17.

Scheidt-Nave, C., Bismar, H., Leidig-Bruckner, G., Woitge, H., Seibel, M. J., Ziegler, R. and Pfeilschifter, J. (2001) 'Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause', *J Clin Endocrinol Metab,* 86(5), pp. 2032-42.

Schlemmer, A. and Hassager, C. (1999) 'Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption', *Eur J Endocrinol,* 140(4), pp. 332-7.

Schomburg, L. (2022) 'Selenoprotein P – Selenium transport protein, enzyme and biomarker of selenium status', *Free Radical Biology and Medicine,* 191, pp. 150-163.

Schomburg, L. and Schweizer, U. (2009) 'Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium', *Biochim Biophys Acta,* 1790(11), pp. 1453-62.

Schomburg, L., Orho-Melander, M., Struck, J., Bergmann, A. and Melander, O. (2019) 'Selenoprotein-P deficiency predicts cardiovascular disease and death', *Nutrients,* 11(8), pp. 1852.

Schomburg, L., Riese, C., Renko, K. and Schweizer, U. (2007) 'Effect of age on sexually dimorphic selenoprotein expression in mice', *Biological chemistry,* 388(10), pp. 1035-1041.

Schomburg, L., Schweizer, U., Holtmann, B., Flohé, L., Sendtner, M. and Köhrle, J. (2003) 'Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues', *Biochem J,* 370(Pt 2), pp. 397-402.

Schousboe, J. T., and Bauer, D. C. (2012) 'Clinical use of bone turnover markers to monitor pharmacologic fracture prevention therapy', *Current Osteoporosis Reports*, 10(1), pp. 56-63.

Schrauzer, G. N. (2000) 'Selenomethionine: a review of its nutritional significance, metabolism and toxicity', *J Nutr,* 130(7), pp. 1653-6.

Schümann, K., Classen, H. G., Hages, M., Prinz-Langenohl, R., Pietrzik, K. and Biesalski, H. K. (1997) 'Bioavailability of oral vitamins, minerals, and trace elements in perspective', *Arzneimittelforschung,* 47(4), pp. 369-80.

Schwarz, K., and Foltz, C. M. (1957) 'Selenium as an Integral Part of Factor 3 Against Dietary Necrotic Liver Degeneration', *Nutrition*, 79(12), pp. 3292-3293.

Schweizer, U., Bräuer, A. U., Köhrle, J., Nitsch, R. and Savaskan, N. E. (2004) 'Selenium and brain function: a poorly recognized liaison', *Brain Res Brain Res Rev,* 45(3), pp. 164-78.

Schweizer, U., Streckfuss, F., Pelt, P., Carlson, B. A., Hatfield, D. L., Köhrle, J. and Schomburg, L. (2005) 'Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply', *The Biochemical Journal,* 386(Pt 2), pp. 221-226.

Schwiebert, C., Kühnen, P., Becker, N.-P., Welsink, T., Keller, T., Minich, W. B., Wiegand, S. and Schomburg, L. (2020) 'Antagonistic Autoantibodies to Insulin-Like Growth Factor-1 Receptor Associate with Poor Physical Strength', *International Journal of Molecular Sciences*, 21(2). DOI: 10.3390/ijms21020463.

Scientific Advisory Committee on Nutrition (2013) Position Statement on Selenium and Health. https://www.gov.uk/government/publications/sacn-statement-on-selenium-and-health-2013 Accessed on: 03/03/22

Scientific Advisory Committee on Nutrition (2016) 'Vitamin D and Health', The Scientific Advisory Committee on Nutrition. https://www.gov.uk/government/publications/sacn-vitamin-d-and-health-report Accessed on: 12/10/22

Scott, D., Blizzard, L., Fell, J., Giles, G., and Jones, G. (2010) 'Associations between dietary nutrient intake and muscle mass and strength in community-dwelling older adults: The Tasmanian older adult cohort study', *Journal of the American Geriatrics Society,* 58(11), pp. 2129-2134.

Scott, J. P., Sale, C., Greeves, J. P., Casey, A., Dutton, J., and Fraser, W. D. (2012) 'Effect of fasting versus feeding on the bone metabolic response to running', *Bone*, 51(6), pp. 990-999.

Semba, R. D., Bartali, B., Zhou, J., Blaum, C., Ko, C. W. and Fried, L. P. (2006) 'Low serum micronutrient concentrations predict frailty among older women living in the community', *J Gerontol A Biol Sci Med Sci,* 61(6), pp. 594-9.

Sempértegui, F., Estrella, B., Vallejo, W., Tapia, L., Herrera, D., Moscoso, F., Cerón, G., Griffiths, J. K., and Hamer, D. H. (2003) 'Selenium serum concentrations in malnourished Ecuadorian children: A case-control study', *International Journal for Vitamin and Nutrition Research,* 73(3), pp. 181-186.

Shafiei Neek, L., Gaeini, A. A. and Choobineh, S. (2011) 'Effect of zinc and selenium supplementation on serum testosterone and plasma lactate in cyclist after an exhaustive exercise bout', *Biol Trace Elem Res,* 144(1-3), pp. 454-62.

Shahar, A., Patel, K. V., Semba, R. D., Bandinelli, S., Shahar, D. R., Ferrucci, L. and Guralnik, J. M. (2010) 'Plasma selenium is positively related to performance in neurological tasks assessing coordination and motor speed', *Mov Disord,* 25(12), pp. 1909-15.

Sharkey, J. R., Giuliani, C., Haines, P. S., Branch, L. G., Busby-Whitehead, J. and Zohoori, N. (2003) 'Summary measure of dietary musculoskeletal nutrient (calcium, vitamin D, magnesium, and phosphorus) intakes is associated with lower-extremity physical performance in homebound elderly men and women', *The American Journal of Clinical Nutrition,* 77(4), pp. 847-856.

Shen, Y., Huang, X., Wu, J., Lin, X., Zhou, X., Zhu, Z., Pan, X., Xu, J., Qiao, J., Zhang, T., Ye, L., Jiang, H., Ren, Y. and Shan, P. F. (2022) 'The Global Burden of Osteoporosis, Low Bone Mass, and Its Related Fracture in 204 Countries and Territories, 1990-2019', *Front Endocrinol,* 13, pp. 882241.

Sheppard, A. D. and Millar, K. R. (1981) 'Stability of glutathione peroxidase in ovine blood samples under various storage conditions and the response of this enzyme to different methods of selenium supplementation', *N Z Vet J,* 29(5), pp. 77-80.

Shigdel, R., Osima, M., Ahmed, L. A., Joakimsen, R. M., Eriksen, E. F., Zebaze, R. and Bjornerem, A. (2015) 'Bone turnover markers are associated with higher cortical porosity, thinner cortices, and larger size of the proximal femur and non-vertebral fractures', *Bone,* 81, pp. 1-6.

Shumway-Cook, A., Brauer, S. and Woollacott, M. (2000) 'Predicting the probability for falls in community-dwelling older adults using the Timed Up & Go Test', *Phys Ther,* 80(9), pp. 896-903.

Siervo, M., Bertoli, S., Battezzati, A., Wells, J. C., Lara, J, Ferraris, C., and Tagliabue, A. (2014) 'Accuracy of predictive equations for the measurement of resting energy expenditure in older subjects', *Clinical Nutrition,* 33(4), pp. 613-619.

Silverman, S. L. and Calderon, A. D. (2010) 'The Utility and Limitations of FRAX: A US Perspective', *Current Osteoporosis Reports,* 8(4), pp. 192-197.

Siris, E. S., Baim, S. and Nattiv, A. (2010) 'Primary care use of FRAX: absolute fracture risk assessment in postmenopausal women and older men', *Postgrad Med,* 122(1), pp. 82-90.

Skrajnowska, D., Jagielska, A., Ruszczyńska, A., Idkowiak, J. and Bobrowska-Korczak, B. (2022) 'Effect of Copper and Selenium Supplementation on the Level of Elements in Rats' Femurs under Neoplastic Conditions', *Nutrients,* 14(6), pp. 1285.

Smith, G. D. and Ebrahim, S. (2003) ''Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?', *Int J Epidemiol,* 32(1), pp. 1-22.

Solovyev, N., Vanhaecke, F., and Michalke, B. (2019) 'Selenium and iodine in diabetes mellitus with a focus on the interplay and speciation of the elements', *Journal of Trace Elements in Medicne and Biology*, 56, pp. 69-80.

Sontakke, A. N., and Tare, R. S. (2002) 'A duality in the roles of reactive oxygen species with respect to bone metabolism', *Clinica Chemica Acta*, 318, pp. 145-148.

Sornay-Rendu, E., Munoz, F., Garnero, P., Duboeuf, F. and Delmas, P. D. (2005) 'Identification of osteopenic women at high risk of fracture: the OFELY study', *J Bone Miner Res,* 20(10), pp. 1813-9.

Spallholz, J. E. (1994) 'On the nature of selenium toxicity and carcinostatic activity', *Free Radic Biol Med,* 17(1), pp. 45-64.

Steinbrenner, H. and Sies, H. (2009) 'Protection against reactive oxygen species by selenoproteins', *Biochimica et Biophysica Acta,* 1790(11), pp. 1478-1485.

Steptoe, A., Breeze, E., Banks, J. and Nazroo, J. (2013) 'Cohort profile: the English longitudinal study of ageing', *Int J Epidemiol,* 42(6), pp. 1640-8.

Sternfeld, B., Ngo, L., Satariano, W. A. and Tager, I. B. (2002) 'Associations of body composition with physical performance and self-reported functional limitation in elderly men and women', *Am J Epidemiol,* 156(2), pp. 110-21.

Steyn, M., Zitouni, K., Kelly, F. J., Cook, P. and Earle, K. A. (2019) 'Sex Differences in Glutathione Peroxidase Activity and Central Obesity in Patients with Type 2 Diabetes at High Risk of Cardio-Renal Disease', *Antioxidants,* 8(12), pp. 629.

Stoffaneller, R., and Morse, N. L. (2015) 'A review of dietary selenium intake and selenium status in Europe and the Middle East', *Nutrients*, 7(3), pp. 1494-1537.

Stranges, S., Sieri, S., Vinceti, M., Grioni, S., Guallar, E., Laclaustra, M., Muti, P., Berrino, F., Krogh, V. (2010) 'A prospective study of dietary selenium intake and risk of type 2 diabetes', *BMC Public Health*, 10, pp. 564.

Studenski, S., Perera, S., Patel, K., Rosano, C., Faulkner, K., Inzitari, M., Brach, J., Chandler, J., Cawthon, P., Connor, E. B., Nevitt, M., Visser, M., Kritchevsky, S., Badinelli, S., Harris, T., Newman, A. B., Cauley, J., Ferrucci, L. and Guralnik, J. (2011) 'Gait Speed and Survival in Older Adults', *JAMA,* 305(1), pp. 50-58.

Suadicani, P., Hein, H. O. and Gyntelberg, F. (1992) 'Serum selenium concentration and risk of ischaemic heart disease in a prospective cohort study of 3000 males', *Atherosclerosis,* 96(1), pp. 33-42.

Suadicani, P., Hein, H. O. and Gyntelberg, F. (2012) 'Serum selenium level and risk of lung cancer mortality: a 16-year follow-up of the Copenhagen Male Study', *Eur Respir J,* 39(6), pp. 1443-8.

Sun, L. L., Li, B. L., Xie, H. L., Fan, F., Yu, W. Z., Wu, B. H., Xue, W. Q., and Chen, Y. M. (2014) 'Associations between the dietary intake of antioxidant nutrients and the risk of hip fracture in elderly Chinese: A case-control study', *The British Journal of Nutrition*, 112(10), pp. 1706-1714.

Sunde, R. A., Paterson, E., Evenson, J. K., Barnes, K. M., Lovegrove, J. A., and Gordon, M. H. (2008) 'Longitudinal selenium status in healthy British adults: Assessment using biochemical and molecular biomarkers', *The British Journal of Nutrition*, 99(Suppl 3), pp. S37-S47.

Sunde, R. A., Raines, A. M. (2011) 'Selenium regulation of the selenoprotein and nonselenoprotein transcriptomes in rodents', *Advances in Nutrition*, 2(2), pp. 138-150.

Sunde, R. A., Thompson, B. M., Palm, M. D., Weiss, S. L., Thompson, K. M., and Evenson, J. K. (1997) 'Selenium regulation of selenium dependent glutathione peroxidases in animals and transfected CHO cells', *Biomedical and Environmental Sciences*, 10(2-3), pp. 346-355.

Sunde, R. A., Raines, A. M., Barnes, K. M. and Evenson, J. K. (2009) 'Selenium status highly regulates selenoprotein mRNA levels for only a subset of the selenoproteins in the selenoproteome', *Bioscience Reports,* 29(5), pp. 329-338.

Suzuki, K. T., Kurasaki, K., Ogawa, S., and Suzuki, N. (2006) 'Metabolic transformation of methylseleninic acid through key selenium intermediate selenide', *Toxicology and Applied Pharmacology*, 215(2), pp. 189-197.

Szulc, P. and Delmas, P. D. (2008) 'Biochemical markers of bone turnover: potential use in the investigation and management of postmenopausal osteoporosis', *Osteoporos Int,* 19(12), pp. 1683-704.

Takada, J., Dinavahi, R., Miyauchi, A., Hamaya, E., Hirama, T., Libanati, C., Nakamura, Y., Milmont, C. E., and Grauer, A. (2020) 'Correction to: Relationship between P1NP, a biochemical marker of bone turnover, and bone mineral density in patients transitioned from alendronate to romosozumab or teriparatide: a post hoc analysis of the STRUCTURE trial, *Journal of Bone and Mineral Metabolism*, 38(3), pp. 310-315.

Tamas, M., Ḿandoki, Z., and Csaṕo, J. (2010) 'The role of selenium content of wheat in the human nutrition. A literature review', *Acta Univ. Sapientiae, Alimentaria,* 3, pp. 5-34.

Tan, L. C., Nancharaiah, Y. V., van Hullebusch, E. D. and Lens, P. N. L. (2016) 'Selenium: environmental significance, pollution, and biological treatment technologies', *Biotechnology Advances,* 34(5), pp. 886-907.

Tarp, U., Thorling, E. B. and Hansen, J. C. (1990) 'Longitudinal study of changes in blood selenium among danish blood bank donors (1974–1988)', *Nutrition Research,* 10(10), pp. 1171-1176.

Taylor, A. H., Cable, N. T., Faulkner, G., Hillsdon, M., Narici, M. and Van Der Bij, A. K. (2004) 'Physical activity and older adults: a review of health benefits and the effectiveness of interventions', *Journal of Sports Sciences,* 22(8), pp. 703-725.

ter Borg, S., Verlaan, S., Hemsworth, J., Mijnarends, D. M., Schols, J. M. G. A., Luiking, Y. C. and de Groot, L. C. P. G. M. (2015) 'Micronutrient intakes and potential inadequacies of community-dwelling older adults: a systematic review', *The British journal of nutrition,* 113(8), pp. 1195-1206.

Tessier, F., Margaritis, I., Richard, M. J., Moynot, C. and Marconnet, P. (1995) 'Selenium and training effects on the glutathione system and aerobic performance', *Med Sci Sports Exerc,* 27(3), pp. 390-6.

Theiler, R., Stahelin, H. B., Kranzlin, M., Somorjai, G., Singer-Lindpaintner, L., Conzelmann, M., Geusens, P., and Bischoff, H. A. (2000) 'Influence of physical mobility and season on 25-hydroxyvitamin D-parathyroid hormone interaction and bone remodelling in the elderly', *European Journal of Endocrinology*, 143(5), pp. 673-679.

Thisse, C., Degrave, A., Kryukov, G. V., Gladyshev, V. N., Obrecht-Pflumio, S., Krol, A., Thisse, B. and Lescure, A. (2003) 'Spatial and temporal expression patterns of selenoprotein genes during embryogenesis in zebrafish', *Gene Expr Patterns,* 3(4), pp. 525-32.

Thompson, K. M., Haibach, H. and Sunde, R. A. (1995) 'Growth and plasma triiodothyronine concentrations are modified by selenium deficiency and repletion in second-generation selenium-deficient rats', *J Nutr,* 125(4), pp. 864-73.

Thomson, C. D. (2003) 'Proposed Australian and New Zealand nutrient reference values for selenium and iodine', *J. Nutr. Suppl,* 133.

Thomson, C. D. (2004a) 'Assessment of requirements for selenium and adequacy of selenium status: a review', *European Journal of Clinical Nutrition*, 58(3), pp. 391-402.

Thomson, C. D. (2004b) 'Selenium and iodine intakes and status in New Zealand and Australia', *The British Journal of Nutrition*, 91(5), pp. 661-672.

Thomson, C. D., Rea, H. M., Doesburg, V. M. and Robinson, M. F. (1977) 'Selenium concentrations and glutathione peroxidase activities in whole-blood of New Zealand residents', *The British Journal of Nutrition,* 37(3), pp. 457-60.

Thomson, C. D., Robinson, M. F., Butler, J. A. and Whanger, P. D. (1993) 'Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase (EC 1.11.1.9) in blood components of New Zealand women', *The British Journal of Nutrition,* 69(2), pp. 577-88.

Thomson, C. D., Robinson, M. F., Campbell, D. R. and Rea, H. M. (1982) 'Effect of prolonged supplementation with daily supplements of selenomethionine and sodium selenite on glutathione peroxidase activity in blood of New Zealand residents', *Am J Clin Nutr,* 36(1), pp. 24-31.

Thomson, M. R. (1986) 'Urinary and fecal excretions and absorption of a large supplement of selenium: superiority of selenate over selenite', *Am J Clin Nutr*, 44(5), pp. 659-665.

Tian, X.-Z., Li, J.-X., Luo, Q.-Y., Wang, X., Xiao, M.-M., Zhou, D., Lu, Q. and Chen, X. (2022) 'Effect of Supplementation With Selenium-Yeast on Muscle Antioxidant Activity, Meat Quality, Fatty Acids and Amino Acids in Goats', *Frontiers in veterinary science,* 8, pp. 813672-813672.

Tolonen, M. (1990) 'Vitamins and minerals in health and nutrition' Woodhead Publishing Series, Food Science, Technology and Nutrition, Elsevier pp. 1-240.

Toppo, S., Vanin, S., Bosello, V. and Tosatto, S. C. E. (2008) 'Evolutionary and structural insights into the multifaceted glutathione peroxidase (Gpx) superfamily', *Antioxidants and Redox Signaling,* 10(9), pp. 1501-1514.

Touat-Hamici, Z., Bulteau, A. L., Bianga, J., Jean-Jacques, H., Szpunar, J., Lobinski, R. and Chavatte, L. (2018) 'Selenium-regulated hierarchy of human selenoproteome in cancerous and immortalized cells lines', *Biochim Biophys Acta Gen Subj,* 1862(11), pp. 2493-2505.

Tranah, G. J., Taylor, B. C., Lui, L.-Y., Zmuda, J. M., Cauley, J. A., Ensrud, K. E., Hillier, T. A., Hochberg, M. C., Li, J., Rhees, B. K., Erlich, H. A., Sternlicht, M. D., Peltz, G., Cummings, S. R. and Study of Osteoporotic Fractures Research, G. (2008) 'Genetic variation in candidate osteoporosis genes, bone mineral density, and fracture risk: the study of osteoporotic fractures', *Calcified Tissue International,* 83(3), pp. 155-166.

Tromp, A. M., Ooms, M. E., Popp-Snijders, C., Roos, J. C., and Lips, P. (2000) 'Predictors of fractures in elderly women', *Osteoporosis International*, 11(2), pp. 134-140.

Tseng, C. K., Ho, C. T., Hsu, H. S., Lin, C. H., Li, C. I., Li, T. C., Liu, C. S., Lin, C. C. and Lin, W.-Y. (2013) 'Selenium is inversely associated with interleukin-6 in the elderly', *The Journal of Nutrition, Health and Aging,* 17(3), pp. 280-284.

Tsujimoto, M., Chen, P., Miyauchi, A., Sowa, H., and Krege, J. H. (2011) 'PINP as an aid for monitoring patients treated with teriparatide', *Bone*, 48(4), pp. 798-803.

Turner, M. R., Camacho, X., Fischer, H. D., Austin, P. C., Anderson, G. M., Rochon, P. A. and Lipscombe, L. L. (2011) 'Levothyroxine dose and risk of fractures in older adults: nested case-control study', *BMJ Clinical Research,* 342, pp. d2238-d2238.

United Nations (2012). In Affairs DoEaS, ed. Population Ageing and Development. New York: United Nations. https://www.un.org/en/development/desa/publications/world-population-prospects-the-2012-revision.html Accessed on: 20/11/22

United Nations (2017) 'World Population Ageing'. https://www.un.org/development/desa/publications/world-population-prospects-the-2017-revision.html Accessed on: 30/10/22

van Dijk, M., Dijk, F. J., Bunschoten, A., van Dartel, D. A., van Norren, K., Walrand, S., Jourdan, M., Verlaan, S. and Luiking, Y. (2016) 'Improved muscle function and quality after diet intervention with leucine-enriched whey and antioxidants in antioxidant deficient aged mice', *Oncotarget,* 7(14), pp. 17338-55.

van Dronkelaar, C., van Velzen, A., Abdelrazek, M., van der Steen, A., Weijs, P. J. M. and Tieland, M. (2018) 'Minerals and Sarcopenia; The Role of Calcium, Iron, Magnesium, Phosphorus, Potassium, Selenium, Sodium, and Zinc on Muscle Mass, Muscle Strength, and Physical Performance in Older Adults: A Systematic Review', *J Am Med Dir Assoc,* 19(1), pp. 6-11.e3.

van Rij, A. M., Thomson, C. D., McKenzie, J. M. and Robinson, M. F. (1979) 'Selenium deficiency in total parenteral nutrition', *The American Journal of Clinical Nutrition,* 32(10), pp. 2076-2085.

Vasikaran, S., Cooper, C., Eastell, R., Griesmacher, A., Morris, H. A., Trenti, T., and Kanis, J. A. (2011) 'International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine Position on bone marker standards in osteoporosis', *Clinical Chemistry and Laboratory Medicine,* 49(8), pp. 1271-1274.

Vasunilashorn, S., Coppin, A. K., Patel, K. V., Lauretani, F., Ferrucci, L., Bandinelli, S. and Guralnik, J. M. (2009) 'Use of the Short Physical Performance Battery Score to predict loss of ability to walk meters: analysis from the InCHIANTI study', *J Gerontol A Biol Sci Med Sci,* 64(2), pp. 223-9.

Vendeland, S. C., Beilstein, M. A., Yeh, J. Y., Ream, W. and Whanger, P. D. (1995) 'Rat skeletal muscle selenoprotein W: cDNA clone and mRNA modulation by dietary selenium', *Proc Natl Acad Sci U S A,* 92(19), pp. 8749-53.

Vendeland, S. C., Deagen, J. T., Butler, J. A. and Whanger, P. D. (1994) 'Uptake of selenite, selenomethionine and selenate by brush border membrane vesicles isolated from rat small intestine', *Biometals,* 7(4), pp. 305-12.

Vertesi, A., Lever, J. A., Molloy, D. W., Sanderson, B., Tuttle, I., Pokoradi, L. and Principi, E. (2001) 'Standardized Mini-Mental State Examination. Use and interpretation', *Can Fam Physician,* 47, pp. 2018-23.

Vilaca, T., Gossiel, F. and Eastell, R. (2017) 'Bone Turnover Markers: Use in Fracture Prediction', *J Clin Densitom,* 20(3), pp. 346-352.

Vinceti, M., Crespi, C. M., Malagoli, C., Del Giovane, C. and Krogh, V. (2013) 'Friend or foe? The current epidemiologic evidence on selenium and human cancer risk', *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev,* 31(4), pp. 305-41.

Voelcker-Rehage, C., Godde, B., and Staudinger, U. M. (2010). 'Physical and motor fitness are both related to cognition in old age', *The European journal of neuroscience*, *31*(1), pp. 167–176.

von Faber, M., Bootsma-van der Wiel, A., van Exel, E., Gussekloo, J., Lagaay, A. M., van Dongen, E., Knook, D. L., van der Geest, S. and Westendorp, R. G. (2001) 'Successful aging in the oldest old: Who can be characterized as successfully aged?', *Arch Intern Med,* 161(22), pp. 2694-700.

von Haehling, S., Morley, J. E., and Anker, S. D. (2010). 'An overview of sarcopenia: Facts and numbers on prevalence and clinical impact', *Journal of Cachexia, Sarcopenia and Muscle*, 1(2), pp. 129-133.

Wade, S. W., Strader, C., Fitzpatrick, L. A., Anthony, M. S., and O'Malley, C. D. (2014) 'Estimating prevalence of osteoporosis: examples from industrialized countries.' *Archives of Osteoporosis*, 9, pp. 182.

Wainwright, S. A., Marshall, L. M., Ensrud, K. E., Cauley, J. A., Black, D. M., Hillier, T. A., Hochberg, M. C., Vogt, M. T. and Orwoll, E. S. (2005) 'Hip fracture in women without osteoporosis', *J Clin Endocrinol Metab,* 90(5), pp. 2787-93.

Walsh, J. S., Jacques, R. M., Schomburg, L., Hill, T. R., Mathers, J. C., Williams, G. R. and Eastell, R. (2021) 'Effect of selenium supplementation on musculoskeletal health in older women: a

randomised, double-blind, placebo-controlled trial', *Lancet Healthy Longev,* 2(4), pp. e212-e221.

Walston, J., Xue, Q., Semba, R. D., Ferrucci, L., Cappola, A. R., Ricks, M., Guralnik, J. and Fried, L. P. (2006) 'Serum antioxidants, inflammation, and total mortality in older women', *Am J Epidemiol,* 163(1), pp. 18-26.

Walzel, E. 'Selenium (Environmental Health Criteria 58). 306 Seiten, 13 Abb., 57 Tab. World Health Organization, Geneva 1987. Preis: 24,— Sw.fr.; 14,40 US'.

Wang, L., Wang, L., Zhang, D., Li, S., Yin, J., Xu, Z. and Zhang, X. (2020) 'Effect of dietary selenium on postprandial protein deposition in the muscle of juvenile rainbow trout (Oncorhynchus mykiss)', *Br J Nutr*, 125(7), pp. 1-11.

Wang, L., Yu, H., Yang, G., Zhang, Y., Wang, W., Su, T., Ma, W., Yang, F., Chen, L., He, L., Ma, Y. and Zhang, Y. (2015) 'Correlation between bone mineral density and serum trace element contents of elderly males in Beijing urban area', *Int J Clin Exp Med,* 8(10), pp. 19250-7.

Wang, Y., Xie, D., Li, J., Long, H., Wu, J., Wu, Z., He, H., Wang, H., Yang, T., and Wang, Y. (2019) 'Association between dietary selenium intake and the prevalence of osteoporosis: A cross-sectional study', *BMC Musculoskeletal*, 20, pp. 585.

Weitoft, T., Larsson, A., Saxne, T., and Rönnblom, L. (2005) 'Changes of cartilage and bone markers after intraarticular glucocorticoid treatment with and without postinjection rest in patients with rheumatoid arthritis', *Annals of the Rheumatic Diseases,* 64(12), pp. 1750-1753.

Whanger, P. D. (2004) 'Selenium and its relationship to cancer: an update', *The British Journal of Nutrition*, 91(1), pp. 11-28.

Whanger, P. D. (2009) 'Selenoprotein expression and function-selenoprotein W', *Biochim Biophys Acta,* 1790(11), pp. 1448-52.

Whanger, P. D., Beilstein, M. A., Thomson, G. D., Robinson, M. F. and Howe, M. (1988) 'Blood selenium and glutathione peroxidase activity of populations in New Zealand, Oregon, and South Dakota1', *The FASEB Journal,* 2(14), pp. 2996-3002.

White, P. J. (2016) 'Selenium accumulation by plants', *Annals of Botany,* 117(2), pp. 217-235.

White, P. J., Broadley, M. R. and Gregory, P. J. (2012) 'Managing the nutrition of plants and people', *Applied and Environmental Soil Science,* 2012. 108826. ISSN 1687-7675

White, S. H., Johnson, S. E., Bobel, J. M. and Warren, L. K. (2016) 'Dietary selenium and prolonged exercise alter gene expression and activity of antioxidant enzymes in equine skeletal muscle', *J Anim Sci,* 94(7), pp. 2867-78.

Wilkinson, D. J., Piasecki, M. and Atherton, P. J. (2018) 'The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans', *Ageing Research Reviews,* 47, pp. 123-132.

Willers, C., Norton, N., Harvey, Nicholas C., Jacobson, T., Johansson, H., Lorentzon, M., McCloskey, E. V., Borgström, Fredrik and Kanis, J. A. (2022) 'Osteoporosis in Europe: a compendium of country-specific reports', *Archives of Osteoporosis,* 17(1), pp. 23.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019) 'Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems', *The Lancet*, 393(10170), pp. 447-492.

Winkel, L. H., Vriens, B., Jones, G. D., Schneider, L. S., Pilon-Smits, E. and Bañuelos, G. S. (2015) 'Selenium cycling across soil-plant-atmosphere interfaces: a critical review', *Nutrients,* 7(6), pp. 4199-239.

Wolf, R. L., Cauley, J. A., Pettinger, M., Jackson, R., Lacroix, A., Leboff, M. S., Lewis, C. E., Nevitt, M. C., Simon, J. A., Stone, K. L., and Wactawski-Wende, J. (2005) 'Lack of a relation between vitamin and mineral antioxidants and bone mineral density: results from the Women's Health Initiative', *The American Journal of Clinical Nutrition*, 82(3), pp. 581-588.

World Health Organization (2004). 'Selenium'. A report of the International Programme on Chemical Safety. Environmental Health Criteria number 58, World Health Organization, Geneva (1987).

World Health Organization (2004) 'Vitamin and mineral requirements in human nutrition', *World Health Organization and Food and Agriculture Organization of the United Nations,* (Second Edition), pp. 1-341.

World Health Organization. (1994) 'Assessment of fracture risk and its application to screening for postmenopausal osteoporosis : report of a WHO study group' [meeting held in Rome from 22 to 25 June 1992]. World Health Organization. https://apps.who.int/iris/handle/10665/39142

Wu, C. C., Wang, C. K., Yang, A. M., Lu, C. S., and Lin, C. Y. (2021) 'Selenium status is independently related to bone mineral density, FRAX score, and bone fracture history: NHANES, 2013 to 2014', *Bone*, 143, pp. 115631.

Wu, Q., Yao, H. D., Tan, S. R., Zhang, Z. W., Zhu, Y. H. and Xu, S. (2014) 'Possible correlation of selenoprotein W with inflammation factors in chicken skeletal muscles', *Biol Trace Elem Res,* 161(2), pp. 167-72.

Wu, Y., Xiang, C., Jia, M., and Fang, Y. (2022) 'Interpretable classifiers for prediction of disability trajectories using a nationwide longitudinal database', *BMC Geriatrics,* 22(1), pp. 627.

Xia, Y., Hill, K. E., Byrne, D. W., Xu, J. and Burk, R. F. (2005) 'Effectiveness of selenium supplements in a low-selenium area of China', *Am J Clin Nutr,* 81(4), pp. 829-34.

Xia, Y., Hill, K. E., Li, P., Xu, J., Zhou, D., Motley, A. K., Wang, L., Byrne, D. W. and Burk, R. F. (2010) 'Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled, double-blind study of selenomethionine supplementation in selenium-deficient Chinese subjects', *Am J Clin Nutr,* 92(3), pp. 525-31.

Xie, D., Liao, Y., Yue, J., Zhang, C., Wang, Y., Deng, C., et al. (2018) 'Effects of five types of selenium supplementation for treatment of Kashin-Beck disease in children: a systematic review and network meta-analysis', *BMJ Open*, 8(3), pp. e017883.

Xie, M., Sun, X., Li, P., Shen, X. and Fang, Y. (2021) 'Selenium in cereals: Insight into species of the element from total amount', *Compr Rev Food Sci Food Saf,* 20(3), pp. 2914-2940.

Yang, C., Wolf, E., Roser, K., Delling, G. and Muller, P. K. (1993) 'Selenium deficiency and fulvic acid supplementation induces fibrosis of cartilage and disturbs subchondral ossification in knee joints of mice: an animal model study of Kashin-Beck disease', *Virchows Arch A Pathol Anat Histopathol,* 423(6), pp. 483-91.

Yang, G., Zhou, R., Yin, S., Gu, L.,Yan, B., Liu, Y., Liu, Y., and Li, X. (1989) 'Studies of safe maximal daily dietary selenium intake in a seleniferous area in China. I. Selenium intake and tissue selenium levels of the inhabitants', *Journal of Trace Elements and Electrolytes in Health and Disease* 3(2), pp. 77-87.

Yang, G.Q. (1987) 'Human selenium requirements in China', *Selenium in Biology and Medicine*, pp. 589-607.

Yao, H. D., Wu, Q., Zhang, Z. W., Li, S., Wang, X. L., Lei, X. G., and Xu, S. W. (2013a) 'Selenoprotein W serves as an antioxidant in chicken myoblasts', *Biochimica et Biophysica Acta ,* 1830(4), pp. 3112-3120.

Yao, H. D., Wu, Q., Zhang, Z. W., Zhang, J. L., Li, S., Huang, J. Q., Ren, F. Z., Xu, S. W., Wang, X. L., and Lei, X. G. (2013b) 'Gene expression of endoplasmic reticulum resident selenoproteins correlates with apoptosis in various muscles of se-deficient chicks', *The Journal of Nutrition*, 143(5), pp. 613-619.

Yao, Y., Pei, F., and Kang, P. (2011) 'Selenium, iodine, and the relation with Kashin-Beck disease', *Nutrition*, 27(11-12), pp. 1095-1100.

Yao, Y.F., Pei, F. X., Li, X. B., Yang, J., Shen, B., Zhou, Z. K., et al. (2012) 'Preventive effects of supplemental selenium and selenium plus iodine on bone and cartilage development in rats fed with diet from kashin-beck disease endemic area' *Biological Trace Element Research,* 146(2), pp. 199-206.

Yarmolinsky, J., Bonilla, C., Haycock, P. C., Langdon, R. J. Q., Lotta, L. A., Langenberg, C., Relton, C. L., Lewis, S. J., Evans, D. M., Consortium, P., Davey Smith, G. and Martin, R. M. (2018) 'Circulating Selenium and Prostate Cancer Risk: A Mendelian Randomization Analysis', *Journal of the National Cancer Institute,* 110(9), pp. 1035-1038.

Yeh, J.Y., Gu, Q., Beilstein, M. A., Forsberg, N. E., Whanger, P. D. (1997) 'Selenium Influences Tissue Levels of Selenoprotein W in Sheep', *The Journal of Nutrition,* 127(3), pp. 394-402.

Yim, S. H., Clary C. B., Gladyshev, V. N. (2019) 'Selenium Deficiency Is Associated with Pro-longevity Mechanisms', *Cell Reports*, 27(9), pp. 2785-2797.e3.

Ysart, G., Miller, P., Crews, H.M., Robb, P., Baxter, M.J., De L'Argy, C., Lofthouse, S.D., Sargent, C.M., and Harrison, N. (1999) 'Dietary exposure estimates of 30 elements from the UK. Total diet study', *Food Additives and Contaminants,* 16(9), pp. 391-403.

Yu, R., Wong, M., Chang, B., Lai, X., Lum, C. M., Auyeung, T. W., Lee, J., Tsoi, K., Lee, R. and Woo, J. (2016) 'Trends in activities of daily living disability in a large sample of community-dwelling Chinese older adults in Hong Kong: an age-period-cohort analysis', *BMJ Open,* 6(12), pp. e013259.

Zachara, B. A., Pawluk, H., Bloch-Boguslawska, E., Sliwka, K. M., Korenkiewicz, J., Skok, Z., and Ryć, K. (2001) 'Tissue level, distribution, and total body selenium content in healthy and diseased humans in Poland', *Archives of Environmental Health*, 56(5), pp. 461-466.

Zamora, A. J., Tessier, F., Marconnet, P., Margaritis, I. and Marini, J. F. (1995) 'Mitochondria changes in human muscle after prolonged exercise, endurance training and selenium supplementation', *European Journal of Applied Physiology and Occupational Physiology,* 71(6), pp. 505-511.

Zeng, H., Cao, J. J. and Combs, G. F., Jr. (2013) 'Selenium in bone health: roles in antioxidant protection and cell proliferation', *Nutrients,* 5(1), pp. 97-110.

Zhang, J., Munger, R. G., West, N. A., Cutler, D. R., Wengreen, H. J., and Corcoran, C. D. (2006) 'Antioxidant intake and risk of osteoporotic hip fracture in Utah: An effect modified by smoking status', *American Journal of Epidemiology*, 163(1), pp. 9-17.

Zhang, W. R., Ku, P. K., Miller, E. R. and Ullrey, D. E. (1986) 'Stability of glutathione peroxidase in swine plasma samples under various storage conditions', *Can J Vet Res,* 50(3), pp. 390-2.

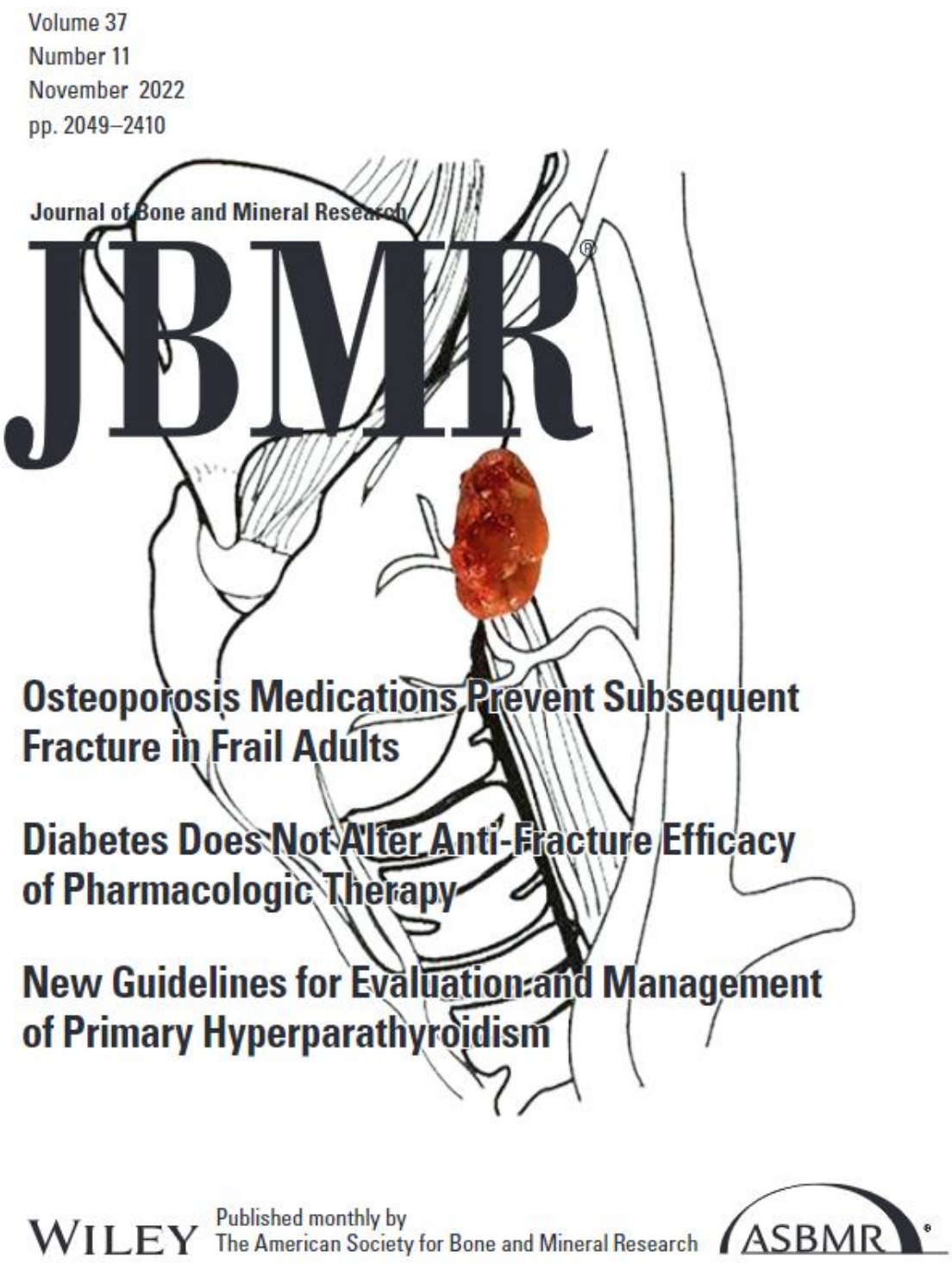
Zhang, Y., Ye, M., Zhao, Y., Xiong, Y., Shen, S., Yu, Q., Lu, Y., Shi, Z., and Lei, X. (2021) 'Higher Dietary Se Intake Is Associated With the Risk of New-Onset Fracture: A National Longitudinal Study for 20 Years', *Front Nutr,* 8, pp. 719147.

Zhang, Z., Zhang, J. and Xiao, J. (2014) 'Selenoproteins and selenium status in bone physiology and pathology', *Biochimica et Biophysica Acta,* 1840(11), pp. 3246-3256.

9. Appendices

A. Papers Published Relevant to this Thesis

Perri G, Hill TR, Mathers JC, Walsh JS, Gossiel F, Winther K, Frölich J, Folkestad L, Cold S, Eastell R. Long-Term Se-yeast Supplementation Does Not Affect Bone Turnover Markers: A Randomized Placebo-Controlled Trial. J Bone Miner Res. 2022 Sep 12. doi: 10.1002/jbmr.4703. Epub ahead of print. PMID: 36093566 (Perri *et al.*, 2022)
Recently published in November 2022 Issue



CLINICAL TRIAL

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Long-Term Selenium-Yeast Supplementation Does Not Affect Bone Turnover Markers: A Randomized Placebo-Controlled Trial

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ABSTRACT

Higher selenium status has been associated with lower bone turnover markers (BTM) in epidemiological studies. However, the long-term impact of selenium supplementation on BTMs has not been studied. We investigated the effects of selenium supplementation on BTMs including osteocalcin (OC), procollagen type I N-terminal propeptide (PINP), collagen type I cross-linked C-telopeptide (CTX), and bone alkaline phosphatase (BALP) in the short (6 months) and long term (5 years). A total of 481 Danish men and women (60–74 years) were randomized to receive placebo-yeast versus 100, 200, or 300 μg selenium as selenium-enriched yeast daily for 5 years. Plasma selenium concentration was measured using inductively coupled plasma mass spectrometry, and BTMs were measured in nonfasted samples at baseline, 6 months, and 5 years. Data were analyzed by ANCOVA to investigate the shape of the dose-response relationships. Covariates included age, body mass index, baseline selenium status, baseline BTM, smoking, alcohol, supplement use, and medication. Plasma selenium concentration (mean 86.5 μg/d at baseline) increased significantly with increasing selenium supplementation to 152.6, 209.1, and 253.7 μg/L after 6 months and remained elevated at 5 years (158.4, 222.4, and 275.9 μg/L for 100, 200, and 300 μg supplemental selenium/d, respectively ($p < 0.001$)). There was no change in plasma selenium concentration in the placebo-treated group. There was no significant effect of selenium supplementation on OC (6 months $p = 0.37$; 5 years $p = 0.63$), PINP (6 months $p = 0.37$; 5 years $p = 0.79$), CTX (6 months $p = 0.91$; 5 years $p = 0.58$) or BALP (6 months $p = 0.17$; 5 years $p = 0.53$). The relatively replete baseline selenium status in the study participants may explain this lack of effect. Testing in more deficient populations may provide further insights into the impact of selenium supplementation on bone health. © 2022 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: BIOCHEMICAL MARKERS OF BONE TURNOVER; GENERAL POPULATION STUDIES; NUTRITION

Introduction

Globally, 0.5 to 1 billion individuals are selenium deficient with blood concentrations below 70 μg/L. Inadequate selenium status in humans impairs the expression of the consortium of selenoproteins that are the biologically active selenium-containing molecules.[1] Selenium intakes vary greatly[1]; residents of Europe, New Zealand, central Africa, and some parts of China have insufficient selenium intake due to low soil selenium. Among Europeans, selenium intakes declined[2] from

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60–63 μg/d to 29–39 μg/d from 1970 to 2000.[2,3] In Denmark, selenium intake fell from 51 to 42 μg/d during 1990–95,[4] and plasma selenium concentration dropped from 42 to 37 μg/L between 1995–2000 and 2002.[5,6] However, among older adults, aged 65 to 75 years, intakes increased from the study period by approximately 25 μg/d between 1995–2001 and 2011–2013.[7]

In humans, selenium deficiency is associated with muscle fatigue, pain, weakness, and increased serum concentration of creatine kinase.[8,9] Compared with selenium-supplemented mice, selenium-deficient mice had higher concentrations of inflammatory markers and bone resorption markers with poorer bone microarchitecture.[10] Similarly, abnormal skeletal growth and poor bone health was observed in selenium-deficient rats,[11-13] whereas selenium supplementation improved bone microarchitecture.[14]

When incorporated into selenoproteins, selenium is important for musculoskeletal function. Most selenoproteins are involved in redox reactions, reducing concentrations of reactive oxygen species (ROS), such as hydrogen peroxide.[15,16] Multiple studies have shown an inverse relationship between selenium status and inflammatory molecules, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).[17] Both ROS and pro-inflammatory molecules (e.g. IL-6, TNF-α) can initiate bone resorption.[18,19] Because selenoproteins are expressed within osteoblasts and osteoclasts,[20] the bone formation and resorption cells, respectively, they may regulate bone resorption by moderating oxidative stress through ROS reduction.[14,21,22] Higher ROS concentrations increase bone loss through the RANK pathway.[23] Participants with lower selenium status have higher concentrations of IL-6[17,24] and supplementation with 200 μg selenium daily for 12 weeks reduced IL-6 concentration.[25] Concentrations of IL-6 are higher in osteoporotic individuals.[23,26,27] In addition, higher selenium concentrations are associated with higher bone mineral density (BMD) and lower bone turnover markers (BTM).[28] NHANES data suggested that higher selenium status (mean 131.1 μg/L) in US individuals, especially postmenopausal women, was positively associated with femur BMD.[29] Consequently, improving selenium status could be an effective and inexpensive approach to reducing the age-related decline in bone health. A recent randomized controlled trial (RCT) in 120 postmenopausal women showed that supplementation with sodium selenite for 6 months did not affect bone turnover or BMD.[30] Our study aims to extend the findings from this investigation by Walsh and colleagues[30] by using both men and women, a larger sample size, and a longer study duration.

This study tested the hypothesis that long-term supplementation with selenium improves bone health in older adults. We investigated the long-term effects of selenium supplementation on biomarkers of bone turnover through secondary analysis of data from a 5-year trial of adults in Denmark who were randomized to supplements providing 100, 200, or 300 μg selenium/d or to a placebo.

Subjects and Methods

PRECISE study

Data was obtained from the Danish Prevention of Cancer by Intervention with Selenium (PRECISE): A Pilot Study. The study began in 1998 and ended in 2004 and was organized by the Selenium Centre, Odense University Hospital, Denmark. It aimed to assess the viability for a full randomized controlled trial and hypothesized that selenium supplementation would reduce cancer risk in healthy adults. The trial was registered (ClinicalTrials.gov ID: NCT01819649) and other outcomes from the study have been published.[31,32] The regional Data Protection Agency and Scientific Ethical Committees of Vejle and Funen counties approved the study (Journal Number 19980186). The present study is a secondary analysis to determine the effect of selenium supplementation on biomarkers of bone turnover.

Participants

Using a random sample from the Danish Civil Registration, invitation letters were sent to 2897 men and women aged 60 to 74 years from the County of Funen between November 1998 and June 1999. Of these, 630 accepted the invitation and were screened for inclusion (Supplemental Table S1). A nonfasted blood sample was collected from those meeting the inclusion criteria, and placebo yeast tablets were provided during a 4-week run-in phase to determine compliance. At the second visit, participant satisfaction and adherence (>80% of tablets taken using tablet counts[31]) were assessed. After this, 491 participants met the inclusion and adherence criteria for continuation. All participants provided written informed consent.

Randomization

The eligible 491 participants were enrolled in a randomized, double-blinded, non-stratified, single-center, parallel clinical trial with four experimental arms distributed as 1:1:1:1 placebo (yeast tablet; $n = 126$), 100 μg selenium/d ($n = 124$), 200 μg selenium/d ($n = 122$), or 300 μg selenium/d ($n = 119$). There were 482 participants wtih BTM measurements. One participant was removed (see Statistical Analyses below); therefore, participants with BTMs at baseline were $n = 124$, 122, 118, and 117 for placebo, 100, 200, and 300 μg selenium/d, respectively (Fig. 1), giving a total of 481 participants. The study used computer-generated, blocked, and non-stratified randomization conducted by the Division of Epidemiology and Biostatistics, Arizona Cancer Centre, University of Arizona. Couples living at the same address were provided with the same intervention supplementation dose for practical reasons, i.e. to prevent mixing of selenium doses. The responsibility of distributing the tablets was placed with pharmacists at Odense University Hospital. Participants, research staff, and investigators were blinded to supplementation doses.[31]

Intervention

The selenium was provided as selenium-enriched yeast (in tablet form) in 100, 200, and 300 μg doses. These doses were suggested to be safe, as the tolerable upper intake level for adversity, set by the Institute of Medicine, is 400 μg/d.[33] The SelenoPrecise tablets (prepared by Pharma Nord ApS, Vejle, Denmark) contained 54% to 60% of total selenium as selenomethionine (SeMet) with unknown seleno compounds providing the remainder.[34] The placebo was identical to the supplementation tablets and consisted of inactive, spray-dried baker's yeast (250 μg yeast placebo, 80 μg cellulose, 65 μg dicalcium phosphate, and ≤5 μg of inactive ingredients). Smell and taste were matched by coating all tablets in titanium oxide, and tablets were packaged as 28-tablet blister packs.

Participant characteristics were determined at baseline. Further evaluations were performed at 6, 12, 18, 24, 36, and 60 months, which included assessment of medical status, tablet count, records of side effects, and the provision of new tablets, as previously described.[31]

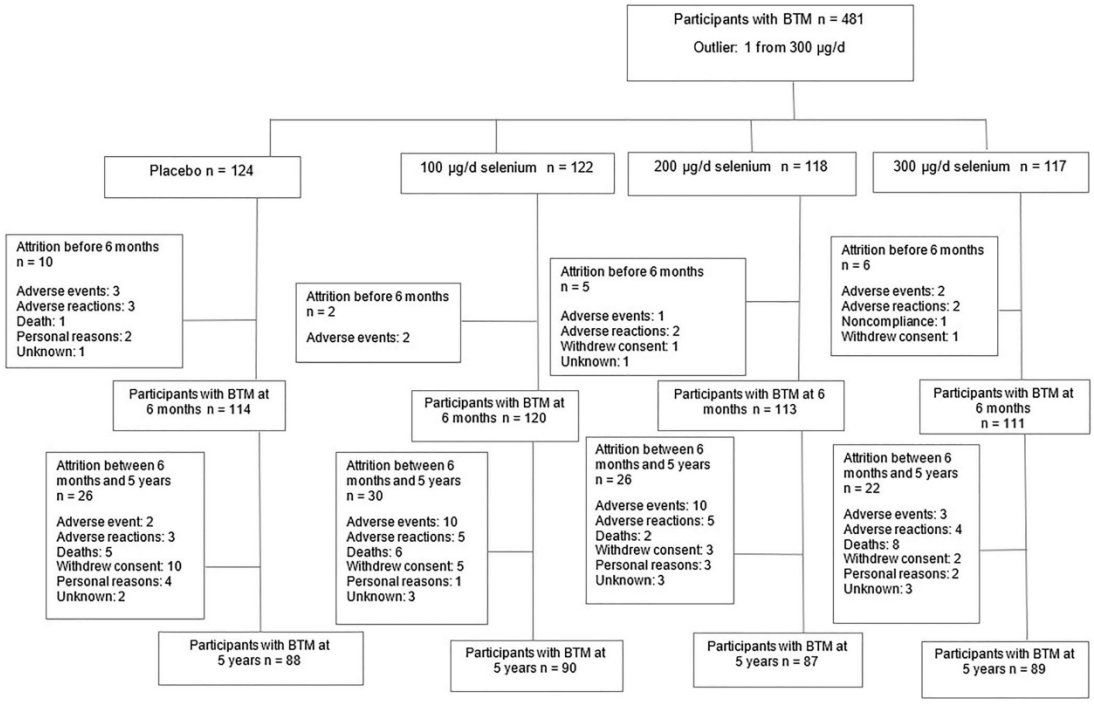

Fig. 1. Number of participants with bone turnover marker data at each stage of the study and dropout reasons.

Biochemical analyses

Nonfasting blood samples were collected at baseline, 6 months, and 5 years. Plasma was prepared and stored at −80°C.

Plasma selenium

Total selenium in plasma (µg/L) was measured at baseline, 6 months, and 5 years by LGC Limited using inductively coupled plasma mass spectrometry with external calibration (described in Cold and colleagues[31]). The selenium concentration for the certified reference standard BCR-637 was 78.3 (SD 2.7) µg/L (16 independent replicates), indicating good accuracy of the method. High-selenium concentrations had an intra-assay coefficient of variation (CV) of 0.5%, whereas low-selenium concentrations had an intra-assay CV of 3%. The interassay CV was 3.4%.

Bone turnover markers

The rationale for choice of the selected BTMs is described in Supplemental Table S2.[35] PINP and CTX were selected because they are the two reference markers recommended by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry for inclusion of all studies using bone turnover markers.[36] Additionally, they have been associated with selenium and selenoprotein P in the OPUS study, [28] where OC was more closely associated with selenoprotein P than PINP.[28] Studies suggest BALP can help identify changes in bone mineralization such as osteomalacia and Paget's disease.[37]

The BTMs were analyzed in 2017, with study recruitment from 1998–1999 until 2004. Thus, the oldest study samples were 19 years old, and the most recent ones were 13 years old. Studies have suggested that BTMs are stable when stored at −80°C for longer periods of time.[38,39] Serum was analyzed from the baseline, 6-month, and 5-year time points for N-MID osteocalcin (OC, measuring the large 1–43 N-mid and the intact OC), procollagen type I N-terminal propeptide (PINP, measuring the trimer only), collagen type I cross-linked C-telopeptide (CTX), and bone alkaline phosphatase (BALP) at the Bone Biochemistry Laboratory, Department of Oncology and Metabolism, University of Sheffield (England). OC, PINP, CTX, and BALP were measured using the IDS-iSYS automated immunoassays (Immunodiagnostic Systems, Boldon, UK). The interassay CVs were 5.0%, 7.2%, 6.5%, and 3.5% for OC, PINP, CTX, and BALP, respectively.

Covariates

Data on covariates were collected during visits with trained research nurses. Body mass index (BMI) was calculated as kg weight/m^2 height (continuous). Participants were classified into education status using surveys based on time spent in education after public school (0 = no, 1 = 1–3 years, 2 = 3–4 years, 3 = above 4 years). Living status was determined as living alone (0 = no, 1 = yes). Smoking status was determined at baseline (0 = never, 1 = previous, 2 = current). Alcohol intake was reported as standard drinks per week (continuous). Medication usage (thyroid,

antiresorptives, glucocorticoids [GC], hormone replacement therapy [HRT]) was classified as a binary variable depending on the medication (0 = no, 1 = yes), with thyroid medication having three categories (0 = none, 1 = levothyroxine, 2 = antithyroid drugs). Supplementation usage (calcium, vitamin D, multivitamins) was classified as a binary variable (0 = no, 1 = yes).

Statistical analyses

Data were analyzed using the IBM (Armonk, NY, USA) statistical software package version 24.0 (SPSS). A p value < 0.05 was considered statistically significant. To determine the normality of the variables, quantile–quantile (QQ) plots were used. Log 10 transformation was applied to all BTM measurements to normalize the data. Participant baseline characteristics are presented according to the supplementation dose. Differences in characteristics across supplementation doses were assessed using chi-square test (categorical) or Kruskal–Wallis (ordered and non-normally distributed). Data were analyzed using intention-to-treat.

For the main analyses, the shape of the dose-response relationship between selenium supplementation and each BTM (OC, PINP, CTX, BALP), at each time (6 months and 5 years, respectively), was investigated separately using an ANCOVA with orthogonal polynomials. Covariates included age (continuous), BMI (continuous), baseline selenium status (continuous), baseline BTM (continuous), smoking (binary), alcohol (binary), supplement use (binary; calcium, vitamin D, and multivitamins), and medication (binary; thyroid, systemic and inhaled GC, antiresorptive, and HRT). These covariates were selected based on previous literature showing their effect on bone health.[40] Outcomes are reported as estimated marginal means with upper and lower 95% confidence intervals after back transformation.

One participant of the 482 with BTMs was removed from the analyses. This participant's BTM concentrations for OC, PINP, CTX, and BALP were 6-, 5-, 7-, and 2-fold higher than mean concentrations. The removal of this participant had no significant effect on the main findings or baseline descriptives (data not shown). Sensitivity analyses were undertaken after excluding those participants receiving systemic GC ($n = 4$) because of their potential to influence BTMs.[35,39,41,42] A second analysis excluded systemic GC, inhaled GC, antiresorptive, and thyroid medication users ($n = 53$). Although some research suggests inhaled GC have minimal effects on bone,[43] a recent article suggested they increased the risk of osteoporosis.[44] Thyroid medication has been shown to have a detrimental effect on bone health.[45,46] Another sensitivity analysis excluded those using HRT ($n = 75$). A further sensitivity analysis removed supplement users ($n = 215$) because calcium, vitamin D, and multivitamins can influence bone metabolism. A final sensitivity analysis removed those using antiresorptives at baseline, 6 months, and 5 years, as well as those who had fractures, as a proxy to estimate those with osteoporosis ($n = 14$). The analyses were also repeated after categorizing baseline plasma selenium concentration into a binary variable, above and below 70 μg/L, based on evidence that this concentration is required to optimize glutathione peroxidase 3 (GPx) activity.[47] Sensitivity analyses are reported in Supplemental Tables S3–S8, respectively.

This was a secondary analysis, sample size was not determined for this particular study. The initial pilot study proposed a sample size of 500 participants.[31] The selenium supplemental trial of the effects on bone turnover markers in 120 postmenopausal women conducted by Walsh and colleagues[30] was 90% powered to be able to detect 20% between-group difference in urine N-terminal cross-linking telopeptide of type I collagen/creatinine ratio.[30] Because our study had similar outcome measures, in 481 participants, we are confident that we have sufficient power to detect any changes in BTMs after selenium supplementation. In addition, a retrospective power analysis was conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA). Least significant changes were set to 20% for OC, 21% for PINP, and 30% for CTX and BALP.[48–50] These calculations showed that we had >90% power to detect least significant changes in OC, PINP, and BALP at 6 months and 5 years, but only 5% and 16% for CTX at 6 months and 5 years, respectively.

Results

Baseline characteristics

Of the 491 participants randomized into the trial, 481 participants had BTM measurements at baseline (Fig. 1). The mean age of participants was 66.2 ± 4.1 years, and there was an almost equal split of male and female participants (52.0 versus 48.0%, $p = 0.476$, respectively) (Table 1). There were significant differences at baseline between supplementation groups for living status ($p = 0.047$), calcium supplementation ($p = 0.028$), and vitamin D supplementation ($p = 0.020$), but, otherwise, the supplementation groups were well matched (Table 1). Overall mean plasma selenium concentration at baseline was 86.5 ± 16.2 μg/d and did not differ between groups ($p = 0.190$; Table 2). Across all supplementation groups, 12% of participants had evidence of selenium inadequacy (plasma concentration < 70 μg/L). Mean ± (SD) baseline concentrations of OC, PINP, CTX, and BALP were 18.7 ± 8.5, 42.7 ± 18.1, 0.20 ± 0.22, and 15.7 ± 5.7 μg/L, respectively (Table 3). Over the 5 years of study, 127 participants were lost to follow-up, leaving 354 for the full study duration (Fig. 1). There were no differences between supplementation groups in loss to follow-up ($p = 0.847$) or reasons for dropout ($p = 0.816$, data not shown). However, participants who dropped out were more likely to have lower selenium status at 6 months ($p = 0.009$) but not at baseline. There were no other significant differences in baseline characteristics between participants who dropped out and those who did not (Supplemental Table S9). BTM concentrations did not differ significantly between those who dropped out and those who remained in the study (Supplemental Table S10).

Effects of increasing doses of supplemental selenium on plasma selenium concentration

Over the 5 years of the study, mean ± (SD) plasma selenium concentration in the placebo group remained unchanged (85.9 ± 15.3, 85.2 ± 14.3, and 87.5 ± 24.1 μg/L at baseline, 6 months, and 5 years, respectively, $p = 0.190$; Table 2). In contrast, at 6 months, plasma selenium concentration increased significantly in a dose-dependent manner with increasing supplemental selenium to reach 152.6, 209.1, and 253.7 μg/L for selenium doses 100–300 μg/d, respectively, and remained elevated at 5 years (Table 2).

Effects of increasing dose of supplemental selenium on concentrations of bone turnover markers

Concentrations of each of the four BTMs in serum at 6 months and at 5 years were similar to those at baseline and there was

no evidence that selenium supplementation altered any of the BTMs at either time point (Table 4). These findings remained robust in sensitivity analyses after excluding (i) users of systemic GC, (ii) combined users of systemic GC, inhaled GC, antiresorptives, and thyroid medication, (iii) users of HRT, (iv) users of nutritional supplements, (v) users of antiresorptives at baseline, 6 months, and 5 years, and those having fractures (Supplemental Tables S3–S7).

Table 1. Baseline Characteristics of Participants with Bone Turnover Marker Measurements, Randomized to Selenium Supplementation (0–300 μg/d)

Characteristic	All participants $N = 481$	Selenium dosage (μg/d) 0 $n = 124$	100 $n = 122$	200 $n = 118$	300 $n = 117$	*p* Value
Male, *n* (%)	250 (52.0)	59 (23.6)	69 (27.6)	64 (25.6)	58 (23.3)	0.476
Female, *n* (%)	231 (48.0)	65 (28.1)	53 (22.9)	54 (23.4)	59 (25.5)	
Age (years), mean (SD)	66.16 (4.10)	65.42 (3.8)	66.49 (4.2)	66.32 (4.3)	66.45 (4.1)	0.155
BMI (kg/m^2) (SD)	26.83 (4.02)	26.51 (4.1)	27.01 (3.8)	27.24 (4.3)	26.51 (4.0)	0.320
Height (m), mean (SD)	1.69 (0.09)	1.68 (0.09)	1.69 (0.08)	1.70 (0.09)	1.69 (0.08)	0.538
Weight (kg), mean (SD)	76.87 (13.5)	75.41 (12.6)	76.84 (11.6)	79.21 (15.4)	75.83 (14.3)	0.255
Alcohol units per week, mean (SD)	7.30 (7.5)	7.75 (8.4)	7.75 (8.0)	7.25 (7.3)	6.37 (6.3)	0.741
Smokers, *n* (%)						
Never	158 (32.8)	36 (22.8)	40 (25.3)	39 (24.7)	43 (27.2)	0.590
Previous	178 (37.0)	45 (25.3)	47 (26.4)	49 (27.5)	37 (20.8)	
Present	145 (30.1)	43 (29.7)	35 (24.1)	30 (20.7)	37 (25.5)	
Education, *n* (%)						
No further education	134 (28.7)	38 (28.4)	41 (30.6)	30 (22.4)	25 (18.7)	0.267
1–3 years	76 (16.3)	13 (17.1)	19 (25.0)	21 (27.6)	23 (30.3)	
3-4 years	212 (45.4)	57 (26.9)	44 (20.8)	53 (25.0)	58 (27.4)	
>4 years	45 (9.6)	12 (26.7)	14 (31.1)	10 (22.2)	9 (20.0)	
Live alone, *n* (%)						
No	400 (85.7)	94 (23.5)	107 (26.8)	99 (24.8)	100 (25.2)	0.047
Yes	67 (14.3)	26 (38.8)	11 (16.4)	15 (22.4)	15 (22.4)	
Thyroid medication, *n* (%)						
None	467 (97.1)	122 (26.1)	118 (25.3)	113 (24.2)	114 (24.4)	0.659
LT4	11 (2.3)	2 (18.2)	3 (27.3)	3 (27.3)	3 (27.3)	
ATD	3 (0.6)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	
Inhaled GC, *n* (%)						
No	450 (96.4)	118 (26.2)	111 (24.7)	110 (24.4)	111 (24.7)	0.374
Yes	17 (3.6)	2 (11.8)	7 (41.2)	4 (23.5)	4 (23.5)	
Systemic GC, *n* (%)						
No	475 (99.2)	122 (25.7)	121 (25.5)	117 (24.6)	115 (24.2)	0.595
Yes	4 (0.8)	2 (50.0)	1 (25.0)	0 (0.0)	1 (25.0)	
Antiresorptives, *n* (%)						
No	461 (98.7)	118 (25.6)	117 (25.4)	113 (24.5)	113 (24.7)	0.884
Yes	6 (1.3)	2 (33.3)	1 (16.7)	1 (16.7)	2 (33.3)	
HRT, *n* (%)						
No	392 (83.9)	98 (25.0)	98 (25.0)	99 (25.3)	97 (24.7)	0.740
Yes	75 (16.1)	22 (29.3)	20 (26.7)	15 (20.0)	18 (24.0)	
Calcium, *n* (%)						
No	419 (89.7)	110 (26.3)	112 (26.7)	95 (22.7)	102 (24.3)	0.028
Yes	48 (10.3)	10 (20.8)	6 (12.5)	19 (39.6)	13 (27.1)	
Vitamin D, *n* (%)						
No	442 (94.6)	115 (26.0)	117 (26.5)	103 (23.3)	107 (24.2)	0.020
Yes	25 (5.4)	5 (20.0)	1 (4.0)	11 (44.0)	8 (32.0)	
Multivitamin, *n* (%)						
No	325 (69.6)	90 (27.7)	86 (26.5)	70 (21.5)	79 (24.3)	0.116
Yes	142 (30.4)	30 (21.1)	32 (22.5)	44 (31.0)	36 (25.4)	
Dropout, *n* (%)						
6 months	23 (18.1)	10 (43.5)	2 (8.7)	5 (21.7)	6 (26.1)	0.133
5 years	104 (81.9)	26 (25.0)	30 (28.8)	26 (25.0)	22 (21.2)	

ATD = antithyroid drugs; BMI = body mass index; HRT = hormone replacement therapy; inhaled GC = inhaled glucocorticoid; LT4 = levothyroxine; SD = standard deviation; systemic GC = systemic glucocorticoid.

Age, alcohol $n = 481$; height, weight, BMI $n = 478$; smoking, sex, thyroid medication $n = 480$; BMI, weight, height $n = 478$; systemic GC $n = 479$; education, live alone, inhaled GC, antiresorptives, HRT and supplement users $n = 467$.

Table 2. Plasma Selenium Concentration at Baseline, 6-Month, and 5-Year Measurements for Participants With Bone Turnover Marker Measurements Randomized to Selenium Supplementation (0–300 μg/d)

		Selenium dosage (μg/d)				
Selenium status (μg/L), mean (SD)	All participants	0	100	200	300	*p* Value
Baseline ($n = 479$)	86.5 (16.2)	85.9 (15.3)	87.8 (16.2)	88.3 (16.4)	84.0 (16.9)	0.190
6 months ($n = 426$)	174.1 (72.4)	85.2 (14.3)	152.6 (23.7)	209.1 (42.2)	253.7 (53.7)	<0.001
5 years ($n = 349$)	185.6 (85.4)	87.5 (24.1)	158.4 (28.4)	222.4 (41.1)	275.9 (78.9)	<0.001

Baseline selenium status $n = 479$: 124, 122, 117, 116; 6 months $n = 426$: 106, 112, 106, 102; 5 years $n = 349$: 88, 88, 86, 87 for 0–300 μg/d selenium, respectively.

Table 3. Plasma Concentration of Bone Turnover Markers at Baseline for Participants Randomized to Selenium Supplementation (0–300 μg/d)

		Selenium dosage (μg/d)				
Bone turnover (μg/L), mean (SD)	All participants ($N = 481$)	0	100	200	300	*p* Value
OC	18.7 (8.5)	19.1 (8.2)	18.3 (8.3)	18.0 (8.8)	19.3 (8.5)	0.321
PINP	42.7 (18.1)	43.4 (19.3)	43.0 (16.4)	41.6 (19.8)	42.7 (16.5)	0.629
CTX	0.20 (0.22)	0.21 (0.13)	0.18 (0.11)	0.22 (0.40)	0.21 (0.14)	0.167
BALP	15.7 (5.7)	15.3 (5.5)	15.8 (5.7)	15.6 (5.4)	16.0 (6.2)	0.901

BALP = bone alkaline phosphatase; CTX = collagen type 1 cross-linked C-telopeptide; OC = osteocalcin; PINP = procollagen type 1 N-terminal propeptide; SD = standard deviation.

OC and PINP $n = 481$: 124, 122, 118, 117; CTX $n = 459$: 118, 117, 110, 114; BALP $n = 479$: 124, 121, 117, 117 for 0–300 μg/d selenium, respectively.

When analyses were limited to participants with plasma selenium status below 70 μg/L, supplementation had a marginal, significant effect on CTX concentrations at 5 years (Supplemental Table S8).

Adverse events

Adverse events for the full study have been reported and consisted of grooved nails, hair loss, and skin reactions.[31,32] During the 5 years, 22 (4.6%) participants with BTM measurements died and 57 (11.9%) withdrew due to nonfatal adverse events and reactions (Fig. 1) with no significant differences between supplementation groups ($p = 0.727$).

Discussion

In this RCT of selenium supplementation in older Danish adults, plasma selenium concentration did not have a significant effect on BTMs.

These results are consistent with findings from a RCT of selenium supplementation for 6 months in older women in the UK.[30] That study recruited 120 osteoporotic and osteopenic postmenopausal women (aged 55 to 83 years) with a baseline plasma selenium concentration (79.4 μg/L) similar to our present study (86.5 μg/L) and found no effect of selenium supplementation on any of the measured BTMs.[30]

The plasma selenium concentration for optimal bone health is not known with certainty. However, if it is assumed that the

Table 4. Estimated Marginal Means From ANCOVA of Bone Turnover Markers by Supplementation Group at 6 Months and 5 Years

	Selenium dosage (μg)				
Bone turnover (μg/L), mean (CI)	0	100	200	300	*p* Value
OC 6 months	17.1 (16.3–17.9)	16.7 (16.0–17.5)	17.5 (16.6–18.3)	16.5 (15.7–17.3)	0.373
OC 5 years	16.9 (15.6–18.2)	17.1 (15.8–18.6)	17.1 (15.7–18.6)	16.0 (14.8–17.3)	0.630
PINP 6 months	38.7 (36.8–40.8)	36.6 (34.7–38.5)	38.4 (36.4–40.5)	38.6 (36.6–40.8)	0.370
PINP 5 years	39.5 (36.1–43.3)	40.0 (36.5–43.8)	39.4 (35.8–43.3)	37.6 (34.3–41.1)	0.793
CTX 6 months	0.16 (0.14–0.17)	0.16 (0.14–0.17)	0.16 (0.15–0.18)	0.15 (0.14–0.17)	0.910
CTX 5 years	0.16 (0.14–0.18)	0.17 (0.15–0.19)	0.16 (0.14–0.18)	0.15 (0.14–0.19)	0.582
BALP 6 months	14.4 (13.8–14.9)	13.7 (13.2–14.3)	13.7 (13.2–14.3)	14.3 (13.7–14.9)	0.170
BALP 5 years	14.0 (13.2–14.8)	14.8 (14.0–15.7)	14.5 (13.6–15.4)	14.5 (13.7–15.3)	0.525

CI = confidence intervals; OC = osteocalcin; PINP = procollagen type 1 N-terminal propeptide; CTX = collagen type 1 cross-linked C-telopeptide; BALP = bone alkaline phosphatase.

Upper and lower 95% confidence intervals are displayed in parentheses.

Covariates in the ANCOVA included age (continuous), body mass index (continuous), baseline selenium status (continuous), baseline bone turnover marker (continuous), smoking (binary), alcohol (binary), supplement use (binary) (calcium, vitamin D, and multivitamins) and medication (binary) (thyroid, inhaled and systemic glucocorticoid [GC], antiresorptives, hormone replacement therapy [HRT]). OC 6 months $n = 404$: 107, 105, 98, 94; OC 5 years $n = 328$: 84, 84, 76, 84; PINP 6 months $n = 403$: 106, 105, 98, 94; PINP 5 years $n = 328$: 84, 84, 76, 84; CTX 6 months $n = 378$: 99, 98, 93, 88; CTX 5 years $n = 299$: 74, 77, 73, 75; BALP 6 months $n = 402$: 106, 104, 98, 94; BALP 5 years $n = 328$: 84, 83, 77, 84 participants for 0–300 μg/d selenium, respectively.

concentration that is adequate for wider aspects of health (≥70 μg/L[47]) is also adequate for bone, then it is likely that only a small minority of PRECISE study participants (12.1%) had potential to benefit from selenium supplementation. Studies that have observed responses to selenium, such as cancer prevalence or BMD, are usually in populations with suboptimal intakes or status, whereas replete populations are less responsive or nonresponsive.[51-53] The relatively high baseline selenium concentration (≥70 μg/L[47]) in the present study may explain the lack of response in BTMs to selenium supplementation.

Specific forms of selenium that have been used for supplementation studies differ in their bioavailability.[54] For example, sodium selenite was used by Walsh and colleagues,[30] whereas selenium yeast was used in our study and the Nutritional Prevention of Cancer Study.[55] Our supplement contained 54% to 60% SeMet[34]; studies suggest that the bioavailability of SeMet is more than 90%, whereas it is around 50% for the inorganic forms, selenite and selenate.[56] Selenium yeast is one of the most bioavailable forms of selenium with high efficiency in increasing selenoenzyme activity.[57,58] Therefore, it is unlikely that the lack of effect of selenium supplementation on BTMs observed in the present study was attributable to low selenium bioavailability. Indeed, supplementation raised plasma selenium concentration in a dose-dependent manner ($p > 0.001$).

Among the strengths of our study, to our knowledge, this is the first, long-term (5 years), large-scale RCT exploring the effects of selenium supplementation on BTMs in older men and women. There was a large dose-dependent increase in plasma selenium concentration with supplementation, suggesting compliance was good. This was gauged by the linear increase in selenium concentration with an increasing daily dose at 6 months, which was maintained at 5 years. In contrast, the plasma selenium concentration remained similar to baseline in the placebo group. Plasma selenium concentration is a robust indicator of selenium status[59] and correlates well with recent intakes of organic selenium.[60] The use of BTMs can help determine metabolic imbalances within bone, fracture risk, and detect nonresponders to treatment. Using a range of biomarkers allowed us to overcome some of the individual limitations of each BTM to provide a more representative finding, as well as using markers suggested by the IOF.[35,36]

A limitation of our study was the use of nonfasted blood samples as feeding can decrease BTMs.[61] Circadian rhythm can also affect BTMs, especially markers of bone resorption, for which concentrations are highest in the morning.[62-65] Consequently, the use of nonfasted samples may increase the variance in BTM measurements, but this is likely to be similar for all supplementation groups. We have no information on dietary intakes of other nutrients that influence bone health, such as calcium and vitamin D,[40,66] although we were able to adjust for supplementation with calcium, vitamin D, and multivitamins. We do not have data on BMI change through the study, but mean values at baseline were similar for all supplement groups, so this is unlikely to have been a confounder. Our participants were aged 60 to 74 years at baseline and so we are unable to generalize our results to older populations among whom osteoporosis and micronutrient deficiencies are more likely.[40,67] Future studies exploring the effects of selenium supplementation on bone health should consider including older people with lower baseline selenium status and/or those with inflammatory conditions who are at greater risk of osteoporosis and who may be more responsive to selenium supplementation.

This was the first long-term (5 years) RCT exploring the effects of selenium supplementation on BTMs in healthy older adults. Selenium supplementation did not have any significant effect on BTMs. We cannot rule out the potential of selenium supplementation to improve bone health in adults with lower selenium status and/or poorer bone health at baseline.

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Author Contributions

Giorgia Perri: Formal analysis; writing – original draft; writing – review and editing. **Tom R Hill:** Supervision; writing – review and editing. **John C Mathers:** Supervision; writing – review and editing. **Jennifer S Walsh:** Conceptualization; data curation; funding acquisition; writing – review and editing. **Fatma Gossiel:** Conceptualization; data curation; investigation; writing – review and editing. **Kristian Winther:** Conceptualization; data curation; investigation; supervision; writing – review and editing. **Jacob Frølich:** Conceptualization; data curation; writing – review and editing. **Lars Folkestad:** Conceptualization; data curation; writing – review and editing. **Søren Cold:** Investigation;

writing – review and editing. **Richard Eastell:** Conceptualization; data curation; funding acquisition; writing – review and editing.

Peer Review

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Data Availability Statement

The data that support the findings of this study are not openly available.

References

1. Combs GF Jr. Selenium in global food systems. Br J Nutr. 2001;85(5):517-547.
2. Rayman MP. The argument for increasing selenium intake. Proc Nutr Soc. 2002;61(2):203-215.
3. Joint Food Safety and Standards Group. Dietary intake of selenium food surveillance information sheet no. 126. London: Ministry of Agriculture, Fisheries and Food; 1997.
4. Bro S, Sandström B, Heydorn K. Intake of essential and toxic trace elements in a random sample of Danish men as determined by the duplicate portion sampling technique. J Trace Elem Electrolytes Health Dis. 1990;4(3):147-155.
5. Andersen N, Fagt S, Groth M, et al. [Food habits in Denmark 1995: main results] [Danish]. Publikation LST Levnedsmiddelstyrelsen (Denmark) no. 235. 1996.
6. Lyhne N, Christensen T, Groth M, et al. Danskernes kostvaner 2000–2002. Hovedresultater (DFVF Publikation nr 11 Danish Institute for Food and Veterinary Research [Dietary habits in Denmark 2000–2002: main results] [Danish]). 2005.
7. Danish National Survey of Dietary Habits and Physical Activity 2003, 2015. [Internet]. Available at: https://www.food.dtu.dk/english/publications/nutrition/danish-national-survey-of-dietary-habits-and-physical-activity. Accessed 15 June 2022.
8. Brown MR, Cohen HJ, Lyons JM, et al. Proximal muscle weakness and selenium deficiency associated with long term parenteral nutrition. Am J Clin Nutr. 1986;43(4):549-554.
9. Lauretani F, Semba RD, Bandinelli S, Ray AL, Guralnik JM, Ferrucci L. Association of low plasma selenium concentrations with poor muscle strength in older community-dwelling adults: the InCHIANTI study. Am J Clin Nutr. 2007;86(2):347-352.
10. Yang C, Wolf E, Roser K, Delling G, Muller PK. Selenium deficiency and fulvic acid supplementation induces fibrosis of cartilage and disturbs subchondral ossification in knee joints of mice: an animal model study of Kashin-Beck disease. Virchows Arch A Pathol Anat Histopathol. 1993;423(6):483-491.
11. Hurt HD, Cary EE, Visek WJ. Growth, reproduction, and tissue concentrations of selenium in the selenium-depleted rat. J Nutr. 1971;101(6):761-766.
12. Ewan RC. Effect of selenium on rat growth, growth hormone and diet utilization. J Nutr. 1976;106(5):702-709.
13. Thompson KM, Haibach H, Sunde RA. Growth and plasma triiodothyronine concentrations are modified by selenium deficiency and repletion in second-generation selenium-deficient rats. J Nutr. 1995;125(4):864-873.
14. Cao JJ, Gregoire BR, Zeng H. Selenium deficiency decreases antioxidative capacity and is detrimental to bone microarchitecture in mice. J Nutr. 2012;142(8):1526-1531.
15. Steinbrenner H, Sies H. Protection against reactive oxygen species by selenoproteins. Biochim Biophys Acta. 2009;1790(11):1478-1485.
16. Hariharan S, Dharmaraj S. Selenium and selenoproteins: its role in regulation of inflammation. Inflammopharmacology. 2020;28(3):667-695.
17. Tseng CK, Ho CT, Hsu HS, et al. Selenium is inversely associated with interleukin-6 in the elderly. J Nutr Health Aging. 2013;17(3):280-284.
18. Harmer D, Falank C, Reagan MR. Interleukin-6 interweaves the bone marrow microenvironment, bone loss, and multiple myeloma. Front Endocrinol. 2018;9:788.
19. Kitaura H, Marahleh A, Ohori F, et al. Osteocyte-related cytokines regulate osteoclast formation and bone resorption. Int J Mol Sci. 2020;21(14):5169.
20. Zhang Z, Zhang J, Xiao J. Selenoproteins and selenium status in bone physiology and pathology. Biochim Biophys Acta. 2014;1840(11):3246-3256.
21. Beukhof CM, Medici M, van den Beld AW, et al. Selenium status is positively associated with bone mineral density in healthy aging European men. PLoS One. 2016;11(4):e0152748.
22. Cervellati C, Bonaccorsi G, Cremonini E, et al. Oxidative stress and bone resorption interplay as a possible trigger for postmenopausal osteoporosis. Biomed Res Int. 2014;2014:569563.
23. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr Rev. 2010;31(3):266-300.
24. Prystupa A, Kicinski P, Luchowska-Kocot D, et al. Association between serum selenium concentrations and levels of proinflammatory and profibrotic cytokines-interleukin-6 and growth differentiation factor-15, in patients with alcoholic liver cirrhosis. Int J Environ Res Public Health. 2017;14(4):437.
25. Salehi M, Sohrabi Z, Ekramzadeh M, et al. Selenium supplementation improves the nutritional status of hemodialysis patients: a randomized, double-blind, placebo-controlled trial. Nephrol Dial Transplant. 2013;28(3):716-723.
26. Scheidt-Nave C, Bismar H, Leidig-Bruckner G, et al. Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab. 2001;86(5):2032-2042.
27. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab. 2003;88(1):255-259.
28. Hoeg A, Gogakos A, Murphy E, et al. Bone turnover and bone mineral density are independently related to selenium status in healthy euthyroid postmenopausal women. J Clin Endocrinol Metab. 2012;97(11):4061-4070.
29. Wu CC, Wang CK, Yang A-M, Lu CS, Lin CY. Selenium status is independently related to bone mineral density, FRAX score, and bone fracture history: NHANES, 2013 to 2014. Bone. 2021;143:115631.
30. Walsh JS, Jacques RM, Schomburg L, et al. Effect of selenium supplementation on musculoskeletal health in older women: a randomised, double-blind, placebo-controlled trial. Lancet Healthy Longev. 2021;2(4):e212-e221.
31. Cold F, Winther KH, Pastor-Barriuso R, et al. Randomised controlled trial of the effect of long-term selenium supplementation on plasma cholesterol in an elderly Danish population. Br J Nutr. 2015;114(11):1807-1818.
32. Rayman MP, Winther KH, Pastor-Barriuso R, et al. Effect of long-term selenium supplementation on mortality: results from a multiple-dose, randomised controlled trial. Free Radicals Biol Med. 2018;1(127):46-54.
33. Institute of Medicine Panel on Dietary Antioxidants and Related Compounds. Dietary reference intakes for vitamin c, vitamin e, selenium, and carotenoids. Washington, DC: National Academies Press; 2000.
34. Larsen EH, Hansen M, Paulin H, Moesgaard S, Reid M, Rayman M. Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies. J AOAC Int. 2004;87(1):225-232.
35. Burch J, Rice S, Yang H, et al. Systematic review of the use of bone turnover markers for monitoring the response to osteoporosis treatment: the secondary prevention of fractures, and primary prevention of fractures in high-risk groups. Health Technol Assess. 2014;18(11):1-180.

36. Vasikaran S, Cooper C, Eastell R, et al. Int Osteoporos. 2011;49(8): 1271-1274.
37. Nizet A, Cavalier E, Stenvinkel P, Haarhaus M, Magnusson P. Bone alkaline phosphatase: an important biomarker in chronic kidney disease-mineral and bone disorder. Clin Chim Acta. 2020;501: 198-206.
38. Park SY, Ahn SH, Yoo JI, et al. Clinical application of bone turnover markers in osteoporosis in Korea. J Bone Metab. 2019;26(1):19-24.
39. Eastell R, Pigott T, Gossiel F, Naylor KE, Walsh JS, Peel NFA. Diagnosis of endocrine disease: bone turnover markers: are they clinically useful? Eur J Endocrinol. 2018;178(1):R19-R31.
40. Aspray TJ, Hill TR. Osteoporosis and the ageing skeleton. Subcell Biochem. 2019;91:453-476.
41. Barahona MJ, Sucunza N, Resmini E, et al. Deleterious effects of glucocorticoid replacement on bone in women after long-term remission of Cushing's syndrome. J Bone Miner Res. 2009;24(11): 1841-1846.
42. Devogelaer JP, Durnez A, Gruson D, Manicourt DH, Bone turnover markers and glucocorticoid treatments. In: Patel V, Preedy V, editors. Biomarkers in bone disease. biomarkers in disease: methods, discoveries and applications. Dordrecht: Springer; 2017 pp 905-932.
43. Loke YK, Gilbert D, Thavarajah M, Blanco P, Wilson AM. Bone mineral density and fracture risk with long-term use of inhaled corticosteroids in patients with asthma: systematic review and meta-analysis. BMJ Open. 2015;5(11):e008554.
44. Chiu KL, Lee CC, Chen CY. Evaluating the association of osteoporosis with inhaled corticosteroid use in chronic obstructive pulmonary disease in Taiwan. Sci Rep. 2021;11(1):724.
45. Karimifar M, Esmaili F, Salari A, Kachuei A, Faragzadegan Z, Karimifar M. Effects of levothyroxine and thyroid stimulating hormone on bone loss in patients with primary hypothyroidism. J Res Pharm Pract. 2014;3(3):83-87.
46. Turner MR, Camacho X, Fischer HD, et al. Levothyroxine dose and risk of fractures in older adults: nested case-control study. BMJ. 2011;342: d2238.
47. Nève J. Human selenium supplementation as assessed by changes in blood selenium concentration and glutathione peroxidase activity. J Trace Elem Med Biol. 1995;9(2):65-73.
48. Schousboe JT, Bauer DC. Clinical use of bone turnover markers to monitor pharmacologic fracture prevention therapy. Curr Osteoporos Rep. 2012;10:56-63.
49. Tsujimoto M, Chen P, Miyauchi A, Sowa H, Krege JH. PINP as an aid for monitoring patients treated with teriparatide. Bone. 2011;48(4): 798-803.
50. Bergmann P, Body JJ, Boonen S, et al. Evidence-based guidelines for the use of biochemical markers of bone turnover in the selection and monitoring of bisphosphonate treatment in osteoporosis: a consensus document of the Belgian Bone Club. Int J Clin Pract. 2009;63(1): 19-26.
51. Duffield-Lillico AJ, Dalkin BL, Reid ME, et al. Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the nutritional prevention of cancer trial. BJU Int. 2003;91(7):608-612.
52. Lippman SM, Klein EA, Goodman PJ, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the selenium and vitamin E cancer prevention trial (SELECT). JAMA. 2009;301(1):39-51.
53. Galvez-Fernandez M, Grau-Perez M, Garcia-Barrera T, et al. Arsenic, cadmium, and selenium exposures and bone mineral density-related endpoints: the HORTEGA study. Free Radicals Biol Med. 2021;162: 392-400.
54. Fairweather-Tait SJ, Collings R, Hurst R. Selenium bioavailability: current knowledge and future research requirements. Am J Clin Nutr. 2010;91(5):1484S-1491S.
55. Clark LC, Combs GF Jr, Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA. 1996;276(24):1957-1963.
56. Thomson CD. Urinary and fecal excretions and absorption of a large supplement of selenium: superiority of selenate over selenite. Am J Clin Nutr. 1986;44(5):659-663.
57. Clausen J, Nielsen SA. Comparison of whole blood selenium values and erythrocyte glutathione peroxidase activities of normal individuals on supplementation with selenate, selenite, L-selenomethionine, and high selenium yeast. Biol Trace Elem Res. 1988;15:125-138.
58. Thomson CD, Robinson MF, Butler JA, Whanger PD. Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase (EC 1.11.1.9) in blood components of New Zealand women. Br J Nutr. 1993;69(2):577-588.
59. Burk RF, Norsworthy BK, Hill KE, Motley AK, Byrne DW. Effects of chemical form of selenium on plasma biomarkers in a high-dose human supplementation trial. Cancer Epidemiol Biomarkers Prev. 2006;15(4):804-810.
60. Hurst R, Collings R, Harvey LJ, et al. EURRECA-estimating selenium requirements for deriving dietary reference values. Crit Rev Food Sci Nutr. 2013;53(10):1077-1096.
61. Clowes JA, Hannon RA, Yap TS, Hoyle NR, Blumsohn A, Eastell R. Effect of feeding on bone turnover markers and its impact on biological variability of measurements. Bone. 2002;30(6):886-890.
62. Schlemmer A, Hassager C. Acute fasting diminishes the circadian rhythm of biochemical markers of bone resorption. Eur J Endocrinol. 1999;140(4):332-337.
63. Christgau S. Circadian variation in serum CrossLaps concentration is reduced in fasting individuals. Clin Chem. 2000;46(3):431.
64. Hannon R, Eastell R. Preanalytical variability of biochemical markers of bone turnover. Osteoporos Int. 2000;11(Suppl 6):S30-S44.
65. Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. Effect of fasting versus feeding on the bone metabolic response to running. Bone. 2012;51(6):990-999.
66. Garnero P, Sornay-Rendu E, Chapuy MC, Delmas PD. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. J Bone Miner Res. 1996;11(3):337-349.
67. De Laet CE, van Hout BA, Burger H, Hofman A, Pols HA. Bone density and risk of hip fracture in men and women: cross sectional analysis. BMJ. 1997;315(7102):221-225.

B. Abstracts Published Relevant to this Thesis

Royal Osteoporosis Society, Osteoporosis Online Conference December 1st 2020: Abstracts. Therapeutic Advances in Musculoskeletal Disease. 2020;12. doi:10.1177/1759720X20969289

Proceedings of the Nutrition Society (2021), 80 (OCE3), E110 doi:10.1017/S0029665121002330

Irish Section Conference, 22–24 June 2021, Nutrition, health and ageing — translating science into practice – Part A

Effect of selenium supplementation on biomarkers of bone turnover

G. Perri[1], T. Hill[1], J.C. Mathers[1], J. Walsh[2], F. Gossiel[2], K. Winther[3,4,5], J. Frölich[3], L. Folkestad[3,6], S. Cold[7] and R. Eastell[2]

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[5]*Department of Molecular Medicine and Surgery, Karolinska Institute, Solna, Sweden,*
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Selenium is an essential trace element with roles in musculoskeletal health[1,2]. Osteoclast inactivation is associated with selenium supplementation *in vitro* and selenium status is correlated negatively with markers of bone health[3,4]. However, the impact of selenium supplementation on bone turnover markers (BTM) has not been studied. This study investigated the effects of selenium supplementation for up to 5 years in older people on BTM including osteocalcin, procollagen type 1 N-terminal propeptide (P1NP), carboxy-terminal collagen crosslinks and bone alkaline phosphatase.

490 Danish men and women (60–74 y) were randomised to receive 0, 100, 200 or 300 μg of selenium daily as selenium-enriched yeast. Plasma selenium concentration was measured using inductively-coupled-plasma mass spectrometry and BTMs were measured using an autoanalyser at baseline, 6 months and 5 years in non-fasted samples. Data were analysed by ANCOVA with polynomial contrasts to investigate the shape of the dose-response relationships. Covariates included: age, body mass index, baseline plasma selenium concentration, baseline BTM, smoking, alcohol, supplement use and medication.

Plasma selenium concentration increased significantly with increasing selenium supplementation at 6 months (84.1, 155.2, 212.3, 258.3 ng/ml for placebo, 100, 200 and 300 μg selenium, respectively) ($P < 0.001$) and remained elevated at 5 years (88.2, 156.4, 223.8 and 270.9 respectively) ($P < 0.001$). At 6 months, there was a significant linear decrease in P1NP ($P = 0.036$, $\eta 2 = 0.019$) with increasing selenium supplementation but this effect was not apparent at 5 years. There was no significant effect of selenium supplementation on any other BTM.

Selenium supplementation reduced P1NP at 6 months but there were no significant effects on other BTM or after 5 years. Since P1NP is a marker of osteoblast function, the fall in PINP with increasing selenium supplementation suggests a reduction in new bone formation 5. The impact of this change in bone turnover on bone health remains to be determined.

References

1. Moreno-Reyes R, *et al.* (2001) *J Bone Miner Res* 16, 1556–63.
2. Zhang Z, *et al.* (2014) *Biophys Acta* 1840, 3246–3256.
3. Hoeg A, *et al.* (2012) *J Clin Endocrinol Metab* 97, 4061–70.
4. Beukhof CM *et al.* (2016) *PLoS ONE* 11, e0152748.
5. Kuo T & Chen CH (2017) *Biomark Res* 5, 18

OSTEOPOROSIS CONFERENCE 2020 ACC LIVERPOOL 30 NOV – 02 DEC

REF No: Hg3/ROS/PERR23NE56 (Please quote in all correspondence)
11th September 2020

Dear Miss Perri

Osteoporosis Conference 2020
30 November - 02 December 2020, ACC Liverpool

On behalf of the Conference Programme Committee, I am pleased to inform you that your abstract (title below) has been accepted for publication at 'Osteoporosis online'.

Abstract Title:

Effect of selenium supplementation on biomarkers of bone turnover

I am delighted to advise you that your abstract has been awarded a commendation.

As you are aware there will not be a face to face meeting this year due to the pandemic COVID-19 but the Society is working hard to work out an online offering and will be in touch with further details as soon as they are determined.

questions

Effect of selenium supplementation on biomarkers of bone turnover

G Perri[1,2], *TR Hill*[1,2], *JC Mathers*[1,2], *J Walsh*[2,3], *F Gossiel*[2,3], *KH Winther*[4,5,6], *J Frölich*[4], *L Folkestad*[4,7], *S Cold*[8], *R Eastell*[2,3]

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Background: Selenium is an essential trace element for human health. Osteoclast inactivation is associated with selenium supplementation *in vitro* and selenium status is correlated negatively with markers of bone turnover (BTM). However, the impact of selenium supplementation on BTM has not been studied.

Objective: To investigate the effects of selenium supplementation on BTM including OC, P1NP, CTX and BAP in the short- and long-term.

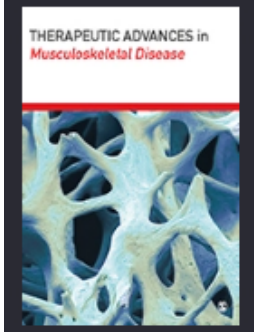
Methods: 491 Danish men and women (60–74 year) were randomised to receive 0, 100, 200 or 300 µg of selenium-enriched yeast daily. Serum selenium concentration was measured using inductively-coupled-plasma mass spectrometry and BTM were measured using an autoanalyser (IDS iSYS) at baseline, 6 months and 5 years in non-fasted samples. Data were analysed by ANCOVA with polynomial contrasts to investigate the shape of the dose-response relationships. Covariates included: age, BMI, baseline selenium status, baseline BTM, smoking, alcohol, supplement use and medication.

Results: Plasma selenium concentration increased significantly with increasing selenium supplementation at 6 months and remained elevated at 5 years ($p < 0.001$). Selenium status remained constant in the placebo-treated group over time. At 6 months serum selenium concentration increased by 79, 145 and 203% for doses 100–300 µg, respectively. At 6 months, there was a significant linear decrease in P1NP ($p = 0.019$) with increasing selenium supplementation (Figure 1). There was no significant effect of selenium supplementation on any other BTM.

Discussion: P1NP is a marker of osteoblast function. The fall in PINP with increasing selenium intake suggests a reduction in new bone formation.

Conclusions: Selenium supplementation reduced P1NP at 6 months but there were no significant effects on other BTM or at later times. The impact of this change in bone turnover on bone health remains to be determined.

Details



Therapeutic Advances in Musculoskeletal Disease Volume 12,

January-December 2020

ARTICLE

Royal Osteoporosis Society, Osteoporosis Online Conference December 1st 2020: Abstracts

NEPG

NORTH-EAST POSTGRADUATE CONFERENCE

2020

DIVERSITY

This certificate is awarded
to certify that

Giorgia Perri

has presented an oral presentation

The Effect of Selenium Supplementation on Biomarkers of Bone Turnover

at the NEPG 2020 Conference on
13th November 2020

28th Nov 2020
Issue Date

Newcastle University

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Giorgia Perri

C3S11T3 | Physical Activity & Nutrition

The Effect of Selenium Supplementation on Biomarkers of Bone Turnover

Background: Selenium is an essential trace element for human health. Osteoclast inactivation is associated with selenium supplementation in vitro and selenium status is correlated negatively with markers of bone turnover (BTM). However, the impact of selenium supplementation on BTM has not been studied.
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Discussion: P1NP is a marker of osteoblast function. The fall in PINP with increasing selenium intake suggests a reduction in new bone formation.

C. Papers and Abstracts Published Not Included in the Thesis

Perri G, Mendonça N, Jagger C, Walsh J, Eastell R, Mathers JC, Hill TR. Dietary Selenium Intakes and Musculoskeletal Function in Very Old Adults: Analysis of The Newcastle 85+ Study. Nutrients. 2020; 12(7):2068. https://doi.org/10.3390/nu12072068 (Perri, 2020)

Plant Based Diets and Bone Health: A Mini-Review of the Scientific Evidence By Giorgia Perri* and Tom Hill. A mini review for The Royal Osteoporosis Society

Proceedings of the Nutrition Society (2020), 79 (OCE2), E611 doi:10.1017/S0029665120005601

The 13th European Nutrition Conference, FENS 2019, was held at the Dublin Convention Centre, 15–18 October 2019

Dietary selenium intakes and their association with muscle strength and function in ≥85 year old adults: the Newcastle 85+Study

Giorgia Perri[1], Tom Hill[1], John Mathers[1], Nuno Mendonça[2], Rachel Duncan[3], Jennifer Walsh[4] and Richard Eastell[4]

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Abstract

Selenium is an essential micronutrient with biochemical and cellular effects through activities of 25 selenocysteine-containing selenoproteins. Selenoproteins are anti-inflammatory and have antioxidant properties. Severe selenium deficiency causes muscle weakness and atrophy in humans however the effects of moderate selenium deficiency are unclear. The aims of this study are twofold: 1) to determine dietary selenium intakes and contributing food sources in very old adults and; 2) to determine whether dietary selenium intakes are associated with 5-year trajectories of muscle function: hand-grip strength (HGS) and Timed-Up-and-Go (TUG).

Cross-sectional (baseline) and prospective (1.5, 3 and 5-year follow-up) analyses of 845 participants aged 85 years from the Newcastle 85 + study were assessed for HGS and TUG performance using standardized protocols (Antoneta et al. 2016). Baseline dietary intakes were assessed using 24-hour multiple pass recall methods on two separate days (Mendonça et al. 2016). The top selenium food contributors (~90%) and the adequacy of intakes were determined i.e. those with intakes < LRNI, between the LRNI and RNI and > RNI. Linear mixed models explored the associations between selenium intake categories and time on the prospective, 5-year change in HGS and TUG in all participants, males and females.

Median intakes of selenium were 39, 48 and 35μg for all participants, males and females, respectively. Selenium intakes were below the LRNI in 51% of participants (median 27μg) whilst 15% had intakes ≥ the RNI (median 85μg). Only 13.3% of females and 16.9 % of males met the RNI. The top selenium contributors were cereals (46%), meat (22%), fish (10%), milk (6%), eggs (4%) and potatoes (3%) making up 91% of selenium intakes. Those with the lowest intakes had 2.72 kg lower HGS and 2.36s slower TUG compared to those with higher intakes ($P < 0.005$). There was no association between selenium intake in HGS or TUG, but time had a significant effect on the rate of change over 5-years in both parameters ($P < 0.001$).

Overall, these results show that poor dietary selenium intakes are common in very old adults and that cereal and cereal products are major sources of selenium in this population. Whilst low selenium intakes are associated with worse HGS and TUG performance in the cross-sectional analysis, no significant associations were observed in the prospective analyses.

Conflict of Interest

There is no conflict of interest.

Abstracts From the December 2019 International Sport + Exercise Nutrition Conference in Newscastle upon Tyne

in International Journal of Sport Nutrition and Exercise Metabolism

Click name to view affiliation

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Resumption of Eumenorrhea After Prolonged Menstrual Dysfunction in an Elite Road-Cyclist Following Increase in Body Weight in Parallel With High Training Load: A 5-Year Case Report

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Chronic low energy availability typically results in menstrual dysfunction and/or low bone mineral density. It is unclear how this condition, known as the female athlete triad, affects physical capacity and what the recovery time-course of menses is with increased energy availability concomitant to high training load. In this case-study, an amenorrheic elite female cyclist (VO_{2max} 3.54 L/min, 64 ml/min/kg, PPO 300 W, 5.4 W/kg and 23 years in 2014) is shown to resume normal menstrual function after a prolonged period of menstrual dysfunction (amenorrhea (2013-2015)/oligomenorrhea (2015-2018)) while engaging in high training load and in competition. Resumption of menses was recorded in June 2018, ~5-6 months after a weight gain of ~10% (~5 kg) displaying regular menses (every 23-35 days) ever since. Training load and volume increased from 2014 to 2019 (26707 to 41945 TSS/year and 584 to 818 h/year, respectively). During the period of menstrual dysfunction weight was 51.3±2.3 kg (mean±95% CL; 80 records), and fat percentage 19% (DXA, 2016), and after an episode of weight gain 56.8±2.6 (35 records) ($p < 0.001$) and fat percentage 25% (DXA, 2019). Crank-based power-meter data was recorded for training sessions and competitions, and mean maximal power (MMP) over the range of 5 seconds to 4 h improvements in absolute power (watts) through the 2014-2019 period, while relative MMP (watts/kg) likely peaked in the 2015-16 season for 5', 20' and 30' and remained mostly unaffected across seasons despite the increase in body mass. The athlete achieved podium placings consistently in national and international competitions and achieved best performances in the 2018-2019 season. These findings suggest that 1) best relative power output of 5'-1 h seemed to be achieved despite low energy availability and menstrual dysfunction, 2) high performance can be achieved at an elite level despite an increase in body weight in a weight-sensitive sport and 3) despite high training loads when coupled with high energy availability.

Dietary Selenium Intakes and Their Association With Muscle Strength and Function in ≥85 Year Old Adults: The Newcastle 85+ Study

G Perri[1], T Hill[1], J Mathers[1], N Mendonça[2]

[1]Institute of Cellular Medicine, University of Newcastle, UK

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Selenium is an essential micronutrient with biochemical and cellular effects through activities of 25 selenocysteine-containing selenoproteins. Selenoproteins are anti-inflammatory and have antioxidant properties. Severe selenium deficiency causes muscle weakness and atrophy in humans but the effects of moderate selenium deficiency are unclear. The aims of this study are: to determine dietary selenium intakes and contributing food sources in very old adults and; to determine whether selenium intakes are associated with 5-year trajectories of muscle function: hand-grip strength (HGS) and Timed-Up-and-Go (TUG). Cross-sectional (baseline) and prospective (1.5, 3 and 5-year follow-up) analyses of 845 participants aged 85 years from the Newcastle 85+ study were assessed for HGS and TUG using standardized protocols (Antoneta et al. 2016). Baseline dietary intakes were assessed using 24-hour multiple pass recall methods on two separate days (Mendonça et al. 2016). The top selenium food contributors (~90%) and the adequacy of intakes were determined i.e. those with intakes < LRNI, between the LRNI and RNI and > RNI. Linear mixed models explored associations between selenium intake categories and time on prospective, 5-year changes in HGS and TUG in all participants, males and females. Median intakes of selenium were 39, 48 and 35 µg, respectively. Selenium intakes were below the LRNI in 51% of participants (median 27 µg) whilst 15% had intakes ≥ the RNI (median 85 µg). Only 13.3% of females and 16.9 % of males met the RNI. The top selenium contributors were cereals (46%), meat (22%), fish (10%), milk (6%), eggs (4%) and potatoes (3%) making up 91% of selenium intakes. Those with the lowest intakes had 2.72 kg lower HGS and 2.36s slower TUG compared to those with higher intakes ($P < 0.005$). There was no association between selenium intake in HGS or TUG, but time had a significant effect on the rate of change over 5-years in both parameters ($P < 0.001$). Overall these results show that poor dietary selenium intakes are common in very old adults and that cereal and cereal products are major sources of selenium in this population. Whilst low selenium intakes are associated with worse HGS and TUG performance in cross-sectional analyses, no significant associations were observed in the prospective analyses.

Treatment Strategies to Reverse Bone Loss in Athletes With Functional Hypothalamic Amenorrhea

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Functional hypothalamic amenorrhea (FHA) is characterized by the loss of the menstrual cycle due to the suppression of the hypothalamic pituitary ovarian axis (HPOA). FHA in athletes can be a symptom of relative energy deficiency in sport (RED-S). RED-S has expanded the concept of the Female athlete triad acknowledging that this syndrome affects many aspects physiological function including metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular function and psychological health. The fundamental issue with RED-S is a mismatch between energy availability (EA) (the amount of dietary energy remaining after exercise training for all physiological functions each day) and energy expenditure (EE). It is well established that the endocrine disruptions and a state of low EA directly impair bone health through the suppression of the key metabolic hormones required for bone formation (leptin, IGF-1 and T3) and the hypoestrogenic environment upregulating osteoclastic bone resorption. This leads to a loss in bone mineral density (BMD), thus increasing the risk of stress fractures and osteoporosis. To support the continued development of clinical guidelines in treating athletes with FHA, a systematic review of the literature was conducted to document the current evidence regarding the efficacy of treatment strategies to address bone loss in athletes with FHA. The primary treatment of FHA in female athletes is the modification of diet and exercise behaviours to enable an increase in EA and weight restoration if required. Restoring energy availability and the return of menses are essential to enable athletes with FHA to reverse bone loss, and this should not be understated. If an athlete experiences further health complication, whilst restoring EA (e.g. stress fractures or if menstruation is not restored after an adequate trial of restoring EA), transdermal oestradiol and cyclic micro-ionized progesterone may be prescribed. This will be used as a short-term treatment to protect bone health until the underlying issue of low energy availability is resolved. Ackerman et al (2018) concluded the oral contraceptive pill should not be prescribed to these athletes due to the hepatic first pass effects on the liver leading to downregulation of insulin growth factor-1, further exacerbating bone loss.

Metabolic Enzyme Adaptations to Long-Term Feeding of Ketogenic Diet Containing Medium-Chain Triglyceride in Rats

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Very low-carbohydrate, high-fat (ketogenic) diets are recently receiving much attention from athletes, because long-term intake of the diet enhances

D. Training

During my PhD programme, I have attended a vast variety of training, courses, webinars and workshops, as well as following the weekly Health and Nutrition Research Centre (HNRC) seminars (Table 9.1). To enhance my statistical knowledge, I have watched Analysis Factor Recordings and undertook multiple statistical courses. One of these was The Sysmic course, this involved learning to use MatLab and R coding software. The course consisted of three topics: 1) Networks (sessions 1 to 4) where biological systems were described and analysed as networks involving vectors and matrices; 2) Maths and Modelling (sessions 5 to 8) where biological systems and mechanisms were modelled and simulated using computer programmes using functions and calculus; 3) Statistics (sessions 9 to 11) where the statistical software package R was introduced and used for and data analysis; 4) Mini projects (session 12) where the skills are applied to build a model. I have also recently received funded places for R for Data Science provided by the University of Liverpool, these were 3-day courses aimed at Beginner and Intermediate levels. Modules consisted of loops and control statements, functions, batch correction, normalisation and multivariate analysis.

Co-Supervision of BSc and MRes Projects

During my PhD programme I have supervised three undergraduate students during their final year dissertation. One student studied Food and Human Nutrition and their project was titled “Dietary Selenium Intake and Cognitive Decline in the Very Old: The Newcastle 85+ Study”. The second student studied Sports and Exercise Science and their project was titled “Physical Activity, Sarcopenia, and Disability in the Oldest Old: A Cross-sectional Analysis”. The final student studied Sports and Exercise Science and their project was titled “ Is Dietary Magnesium Intake related to Muscle Strength and Function in Very Old Adults? Analysis of The Newcastle 85+ Study”. I guided the students through the statistical analysis of The Newcastle 85+ Study data set. This involved creating new variables, performing basic descriptive tests (t-tests, normality, transformations, chi-square, non-parametric tests), and guiding the students through their learning and understanding of linear and logistic regressions, correlations, linear mixed models and helping them with the write up of their dissertation. These supervisory experiences were valuable in improving my leadership skills, as I was the first point of call for the students, before approaching our joint supervisor, Prof Tom Hill. The

experience also enhanced my project management skills as I had my own PhD programme to work on, as well as ensuring they were meeting their dissertation deadlines.

Table 9.1: Training and courses during the MRes and PhD.

Date	Training	Skills
26/02/19	Systematic Review	A1, A2, A3, C2
07/03/19	Recognising and Building your Resilience to the Rigours of Research	B1, B2, B3, D1
08/03/19	Procrastination	A3, B1, B2, B3, C1
13/03/19	How to Manage Anxiety and Stress Part II	B1, B3, C1, D1
17/04/19	Resilience for PGR's	B1, B2, C1, D1
20/03/19	Assertion – How to Communicate Well and Improve Relationships	B1, B2, B3, C1, D1
01/10/19	Managing your PhD/MPhil for FORMER Masters students	B1, B2, B3, C1, C2, D1, D2, D3
08/01/20	Introduction to Learning and Teaching one day workshop - Demonstrator	A1, B1, B2, B3, D1, D2, D3
20/01/20	CIMA – Epigenetics Conference	A1, A2
26/02/20	Writing Your First Year Report	A1, A3, B2, C2, D2
28/02/20	National Innovation Centre for Ageing - Unequal Ageing	A1
20/04/20	Online: Creating Data Management Plan	C3
23/04/20	British Nutrition Foundation - Processing the Nutribabble – Is natural always Best?	A1
12/05/20	UK Data Service - Being a Computational Social Scientist	A1, A2, B3
18/05/20	HASS - Presentation Skills	A1, B1, D2
19/05/20	Video Practice for Presentation Skills	A1, B1, D2
20/05/20	Public Health England - Impact of COVID-19 on Musculoskeletal Health and Mental Well-being	A1
22/05/20	Writing for Publication and the Publication Process	A1, C1, C2, D1, D2
28/05/20	VOICE - Research Coffee Morning: Exercise and Muscle Ageing	A1, D2, D3
05/06/20	NUGO - Microbiota Analysis for Nutritional Research: Concepts and Tips to Get Started	A1, A2
18/06/20	DiMeN - How to Maximise Twitter to Communicate your Research	A1, A3, D2, D3
24/06/20	Your Personal Development Plan - Preparing for the Progress Review Panel	B1, B2, B3, C1, C2
25/06/20	British Society of Animal Science – Early Careers Writing Skills	A1, B3
30/06/20	Digital Health – Nail your PhD	A1, B3
02/07/20	VOICE - Research Coffee Morning: Diet and Nutrition	A1, D2, D3
02/07/20	International Osteoporosis Foundation - Osteosarcopenia	A1
08/07/20	Academic Writing: Accessible Abstracts	B3, D1, D3
09/07/20	British Society of Animal Science – Early Careers Tips for Writing in Word	A1, B3
29/07/20	Enago Academy's Webinar - Writing Review Articles	A1, A2, C2
29/07/20	Biochemistry Focus Webinar Series - From Diagnosis to Therapy in Duchenne Muscular Dystrophy	A1
31/07/20	NUGO - Ever Wondered what Happens to your Proposal when you Submit it?	A1, B3, D2
10/08/20	Digital Health - Writing: How to be More Productive without Procrastinating or Bingeing	A1, A2, C2
25/08/20	University of Aberdeen - Appetite Workshop in Older Adults	A1 Application Process
26/08/20	VOICE - Research Coffee Morning: Nutrition and Brain Ageing	A1
02/09/20	Digital Health - Education Productive Writing - How to be More Productive without Procrastinating or Bingeing	A1, A2, C2, D2
09/09/20	MRC Circadian Webinar	A1
10/09/20	DiMeN – Royal Society of Biology	A1
10/09/20	SheCodes – Introductory Coding Class	A1, A2
17/09/20	Biochemistry Focus Webinar Series - Genes Regulating Ageing and the Quest for Immortality	A1
23/09/20	DiMeN – PhD on Frontline	A1
24/09/20	British Society of Animal Science – Early Careers Mental Health	B1, B2
14/10/20	Digital Health - Plan your Journey and Audit your Time During your Research Degree	A1, B2, C2
19/10/20	Digital Health – Organise your Thoughts	A1, B2, B3
20/10/20	Stanford Online - Data Overload: Making Sense of Statistics in the News	A1, A2, C2
21/10/20	Public Speaking	A1, A3, B3, C2, D2, D3
23/10/20	Storing Research Data during and after a Project	A1, B3, C1, C2
23/10/20	Science about Science - Quality and Peer Review	A1, A2, B3
11/11/20	Data Analysis R	A1, A2, B3
13/11/20	NEPG – Questions on Oral Ppt	A1, A2, D1, D2, D3
16/11/20	Population Health Science Institute - Annual Meeting	A1, D1
25/11/20	How to Write a Great Research Paper and Get it Accepted by a Good Journal	A1, B3, C2

25/11/20	NUGO - Nutrigenomics Debate	A1, A2
02/12/20	Elsevier - Getting the Most Out of Scopus	A1, B3, C2
07/12/20	MatLab	A1, A2
17/12/20	Teaching and Supervising Project Students	A1, B1, B3
07/01/21	Eventbrite - Helping to Help Postgraduate students	A1
18/01/21	Centre for Longitudinal Studies (CLS) - Handling Missing Data in British Cohort Studies	A1, A2, B3, C2
20/01/21	Building Confidence in Social Situations	B1, B2, C1
20/01/21	How to Attend to Stress and Anxiety – Practical Approaches	B1, B2
21/01/21	MRC – Online Careers Talks	A1, B3
26/01/21	Managing Unhelpful Thoughts	B1, B2
26/01/21	IFIS: Best Practice in Food Science and Nutrition Literature Searching: What is Indexing, What is an A&I Database, and Why Should I Care?	A1, A2, C2
28/01/21	Biochemistry Focus Webinar: Obesity - from Genes to Biochemistry of a Global Pandemic	A1
03/02/21	Microsoft - LinkedIn Profile	A1, A3, B3, D3
03/02/21	MRC Networking	B3, D1, D3
03/02/21	Microsoft - Job Interview Success	A1, B1, B2, C1
04/02/21	Biochemistry Focus ECR Webinar Series - Careers in Science Communication, Medical Writing and Engagement	A1, A3, D2, D3
10/02/21	Nutrition Society Journal Club	A1, A2
12/02/21	Thesis Writing	A1, C2, D2
15/02/21	CIMA Student Session and Spring Meeting	A1, A3, B3, D1, D2, D3
16/02/21	CIMA Writing for the PhD and Beyond	A1, A2, B3, C2
17/02/21	Nutrition Society Journal Club	A1, A2
24/02/21	DiMeN – Being an Ally	A1
25/02/21	Science Communication	A1, A3, D2, D3
04/03/21	NUGO - Role of the Gut Microbiota-Brain Axis for Human Health	A1, A2
05/03/21	Thesis Writing	A1, C2, D2
05/03/21	Learn It - Dealing with Conflict and Difficult People	A1, B1, B2, B3, B1, D1
08/03/21	Second Year Annual Review: Your Research Outputs	A1, B3, D2
15/03/21	MRC - Using Health and Social Care Datasets in Research – Opportunities, Assets and Examples	A1, A2, C2
16/03/21	Careers Service - Science Careers Outside the Lab: Science Communication, Education & Outreach	A1, B3, C1
17/03/21	Genesis Research - Introduction to Medical Writing for PhDs	A1, B2, B3
18/03/21	MRC – Circadian Rhythms: Everything you always wanted to know about Jet Lag	A1
23/03/21	MRC – Social Media, Online Profile	A1, A2
23-24/03/21	Advanced Systematic Review	A1, B3, C2
24/03/21	MRC – Effective in Online Meetings	A1, A2
24/03/21	Nutrition Society – Careers Talk	A1, A2
29-31/03/21	MRC Podcast	A1, A3, B1, D2, D3
29/03/21	Wellness Uni	B1, B2
08/04/21	Web of Science: Fine-tune your FSTA search with Phrase and Proximity Searching	A1, A2, B3, C2
12-13/04/21	MRC NRP - Best Practice in Human Nutrition	A1
15/04/21	Canvas: Careers for Researchers - Presentation Skills: Storytelling for Researchers	A1, A3, D2, D3
15/04/21	PROMISS Webinar - Prevention of Malnutrition in Senior Subjects in the EU	A1
16/04/21	NUGO Meet Up	A1, A2
20/04/21	NIHR - Clinical Research in Older People	A1
23/04/21	Eventbrite - Evidence Based Nutrition – Research to Practice	A1
28/04/21	Research Retold - Bursting the Bubble: Making your Research Accessible beyond Academia	A1, A3, B3, D2, D3
29/04/21	The Viva and Beyond	A1, B3
29/04/21	Nutrition Society – Student Session and Careers	A1, B3
11/05/21	Coping with Change	B1, B2
12/05/21	Stress Management	B1, B2
14/05/21	NUGO Meet Up and Data Presentation	A1, A2, A3, D2
18/05/21	Wellbeing4all: Coping with Imposter Syndrome	B1, B2
23/06/21	Psychological Insights into Coaching Practice	A1, A3, B1
25/06/21	Mind Management Skills Workshops for Postgraduate Students	A1, B1, B2
29/06/21	Patient and Public Involvement & Engagement in FMS	A1, B1, B3, D2, D3
30/06/21	NCL+ Advanced Award in Career Preparation	B1, B2, B3
23/08/22	Social Media and Science	A3, B3, D2, D3
15/09/22	Scriptoria CV Course	A2, A3, B1, B3, C1, D2
24/11/22	Careers in Dietetics	A1, B3

Throughout	Nutriweb Webinars	A1
	External Training	
02/05/19	Nutrition Society Advanced Statistical Analysis	A1, A2, B3, C2
19/09/19	Level 2 Food Safety and Hygiene	A1, C1
12/10/19	Presentation Training	A1, A3, B1, D2, D3
31/01/20	Good Clinical Practice	A1, A3, B3, C1, C2, D1
24-29/04/20	R Course Twitch	A1, A2
02/21	Smart Resourcing Solutions – Employability Webinars	A1, B2, B3, C1
27/05/20	Sysmic Computing and Programming Course - Completed May 2021	A1, A2, A3, B3, C2
03/22	IUNS/FENS Volunteer	A3, B3, D1, D2, D3
12/10/22	R for Data Science: Beginner – University of Liverpool – Funded Place	A1, A2, A3, B3, C2
15/11/22	R for Data Science: Intermediate – University of Liverpool – Funded Place	A1, A2, A3, B3, C2

A1: Knowledge base, A2: Cognitive abilities, A3: Creativity, B1: Personal qualities, B2: Self-Management, B3: Professional and Career Development, C1: Professional Conduct, C2: Research Management, C3: Finances, Funding and Resources, D1: Working with Others, D2: Communication and Dissemination, D3: Engagement and Impact. NUGO - molecular nutrition, personalised nutrition, nutrigenomics and nutritional systems biology. CIMA - The MRC-Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing. NEPG – Northeast Post Graduate conference, MRC – Medical Research Council, DiMeN - Discovery Medicine North, IFIS - International Food Information Services; IUNS: International Union of Nutrition Societies; FENS: Federation of European Nutrition Societies

E. Conferences

During my PhD programme I have had the opportunity to present my work at various conferences aimed at academic, or lay audiences (Table 9.2).

Table 9.2: List of conferences with dates and type of presentation.

Date	Conference	Type of Presentation
23/10/19	HNRC 25th Anniversary Conference "Global Nutrition Challenges in the Next 25 Years"	Poster
17/12/19	ISENC 2019	Poster, Abstract Published
14-15/07/20	Nutrition Society Live	Awarded Funded Position – Prof Phillips Bursary
12/10/20	CIMA Annual Meeting	Oral 3-minute thesis
13/11/20	NEPG Conference	Oral ppt
01/12/20	ROS Conference	Oral ppt, Abstract Published
15/02/21	CIMA Spring Meeting	Poster ppt
10/06/21	ISTRC - Sarcopenia	Awarded Funded Position
22-24/06/21	Nutrition Society – Irish Section Conference	Poster and Oral Ppt, Abstract Published
06-07/09/21	Nutrition Society – Futures	Awarded Funded Position – Quorn Nutrition
18/10/21	CIMA Annual Meeting	Oral Ppt
05/07/22	PHSI Research Day	Oral Ppt
6-7/09/22	Nutrition Society – Futures	Awarded Funded Position and Travel Grant
3-5/10/22	CIMA Annual Meeting	Oral Ppt

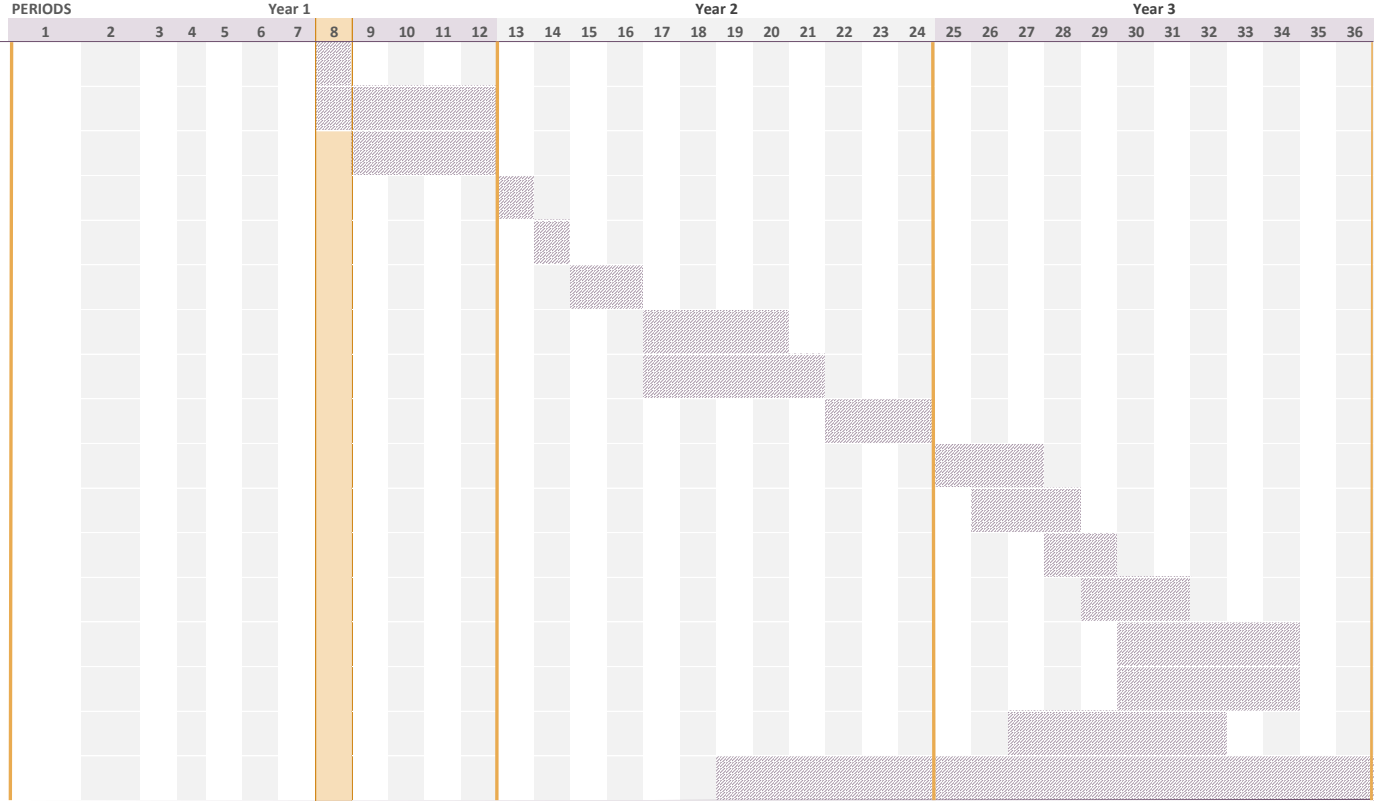
ISENC – International Sport and Exercise Nutrition Conference; HNRC – Human Nutrition and Research Centre; CIMA – The; MRC-Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing; NEPG – Northeast Post Graduate conference; ISTRC - International Sarcopenia Translational Research Conference; ROS – Royal Osteoporosis Society; PHSI – Population Health Science Institute; Ppt: PowerPoint

F. Initial PhD Gannt Chart

Project Planner

Period: 8 — Plan Duration

ACTIVITY	PLAN START (mo)	PLAN DURATION (mo)
Organise Work	8	1
SYSMIC Computing/Programming	8	5
PRECISE Manuscript Write Up	9	4
Organise Work	13	1
RCT Lab Visit - COVID Dependent	14	1
RCT Data Organise	15	2
RCT Analyses	17	4
RCT Write Up	17	5
Selenium Blood Samples	22	3
Analyses of Se Status	25	3
Write Up of Se Status	26	3
Literarature Review for Se and Epigenetics	28	2
BFU Analyses	29	3
DNA Methylation	30	5
Gene Expression	30	5
LiLACs if time permits	27	6
Thesis Writing	19	18



H. Annual Progress Review

2022

Supervisor

Progress on Project Plan

RCT trial: Giorgia is close to submitting her RCT paper to JBMR and her linked thesis chapter is almost complete.

Newcastle 85+ Prospective cohort study: She awaits the selenium biomarker data from Prof Lutz Schomburg in Berlin and hopes to have this by the end of May. Giorgia also plans a short trip to Berlin to meet Prof Schomburg and gain an insight into the methods used to measure Se biomarkers. This data will form the basis of two thesis chapters. In the meantime, Giorgia has prepared drafts of these chapters mainly focusing on the introduction, hypothesis methods and approach to the results.

Thesis: Giorgia has a clear understanding of the structure of her thesis. She is aware of the time pressures she will face over the coming 5 months in order for her to submit on time. She continues to have weekly meetings with her supervisors to ensure that she remains on track.

I am confident that provided Giorgia receives the selenium data in May that she will produce an excellent thesis.

Risks Prof Schomburg has reassured Giorgia that the selenium biochemical data for the cohort study will be available by the end of May. Receiving this data in good time is the only risk associated with the timely completion of the PhD.

Form submitted by Thomas Hill - April 20, 2022, 9:06 p.m

Panel Reviewer

Giorgia presented to the panel her progress so far, the training she followed, and the current structure of her planned thesis. She discussed her most recent research work and the

writing of a publication. She also showed her plan for her PhD thesis writing and for completing her research work.

Giorgia's presentation skills are excellent, and her progress is very good.

The panel was pleased to see that Giorgia's work will result in a publication for which she will be first and corresponding author. This reflects the high quality of Giorgia's work.

The panel however suggested that Giorgia should check if she could get a short extension (e.g. 3 months) to give her time to write her research paper and finalise her data analysis. This would also enable her to potentially visit a lab in Berlin where some of her sample analysis is carried out and therefore provide her with a greater understanding of the techniques used.

The panel strongly supports the visit in Berlin and the panel would recommend that a short extension should be given to Giorgia based on the fact that, as a result of COVID, she had to change part of her project, and that she would benefit from producing a very good quality analysis of the data. It is worth noting that as the sample analysis is produced in another lab, Giorgia has little control over when she will access the data for analysis and therefore this fully justifies this extra-time.

The panel discussed with Giorgia the support she receives and was pleased that Giorgia felt she receives adequate support from her supervisory team in Newcastle.

Form submitted by Richard McNally - May 4, 2022, 5:28 p.m.

Head of School

Panel decision i. The candidate's performance is satisfactory, and the candidate can proceed to the next stage. (Note: This will allow Year 1 candidate-elect students to have their candidature confirmed)

I agree with the panel that requesting a 3-month extension on Covid grounds would be sensible. Best of luck on the run in to submission, Giorgia

Form submitted by Elaine McColl - May 5, 2022, 8:13 a.m.

Dean recommendation

Proceed

Agree with advice provided. Happy to approve.

Form submitted by Alison Tyson-Capper - May 18, 2022, 6:31 p.m.

2021

Supervisor

Giorgia is progressing very well with her PhD studies. She has prepared a draft manuscript on the PRECISE RCT study which will be submitted to JBMR after it receives feedback from Sheffield and Danish co-authors. Giorgia will send serum from the N85+ Study participants to Prof Lutz Schomburg's lab in Berlin for Se biomarker analysis and this data will be used for the following purposes:

1) To examine the associations between dietary Se and Se biomarkers in the 85+ cohort

2) Explore the relationship between serum Se and SEPP on

(i) prospective measures of muscle strength and function from the 85+ cohort

(ii) Disability trajectories over 5 years in the 85+ cohort

(iii) Incidence of sarcopenia, falls and fractures in the 85+ cohort

Giorgia is keen to get at least some laboratory exposure during her PhD and this will not only be facilitated by the CIMA collaboration with Sheffield supervisors, but she hopes to visit Prof Schomburg's lab in Berlin to learn about Se status assessment.

Appreciably Giorgia's PhD plan has changed to include more scope on the N85+ dataset and now does not involve molecular work on the BFU samples. This decision was made in consultation with Giorgia and her supervisors.

Risks Highlight any major risk to the on-time completion of this research project and submission of a satisfactory thesis, including any knowledge you have of relevant Personal Extenuating Circumstances (PECs) significantly affecting the candidate's studies.

The only risk at the moment is ensuring that the N85+ samples can be safely transported to Berlin for Se analysis.

Form submitted by Thomas Hill - June 11, 2021, 10:59 p.m

Panel Reviewer

i. The candidate's performance is satisfactory, and the candidate can proceed to the next stage. (Note: This will allow Year 1 candidate-elect students to have their candidature confirmed)

The panel met with Giorgia on a zoom meeting due to COVID-19 restrictions. Giorgia presented her work and work plan, demonstrating a good understanding of the topic. The panel congratulates Giorgia on her the excellent presentation skills.

Giorgia has made significant progress since our last meeting, as evidenced by the production or 2 manuscripts (one published in the Nutrients Journal, the other one being in preparation), and her contribution at national conferences. In addition, she has managed to follow several training sessions and develop a large array of skills in statistics and other transferrable skills. She has also contributed to the supervision of an undergraduate project.

Although Giorgia managed to carry out some of the statistical analysis for her project and was able to continue working, COVID-19 restrictions have impacted on her ability to carry out lab work. Additionally, some changes to her original work plan were made and include 1) carrying out a secondary analysis on data from The PRECISE Study instead of working on the Sheffield RCT, 2) additional measurements in samples from the 85+ Study, and 3) not using samples from the BORICC study.

The secondary analysis of data from The PRECISE Study is relevant to her PhD work and will nicely complement the work she has carried out on the 85+ cohort. However, it is unfortunate that she could not contribute to the data analysis on the Sheffield RCT, especially given

the fact it was in her original plan and that the work was recently published. This would have given her a chance to participate in the data analysis of the first RCT in the field and would have been a key part of in her PhD thesis.

As Giorgia will not anymore perform DNA methylation assays in the BORICC samples and given the fact that she is carrying out a secondary analysis on data from PRECISE study, we strongly recommend that, to strengthen both her PhD and her skills portfolio, she should be given an opportunity to carry out some of experimental work on the samples from the 85+ Study. This would give her a wider understanding of the topic and techniques used in the field and would broaden her future professional prospects. The panel strongly recommends that Giorgia discusses with her supervisory team which experiments she would like to carry out, and whether this could be achieved by her spending for example 6 months in a lab looking at bone metabolism or inflammatory biomarkers.

Form submitted by Djordje Jakovljevic - June 12, 2021, 12:35 p.m.

Head of School

Panel decision i. The candidate's performance is satisfactory, and the candidate can proceed to the next stage. (Note: This will allow Year 1 candidate-elect students to have their candidature confirmed)

Giorgia and her supervisors have adapted the plan of work in view of constraints imposed by COVID - well done. Giorgia is also be congratulated on work on publications, an excellent achievement at this stage in her research. The suggestions of the panel in respect of alternative lab work merit consideration by Giorgia and her supervisors but should not be allowed to unduly delay completion and submission of a good quality thesis. Good luck with the next stage in your work, Giorgia.

Form submitted by Elaine McColl - July 29, 2021, 5:09 p.m.

Dean recommendation

Proceed

Well done, Giorgia.

Form submitted by Alison Tyson-Capper - Aug. 18, 2021, 4:29 p.m.

2020

Supervisor

Giorgia has made excellent progress on her PhD plan to date with no concerns whatsoever.

Giorgia is very motivated and dedicated to her PhD. She always takes the initiative with tasks and generates good quality work.

Risks None at this stage as the work is desk based and will be for the first two years of her PhD.

Form submitted by Thomas Hill - May 27, 2020, 8:05 a.m.

Panel Reviewer

i. The candidate's performance is satisfactory, and the candidate can proceed to the next stage. (Note: This will allow Year 1 candidate-elect students to have their candidature confirmed)

The panel met with Giorgia on a zoom meeting due to COVID-19. The panel congratulates Giorgia for the excellent presentation of her wok so far and her work plan. She demonstrated a very good understanding of the subject and has already completed a large part of the work. Giorgia also informed us that she has submitted a research article for her MRes work, showing that she is progressing well.

As most Giorgia's work at present is based on statistical analysis of data, she is able to work remotely, and her work progress are not impeded by the current COVID-19 restrictions. She is supported by her supervisory team and is confident in the work she is currently carrying out.

The panel recommended the student progression to the next stage. The work plan suggests that Giorgia has access to very interesting data sets to address her research question. The panel emphasised that a scientific justification of the progress between one study to the other would strengthen the coherence of the overall PhD thesis.

As most of the planned work will involve analysing data from data sets that have already been collected (without an input from the student), the panel strongly recommended that the student should be given the opportunity to carry out some experimental work, so that some of the data presented in the PhD thesis will have been directly produced by the student. At present, it seems that the most relevant part of the project for this would be the determination of DNA methylation pattern (3rdyear of the project). Thus, the panel strongly recommended that the student discuss with her supervisors how she could be involved in data collection.

Furthermore, the panel strongly recommended that the student should discuss in detail with her supervisory team the last part of her project (3rdyear). The panel was concerned that studying differences in DNA methylation in rectal biopsies from the BORRIC study may not be relevant to other tissues such as bones. The panel would also advise to only focus on healthy volunteers with either suboptimal or optimal Se status, rather than colorectal cancer patients.

Form submitted by Catherine Meplan - June 29, 2020, 11:35 a.m.

Head of School

Proceed

Happy to support Giorgia's progression. Both supervisory team and panel raise no concerns and agree that excellent progress has been made over the past year. The panel make some suggestions that the supervisory team and student may want to consider. Well done, Giorgia.

Form submitted by Luke Gaughan - June 29, 2020, 1:51 p.m.

Dean recommendation

Proceed

Excellent! well done Giorgia

Form submitted by Alison Tyson-Capper - June 29, 2020, 3:50 p.m