Trends in vitamin D and its influence on outcomes following total hip and knee arthroplasty

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Abstract

There is increasing interest in the extra-skeletal effects of vitamin D. In patients undergoing total hip (THR) or knee (TKR) replacement surgery, insufficiency has been linked to adverse outcomes including longer length of stay, lower patient reported outcome measure scores (PROMs), and increased complication rates. This work aims to explore this further.

Using vitamin D data from an NHS hospital trust, the temporal trends in the local population were explored. Vitamin D levels in patients undergoing THR and TKR were measured and linked to Oxford and EQ-5D-3L scores to determine if baseline vitamin D status is associated with post-operative outcome. The current evidence base for optimising vitamin D levels with supplementation prior to arthroplasty surgery was sought through a systematic review. Finally, a randomised trial to establish the feasibility of supplementing patients with insufficiency prior to surgery to improve outcomes was completed. The influence of this feasibility study in the design of future interventional trials is discussed.

This work has shown increasing trends in vitamin D testing in the local population. Pre-operative deficiency is associated with lower PROM scores following THR/TKR. There is a paucity of evidence for supplementation prior to arthroplasty surgery, but a randomised trial investigating the role of optimising vitamin D levels with supplementation is feasible. Suggestions for how this could be done in the NHS setting are discussed, and future studies should develop this work through adequately powered multicentre trials.

Dedication

I dedicate this work to my wife Jane and sons Benjamin and Oscar, whose encouragement and understanding of my absences through early mornings and late nights has enabled me to complete this work alongside my clinical commitments. In addition, I dedicate this thesis to my mother and father in recognition of their love and support throughout my career.

Acknowledgements

I'd like firstly to acknowledge Professor Mike Reed and Mr. Kenny Rankin for their supervision of this work. Their invaluable support, advice and guidance throughout my PhD has been greatly received.

Thanks to the research nurses and Research and Development support staff at both Northumbria Healthcare NHS Foundation Trust and South Tees Hospitals NHS Foundation Trust, who enabled the VASO clinical trial to run so effectively. Thank you to the Biobank staff at Newcastle University for their assistance in storing patient samples.

I'm incredibly grateful to the patients who agreed to take part in these studies and in doing so, have done their bit to help improve outcomes for others undergoing joint replacement surgery. In particular I'd like to thank the members of the Total Hip User Group for their input and advice from a patient perspective, in the development of the VASO feasibility trial in chapter 5.

My thanks are extended to Inez Schoenmakers, Jonathan Tang and the wider team in the Bioanalytical Facility at the University of East Anglia who performed the mass-spectrometry analysis of vitamin D samples reported in chapter 3. I'm very grateful for their hospitality and teaching when I visited their unit.

Finally, I'm indebted to Orthopaedic Research UK who provided grant funding, without which this research would not have been possible. Likewise, to the Shears family for providing their support for the one-year Research Fellowship through the Royal College of Surgeons of England.

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Declaration

I confirm this is an original piece of work that has not been submitted for consideration of a higher degree award with another institution or university. This work has been carried out by the candidate under the supervision of the supervising research team as per the guidance for doctoral thesis laid out by Newcastle University.

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Awards, papers and presentations derived from this thesis

Awards:

Shears Research Fellowship, awarded by the Royal College of Surgeons of England, 2017-18

George Feggetter Medal for best presentation at the North of England Surgical Society Meeting, May 2018

Best Poster Award at the British Orthopaedic Association Meeting, 2018

Invited Presentations:

Vitamin D - supplementation to enhance outcomes? *Quality Improvement for Surgical Teams Conference, Northumbria, October 2016*

Vitamin D and surgery. *Grand Round Lecture, Hexham General Hospital, January 2017*

Vitamin D and joint replacement outcomes. *Quality Improvement for Surgical Teams Conference, Northumbria, October 2018*

Podium Presentations:

Does supplementation for vitamin D deficiency improve outcomes following total hip or knee replacement? The VASO trial. Morrison RJM, Bunn D, Gray WK, Baker PN, White C, Rangan A, Rankin KS, Reed MR. *Society of Academic and Research Surgery Conference, Nottingham, January 2018.*

Vitamin D insufficiency may be linked to lower patient reported outcome measure (PROM) scores following total hip or knee replacement. RJM Morrison, KS Rankin, MR Reed. *Society of Academic and Research Surgery Conference, Nottingham, January 2018.*

Vitamin D and outcomes following total hip or knee replacement. VASO trial – early results. Morrison RJM, Bunn D, Gray WK, Baker PN, White C, Rangan A, Rankin KS, Reed MR. *Northern England Surgical Society Meeting, Newcastle, May 2018*

Vitamin D and outcomes following total hip or knee replacement. VASO trial – early results. Morrison RJM, Bunn D, Gray WK, Baker PN, White C, Rangan A, Rankin KS, Reed MR. *Kreibich Meeting, June 2018*

Vitamin D deficiency may be linked to lower patient reported outcome measure (PROM) scores following total hip or knee replacement. RJM Morrison, KS Rankin, MR Reed. *British Orthopaedic Association Congress, Birmingham UK, September 2018.*

Poster Presentations:

Successful recruitment to a feasibility trial investigating if supplementation for vitamin D deficiency improves outcomes following total hip or knee replacement? The VASO feasibility trial – early results. *21st Workshop on Vitamin D, Barcelona, May 2018*

Vitamin D deficiency may be linked to lower patient reported outcome measure (PROM) scores following total hip or knee replacement. *21st Workshop on Vitamin D, Barcelona, May 2018*

Does supplementation for vitamin D deficiency improve outcomes following total hip or knee replacement? The VASO Trial. Morrison RJM, Bunn D, Gray WK, Baker PN, White C, Rangan A, Rankin KS, Reed MR. *British Orthopaedic Association Congress, Birmingham UK, September 2018.*

Publications:

VASO (Vitamin D and Arthroplasty Surgery Outcomes) study – supplementation of vitamin D deficiency to improve outcomes after total hip or knee replacement: study protocol for a randomised controlled feasibility trial. Morrison RJM, Bunn D, Gray WK, Baker PN, White C, Rangan A, Rankin KS, Reed MR. *Trials* 2017;18(1):514.

The effect of vitamin D on outcomes following total hip or knee arthroplasty surgery: a rapid systematic review of current evidence. Morrison RJM, Fishley WF, Rankin KS, Reed MR. EFORT Open Rev. 2022;7(5):305-311.

Published Abstracts:

Vitamin D insufficiency may be linked to lower patient reported outcome measure (PROM) scores following total hip or knee replacement. RJM Morrison, KS Rankin, MR Reed. *Br. J Surg 2018;105(S1):27*

Does supplementation for vitamin D deficiency improve outcomes following total hip or knee replacement? The VASO trial. Morrison RJM, Bunn D, Gray WK, Baker PN, White C, Rangan A, Rankin KS, Reed MR. *Br. J Surg 2018;105(S1):28.*

List of Abbreviations

1,25-OHD	1,25-dihydroxy vitamin D
24,25-OHD	24,25-dihydroxy vitamin D
25-OHD	25-hydroxy vitamin D
ANOVA	Analysis of Variance
API	Associate Principle Investigator
BMI	Body Mass Index
DEQAS	Vitamin D External Quality Assessment Scheme
EDTA	Ethylenediaminetetraacetic Acid
EQ-5D-3L	European Quality of Life 5-dimension, 3-level Questionnaire
GCP	Good Clinical Practice
HES	Hospital Episode Statistics
HHS	Harris Hip Score
HRA	Health Research Authority
ICE	Integrated Clinical Environment
IOM	Institute of Medicine
IQR	Interquartile Range
ISRCTN	International Standard Registered Clinical/soCial sTudy Number
JLA	James Lind Alliance
KSS	Knee Society Score
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LOS	Length of Stay
MCID	Minimal Clinically Important Difference
NHS	National Health Service
NICE	The National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NIST	National Institute for Standards and Technology
NJR	National Joint Registry
NS	Not significant

OHS	Oxford Hip Score
OKS	Oxford Knee Score
ONS	Office for National Statistics
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROMs	Patient Reported Outcome Measures
PTH	Parathyroid Hormone
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RCT	Randomised Controlled Trial
RIA	Radioimmunoassay
ROB-2	Risk Of Bias 2 Assessment
ROBINS-I	Risk Of Bias In Non-randomised Studies of Interventions
RXR	Retinoid X Receptor
SACN	The Scientific Advisory Committee on Nutrition
SWAT	Study Within A Trial
THR	Total Hip Replacement
THUG	Total Hip User Group
TKR	Total Knee Replacement
TWIC	Trials Within Cohorts
UEA	University of East Anglia
UV	Ultraviolet
VAS	Visual Analogue Scale
VASO	Vitamin D and Arthroplasty Surgery Outcomes
VDBP	Vitamin D Binding Protein
VDR	Vitamin D Receptor
VDRE	Vitamin D Response Elements
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

Chapter 1: Introduction

1.1 Vitamin D

1.1.1 Discovery of vitamin D

Skeletal deformities in children, subsequently described as rickets, were first reported in the mid-1600s by Daniel Whistler of the Netherlands and then by Francis Glisson of England.[1] As the Industrial Revolution progressed through 18th Century Northern Europe, so too did the observed incidence of rickets. Sniadecki noted in 1822 the increase in cases was largely confined to inner-city areas rather than rural populations, surmising that sunlight and being outdoors was important for prevention.[1,2]

Recognising the high incidence of rickets in the UK and in particular Scotland, Sir Edward Mellanby postulated in 1918 that diet may influence the development of rickets.[3] He fed puppies, he had inadvertently kept indoors, the *"Scottish diet*" of oatmeal and milk and noted development of the disease. The skeletal deformity was reversed with cod liver oil, and so Mellanby concluded that vitamin A, which had been isolated from cod liver oil at that time, was responsible for this effect. Cod liver oil as a treatment for rickets was also noted by Chick in her 1922 study on rachitic children in Vienna.[4]

The favourable effect of cod liver oil on the prevention of xeropthalmia had already been discovered, and this was attributed to an unknown fat-soluble nutrient which had been named vitamin A.[5] In 1922 McCollum and colleagues noted that when cod liver oil was heated with oxygen, its beneficial effect on vision was lost despite it still being able to cure rickets, leading them to suggest "…*that oxidation destroys fat-soluble A without destroying another substance which plays an important role in bone growth"*. As vitamins B and C had now been discovered, then this new unknown substance was named vitamin D.[6]

In 1919, at a similar time to the studies described above, Huldschinsky exposed children with rickets to a quartz-mercury lamp, noting that this was successful in curing rickets and increasing skeletal mineralisation when observed with x-ray. He subsequently stated that exposure to ultraviolet (UV) light was *"… an infallible remedy"* for rickets.[2] The beneficial role of natural light for the treatment of rickets was reported in 1921[7], before Chick and colleagues noted the effect of season on the incidence of rickets, with no cases developing during summer months.[4]

Steenbock found that irradiating rats and their food with UV light cured rickets, and so concluded that an inactive lipid present in both foodstuffs and skin became active on UV exposure.[8] It was in 1932 that vitamin D2, derived from UV-activated ergosterol, was first discovered. However as ergosterol is not present in animals, and it was known that cod liver oil was effective in curing rickets without direct UV irradiation, then another pathway of vitamin D synthesis was thought to exist. This was elucidated in 1935, when 7-dehydrocholesterol was discovered in the skin of pigs, before the discovery of vitamin D3 in 1937, although it was not until the 1970s before it was proven than irradiation of the former produced the latter.[5] It is now known that vitamin D is a secosteroid, and as it can be synthesised in the skin it is therefore not, by definition, a vitamin.[9]

1.1.2 Cutaneous synthesis of vitamin D

An overview of vitamin D activity is provided in figure 1.1. In the presence of ultraviolet-B light of wavelength 290 - 315nm, 7-dehydrocholesterol in the skin, most of which is found in the epidermis, is converted to pre-vitamin D3. This is inherently unstable and therefore undergoes a thermosensitive isomerisation to the much more stable vitamin D3.[10] Vitamin D3 exits the dermal cells and is drawn into the capillary bed by vitamin D binding protein (VDBP), to be transported around the body either for storage in fat cells, or to be activated in the liver and kidneys. Cutaneous synthesis usually accounts for the majority of circulating serum vitamin D.[2]

Prevention of toxicity from prolonged UV exposure is regulated by the conversion of excess pre-vitamin D3 and vitamin D3 to lumisterol-3 and tachysterol-3, both of which have no effect on calcium metabolism.[10]

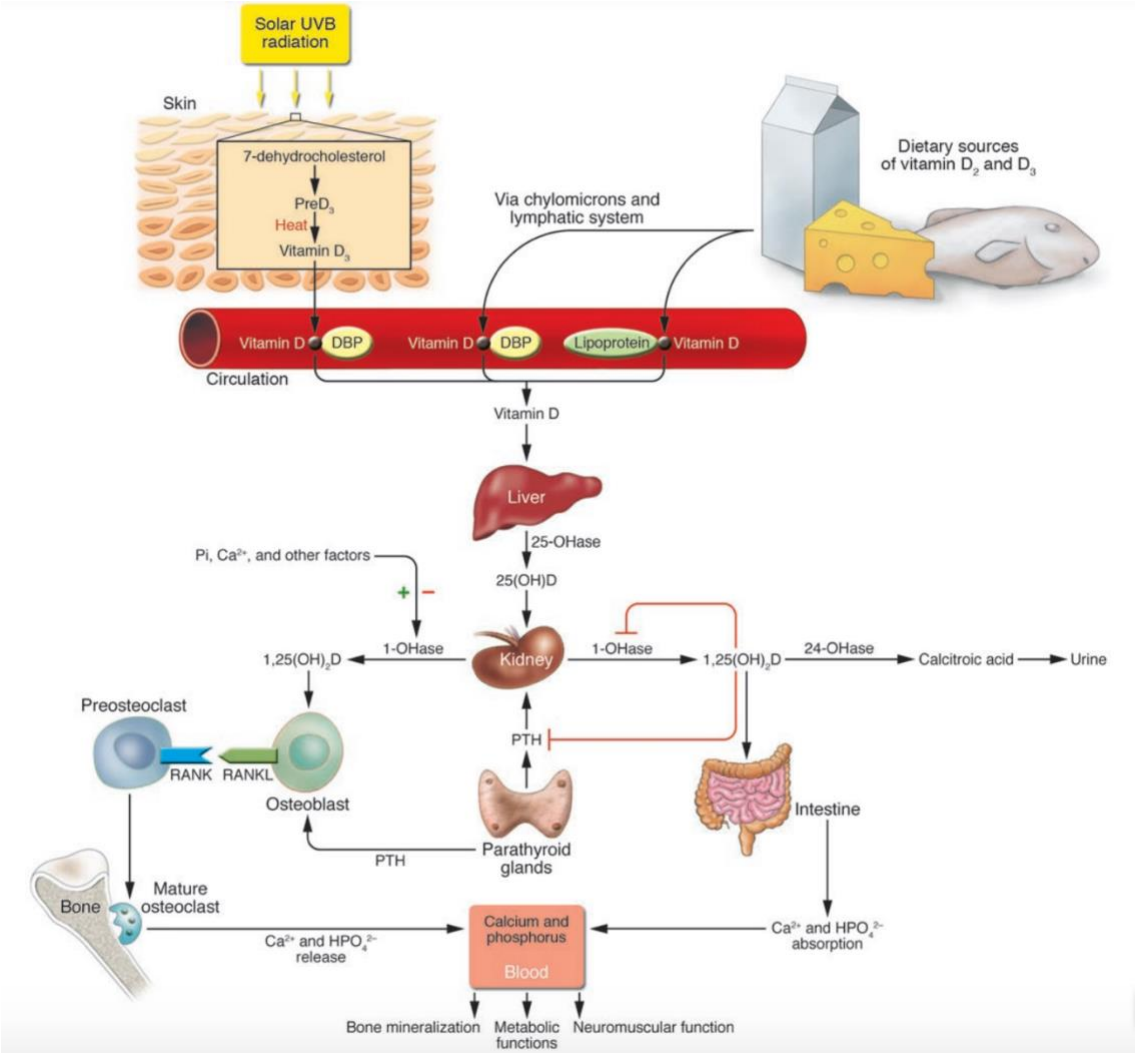


Figure 1.1: An overview of vitamin D activity *(from figure 3 in [2])*

1.1.3 Dietary sources of vitamin D

Less than 20% of serum vitamin D is obtained through diet, although this may become the only source when sun exposure is insufficient for cutaneous synthesis. Natural dietary sources

of ergocalciferol (vitamin D2) may be found in mushrooms, whilst oily fish, red meat and egg yolks are sources of cholecalciferol (vitamin D3). Fortification of food products with vitamin D in the UK is limited to margarine, some breakfast cereals, milk powders and infant formula milk.[11,12]

Vitamin D is fat soluble and absorbed with about 70% efficacy from the small intestine along with lipoproteins, triglycerides and other lipids, where it is taken up by chylomicrons and transported firstly via the lymphatic system and then by the general circulation to the liver. Disorders leading to intestinal malabsorption of lipids may therefore contribute to vitamin D insufficiency. Skeletal muscle and adipose tissue possess lipoprotein lipase and can hydrolyse chylomicrons. A small amount of vitamin D released during this process is thus taken up into these tissues, and may account for the lower vitamin D levels recorded following food consumption, as well as a possible explanation for the lower circulating vitamin D levels measured in obesity.[13]

1.1.4 Vitamin D binding protein

Vitamin D binding protein (VDBP), from the albumin gene family, is produced primarily in the liver, and is the main transport protein for all vitamin D metabolites. Approximately 85% of circulating vitamin D metabolites are highly bound to VDBP at a single binding site. The remaining 15% of vitamin D metabolites are bound to albumin, although with a lower affinity than seen with VDBP.[14] Less than 0.5% are unbound vitamin D metabolites and these are considered to be 'bioavailable' to enter cells and exert their action – the so-called 'free hormone hypothesis'.[14] In addition, some of the 15% of metabolites bound by albumin are also considered to be 'bioavailable' due to the lower binding affinity with albumin, although the extent of this availability is not clear.[15]

Some VDBP-bound vitamin D can be transported across cell membranes via the membrane receptor megalin and its co-receptor cubilin. This megalin-cubilin complex is also responsible

for the transport of other low molecular weight proteins, and is most commonly found in the renal tubules, where transport of VDBP allows for filtered metabolites to be retrieved back into the circulation preventing uncontrolled loss of vitamin D.[14,16]

VDBP is highly polymorphic, and different subtypes of VDBP are reported. These subtypes have variable binding affinity, according to the amino acid sequence, and therefore influence bioavailability of vitamin D. Expression of subtypes varies by ethnicity and subtype GC1F, which has the highest affinity for vitamin D, is most common in Black and Asian populations, whilst subtype GC2, which has the lowest affinity, is most common in Europeans.[17]

In addition to its role in transporting vitamin D, VDBP also serves as a chemotactic factor and can recruit neutrophils.[17] Furthermore, it binds actin released from damaged cells, whether as a consequence of trauma, surgery or infection, preventing disseminated intravascular coagulation and multiorgan failure. These VDBP-actin complexes are rapidly cleared from the circulation by the liver, lungs and spleen and as a consequence a fall in VDBP levels is seen with a subsequent reduction in the bioavailability of vitamin D.[14] A low VDBP level can therefore be a prognostic indicator of disease severity.[18]

1.1.5 Conversion to 25-hydroxy vitamin D [25(OH)D]

Vitamin D requires activating to become metabolically effective, and the first stage of this process occurs primarily in the liver. Hydroxylation at the C-25 position produces 25-hydroxyvitamin D [25(OH)D] which is the major form of vitamin D found in the circulation.[19] As circulating levels of 25(OH)D are not tightly regulated and have a half-life of two to three weeks, it is used as the biomarker for measuring vitamin D status.[12,19]

Cytochrome P-450 enzymes in the liver are responsible for hydroxylation of vitamin D, specifically CYP2R1 which provides equal hydroxylation of both D2 and D3.[12] As patients with a mutation of the CYP2R1 enzyme have been found to have vitamin D deficiency and

symptomatic rickets, it is suggested this is the key hydroxylase. However, in studies of CYP2R1 null mice, whilst measured vitamin D levels are reduced, 25OHD levels do not fall to zero, suggesting other hydroxylases have similar activity, and possible candidates include CYP27A1 CYP3A4, and CYP2D25.[17]

Following this first stage of hydroxylation, 25-OH vitamin D is released into the circulation to be transported, bound to VDBP, to the kidney for the second stage of activation.[12]

1.1.6 Conversion to 1,25-dihydroxy vitamin D [1,25(OH)$_2$D]

25(OH)D, bound to VDBP, enters the kidney and is filtered by the glomeruli, before being taken up into the proximal renal tubule by two surface receptors, megalin and cubilin, through endocytosis. In the proximal renal tubule hydroxylation at the C-1 position occurs, forming 1,25(OH)$_2$D which is the only hormonally active form of vitamin D responsible for metabolic action.[17] This second hydroxylation is mediated by the mitochondrial P450 enzyme CYP27B1 through 1α-hydroxylase activity.[12]

CYP27B1 is the exclusive enzyme responsible for production of 1,25(OH)$_2$D in the kidney, and mutations in this are responsible for pseudovitamin D deficiency, which manifests as vitamin D dependent rickets despite normal circulating 25OHD levels.[17,19,20] The activity of CYP27B1 is tightly regulated to finely control 1,25(OH)$_2$D circulating vitamin D levels. Parathyroid hormone (PTH), in response to low calcium levels, upregulates CYP27B1 production, whereas fibroblast growth factor 23 (FGF23), and 1,25(OH)$_2$D itself downregulate production.[20]

Whilst the kidney is the main source as described above, a number of extra-renal tissues are also able to produce 1,25(OH)$_2$D, including bone, macrophages, lymphocytes, endothelial cells and endocrine tissues.[12,17,19] However, the effect of extra-renal 1,25(OH)$_2$D production is thought to be limited to an autocrine or paracrine function within these tissues producing

CYP27B1, rather than having an influence on systemic levels.[12] This would suggest activated vitamin D is of importance to the function of these extra-renal tissues.

1.1.7 Cellular effects of vitamin D

Activated vitamin D is able to exert its effect on target tissues by binding with high-affinity to a vitamin D receptor (VDR).[12] VDR's are part of the steroid receptor family, and the gene responsible for their production is located on chromosome 12.[17] Activated vitamin D binds to VDRs inducing phosphorylation[21], which enables the VDR to form a heterodimeric complex with retinoid X receptor (RXR) within the cell nucleus. This VDR/RXR complex then binds to specific DNA repeat sequences, known as vitamin D response elements (VDRE),[17] leading to the recruitment of transcriptional coactivators and subsequent changes in gene expression. VDR's are expressed on nearly every nucleated cell in the body,[21] and it has been noted that vitamin D may modulate up to 10 per cent of the human genome.[22]

Vitamin D can also induce non-genomic effects,[23] and these changes occur more rapidly (within minutes) compared to genomic changes which may take some hours.[24] VDRs and membrane-associated rapid-response steroid-binding protein receptors (MARRS) interact with cell membrane invaginations, known as caveolae, activating signal transduction pathways.[24,25] Through secondary messengers, such as cyclic adenosine monophosphate (cAMP) or mitogen-activated protein kinase (MAPK), vitamin D can produce a rapid response in calcium channels, chloride channels and alter membrane lipid turnover, prostaglandin production, and protease activity.[24]

An overview of both the genomic and non-genomic effects of $1,25(OH)_2D$ is shown in figure 1.2.

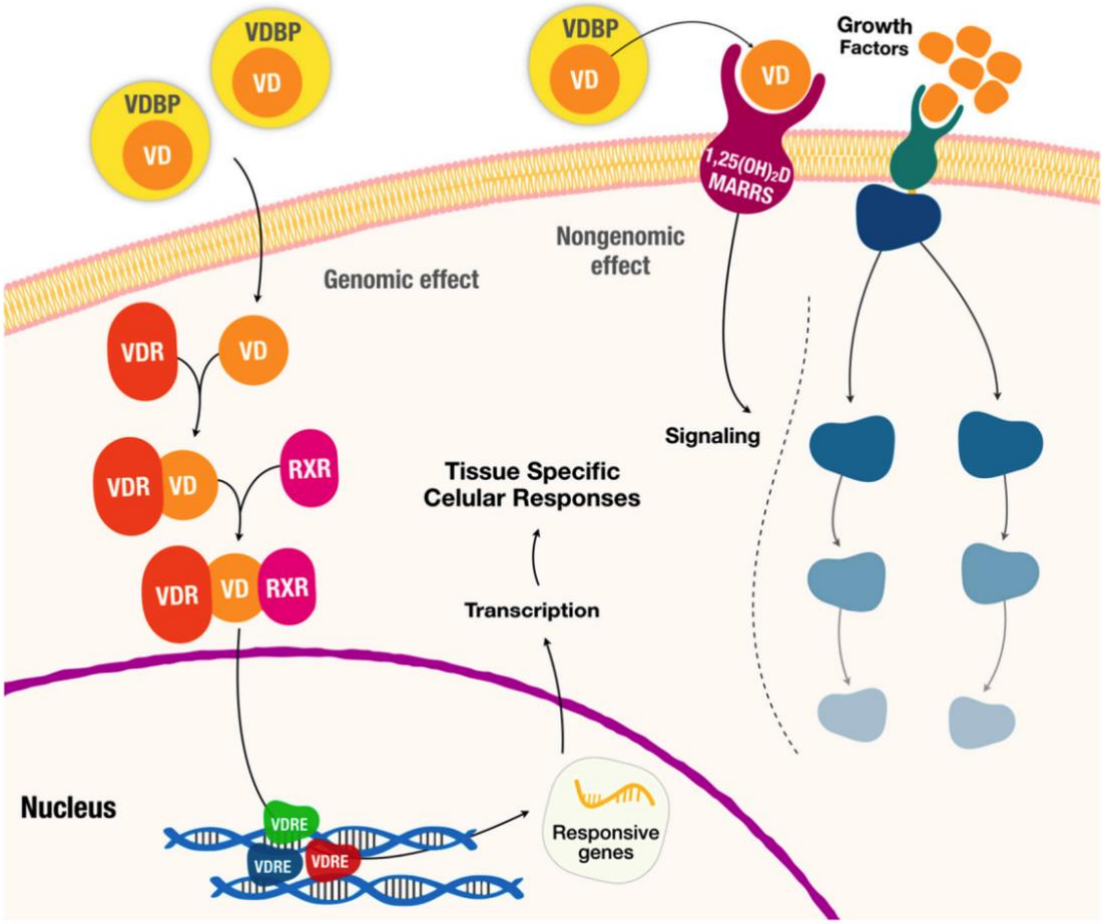


Figure 1.2: The genomic and non-genomic effects of $1,25(OH)_2D$ *(from figure 2 in [26])*

MARRS = membrane-associated, rapid-response steroid-binding protein receptors; RXR = retinoic X receptor; VD = vitamin D; VDBP = vitamin D binding protein; VDR = vitamin D receptor; VDRE = vitamin D response elements.

1.1.8 Skeletal effects – the 'traditional' role of vitamin D

The traditional function of vitamin D is in calcium and phosphate homeostasis. Calcium has physiological roles in neural transmission, muscle contraction, blood coagulation, hormone secretion and skeletal mineralisation, and serum calcium levels are tightly regulated. Over 99% of calcium in the body is stored in the skeleton as calcium hydroxyapatite, and the remaining 1% is found in the extra-cellular fluid, 50% of which is bound to albumin and the remaining 50% as unbound or ionised calcium. A negligible amount is found intracellularly.[27]

Calcium homeostasis is maintained by the interaction of PTH, calcitonin, and $1,25(OH)_2D$ (figure 1.3). Changes in circulating calcium levels are identified by calcium-sensing receptors on the surface of chief cells of the parathyroid glands. In response to low calcium levels, PTH is secreted from these chief cells and acts on the kidneys and bone to increase serum calcium levels.

In the kidney, PTH increases the resorption of filtered calcium in the distal convoluted tubule, and promotes the excretion of phosphate. It stimulates the activation of 25OHD to $1,25(OH)_2D$ in the proximal tubules, as well as preventing the breakdown of $1,25(OH)_2D$ by inhibiting the function of the degrading enzyme CYP24A1.[28]

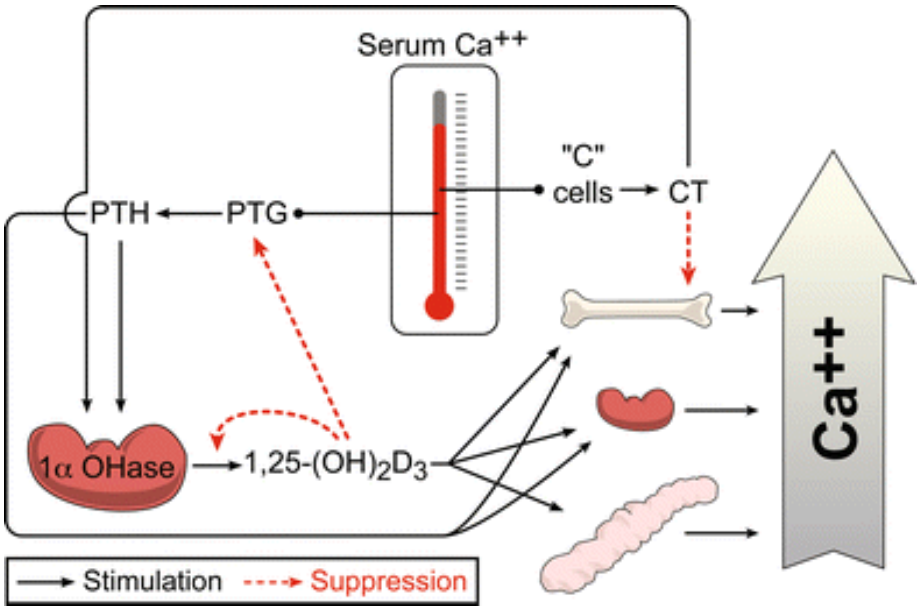


Figure 1.3: Calcium homeostasis *(from figure 1 in [29])*

PTG = parathyroid gland; PTH = parathyroid hormone; C-cells = parafollicular c-cells; CT = calcitonin; Ca^{++} = calcium; 1α-OHase = 1α-hydroxylase.

PTH has no direct effect on the gut. Instead, activated vitamin D stimulates VDRs on the surface of enterocytes throughout the large and small intestines, to increase calcium

absorption, with the most rapid uptake occurring in the duodenum.[30] 1,25$(OH)_2$D regulates the transport of calcium from the gut through both paracellular and transcellular routes, the latter of which occurs through three mechanisms (figure 1.4). Firstly, 1,25$(OH)_2$D induces the expression of TRPV6 calcium channels on the surface of enterocytes, enabling increased uptake into the cell. Secondly, it induces the expression of calbindin-D which are calcium binding proteins in the enterocytes, enabling transport through the cell. Finally, it stimulates the production of the plasma membrane ATPase PMCA1b which transports calcium through the basal membrane into the circulation.[27,30]

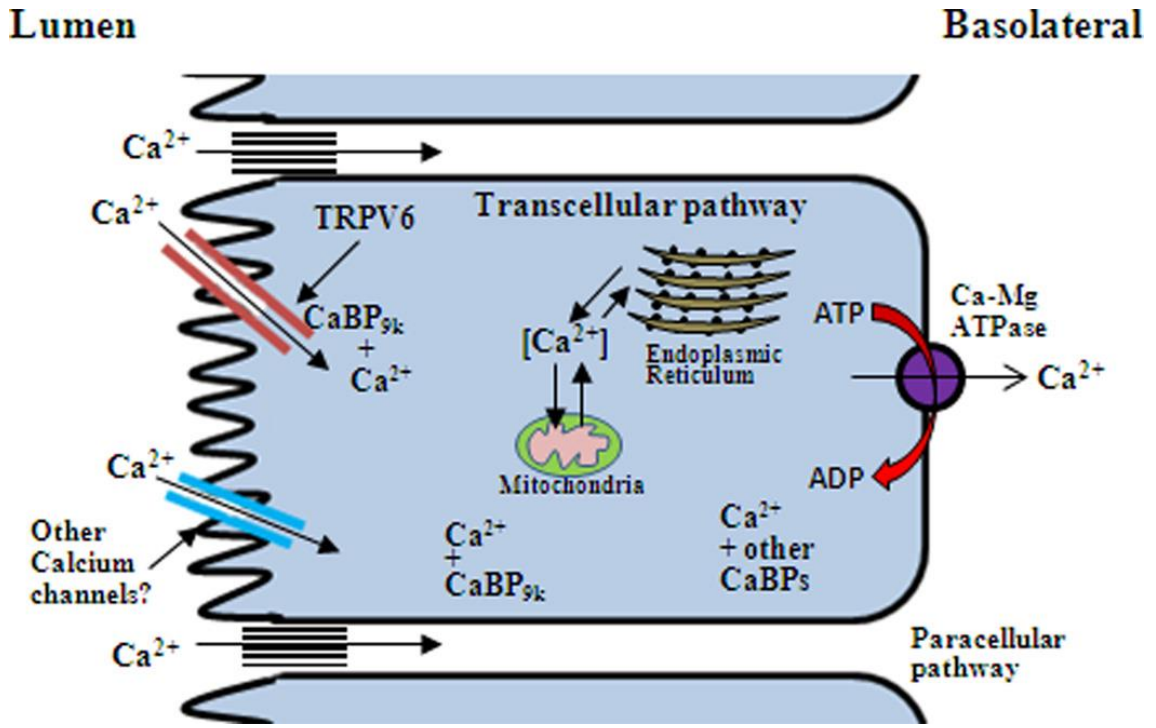


Figure 1.4: Vitamin D dependent calcium absorption in enterocytes *(from figure 2 in [30])*

ADP = Adenosine Diphosphate; ATP = Adenosine Triphosphate; Ca2+ = Calcium; CaBP = Calbindin calcium binding proteins; TRPV6 = Transient Receptor Potential Vanilloid subfamily member 6.

Lastly, PTH and 1,25$(OH)_2$D, along with prostaglandins and interleukins, act on bone to release stored calcium. Instead of stimulating osteoclasts directly, both PTH and 1,25$(OH)_2$D bind to receptors on the surface of osteoblasts (figure 1.5). This triggers the formation of receptor activator of nuclear factor kappa-B ligand (RANKL), which induces differentiation of osteoclast precursors into osteoclasts.[31] RANKL is also able to activate these newly formed osteoclasts to resorb bone and release calcium.

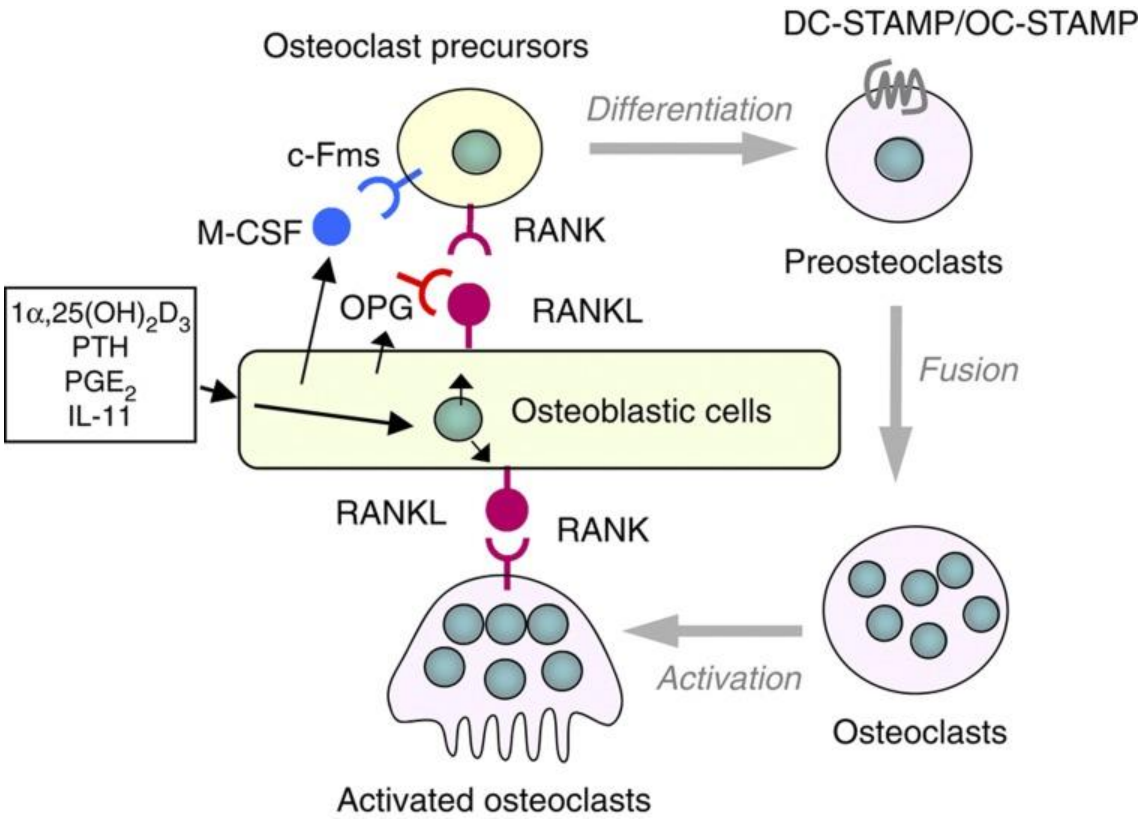


Figure 1.5: The process of osteoclast activation *(from figure 3 in [31])*

c-Fms = Colony-stimulating Factor-1 Receptor; DC-STAMP = Dendritic Cell Specific Transmembrane Protein; IL-11 = Interleukin-11; M-CSF = Macrophage Colony-Stimulating Factor; OC-STAMP = Osteoclast Stimulatory Transmembrane Protein; OPG = Osteoprotegerin; PGE_2 = Prostaglandin E2; PTH = Parathyroid Hormone; RANK = Receptor Activator of Nuclear factor Kappa-B; RANKL = RANK-Ligand.

The net result of the actions of vitamin D and PTH as described above is to increase serum calcium. In contrast, when calcium levels are too high, calcitonin is released from parafollicular C cells which are located in the thyroid gland. Its main action is to bind to calcitonin receptors on osteoclasts, inhibiting the breakdown of bone to prevent further release of calcium, and also has a minor function in inhibiting calcium reabsorption in the renal tubules. Whilst calcitonin may have a role to promote calcium deposition in bone due to the presence of calcitonin receptors on osteocytes, limited evidence for this exists. No metabolic or bone syndromes have been associated with either excess or deficiency of calcitonin.[32] PTH and $1,25(OH)_2D$ inhibit their further release via a negative feedback loop which serves to prevent an increase in calcium by inhibiting breakdown of bone, increasing the renal loss of calcium, and inhibiting renal phosphate loss which allows the formation of insoluble salts with calcium ions.

1.1.9 Inactivation and excretion

Inactivation of 25(OH)D and $1,25(OH)_2D$ occurs through the mitochondrial P450 enzyme CYP24A1, which is located in the proximal convoluted tubule, as well as in all cells expressing the VDR.[17,33] In response to elevated vitamin D levels and fibroblast growth factor 23 (FGF23),[34] CYP24A1 inactivates $1,25(OH)_2D3$ through 24-hydroxylation, forming calcitroic acid which is excreted primarily through the bile into faeces, with negligible renal excretion.[33] In addition, 24-hydroxylation of 25(OH)D3 reduces the overall pool of vitamin D available for conversion to the active metabolite.[17]

Due to its expression in all cells with a VDR, CYP24A1 may modulate cellular levels of vitamin D and can therefore be seen as a marker of vitamin D response within a cell, with a role in preventing toxicity.[19] CYP24A1 activity is suppressed by PTH.[34] It has been suggested that the ratio of serum 25(OH)D to $24,25(OH)_2D$ can be used as an indicator of catabolic state,[34] and that mutations in CYP24A1 may be responsible for long-standing hypercalcaemia and hypercalciuria, particularly in those receiving vitamin D supplementation.[17] An increase in the incidence of idiopathic infantile hypercalcaemia was noted following widespread fortification

of milk products with vitamin D, and subsequently the presence of the CYP24A1 mutation was found to explain this[35]. An overview of the metabolic pathways for vitamin D is shown in figure 1.6.

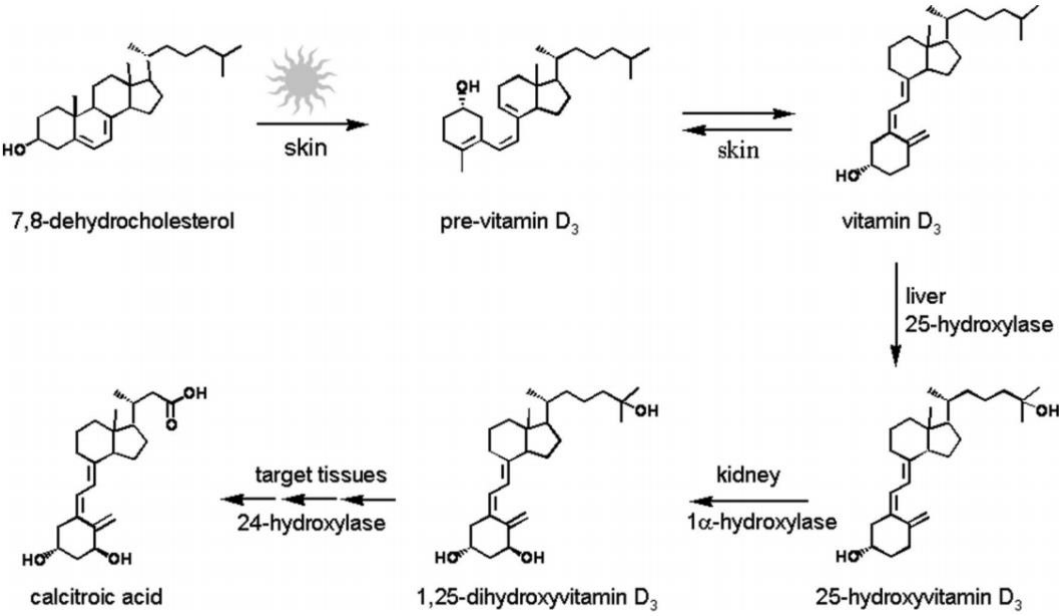


Figure 1.6: The metabolic pathway of vitamin D *(from figure 1 in [24])*

1.1.10 Measurement of vitamin D

Whilst it would at first seem logical to measure 1,25$(OH)_2$D given that it is the biologically active metabolite, it is not routine to do so when assessing overall vitamin D status, although its measurement can be of use in helping diagnose calcium and phosphate metabolism abnormalities. 1,25$(OH)_2$D levels are tightly regulated and reflect activation in response to PTH and calcium levels and the upregulation of CYP27B1, rather than representing overall vitamin D status. Consequently, normal 1,25$(OH)_2$D levels can be sustained even when body stores of vitamin D are deplete.[36] The half-life of 1,25$(OH)_2$D is short, at less than four hours, and serum concentrations are one thousand-fold less than 25(OH)D.[12]

Vitamin D which is formed in the skin as the inactive precursor to 25(OH)D has a short half-life in the circulation as it is either rapidly metabolised in the liver to the partially active 25(OH)D, or taken up into adipose tissue and therefore cannot be quantified in serum analysis.[12] Consequently, it is accepted universally that a patient's vitamin D status is measured using total 25(OH)D. The total 25(OH)D biomarker comprises both D2 and D3 levels and reflects cutaneous synthesis and that derived from dietary sources. It has a half-life of two to three weeks, and is the principle form in which vitamin D is stored and transported in the body, ready for conversion to the active form of $1,25(OH)_2D$ as described in section 1.1.6.[12]

However, there are some limitations to using 25(OH)D which should be considered. Although 25(OH)D is the best marker of overall vitamin D status, it is a precursor to the active metabolite and so its use to indicate biological 'effect' and therefore causal relation to health outcomes, remains to be seen.[13] Furthermore, 25(OH)D levels can vary widely and may reflect recent vitamin D exposure which can be influenced by season, latitude, outdoor activity status, use of sunscreen and institutionalisation. Physiological factors such as BMI, VDBP concentration and genetic variation in VDBP expression, as well as analytic variability according to the method of measurement used, can also influence measured 25(OH)D levels.[13,37] Whilst other metabolites of vitamin D have been suggested as possible biomarkers which, in time, may be important to study,[38,39] and accepting the limitations discussed above, 25(OH)D currently remains the accepted biomarker to be measured.

Both plasma and serum samples can be used for analysis,[40–42] and except for limiting direct exposure of samples to sunlight,[43] no special processing or storage requirements for samples are indicated; vitamin D is stable, and can undergo multiple freeze-thaw cycles with no deterioration in measured levels.[42–46] Indeed, vitamin D has been described *"as solid as a rock".*[47]

The first assays to quantify vitamin D level were often developed 'in-house' and utilised VDBP obtained from rats as a competitive binding protein.[36,48,49] These assays measured all vitamin

D metabolites, whether inactive or active, and therefore vitamin D level was usually overestimated.[36]

The first manual radioimmunoassay (RIA) to measure 25(OH)D was developed in 1985 (DiaSorin Ltd., UK). This used an antibody specific to 25(OH)D, along with a radioactive iodine tracer to determine vitamin D level.[36] It was originally considered the 'gold-standard' method of analysis until the early 2000s, and the vitamin D cut-offs used in practice today were defined from studies using this method.[50,51] However, due to the requirement for radioactive labels and the associated problems with their storage and handling, RIA methods have now largely been superseded by chemiluminescent or enzyme labelled assays.[48,50]

Manual versions of these newer chemiluminescent and enzyme immunoassays exist, but automated versions are typically utilised in clinical laboratories due to the increasing demand for vitamin D testing, with a number of manufacturers including Abbott Architect, Immunodiagnostics Systems Ltd., DiaSorin Ltd., and Roche Diagnostics Ltd.[48] Automated assays are simple to operate and can provide high-throughput at reasonable cost. However, they are susceptible to matrix effects which is the over- or under-estimation of the measured result due to the effect of other sample components on measurement of the analyte, in particular plasma phospholipids[52]. Furthermore, they do not discriminate between D2 and D3 metabolites, display cross-reactivity with other vitamin D metabolites such as 24,25(OH)D leading to overestimation of vitamin D status, and are reported to have significant inter-assay and inter-laboratory differences.[48–50,53–55]

In an attempt to improve the accuracy of vitamin D analysis, chromatography to separate 25(OH)D from other competing vitamin D metabolites has been utilised, and the first methods applied high performance liquid chromatography (HPLC) followed by UV absorption of 25(OH)D to measure its concentration.[36] This technique lacked sensitivity to detect very low vitamin D levels,[50] and due to it being so cumbersome, was only available to research laboratories.[36,48] More recent methods combine liquid chromatography with a mass detector to give the technique of 'liquid-chromatography tandem mass spectrometry' (LC-MS/MS).[48]

Following extraction and chromatographic separation, mass analysis enables several metabolites including both D2 and D3, at a wide range of concentrations to be measured with excellent sensitivity. LC-MS/MS is now considered the 'gold-standard' reference technique for vitamin D analysis.[40,45,50,56,57] However, LC-MS/MS methods require the use of expensive equipment and specialist staff to perform analysis and interpret the results. Due to the time required for sample processing, throughput is limited compared to automated immunoassays and so this may influence the method of analysis chosen by a busy clinical laboratory. Finally, LC-MS/MS results can be adversely affected by the presence of the C-3 epimer.[50]

Epimerisation changes the orientation of the C-3 hydroxyl group in vitamin D.[19] The resultant C-3 epimer of 25(OH)D has reduced binding affinity to VDBP, and the C-3 epimer of $1,25(OH)_2D$ has reduced affinity for the VDR, with consequential reductions in biological activity.[19,50] Immunoassays do not recognise the C-3 epimer whereas LC-MS/MS techniques do.[55] As the molar mass of the C-3 epimer is identical to non-epimeric forms, unless specific chromatography is used to separate the C-3 epimer prior to mass spectrometry analysis, the resultant vitamin D level reported with LC-MS/MS will therefore include both forms and so overestimate the true vitamin D level available.[19,50,55,58]

To monitor the analytical reliability of vitamin D assays, the UK Vitamin D External Quality Assessment Scheme (DEQAS) was set-up in 1989. Based at Charing Cross hospital in London, DEQAS distributes five samples of unprocessed serum four times per year to participating laboratories worldwide. Those laboratories who return 25(OH)D measurements within +/- 25% of the 'target value' for at least 75% of sample results are issued with a certificate of proficiency.[59] The target values are set by the National Institute for Standards and Technology (NIST) Reference Measurement Procedure (RMP),[60,61] which are measured using a validated LC-MS/MS vitamin D assay, developed by NIST and accepted as the RMP in 2010.[49,62] Whilst these are the accepted standards, the target of +/- 25% for at least 75% of sample results does not seem very accurate compared to other measurement assays.

These NIST targets are used as part of the 'Vitamin D Standardisation Programme' (VDSP), which was initiated by the National Institute of Health's (NIH) Office of Dietary Supplements (ODS) in 2010,[12,60,61] and now as part of an international collaborative has the following objectives:

1. *Standardise measured 25(OH)D concentrations in national health surveys to the recently developed NIST-Ghent University reference measurement procedures (RMP).*
2. *Evaluate differences in measured 25(OH)D concentrations among standardised national health surveys.*
3. *Expand standardisation services from national surveys to include assay manufacturers and clinical and research laboratories.*
4. *Promote the standardisation of emerging metabolites of vitamin D status.*
5. *Enable the use of standardised data in patient care and public health activities*.[63]

The VDSP aims to ensure that manufacturers development of assays and the subsequent reported measurements of vitamin D are standardised over *"time, location, and laboratory procedure".*[61]

Vitamin D is a negative acute phase reactant and lower levels combined with higher markers of inflammation may be seen in critical illness.[64,65] This correlation has generated recent widespread publicity and interest during the COVID-19 pandemic.[66] A systematic review to determine if 25(OH)D levels fall during the acute-phase response has identified mixed results; six of eight of studies showed a relationship whilst two did not. The authors suggest further studies are recommended to determine if low vitamin D levels are a cause or consequence of inflammation.[67] One study in critical care has suggested haemodilution may account for the high levels of vitamin D deficiency recorded in this cohort of patients.[68]

Reid *et al*.[69] have demonstrated the importance of timing in peri-operative vitamin D sampling. When measured post-operatively following total knee replacement (TKR), vitamin D levels were up to 40% lower compared to pre-operative levels and remained lower for at least the following three months. A similar finding by another study team was noted in patients undergoing elective total hip replacement (THR),[70] although this time vitamin D levels

returned to pre-operative levels at six weeks. It has been suggested that whilst changes in VDBP may account for the lower 25(OH)D levels recorded post-operatively, this mechanism is not yet fully understood and that 25(OH)D may be an unreliable biomarker after an acute inflammatory insult.[71] It is therefore important that in order to obtain a true reflection of baseline vitamin D status that this is measured prior to the commencement of any surgical procedure.

1.1.11 Defining normal levels

The definition of a 'normal' vitamin D level remains contentious. Some authors suggest using the inverse relationship between PTH and vitamin D, and that plateauing of PTH levels occur with a vitamin D level equivalent to 75nmol/L, suggesting this to indicate a normal level.[72–74] However, the Institute of Medicine considers there to be inconsistencies in this relationship.[13] Nevertheless, a level greater than 75nmol/L is suggested by The Endocrine Society to denote sufficiency.[75] In contrast, with regards to bone health, a level of 50nmol/L is deemed sufficient for most, and this cut-off has been adopted by The Royal Osteoporosis Society[76], National Institute for Health and Care Excellence[77] and the Institute of Medicine.[13] Deficiency has been defined as <25nmol/L, and this is due to an increasing incidence of rickets and osteomalacia below this level.[12]

No clear agreement exists on the optimal level for non-skeletal outcomes,[78] and some studies have highlighted adverse outcomes and an association with increased mortality in an elderly population with increasing vitamin D levels >75nmol/L.[79,80]

Whichever cut-off is chosen, it is important to recognise that vitamin D level is affected by latitude and season,[81–84] with traditional-living populations towards the equator in East Africa having a far higher mean vitamin D level of 115nmol/L than that seen in the UK,[85] with sunlight being the most important determinant.[86] Rather than using a single timepoint measure, the

use of an average annual vitamin D level is suggested, and models exist to calculate this accounting for seasonal variation.[87,88]

1.1.12 Supplementation for deficiency

Vitamin D2 has a shorter half-life and lower potency than D3 in effectively normalising 25OH-D levels, and consequently supplementation with cholecalciferol is recommended.[89–92] This may be through oral or intramuscular routes and whilst the latter for large bolus-doses ensures adherence, oral administration is recommended.[76] Due to the long half-life of vitamin D, daily dosing may not be required and this may help with adherence.

Most sources recommend a loading dose with high-dose supplementation in deficient patients, e.g. 20,000 units twice weekly for 6-8 weeks, followed by maintenance with 800-2,000 units daily thereafter.[76,93] The maintenance dose is also recommended if 'treatment' of insufficiency is necessary, and to achieve levels greater than 50nmol/L through supplementation only, a daily intake of 1,100IU is suggested.[94]

Supplementation is well tolerated, but is not regulated by the usual mechanisms which limit excess natural vitamin D production through UV light. Consequentially there is a risk of developing hypercalcaemia, calcification of soft tissues or renal stones, as well as unmasking primary hyperparathyroidism. A daily dose of 4,000 units has been deemed as a safe upper tolerable limit,[95] although much higher tolerated doses have been reported, with intoxication noted only with serum vitamin D levels >300nmol/L.[12,96]

1.1.13 Extra-skeletal effects of vitamin D

Whilst the traditional role of vitamin D was in calcium homeostasis, there are increasing reports of its extra-skeletal effects, modulated by the presence of the VDR and 1α-hydroxylase

enzyme in multiple cells throughout the body. Numerous observational studies have linked deficiency with cancers, cardiovascular disease, autoimmune disease, asthma, tuberculosis, falls and mortality.[97–101] Deficiency has also been linked to a number of negative outcomes following arthroplasty surgery, and these will be discussed in detail in section 1.4.

Observational studies are limited by confounding variables which cannot be accounted for, and causality should not be assumed. The outcome of RCTs is mixed, with a number not demonstrating a beneficial effect of supplementation on the health condition being assessed.[101] However, a large systematic review has noted most interventional RCTs were of small size, had a short follow-up period and most included individuals without vitamin D insufficiency and so 25OHD levels were largely unchanged following supplementation.[102] There is therefore currently insufficient evidence to draw firm conclusions on the role of supplementation for extra-skeletal benefits.[12]

1.1.14 Vitamin D deficiency – a risk factor for osteoarthritis

A number of studies have linked osteoarthritis and vitamin D, and report a high prevalence of deficiency in patients with hip and knee osteoarthritis.[103–106] Deficiency may also be related to radiographic progression of osteoarthritis (OR 2.8), with loss of joint space (OR 2.3) and osteophyte growth (OR 3.1) being significant in those with the lowest vitamin D levels (<82.5nmol/L) compared to those with highest levels (>90 nmol/L).[107] Similarly, Bergink *et al*.[108] report the highest rates of radiographic progression of arthritis were seen in those with the lowest vitamin D levels (OR 7.7), particularly in those with low bone mineral density, and the authors suggest that improving vitamin D levels may help to protect against progression of the disease.

The vitamin D receptor (VDR) genotype has been shown to be implicated in osteophyte formation, with the VDR allele 1 associated with a 2.3-fold increase, compared to other alleles.[109] The VDR is prevalent in osteoarthritic cartilage, and almost absent in normal cartilage, and may have a role in the regulation of matrix metalloproteinase and prostaglandin

E_2 production. Vitamin D may affect cartilage metabolism by promoting proteoglycan synthesis, however, further *in vitro* work is required to investigate if vitamin D treatment has a restorative effect on arthritic cartilage.[110] Promisingly, in a randomised clinical trial of 107 deficient patients, supplementation with oral cholecalciferol improved WOMAC scores and pain scores in knee osteoarthritis at 12-months, compared to the placebo group, although the effect size was small.[111]

Like most studies reporting on the relation between disease and vitamin D, however, there are mixed results. Three large-scale population studies, with long-term follow-up, did not find a correlation between vitamin D level and the development of arthritis, nor was there an association with radiological progression.[112–114] In a systematic review of the current literature, Cao and colleagues[115] conclude that deficiency is only associated with structural changes in knee arthritis rather than symptoms, and there is currently insufficient evidence to determine an association with hip arthritis.

1.2 Osteoarthritis

1.2.1 Defining osteoarthritis

Osteoarthritis is a degenerative process of articular cartilage, with subsequent structural changes to surrounding joint structures including subchondral bone, synovium and ligaments, although more recent opinion is that it constitutes an inflammatory disease.[116] It is universally the most common form of arthritis, the most common chronic disease of the elderly,[117] and one of the leading causes of disability worldwide.[118] Its incidence increases with age, and current estimates from the UK are that 18% of adults over 45 years of age have knee arthritis, and 11% have hip arthritis.[119] A number of local and systemic factors, in addition to age, have been associated with osteoarthritis. These include female sex, obesity, occupation, a history of previous joint injury, genetics, ethnicity, smoking, altered joint alignment or morphology, sarcopenia and vitamin D deficiency.[103–106,120–122]

1.2.2 Presentation and investigation

The hallmark of osteoarthritis is joint pain, but other symptoms may include stiffness, joint swelling, deformity and subsequent loss of function affecting activities of daily living. On clinical examination, deformity, swelling, crepitus, and reduced movement may be apparent. Along with clinical symptoms, radiographic changes (figure 1.7) are observed and four typical features may be noted; loss of joint space, subchondral sclerosis, osteophyte formation and the presence of bone cysts.[123] The Kellgren and Lawrence system[124] is commonly used to grade osteoarthritis and scored from 0 (no radiological evidence of arthritis) to 4 (severe changes noted).

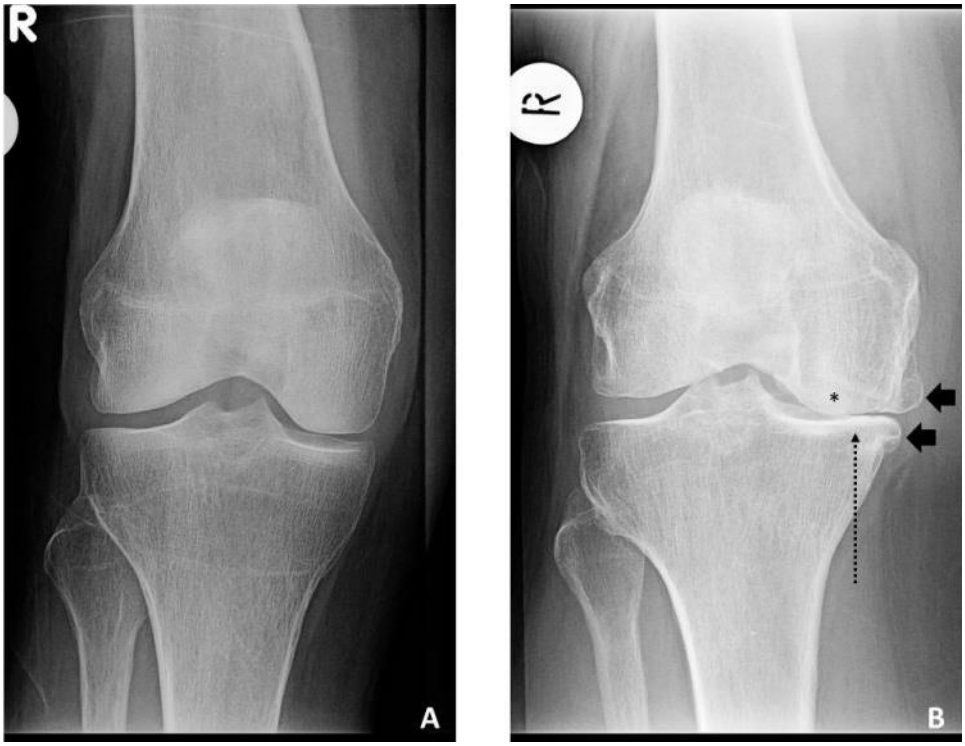


Figure 1.7: Comparison of the radiographic appearance of a normal knee (A) and an osteoarthritic knee (B) *([125])*
Star = loss of joint space; dotted arrow = subchondral sclerosis, thick arrows = osteophyte formation.

1.2.3 Management of osteoarthritis

The initial management of osteoarthritis is typically conservative, and options include weight-loss, physiotherapy, walking aids and analgesia. Intra-articular steroid injections may also be offered. For those patients with advanced disease or continuing symptoms, not amenable to conservative management and affecting quality of life, then surgical intervention may be required and joint arthroplasty is the most common.[126]

From National Joint Registry data, which covers England, Wales and Northern Ireland, there were 106,116 primary total hip replacements and 109,540 primary total knee replacements in 2018. The majority of these (>90%) were for osteoarthritis, and on average the number of procedures performed each year has been increasing.[127]

Joint replacement is largely successful, and in 1991 total hip replacement was termed *"the operation of the century".*[128] Although implant longevity depends on the material and bearing used (metal, ceramic or polyethylene), the fixation method (cemented, uncemented or a hybrid technique), and the age of the patient at surgery, the risk of requiring a revision procedure by 12 years for most patients is 5% for THR and 5.2% for TKR.[127]

The 'requirement for revision' may be used as an end-point measure to judge outcome and longevity of an implant, alongside others such as radiographic appearance and surgeon-completed scores. However, given that the indication for arthroplasty surgery is typically the reduction in quality of life because of symptoms from arthritis, then patient-reported scoring methods should be used as the best evaluation of success.[117]

1.3: Patient Reported Outcome Measures (PROMs)

Patient-reported measures can be used as one indicator of surgical success, and it is known that a discrepancy between a surgeons' and patients' opinion of satisfaction following surgery exists.[129] Satisfaction can differ between operation type,[130] with over 90% of patients reporting a good outcome following THR[131] in comparison to 80% following TKR.[132,133] The cause of dissatisfaction may be multifactorial, and studies using large joint registry data sets highlight a number of potential contributory causes.[134–136] The key factors are highlighted in Table 1.1

Table 1.1: Patient and surgical factors which may be associated with dissatisfaction following TKR.

Patient factors	
Age	This is not clear with some studies reporting better outcomes in younger patients, perhaps due to greater satisfaction,[135] whereas others have reported poorer outcomes in younger patients[137,138]. Age may therefore not be a good predictor of outcome following TKR.
Gender	On multivariate regression modelling of NJR data, Baker *et al*. noted women were less satisfied with their TKR compared to men.[133]
Expectations	Pre-operative expectations influence post-operative outcome and the inability to squat or kneel following TKR has been shown to influence post-operative satisfaction.[135] Managing expectations prior to surgery may therefore influence post-operative outcomes.
Diagnosis	Osteoarthritis as the indication for surgery has been associated with poorer outcomes compared to other diagnoses such as rheumatoid arthritis.[133] This may be attributable to patient expectations following surgery.

Depression/anxiety	Patients with depression or anxiety are more likely to report lower satisfaction rates following TKR.[132,136]
Other joint pain	The presence of other joint pain, and in particular back pain, has been associated with post-operative dissatisfaction following TKR.[132]
Surgical / implant factors	
Implant brand	Post-operative PROMS vary with implant brand, with the NexGen implant having been shown to have the highest PROM scores compared to other common implant brands used in the UK.[136]
Bearing type	The use of a fixed bearing insert is associated with better outcomes compared to a mobile bearing.[136]
Tourniquet use	Use of a tourniquet may be associated with increased post-operative pain scores and longer length of stay[139], but there is still debate whether this influences longer-term outcomes.
Hospital type	Poorer outcomes have been reported on procedures performed in the NHS compared to the independent sector.[136]

1.3.1 The use of PROMs in the NHS

Since 2009, there has been mandatory collection of PROMs data for four NHS-funded surgical procedures - THR, TKR, varicose vein surgery and inguinal hernia repair – although collection for the latter two ceased in 2017.[140,141] Using a disease-specific questionnaire and a general health status measure prior to and following surgery, the effect of an intervention on a patients' health status, as determined by them, can be evaluated. In addition, PROM scores are used to create benchmarks for auditing and commissioning needs, to highlight units where

practice may be exemplar or require development, for research purposes, and to establish referral thresholds for consideration of surgery.[140–142]

Patient consent is required to participate in the PROMs programme. Pre-operative questionnaires are sent to patients by the provider who is completing the episode of care, and this typically occurs following the pre-operative anaesthetic assessment but before surgery takes place. The completed forms, which include the patient's NHS number, are then transferred to appointed contractors who are responsible for collating the information and linking the pre-operative questionnaire responses to HES data.[140]

Post-operative questionnaires are independently sent to the patients' home by the PROMs contractor, so that responses to the questionnaires cannot be influenced by the care provider. For patients undergoing THR and TKR, these questionnaires are sent out six months following surgery. Post-operative questionnaires are linked to those completed pre-operatively through individual serial numbers. Using the difference between pre- and post-operative scores, the 'health gain' from an intervention can be determined. This data is then statistically adjusted for a hospital's casemix, recognising the difference in complexity of patients being treated, and therefore ensuring a fair comparison can be made between providers.[143] Patients may use this published PROMs data to guide their choice of health provider, whilst commissioners can use the information to determine the benefit of health interventions.[144]

PROMs data are reported as funnel plots (figure 1.8) and may indicate either unit-level or individual surgeon-level data. The average health gain for a service provider is plotted against the number of cases they perform. The UK average health gain is also shown, as are lines to denote +/- 2 standard deviations (95% limit) and +/- 3 standard deviations (99.8% limit) from the average. These can be used to identify outliers in practice, with current policy that those in the 95% limit generate an "alert" and those in the 99.8% limit generate an "alarm" for providers to assess their practice and see where improvements may be made. Furthermore, PROMs data now contributes to Best Practice Tariff (BPT) payments, which are used to remunerate service providers. Those whose practice falls significantly below the national

average, deemed as three standard deviations (the 99.8% limit), do not receive BPT.[145] Therefore, improving PROM scores is not only important for patients, but also financially essential for service providers.

For total hip and knee replacement, the PROMs programme uses the Oxford hip or knee scores as the disease specific measure, and the EQ-5D-3L as the general health measure.[140]

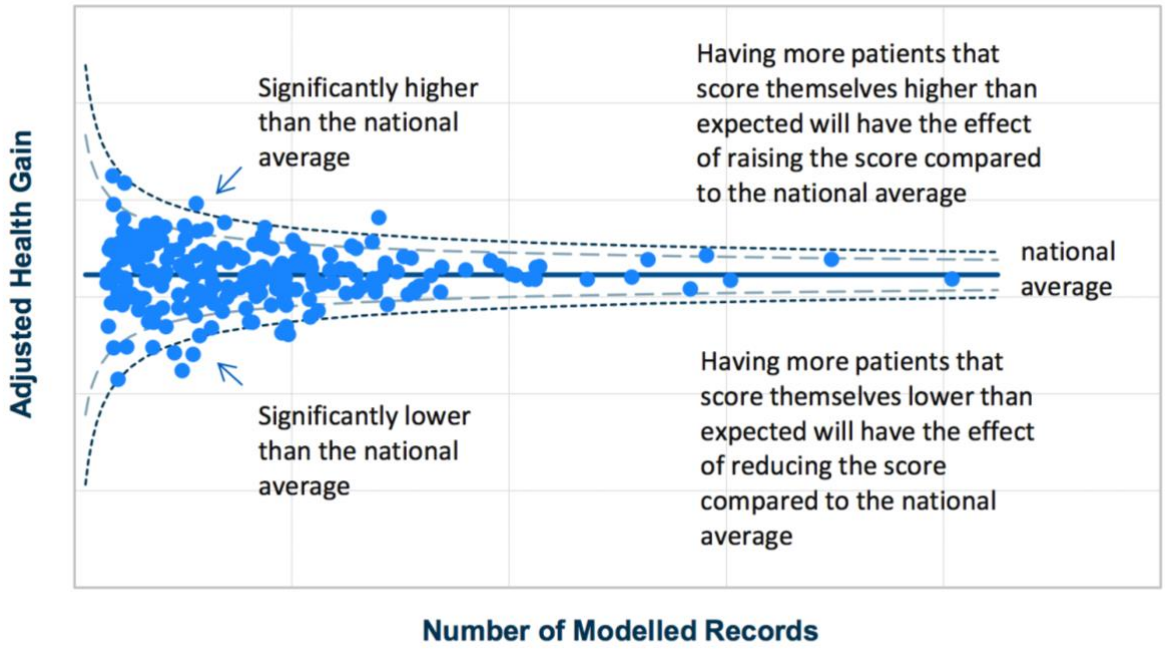


Figure 1.8: An example funnel plot depicting adjusted health gain PROM scores comparing individual units *(from* [146]*)*

1.3.2 Oxford Scores

The Oxford hip (OHS) and Oxford knee (OKS) scores are separate 12-item joint-specific questionnaires completed by patients (Appendix A and Appendix B). Used before and after THR and TKR, these short questionnaires enable the outcome of surgery to be evaluated by the patient, with regard to their hip or knee symptoms and the impact of these on activities of daily living.

First published in 1996 and 1998 for the OHS[147] and OKS[148] respectively, the questionnaires were developed through patient focus groups, to create a measure assessing symptoms and function important to patients. The questionnaires assigned a score from 1-5 for each item, with a higher score indicating the patient having more difficulty with tasks, or greater severity of their symptoms. The minimum score was 12 and the maximum 60. The score became widely used, but over time the use of the score for assessment of outcomes other than that which was originally intended, along with modifications to the scoring made by other authors, has caused confusion. Therefore, to address this, in 2007 the original authors published amendments to the scores.[149] These amendments included describing how to treat missing data, addressing statistical issues in analysing data, and reporting a new scoring method. The present versions now evaluate each of the 12 items with a score from 0-4, with 0 representing significant symptoms or an inability to perform activity, and 4 representing normal function. Consequently, the total minimum score is now 0 (very poor function) and the maximum 48 representing the best outcome and the following outcome categories have been suggested based on the score: >41 excellent, 34–41 good, 27–33 fair, and <27 as poor.[149] These outcomes were based on the Harris Hip score, to which the OHS has good correlation.[150] As well as determining post-operative outcome, PROM scores may predict future events and low scores have been suggested as a predictor of the requirement for early revision.[150]

The minimal important difference for both the OHS and OKS is 5 points when comparing groups,[151] and whilst it has been reported that there are low levels of floor effect for the scores, there may be limitations by post-operative ceiling effects.[152,153] However, as the aim of joint arthroplasty is to return the patient to normal function, then high scores, comparable to a 'normal joint', would be expected following successful surgery.

1.3.3 EQ-5D-3L measure

Developed by the EuroQol group in 1990, the EQ-5D-3L is a generic quality of life measure of health status (Appendix C). The score comprises two parts; a descriptive system which assesses five domains of health and wellbeing (5D), with three levels of response per question (3L), and a Visual Analogue Scale (VAS).[154]

The five dimensions assessed are mobility, self-care, usual activities, pain/discomfort, anxiety/depression, and patients are asked to score each dimension with one of the three response levels - 'no problems', 'some problems', or 'extreme problems'. The VAS element asks patients to assess their *"health state today"* on a scale from 0-100.

The score (1-3) for each of the five dimensions is combined to give a five-digit number which indicates an overall health state, e.g. 11111 indicates 'no problems' in all domains whereas '33333' indicates 'extreme problems' in all domains. This is not an arithmetic score, so instead, group scores can be presented simply by reporting the frequency of each level of response for each domain. Similarly, the average VAS score with a standard deviation or interquartile range, as appropriate, can be reported. The final way of reporting EQ-5D data is by using a formula, with value sets provided for different countries, to convert the overall health state into an index score which weights each of the domain responses. This is presented as the EQ-5D index score.[155] A perfect state of health is 1, whereas 0 represents death. Negative scores are possible and suggest the patients' health status is worse than death.[156,157] EQ-5D scores can be used for health economic evaluation.

Published minimal important differences for the EQ-5D-3L index score vary,[158,159] but a mean difference of 0.074 has been reported.[160] Problems with using the EQ-5D have been identified, including the finding of a bimodal rather than normal distribution of scores thought to be due to the formula used to create the index, rather than any true population differences, as well as ceiling effects.[156,161,162] To negate the ceiling effects and increase the sensitivity, the more responsive -5L version of the score has since been developed.[163] Nevertheless, the -3L version of the score is widely accepted in clinical practice and is utilised as part of the PROMs programme.

1.3.4 Using PROMs to change practice

There are a number of examples where PROM scores have been used as the incentive to drive changes in clinical practice, at both service provider level and through Clinical Commissioning Groups. For example, both Barnsley Hospitals and Derby Hospitals NHS Trusts were below the 95% limit for THR/TKR PROM scores, and used this as the stimulus to change their peri-operative pathways, enhancing physiotherapy and rehabilitation services, and reducing overall length of stay. These strategies lead to an improvement in PROM scores, such that both trusts were subsequently no longer deemed negative outliers.[164]

PROM scores and National Joint Registry data were used to justify a change in implant choice for TKR at one trust. This unit-wide change in implant led to a significant improvement in OKS from 14 to 16.7 points ($p < 0.001$). Furthermore, by then altering surgical technique to retain the infra-patellar fat pad during surgery, there was a further increase in score to 17.3 points (95% CI 16.4 – 18.2, $p = 0.208$).[165]

1.4: Vitamin D and outcomes following arthroplasty surgery

Vitamin D insufficiency is common in patients undergoing orthopaedic surgery, with varying reports suggesting up to 84% of patients may be affected, depending on the age of the patient, season, location or the definition for deficiency used.[105,166–168] Deficiency appears to be particularly common in females undergoing THR, despite no correlation to bone mineral density or a diagnosis of osteoporosis in that group.[169] Insufficient levels have been suggested as a factor adversely affecting outcome following total joint replacement, including poorer PROM scores, longer length of hospital stay and increased infection rates.[170,171]

1.4.1 Vitamin D and outcome scores following TKR

The first report to link vitamin D and outcome following TKR was in 2008.[172] Stimulated by increasing evidence of the extra-skeletal effects of vitamin D, as well as the prevalence of vitamin D deficient patients with osteoarthritis, the authors from Bristol, UK undertook a prospective observational study involving 92 adults aged 65 years and older, to investigate firstly the incidence of vitamin D deficiency in those undergoing TKR, and secondly to see if there was a link to post-operative outcomes.

They observed 36% of patients had insufficient vitamin D levels (<50nmol/L), and that deficiency (<30nmol/L) was associated with statistically significantly worse stiffness and overall Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores post-operatively, when compared to insufficient or replete patients.

However, the authors selected patients for this study randomly from a list of those awaiting TKR surgery. They then invited patients to participate in the trial, so there may be selection or participation bias associated with this method, rather than if they had approached all consecutive patients, for example. The authors also acknowledge that the group size was inadequate to fully establish the link between vitamin D level and post-operative outcomes.

Nevertheless, similar results were reported five years later by Jansen and Haddad in London, UK.[173] In 139 elderly patients (mean age 71.4 years; range 44-88), deficiency was associated with lower outcome scores compared to replete subjects (31.5 vs. 37.1, p = 0.047), as assessed by the Knee Society Score (KSS) which has both patient- and clinician-completed parts, with both parts reported separately. The distinction was not reported by the authors.

Post-operative scores showed improvement compared to baseline, but were still lower in the deficient group, although this didn't reach statistical significance (74.6 vs. 80.4, p = 0.075). Of note is that only 102 of the 139 patients (73%) had completed post-operative scores, so missing data may have affected the result.

The prevalence of insufficiency in this cohort of patients presenting for TKR over a 1-year period was 24%. The reduced prevalence reported may be accounted for by the lower definition used in this study (<40nmol/L) compared to that by Allain and colleagues (2008) (<50nmol/L), rather than due to any difference between the patient populations.

Shin *et al*.[174] also highlight differences in post-operative outcomes in 87 prospective patients undergoing TKR. They found no pre-operative differences between sufficient (n=44) and deficient (n=43, 25OHD <30nmol/L) patients for either the American Knee Society Score (KSS), or alternative step (AST), six-metre walk (SMT), sit to stand (STS) and timed up and go (TUGT) tests. At three months following surgery, they demonstrate a significantly lower KSS functional score, as well as reduced times in the SMT and AST tests in deficient patients ($p < 0.05$). However, there were no significant differences in either the KSS clinical score, or the STS and TUGT tests. All post-operative outcomes that were measured showed an improvement from baseline. They conclude, along with other previous papers, by recommending pre-operative testing of vitamin D and that there may be value in offering pre-operative supplementation.

Maniar *et al*.[175] are the first authors to state a benefit of offering vitamin D supplementation to improve outcome following TKR. In their 2016 review of 120 patients undergoing TKR at a

single unit in India, those with deficiency (<75nmol/L) had poorer WOMAC scores pre-operatively compared to those with normal vitamin D levels. On the 14th post-operative day, all patients (sufficient and deficient) were given supplementation with 0.5μg (20 units) of oral vitamin D, daily for four weeks. Post-operative WOMAC scores were obtained at three months and there was no difference found between the two groups. The authors conclude that "…*TKA should not be delayed in vitamin D deficient patients; rather, supplementation during the post-operative period is preferable to achieve functional outcomes comparable to vitamin D sufficient patients*".[175]

Their conclusions, however, perhaps should be tempered. The level chosen to classify deficiency (<75nmol/L) is far higher than that in the UK (<50nmol/L), so some patients in the study may have been given supplementation unnecessarily. Post-operative and post-supplementation vitamin D levels were not checked, so any improvement in vitamin D level as a consequence of supplementation is not known, and therefore a correlation with outcome cannot be made. There was no control group used, as all patients received supplementation, so one cannot assume the benefit seen was just due to supplementation. The dose of vitamin D given was far smaller than that which is usual in the UK, and only for a short duration. Finally, the work by Jansen and Haddad[173] showed that, without supplementation, post-operative KSS scores were not significantly different between deficient and sufficient patients, despite being so prior to surgery. This observation has also been reported with WOMAC scores.[176] Neither of these studies were referred to by Maniar *et al*., nor were the others described here, which report variable effects of deficiency on post-operative scores.

To date, no reports have been published to assess the effect of vitamin D deficiency using the OKS as an outcome measure.

1.4.2 Vitamin D and pain following TKR

Lee *et al*.[176] have suggested a link between vitamin D deficiency and post-operative pain. The authors found those patients with moderate to severe deficiency (<30nmol/L) had increased pain scores up until 10-hours post TKR surgery, compared to vitamin D replete patients (p = 0.03). This, however, did not affect morphine requirements or the Quality of Recovery questionnaire scores (p > 0.05). At three months, 13.8% of those patients with pre-operative deficiency were more likely to report ongoing moderate-to-severe pain compared to those with sufficient levels (5.9%, p = 0.05), and the role of vitamin D in the modulation of anti-inflammatory cytokines was suggested as a possible reason for this. However, the authors do concede that they cannot currently suggest supplementation will improve chronic pain, a finding which has been corroborated by a Cochrane review.[177]

1.4.3 Vitamin D and outcome scores following THR

Nawabi *et al*.[178] were the first to report the association between deficiency and outcome scores in 62 patients undergoing elective THR. Pre-operative Harris hip scores (HHS) were significantly poorer in those with deficiency (<40nmol/L) compared to those with normal vitamin D levels (32 vs. 42, p = 0.018). Scores improved in both groups following surgery, and whilst they were still lower in the deficient group, the difference between groups was no longer statistically significant (85 vs. 89, p = 0.067).

Lavernia *et al*.[179] confirmed the finding that pre-operative HHS was lower in deficient patients, but also demonstrated post-operative scores were significantly lower too. This finding was also true for a second hip score they measured – the Merle d'Aubigné-Postel score. However, their observations were only true when deficiency was defined as <75nmol/L. Significant differences between deficient and insufficient patients were no longer seen when <50nmol/L was used as the cut-off, but this is likely to be attributable to the small sample size in their study (n=60). The authors do, however, acknowledge that obtaining a consensus on what defines vitamin D deficiency, and thus any effects on outcome, would be beneficial to future work.

In contrast, Unnanuntana *et al*.[180] found no significant difference between sufficient (≥80nmol/L), insufficient (<80nmol/L), or deficient patients (<50nmol/L) following THR when assessing a number of functional outcomes on the day of discharge including transfer in/out of bed, sit-to-stand ability, or competence to climb stairs. Ambulation distance was significantly lower in the deficient group (p = 0.027) but when using multivariable analysis to control for confounding variables such as BMI and age which were found to affect ambulation, there was no significant difference (p = 0.386). The conclusions from this 200-patient study should be considered however, in the context that it was a retrospective review of relatively younger patients, and that all measurements and operations occurred in the summer months when vitamin D levels are known to be highest.

The same group subsequently report no differences were found between deficient (this time <75nmol/L) and sufficient patients with respect to WOMAC or SF-36 scores pre- or post-operatively. Furthermore, there were no differences when assessing function through the two minute walk or timed get-up-and-go performance-based tests.[181] However, these tests were performed at six weeks following surgery, and therefore give no indication of the effect on longer recovery, at six months for example when the National PROMs programme measures post-operative function.

1.4.4 Vitamin D and length of stay

Deficiency has been reported as an independent risk factor to increased length of stay following THR/TKR.[182] In a study of 1,083 consecutive patients admitted to a unit in Germany for elective THR/TKR, vitamin D level was inversely correlated with length of stay (r=-0.16, p = 0.008). Those with deficiency (<50nmol/L) had a mean length of stay 4.3 days longer than those with sufficient levels (15.6 vs. 11.3 days, p = 0.014), and deficiency remained an independent risk factor to increased length of stay after adjusting for age, sex, BMI and comorbidities with multivariable analysis (p = 0.002).

Although the correlation value was -0.16 which suggests only a weak association, a difference of 4.3 days in length of stay is certainly clinically significant, but this should be considered in the context of a UK population where a much shorter mean length of post-operative stay is usual; about four days following THR, and three days following TKR, although some units are now performing day case joint replacement surgery.[183,184]

However, the association between deficiency and longer length of stay has been suggested in other clinical settings including critical care,[185,186] cardiac surgery,[187] burns injuries[188] and in elderly care admissions.[189] Further work would therefore be of benefit to corroborate the relationship between vitamin D and length of stay in the UK elective arthroplasty population.

1.4.5 Vitamin D and prosthetic joint infection

Prosthetic joint infection can be a devastating complication following surgery, with significant morbidity for patients, an increase in mortality, and substantial financial costs for trusts.[190–193] Patient risk factors for infection may be multifactorial, and include smoking, obesity, inflammatory diseases such as rheumatoid arthritis and the medications used in their treatment, and the carriage of MRSA.[194]

Vitamin D is known to modulate the immune system, in particular the control of macrophages, lymphocytes and cytokines,[195] and it has been suggested that deficiency may influence prosthetic joint infection. Maier *et al.*[196] evaluated three groups of patients; those presenting for primary arthroplasty (n=109), those presenting for revision surgery due to aseptic loosening (n=31), and those presenting for revision surgery due to infection (n=50). A greater percentage of those patients requiring revision for infection were vitamin D deficient (86%), compared to those for aseptic loosening (52%, $p < 0.001$), or those presenting for primary surgery (64%, $p < 0.001$). Analysis of Variance (ANOVA) testing found no significant association with other potential cofounders such as age, obesity or diabetes ($p > 0.05$).

Whilst the overall number of patients included in the study was small, the results are similar to those more recently reported by Traven *et al*.[197] who found that patients presenting for revision surgery due to periprosthetic joint infection were more likely to be vitamin D deficient compared to those with aseptic loosening (p = 0.016), even when controlling for other nutritional deficiencies such as hypoalbuminaemia (p = 0.034). Furthermore, following revision surgery, deficient patients were likely to have a non-significant longer LOS (5 vs. 3.6 days, p = 0.097), post-operative complication (p < 0.043), post-operative infection (p < 0.001), or return to theatre within 90-days (p < 0.001).

Genetic differences have been found in patients presenting for revision surgery, and both the T-allele and the T/T genotype for the vitamin D receptor (VDR) have been suggested as possible candidates associated with osteolysis due to deep infection requiring revision surgery compared to control subjects (p = 0.007, OR 1.76 95% CI 1.16 – 2.66)[198] and further investigation of the association between vitamin D and periprosthetic infection has been recommended.[199]

1.5 Conclusions

Vitamin D has an important role in skeletal and non-skeletal tissues, yet due to societal and geographical issues deficiency is common and has been linked to several adverse health outcomes. As a patients' vitamin D status can be easily measured, and treatment to correct deficiency with supplementation is cheap, safe, and effective, there is growing interest in whether vitamin D deficiency is a modifiable risk factor to improve health outcomes.

The incidence of osteoarthritis is increasing, as is the consequential demand for joint replacement surgery. Published studies suggest vitamin D status may have an influence on outcomes following hip and knee replacement, although the results of these are mixed, and do not include the current PROM scores used in the NHS following arthroplasty surgery. Furthermore, the role of supplementation to correct deficiency and the effect this has on outcomes in this cohort of patients is not clear. The effect of pre-operative correction may be multi-factorial due to the extra-skeletal effects of vitamin D, for example on the immune system, tissue healing, muscle strength and function, and mood. As these factors all influence post-operative recovery, and vitamin D deficiency has been linked to adverse outcomes in these areas, then it may be that pre-operative correction is of benefit.

1.6 Aims

1. To determine the trends in testing for, and the reported levels of, vitamin D in the local population.
2. To determine if vitamin D level measured on the day of surgery is linked to post-operative outcomes following total hip and knee replacement, using national PROM scores.
3. To perform a systematic review to determine if supplementation with vitamin D has an influence on post-operative outcomes following total hip and knee replacement.
4. To conduct a feasibility randomised controlled trial investigating the influence of vitamin D supplementation on outcomes following total hip and knee replacement.
5. Consider how the results of the feasibility study will influence the design of a large scale multi-centre trial in the NHS.

Chapter 2: Temporal changes in local population vitamin D levels and testing patterns, using data from a large NHS Foundation trust

2.1 Introduction

Cutaneous synthesis usually accounts for the majority of circulating serum vitamin D, and a number of factors can influence this, including ethnicity, age, season and geography, with sun-exposure often the greatest contributor.[2] Hyppönen and Power measured vitamin D levels in nearly 7,500 45 year olds, who were part of the 1958 British birth cohort study,[200] and demonstrated clear seasonal variation, with higher vitamin D levels at the end of summer, and lower levels in winter (figure 2.1).

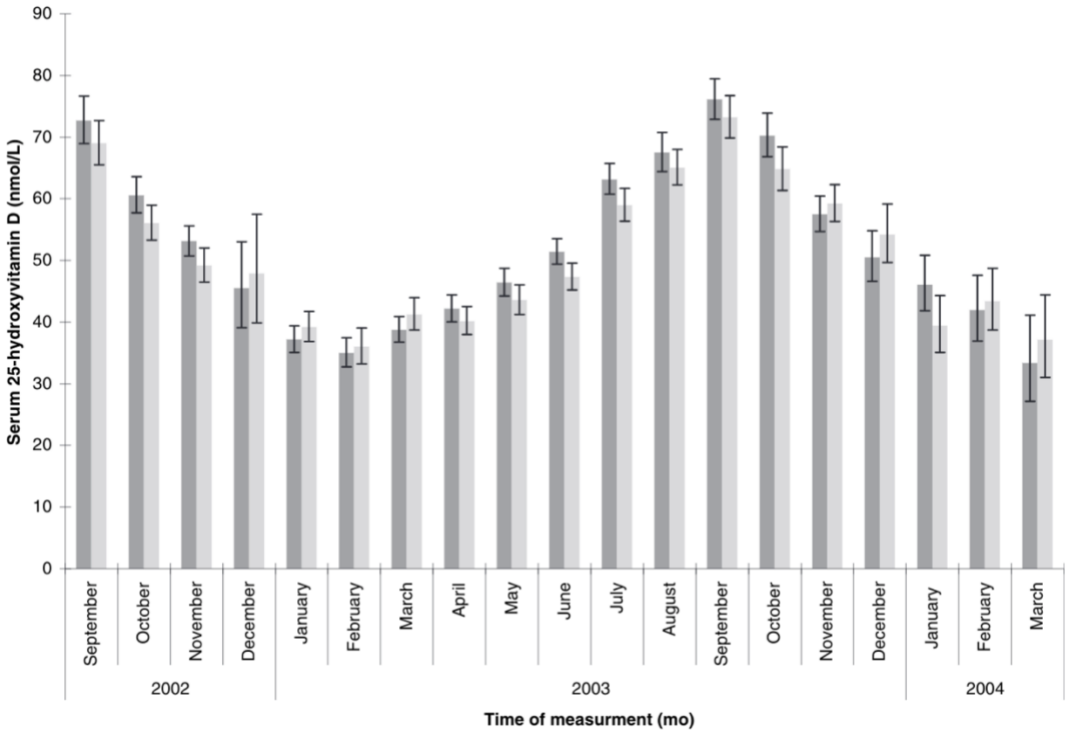


Figure 2.1: Monthly variation in serum 25-hydroxyvitamin D concentrations by gender *(from figure 1 in [200]). Dark bar = male; Light bar = female*

The same authors showed the effect of season and latitude on the prevalence of vitamin D deficiency in the UK, defined as <40nmol/L, with a noted difference between the south of England and the north of Scotland (figure 2.2). In the UK as a whole, UV exposure is insufficient to synthesise vitamin D between October and April,[12] and therefore vitamin D levels vary with deficiency reported in between one-quarter and one-third of patients, depending on the location and season of measurement.[200–202]

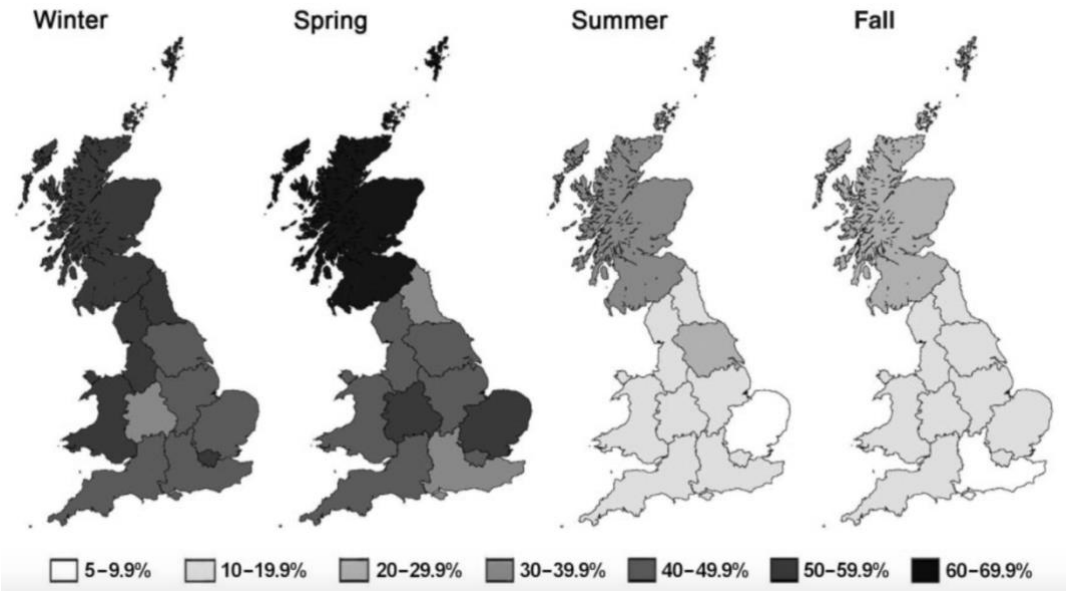


Figure 2.2: Seasonal and geographical variation in the prevalence of hypovitaminosis D (<40nmol/L) *(from figure 3 in [200])*

Internationally, vitamin D synthesis throughout the whole year is only achievable at latitudes below 37 degrees, and is impossible during the winter months at higher latitudes and therefore lower vitamin D levels are seen further away from the equator.[81,203] Reported vitamin D levels for equatorial countries is limited (figure 2.3), although one study has identified traditional-living populations in East Africa having a higher mean vitamin D level of 115nmol/L than that seen in the UK.[85] Sunlight is the most important determinant on vitamin D status,[86] and so to assess the influence of vitamin D on post-operative outcomes, data from

patients in these areas could be compared to that from the UK. Australia has a sunnier climate in comparison to the UK, and has similar healthcare provider models, and monitoring of THR/TKR outcomes through the Australian Joint Registry,[204] and so may be an obvious choice to compare with. However, large cohort studies have shown vitamin D deficiency to be common throughout Australia, with an increased prevalence in urban areas, and public health messages regarding sun exposure and skin cancer in particular are thought to contribute to this.[80,205,206] A comparison in post-operative outcomes in relation to vitamin D status between two countries is therefore more nuanced and will be difficult to compare due to the effect of confounders, and so studies should investigate the influence of vitamin D using data obtained at a more local level.

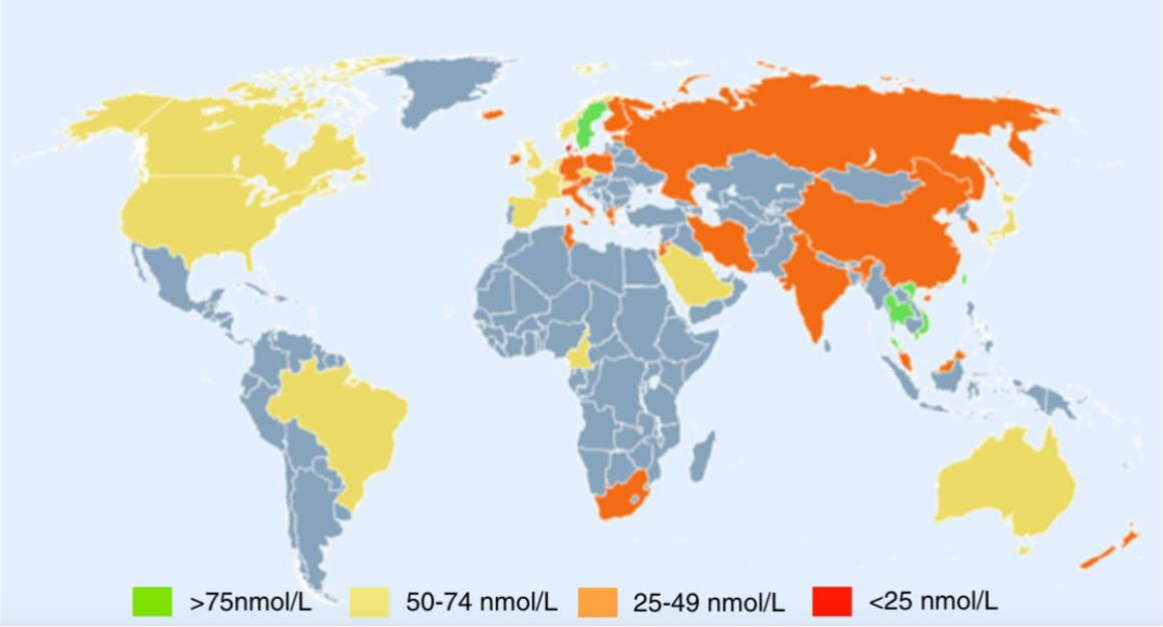


Figure 2.3: Vitamin D levels in adults around the world when available; winter values used to calculate mean 25(OH)D levels *(from figure 2 in [203]) Unshaded (blue) countries = insufficient data available*

2.2 Aim

The aim of this chapter is to explore the temporal trends in vitamin D testing in the local population served by Northumbria Healthcare NHS Foundation Trust. Patterns of vitamin D requests, as well as the influence of season and the effect of patient age on measured vitamin D levels will be investigated. This will enable a comparison to UK and international data, to determine if the results of clinical trials investigating the influence of vitamin D using patients recruited from the Northumbria population are generalisable.

2.3 Methods

2.3.1 Hospital approval and data extraction

Caldicott approval was obtained from Northumbria Healthcare NHS Foundation Trust to review the results of all 25-OH vitamin D test samples analysed in the biochemistry department since 2011. Anonymised vitamin D results were retrieved from the Sunquest Integrated Clinical Environment (ICE) system (Sunquest Information Systems (Europe) Ltd., Norwich, United Kingdom), along with patient age and the date the sample was obtained.

Retrieved data were stored on the trust computer network, and processed using Microsoft Excel (Microsoft Inc., Redmond, WA, USA). Statistical analysis was performed using IBM SPSS Statistics for Macintosh (IBM, Armonk, NY, USA).

2.3.2 Analysis of vitamin D data

The annual number of vitamin D tests recorded in the trust was reviewed to determine the pattern of testing since 2011. Average yearly vitamin D levels were calculated to establish if these have changed over time, as well as the distribution of test results across three categories of vitamin D status, defined by local guidelines and the Royal Osteoporosis Society[76] - deficiency (<25nmol/L), insufficiency (25-49nmol/L) and sufficiency ($\geq$50nmol/L). To investigate the known influence of season on measured levels, average levels per month were reviewed, as well as the relative proportion of results in each vitamin D status category. Finally, the influence of age on measured levels was evaluated.

Linear regression was used to determine the relationship between vitamin D level as the dependent variable and year as the independent variable. Data were tested for normality using the Shapiro-Wilk test, and data not following the normal distribution was reported with median and interquartile range, and displayed using box and whisker plots. Statistical

comparisons of two groups used the Mann Whitney U test, whilst comparison of more than two groups used the Kruskal-Wallis test for non-parametric data. Statistical significance was defined as $p = 0.05$.

2.4 Results

Northumbria Healthcare Trust is situated in the North East of England at a latitude of 55° north. Between 2011 and 2016, 69,414 25-OH vitamin D tests were analysed in the trust biochemistry department. Samples were received from a spectrum of clinical settings including outpatient clinics, inpatient wards and general practice.

25-OH vitamin D levels were quantified using the DiaSorin LIAISON immunochemiluminesence assay (DiaSorin S.p.A, Saluggia) until August 2012, before switching to the Cobas e-601 total 25-OH vitamin D immunochemiluminesence assay (Roche Diagnostics GmbH, Mannheim) which is still in use today. The reference ranges for both assays were the same.

2.4.1 Number of 25-OH vitamin D test requests per year

From 2011 to 2016 there was a linear increase in vitamin D tests analysed annually ($R^2 = 0.993$) with the number of tests increasing six-fold from 3,291 to 20,927 over the time period (figure 2.4).

Northumbria Healthcare Trust serves a population of just over 500,000, and therefore the vitamin D test rate in 2016 was approximately 4,200 per 100,000 population per year, equivalent to 1 in 24 people being tested. In contrast, prior to 2007 there were fewer than 100 tests in total performed annually (personal communication, Northumbria clinical biochemistry department).

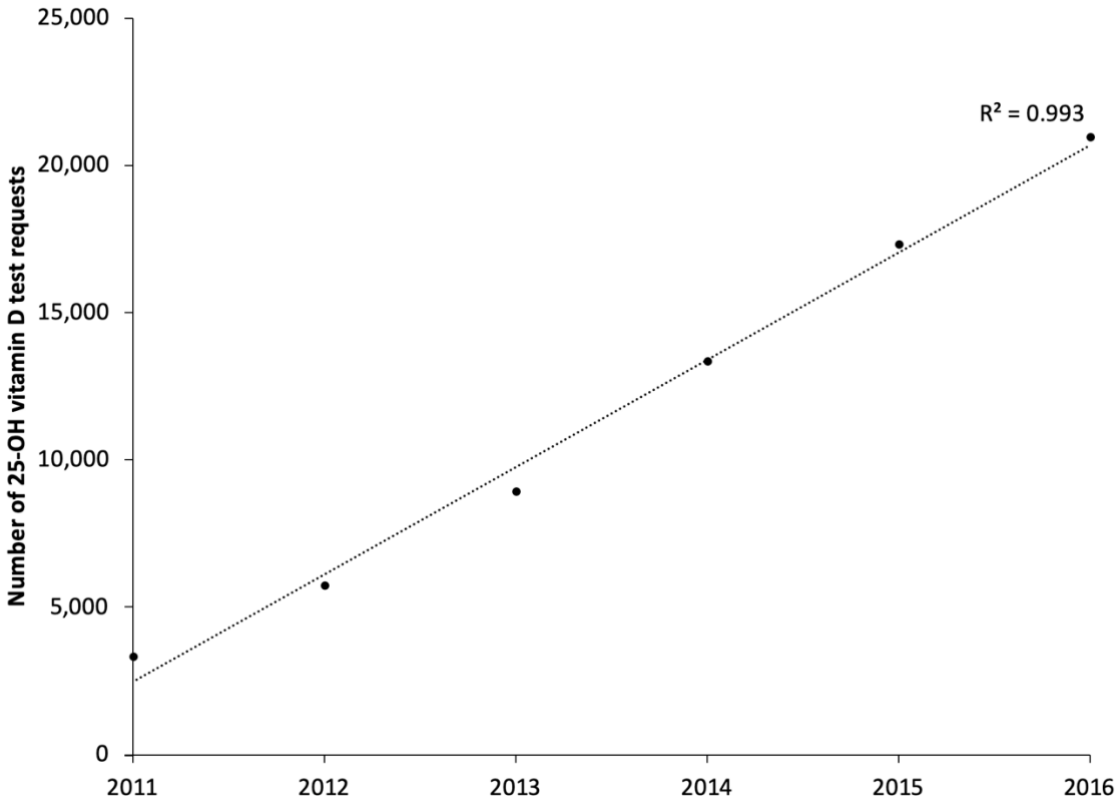


Figure 2.4: Total number of 25-OH vitamin D tests analysed per year at Northumbria Healthcare NHS Foundation Trust between 2011 and 2016.

2.4.2 Average yearly vitamin D levels

The average vitamin D level per month was calculated and plotted against year (figure 2.5). As vitamin D levels were not normally distributed then median values were used. A linear regression line was fitted, with year as the independent variable and vitamin D level as the dependent variable. This suggested an upward trend over the time-period ($R^2 = 0.174$, $p < 0.001$), and median yearly vitamin levels increased from 38nmol/L (IQR 42) in 2011 to 49nmol/L (IQR 49) in 2016 ($p < 0.001$).

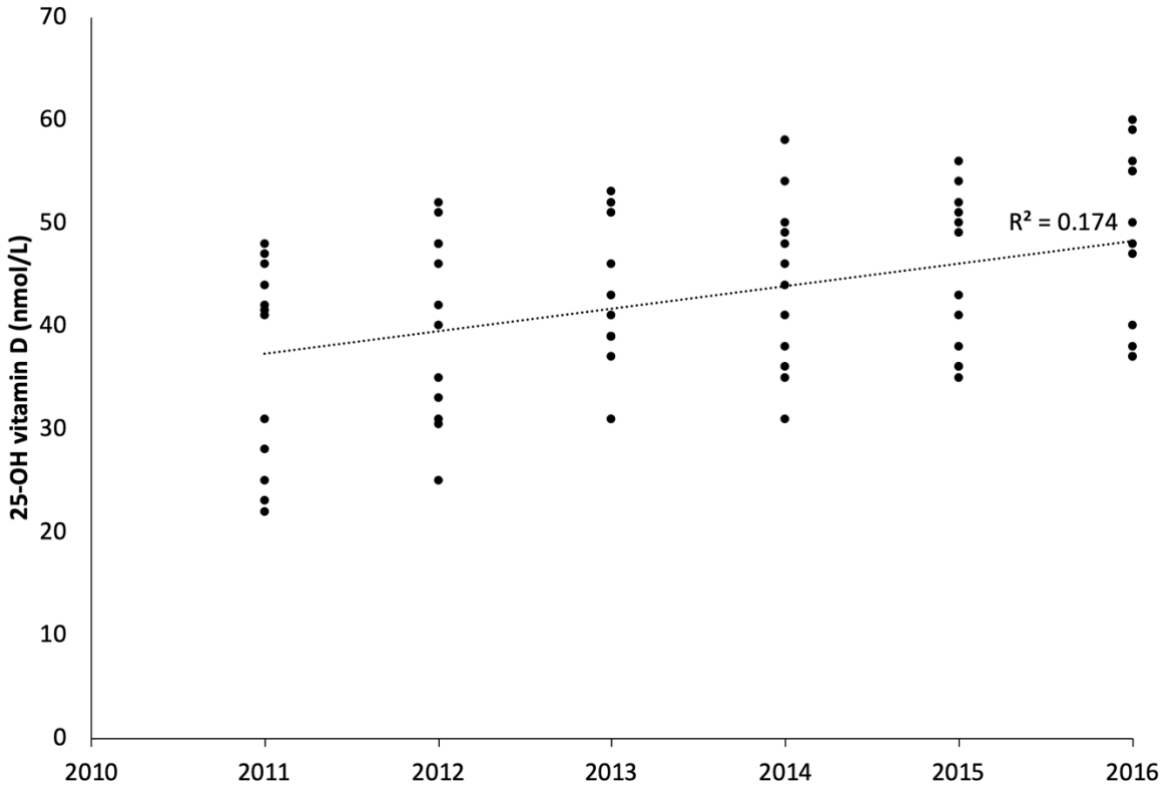


Figure 2.5: Median monthly vitamin D levels each year, with average yearly level (dotted line)

2.4.3 Distribution of test results per year according to vitamin D status

There has been a linear increase in the proportion of tests recorded as 'sufficient' (R^2 = 0.987, *p* = 0.001), along with a corresponding linear decrease in the proportion of tests recorded as 'deficient' (R^2 = 0.949, *p* = <0.001) between 2011 and 2016 (figure 2.6). There was a reduction in the number of tests recorded as 'insufficient', although this was not to the same degree as those who were 'deficient' (R^2 = 0.656, *p* = 0.051).

Figure 2.6: Percentage of samples in each vitamin D status category per year *Red = deficient (<25nmol/L); orange = insufficient (25-49nmol/L); green = sufficient (≥50nmol/L).*

2.4.4 Average vitamin D levels per month for a one-year period

The variation in vitamin D levels per month over a one year period was recorded to determine the influence of season. As the average level has increased in the local population between 2011 and 2016, this last timepoint was used to provide the most contemporaneous data. Vitamin D levels were not normally distributed, and so median and interquartile range values were reported. A box and whisker plot (figure 2.7) was used to show median monthly values, interquartile range, minimum and maximum values, as well as 'outliers', with the upper whisker set at 1.5x the IQR.

Measured vitamin D levels demonstrated clear seasonal variation, with higher levels recorded at the end of the summer, and lower levels at the end of the winter months. Half of the measured results were not above a sufficient level of 50nmol/L for six months of the year. At all timepoints, there were values recorded at both the lowest (7nmol/L) and highest levels (176nmol/L) detectable by the assays.

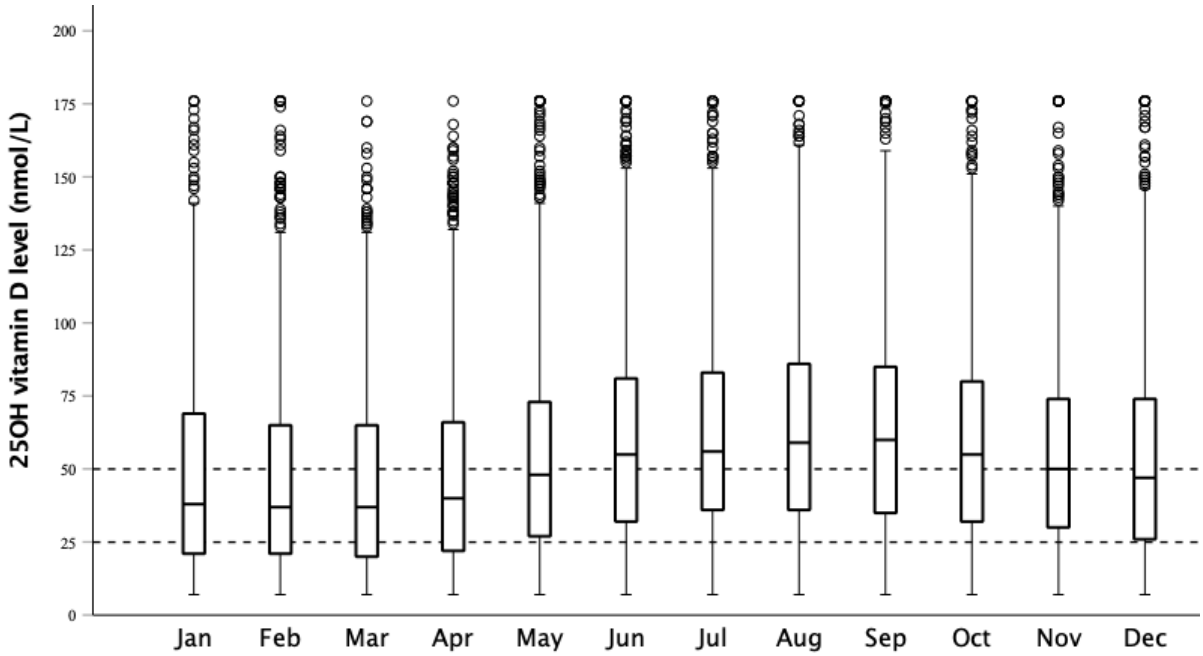


Figure 2.7: Box and whisker plot of 25-OH vitamin D levels per month in 2016 *Reference lines added at 25nmol/L (boundary of deficiency/insufficiency) and at 50nmol/L (boundary of insufficiency/sufficiency). Range of values 7 – 176nmol/L. Upper whisker extends to 1.5x interquartile range, and outliers represented beyond this.*

2.4.5 Distribution of test results per month according to vitamin D status

The percentage of patients comprising the three vitamin D status categories per month is displayed in figure 2.8. The data shown is from 2016 (*n* = 20,927), with highest percentages of deficiency noted in winter (32%), and lowest in the summer (13%). There were corresponding changes in the numbers of patients defined as sufficient (range 38-61%). The number of patients categorised as 'insufficient' remained stable across the year (25 – 30%). This pattern of seasonal variation was observed every year from 2011 and was not unique to 2016.

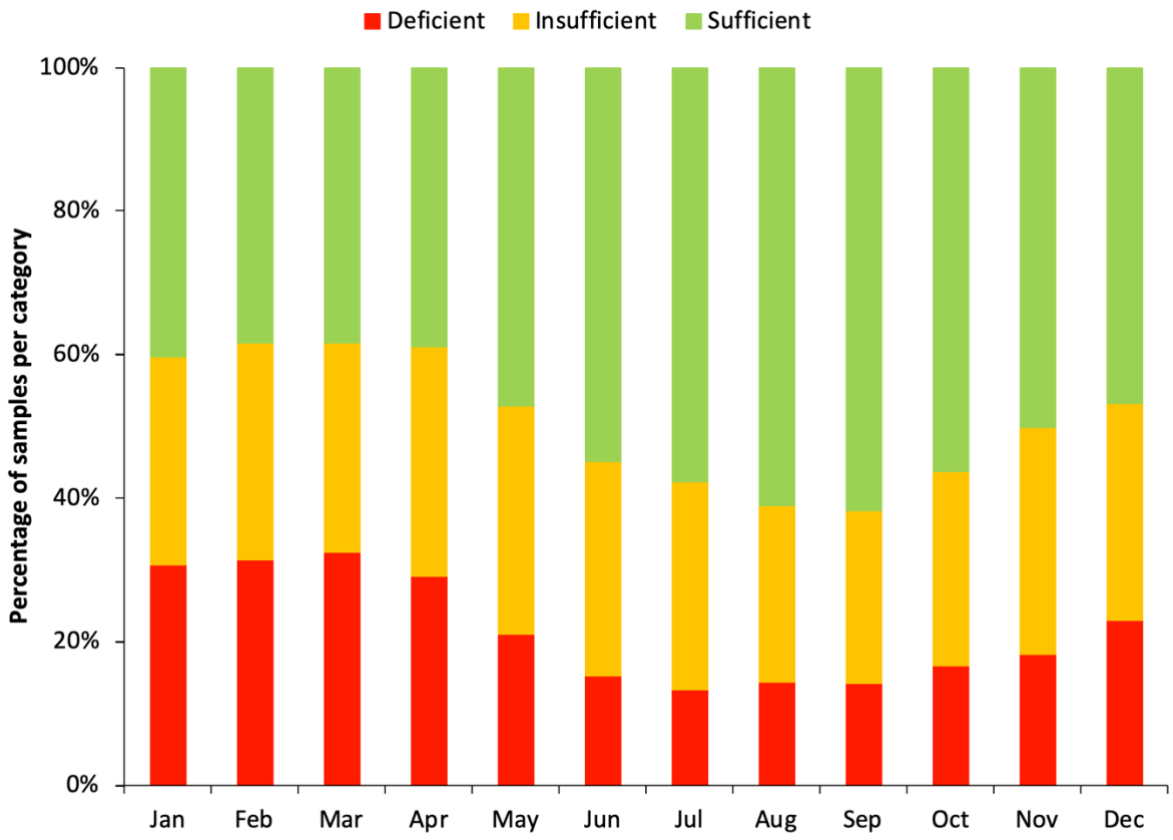


Figure 2.8: Percentage of samples in each vitamin D status category per month in 2016
Deficient (<25nmol/L); insufficient (25-49nmol/L); sufficient (≥50nmol/L)

2.4.6 Distribution of vitamin D results by age

The median age of patient was 60 (IQR 31), ranging from birth to 106 years. Age was not predictive of vitamin D level, with a similar average vitamin D level recorded in each age group (Table 2.1). While there was an increasing percentage of patients older than 75 with deficient vitamin D levels compared to younger patients, this was not statistically significant ($p > 0.05$).

Table 2.1: Breakdown of vitamin D levels by age

Age (years)	% total tests	Average 25OHD nmol/L	% <25nmol/L	% 25-50nmol/L	% >50nmol/L
<30	9.5	44	21.8	35.6	42.6
30-59	38.7	47	20.6	32.5	46.9
60-74	24.5	48	23.3	27.9	48.8
75+	27.1	49	34.7	23.2	42.2

2.5 Discussion

This work has demonstrated that the number of tests analysed locally since 2011 has increased significantly, with a corresponding increase in the average vitamin D level recorded. Seasonal variation exists, with higher vitamin D levels seen in the summer months compared to winter months. There was no influence of age on vitamin D status using the data in this analysis.

2.5.1 Strengths and limitations

The strengths of the results reported in this chapter include all 70,000 unselected vitamin D levels recorded in the trust continuously between 2011 and 2016, thus removing any selection bias. They encompass the whole spectrum of population age from birth to centenarian. Tests were performed at a single laboratory site, thus removing the effect of inter-laboratory variation in measured results. Furthermore, the laboratory is a member of the DEQAS scheme (laboratory reference number 1,723), and has been issued with a proficiency certificate.[60,61]

However, there are some limitations to the data which must be considered. In UK general practice, there has been a substantial increase in the number of all clinical tests requested since 2000, with vitamin D testing showing the second largest increase.[207] This likely reflects the growing interest in vitamin D, particularly its influence on extra-skeletal pathology. However despite increased testing, with an associated increase in annual spending, there has been no corresponding improvement in population health.[208] Clinical details to determine the reason for testing were not available for the data used in this chapter. It may be that these tests were requested because of clinical concern for vitamin D insufficiency, and so the results obtained are lower than what would be predicted in the average population where insufficiency was not suspected. However, Woodford *et al*. have demonstrated a high proportion of vitamin D tests are without a valid clinical reason, including 'tiredness' or 'fatigue' in up to 31%.[209] These perceived expanding indications for testing may therefore account for the increase at Northumbria since 2011.

The pharmacological history of those patients who had their vitamin D level checked was not given, and therefore it is unknown if this data represents those with naturally acquired or supplemented levels. The use of a daily 1,000 IU supplement can raise serum vitamin D levels by 15-25nmol/L over a period of weeks to months.[65] Spending on vitamin D supplementation at Northumbria Healthcare NHS Foundation Trust has increased significantly from 2012 (figure 2.9), with the total cost of testing and treatment with high-dose vitamin D between 2012 and 2016 costing the trust approximately £700,000.[209]

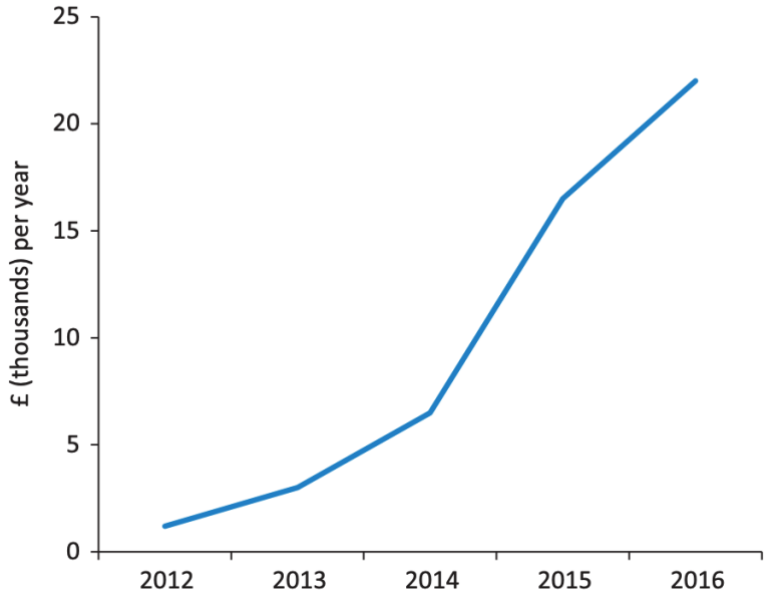


Figure 2.9: Spending on cholecalciferol 20,000 units within the Northumbria Healthcare NHS trust *(from figure 2 in [209])*

As aging reduces vitamin D production in the skin, due to a reduction in the concentration of 7-dehydrocholesterol and a reduced response to UV light, it is generally accepted that vitamin D levels are lower in older age.[210] Given that there was no effect of age on measured vitamin D levels in this dataset, then it could be reasonable to surmise that vitamin D levels in some older patients may have been influenced by supplementation. However, as demonstrated in figure 2.8, 13% of patients in July 2016 had vitamin D levels <25nmol/L, and this increased in winter months. This would suggest that, assuming adherence and adequate absorption, this subset of patients were not taking supplements.

The younger patients in this cohort may have had a valid clinical indication to have their vitamin D levels tested, such as ethnicity or endocrine disorders, and therefore may have had lower vitamin D levels than expected compared to the normal population. As the clinical indication for the test request was not available, this needs to be considered and may have been a reason for the lack of differentiation seen between older and younger patients. A similar finding has been reported by Woodford *et al.*[209]

Average vitamin D levels increased over the time period (figure 2.5), and this trend has also been observed by McKenna *et al*. who reported the increase in vitamin D levels in Ireland between 1993 and 2013,[211] attributing this to the introduction of food fortification and increasing use of supplements. They used the data trends to perform a time series analysis to enable forecasting of future vitamin D levels, and subsequently validated this forecast after three years by demonstrating near perfect agreement to actual recorded values.[212] Similar forecasting could be done to predict future levels in the local population. However, as the time period reported in this work precedes the guideline released by Public Health England in 2016 that all adults should consider taking a supplement in winter months,[213] average population levels may have increased further and so forecasts based on this data may need to be adjusted.

The influence of sunshine on vitamin D levels should also be considered, and the trend in average annual sunshine hours per year in the UK has shown a yearly increase from the 1980s, including over this study period from 2011 to 2016 (figure 2.10). This increase may be a contributing factor to the increase in recorded vitamin D levels seen.

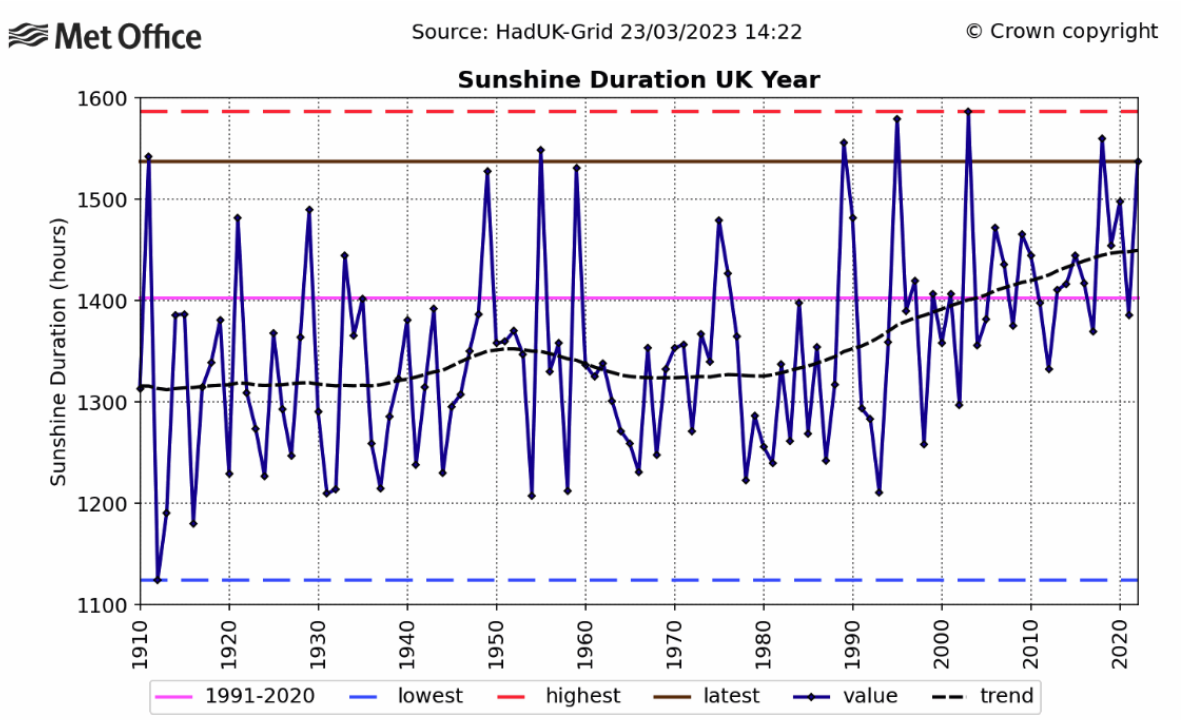


Figure 2.10: Average annual sunshine hours per year in the UK.[214]

Information on BMI was not available, and it is known that this influences vitamin D, with lower levels seen in those with obesity.[215] This would have been useful data to obtain, as The Health Survey for England 2019[216] revealed the North East has the highest prevalence of obesity, and this may be an important confounder to adjust for in recorded vitamin D levels.

Similarly, ethnicity data was not given, and skin tone is known to affect the degree of cutaneous synthesis of vitamin D.[217] Office for National Statistics (ONS) shows the population served by Northumbria Healthcare NHS Foundation trust comprises 99% classed as '*White British*' or '*All Other White'*, in comparison to 85% for the whole of England.[218] This discrepancy may limit the applicability of data derived from the local population if comparing nationally and so should be considered.

It is acknowledged that whilst this data is obtained from a single-site laboratory, the analyser used to measure vitamin D levels changed in August 2012. The detectable change in the upper limit of analysis reduced from 313nmol/L to 175nmol/L. There were 55 patients with vitamin D levels recorded above 175nmol/L during the period when the first analyser was in use, and

so for analysis these values were adjusted to 176nmol/L to reflect the upper limit of the new analyser, with no difference in yearly average values when doing so. The reporting thresholds between analysers were the same, and the pattern of increasing vitamin D levels has continued beyond 2013.

2.6 Conclusion

The patterns in vitamin D testing and the measured levels in the Northumbria population reflect those which are already published. Whilst this is not a novel finding, it is important to validate the data derived from the local population so that future trials investigating the influence of vitamin D, which are run locally and recruit patients from the Northumbria area, can be considered to likely produce results which are generalisable to the wider UK population. The influence on recorded vitamin D levels due to the high proportion of people identifying as '*White British*' or '*All Other White*' compared to the UK average should, however, be accounted for in future studies.

The proportions of patients in each vitamin D status category across the year may be used as a guide to anticipated recruitment of study participants with specific vitamin D levels, and can therefore help plan trial timelines. Likewise, the average vitamin D levels measured in patients participating in a trial can be compared to this local population data, to ensure the data generated through the trial is representative.

Chapter 3: Vitamin D level and post-operative outcomes using data from the UK national PROMs programme

3.1 Introduction

Insufficient vitamin D levels have been linked to a number of adverse outcomes following THR or TKR surgery including longer length of stay, increased need for revision surgery, and lower post-operative PROM scores. This association was discussed in chapter one.

To date there are no studies reporting the relationship between vitamin D level and Oxford hip or knee scores, which are used to assess outcome as part of the NHS PROMs programme. Furthermore, the majority of previously reported studies used immunoassays to measure vitamin D levels, rather than the gold-standard technique of mass spectrometry. Therefore, the primary aim of this chapter is to investigate if pre-operative vitamin D level, measured using mass spectrometry, is related to post-operative outcome according to Oxford score. EQ-5D scores, length of stay and complication data are secondary outcomes.

3.2 Methods

3.2.1 Sample acquisition and storage

This study utilised plasma samples obtained from participants enrolled in a previous clinical trial conducted at Northumbria Healthcare NHS Foundation Trust, with surgery taking place at one of four orthopaedic units; Hexham General Hospital, North Tyneside General Hospital, Northumbria Specialist Emergency Care Hospital, and Wansbeck General Hospital. The samples were stored in the HTA-approved Biobank at Newcastle University. I am grateful to Ramsay Refaie for allowing me to use these samples, which had been collected as part of his PhD study investigating periprosthetic joint infection.[219]

For this previous trial, participants undergoing THR or TKR between 7th January 2014 and 30th June 2016 were invited to provide blood samples pre- and post-operatively, to evaluate the use of CD64 as a marker of joint infection. Written consent was obtained from research nurses, as per Good Clinical Practice guidelines, including for additional blood samples to be obtained and stored anonymously in the university biobank, and utilised in future research studies.

A pre-operative sample of venous blood in an EDTA tube was obtained from patients on the day of surgery. This sample was transferred to Newcastle University, where it was spun for 10 minutes at 1,000g, with excess plasma aliquots stored in the biobank at -80°C. Aliquots were labelled using a unique anonymised study identifier. These stored samples were used for the present work reported in this chapter.

3.2.2 Identification of a laboratory for vitamin D analysis

Attempts were made to set-up a link with laboratories at Newcastle University for analysis of vitamin D using liquid chromatography tandem mass spectrometry (LC-MS/MS). However as none were readily equipped for this, and the time and costs required for set-up and validation for the purpose of this PhD project were not feasible, an external site was sought.

The Bioanalytic Facility at the University of East Anglia (UEA) specialises in LC-MS/MS analysis of vitamin D, has achieved Clinical Pathology Accreditation (CPA), is certified for Good Clinical Laboratory Practice (GCLP), and participates in the DEQAS scheme. As well as publishing widely on vitamin D, the laboratory has developed an analysis technique to measure both 25OH vitamin D and 24,25$(OH)_2$ vitamin D using LC-MS/MS.[34]

The team running the UEA laboratory were contacted about this study, and a consultancy contract was created. A Material Transfer Agreement was completed for the transfer of samples by specialist courier from the Newcastle University Biobank to UEA for the analysis of vitamin D levels by LC-MS/MS. A personal visit was made to the laboratory in April 2018 for educational purposes, to meet with the team to observe their practice, and to learn about their LC-MS/MS methods.

3.2.3 LC-MS/MS analysis of vitamin D

The published technique by the UEA group for the analysis of 25OH vitamin D levels using LC-MS/MS was used by their technicians for obtaining the vitamin D levels reported in this chapter. The following is taken from their published description of their method:[34]

Materials, calibration standards and controls:

SRM972a traceable 25(OH)D_3 and 25(OH)D_2 serum based calibrators (Chromsystems, München, Germany) and internal quality controls (IQC) (UTAK Laboratories, CA, USA) were analysed in each run. Certified pure standards for 24,25(OH)$_2D_3$, 24,25(OH)$_2D_2$ were used for preparation of spiked standards and deuterated standards 25(OH)D_3-[$^2H_6$] and 24,25(OH)$_2D_3$-[$^2H_3$] (IsoSciences, King of Prussia, PA, USA) were used as internal standards. Deionised water, methanol, acetonitrile and formic acid were LCMS grade, n-heptane and isopropanol were analytical grade (Fisher Scientific, Loughborough, UK). 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and methylamine (Sigma-Aldrich, Dorset, UK) were used for derivatization and adduct formation.

Sample preparation procedure for LC–MS/MS:

Sample preparation and extraction were processed using the Extrahera™ automation system (Biotage, Uppsala, Sweden), under positive pressure supplied from a nitrogen generator (Peak, Scotland, UK) at flowrate of 30 L/min. In a 96 position 2 mL deep well plate, 100 µL of calibration standards, IQC materials or serum samples were diluted with 200 µL of pre-treatment solution consisting of deuterated internal standards in isopropanol:water 50:50 (v/v). After mixing, the samples were loaded onto ISOLUTE® supported liquid extraction (SLE+) 400 µL plate (Biotage). Elution was carried out by adding two cycles of 750 µL of n-heptane, both cycle of eluents were collected into corresponding deep well plate. Positive pressure was applied at each stage to remove residual solvent. Samples were then dried under a gentle stream of nitrogen gas heated to 45°C. Derivatization took place by adding 50 µL of 1.1 mmol/L PTAD in acetonitrile, into all wells. The plate was vortexed and allowed to incubate for 30min at room temperature in the dark. 50 µL of water was then added and mixed to stop the reaction. 20 µL of the derivatized extracts was injected into the LC–MS/MS. Using this sample preparation procedure, a batch of 96 samples can be processed in one hour.

Liquid chromatography:

Extracted samples were injected into LC–MS/MS by Waters® 2777 Sample manager (Waters Corp., Milford, MA, USA) equipped with 3-drawer cooler stack regulated at 10°C. Chromatographic separation was achieved using a core-shell C18 50Å~2.1 mm, 2.6 µm, reversed-phase (Restek, Bellefonte, PA, USA) column heated at 55°C. An in-line 2µm, 6.35mmÅ~24mm guard filter was used to protect the column. A gradient elution profile was set up using a binary UPLC pump (Flux Instruments, Switzerland) to deliver mobile phase at flow rate of 0.4mL/min. At the start of the gradient the mobile phase consisted of 50:50 (v/v) of (A) water containing 0.2mM methylamine in 0.1% formic acid and (B) methanol containing 0.2mM methylamine in 0.1% formic acid. The gradient was gradually increased to 99% of methanol mobile phase (B) then returned to starting gradient at 4 min. Solvent divert was employed to divert ion suppression regions of the separation to waste in order to minimize contamination to the source of the mass spectrometer.

Tandem mass spectrophotometry analysis:

LC–MS/MS analysis of vitamin D metabolites was performed using Micromass Quattro Ultima Pt electrospray ionisation (ESI) tandem mass spectrometer (Waters Corp., Milford, MA, USA). MassLynx version 4.1 and QuanLynx software (Waters Corp., Milford, MA, USA) were used for system control, data acquisition, baseline integration and peak quantification. Optimisation of MS/MS parameters was accomplished by direct infusion of derivatized standards. Capillary voltage was set at 3.0 kV and RF lenses 1 and 2 were set at 0.1. Source temperature was maintained at 90°C. Nitrogen was used as both nebuliser gas at flow rate of 30 L/h and as desolvation gas at flow rate of 850 L/h at 120°C. Sample cone voltage and collision energy for all vitamin D metabolites were 35 kV and 25 kV respectively. Argon gas was applied to the collision cell during the Collision Induced Dissociation (CID) process. The precursor to product ion transitions for each of the compounds were ascertained based on the molecular weight of the methylamine adduct of PTAD derived products.

3.2.4 Linkage to demographic data, and PROM scores

The anonymised study identifier enabled each sample to be linked to Microsoft Excel (Microsoft Inc., Redmond, WA, USA) databases held at Northumbria Trust which included baseline demographic data, the operation performed, length of stay, medical complication data and pre- and post-operative Oxford and EQ-5D-3L scores. These databases were collated and processed to allow for analysis relevant for this study. Caldicott Approval (reference RPI-390) was obtained from the trust to use this data.

For primary analysis, vitamin D status for the study was defined according to SACN[12] and local guidelines[220] as sufficient ($\geq$50nmol/L), insufficient (25-49nmol/L) and deficient (<25nmol/L). Accordingly, outcome data for the three groups were compared using one-way ANOVA with Bonferroni correction for normally-distributed data, and Kruskal-Wallis with Bonferroni correction for non-parametric data. Chi-square cross-table testing was used for categorical data, with Fisher's Exact test used where the number of observed events were less than five. Statistical significance was defined as $p = 0.05$. Baseline vitamin D was correlated to Oxford and EQ-5D scores to determine the relationship between the two variables (R^2). The season in which surgery was performed was defined as spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February).

Given the lack of consensus in the definition of vitamin D deficiency, as discussed in chapter one, the data was analysed further by dichotomising into 'deficient' and 'sufficient', with deficiency defined at three different thresholds of <25nmol/L, <50nmol/L and <75nmol/L. The difference in pre-operative, post-operative and change scores for Oxford and EQ-5D questionnaires at each of these thresholds was determined. An independent samples T-test was used to compare groups with normally distributed data, and a Mann-Whitney U test was used for non-parametric data.

3.3 Results

There were 475 plasma samples analysed which were linked to complete outcome data. Patients had their surgery between April 2014 and September 2015 at Northumbria Healthcare NHS Foundation Trust. Measurement of vitamin D levels was performed using mass spectrometry by the UEA laboratory team in April and May 2018.

3.3.1 Vitamin D groups and baseline demographic data

Three groups were established, when defining baseline vitamin D status using SACN and local guidelines – 54% had sufficiency, 33% had insufficiency and 13% had deficiency. Average vitamin D levels varied throughout each season (figure 3.1), with highest levels in the summer and lowest levels in winter and spring, as per the pattern noted in chapter two. The season in which surgery was performed had no influence on pre-, post- or change in Oxford or EQ-5D scores (all $p > 0.05$).

Baseline demographic data for the three groups are recorded in table 3.1. The group with deficiency comprised fewer males ($p = 0.036$) and were more likely to be classed as obese ($p = 0.055$) when compared to those with insufficiency or sufficiency. There was no other difference in baseline demographic or comorbidity data between groups.

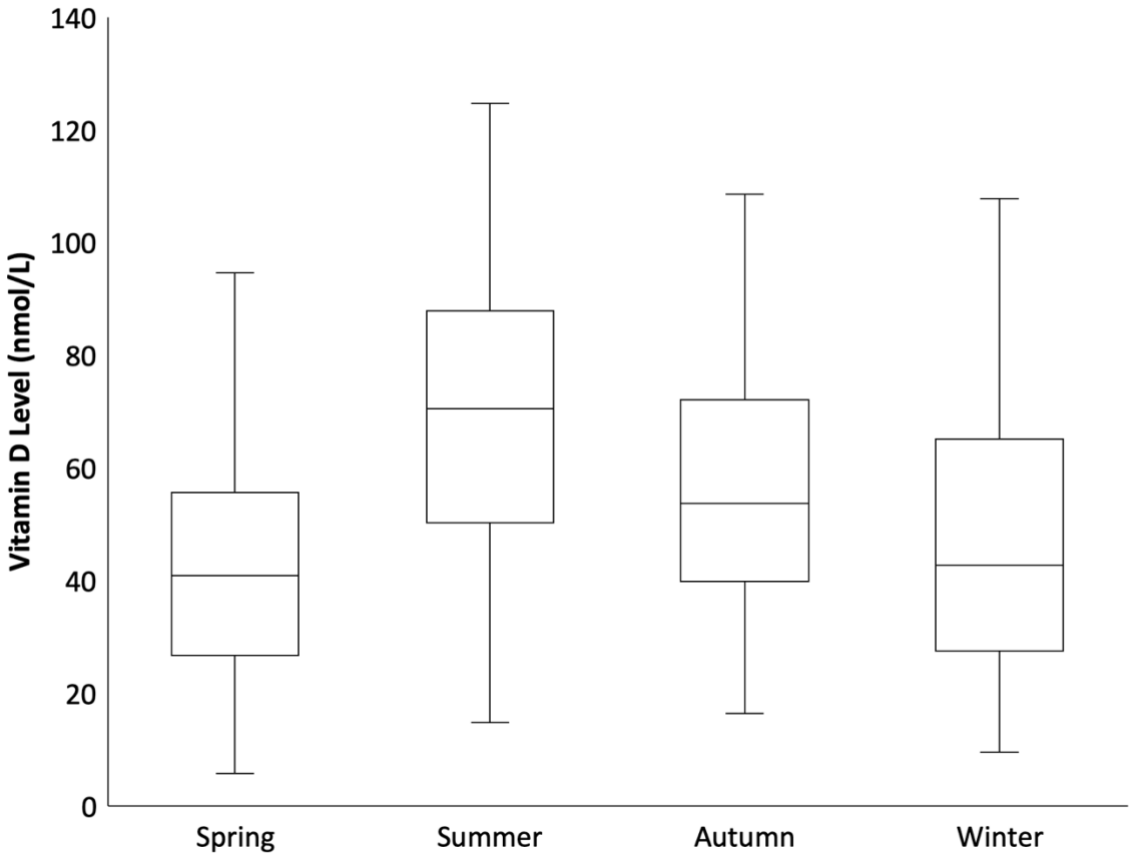


Figure 3.1: Box and whisker plot of average measured vitamin D levels by season

Table 3.1: Baseline demographic and comorbidity data

	Deficient (<25nmol/L)	**Insufficient (25-49nmol/L)**	**Sufficient (≥50nmol/L)**	**p-value**
n (%)	62 (13.1)	156 (32.8)	257 (54.1)	-
Median 25OH vitamin D level (±IQR, nmol/L)	18.9 (8)	37.6 (13)	71.8 (26)	<0.001
Mean age (±SD, years)	70.1 (10.5)	68.6 (9.3)	68.1 (9.0)	0.329
Male (n, %)	22 (35.4)	85 (54.5)	132 (51.4)	0.036*
THR (n, %)	26 (41.9)	69 (44.2)	120 (46.7)	0.757
Obesity (n, %)	33 (53.2)	69 (44.2)	96 (37.4)	0.055
Smoker (n, %)	5 (8.1)	15 (9.6)	16 (6.2)	0.446
Comorbidity:				
Hypertension (n, %)	38 (61.3)	82 (52.6)	130 (50.6)	0.317
Atrial fibrillation (n, %)	4 (6.5)	9 (5.8)	14 (5.4)	0.953
Ischaemic heart disease (n, %)	8 (12.9)	13 (8.3)	33 (12.8)	0.346
Thyroid disease (n, %)	7 (11.3)	12 (7.7)	25 (9.7)	0.429
Diabetes (n, %)	5 (8.1)	15 (9.6)	30 (11.7)	0.639
Peripheral vascular disease (n, %)	2 (3.2)	7 (4.5)	13 (5.1)	0.823
COPD (n, %)	6 (9.7)	5 (3.2)	16 (6.2)	0.152
Rheumatoid arthritis (n, %)	1 (1.6)	2 (1.3)	7 (2.7)	0.588

* *significance = <0.05*

3.3.2 Oxford Scores

Patients with vitamin D deficiency had statistically significant pre-operative scores 3.5 points lower than those with insufficiency and sufficiency (15.5 versus 19). Similarly, post-operative scores were significantly lower by 4.5 points (36.5 versus 41). There was no difference in scores between those with insufficiency and sufficiency at either timepoint. There was no difference in the change scores between all three groups, suggesting all patients benefitted equally from surgery (figure 3.2).

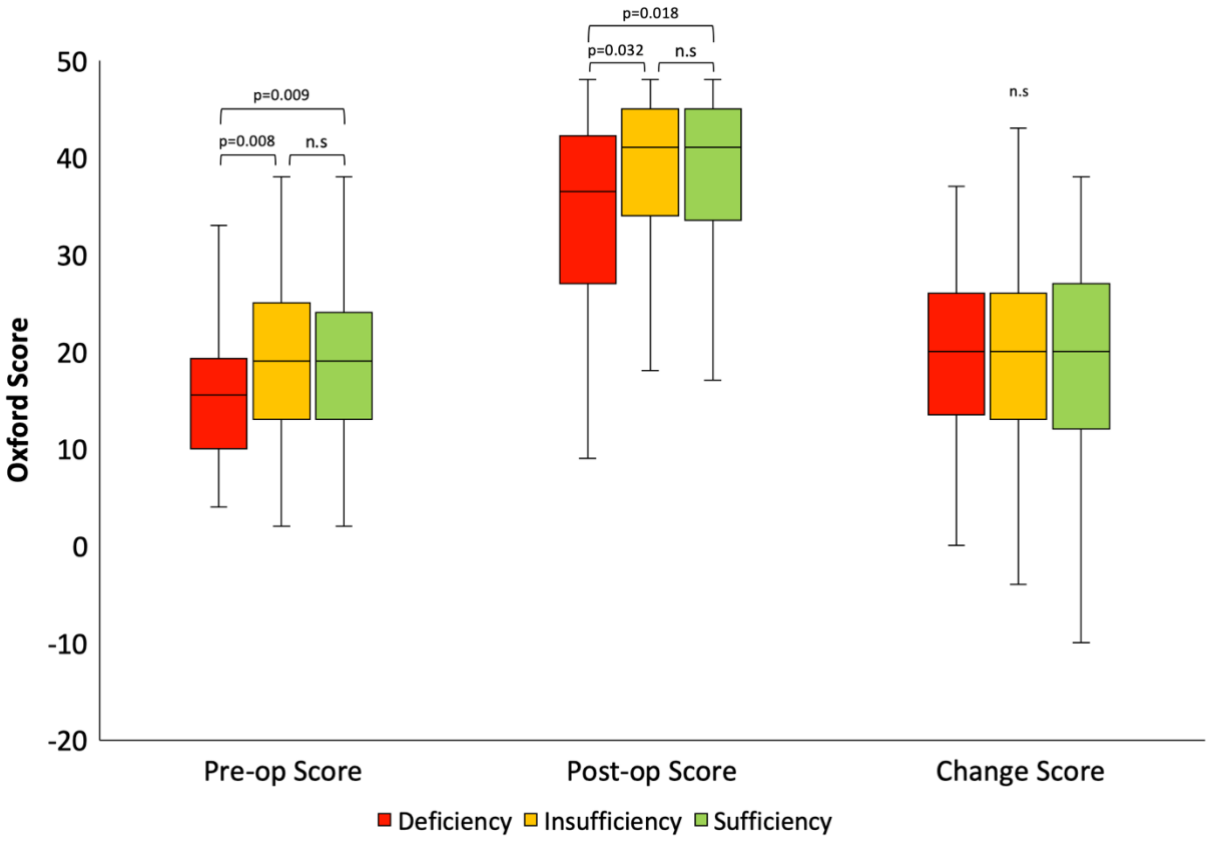


Figure 3.2: Oxford pre-operative, post-operative and change scores *n.s = not significant*

3.3.3 EQ-5D Index Scores

The median pre-operative EQ-5D index score was lower in the deficient group compared to the insufficient and sufficient groups (0.159 versus 0.516), although this was not statistically significant (*p = 0.101*). Post-operative and change scores were similar across all three groups, with no significant difference noted (figure 3.3).

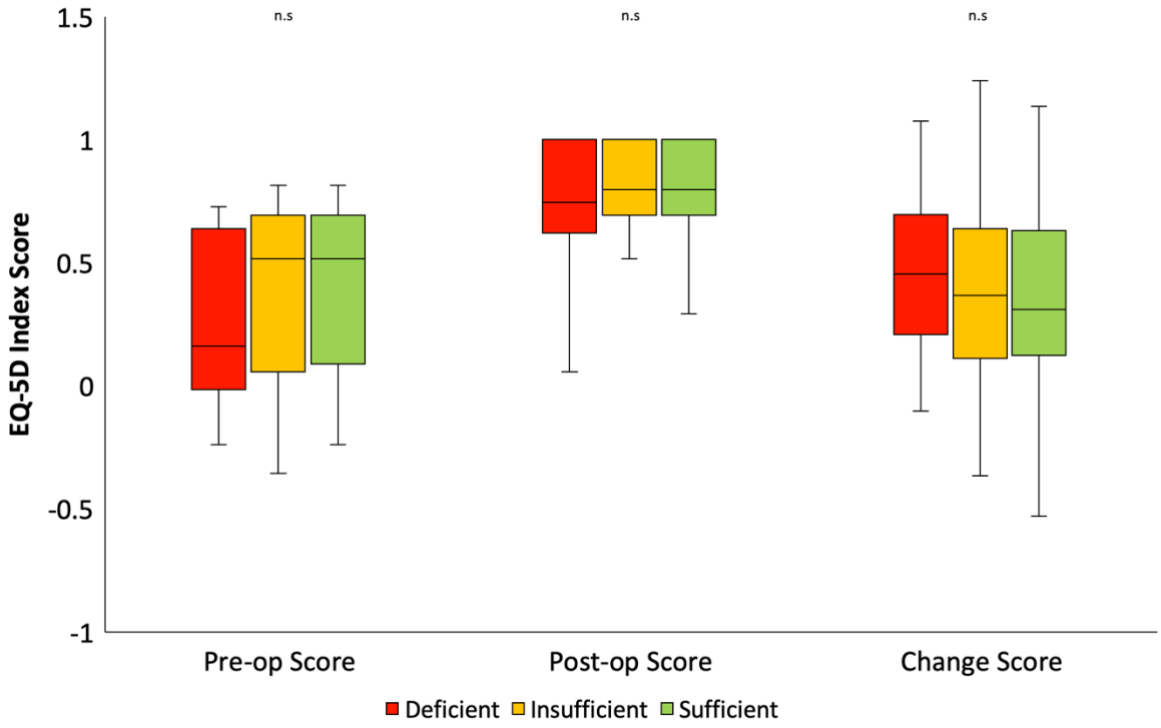


Figure 3.3: EQ-5D Index pre-operative, post-operative and change scores *n.s = not significant*

3.3.4 EQ-5D Visual Analogue Scale Scores

On a scale of 0-100, those with deficiency had significantly lower scores pre-operatively (VAS = 55) compared to those with insufficiency (VAS = 67, *p = 0.047*) and sufficiency (VAS = 70, *p = 0.005*). The same pattern was seen post-operatively in those with deficiency reporting significantly lower VAS scores. There was no difference in the change score between the three groups (figure 3.4).

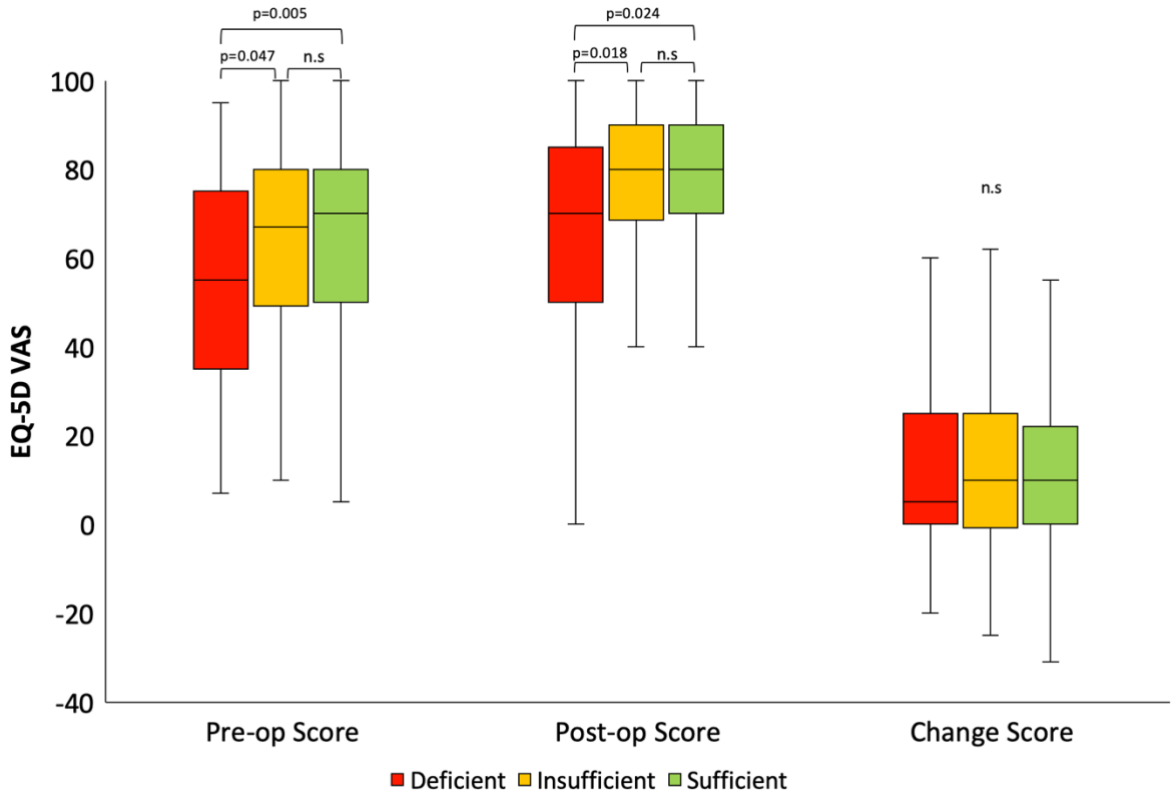


Figure 3.4: EQ-5D Visual Analogue Scale pre-operative, post-operative and change scores *n.s = not significant*

3.3.5 Length of stay

Length of stay data for each group did not follow a normal distribution and so median and interquartile ranges are displayed. The median lengths of stay were three days (total range 1-

11 days; IQR 3) for those with deficiency, two days (total range 1-9 days; IQR 1) for those with insufficiency, and three days (total range 1-74 days; IQR 2) for those with sufficiency. Kruskal-Wallis testing, with Bonferroni correction, indicated statistically significant differences between the three groups (figure 3.5). For the purposes of providing visual clarity in figure 3.5, the outlying patient with a length of stay of 74 days is not displayed, but their data was included in overall analysis.

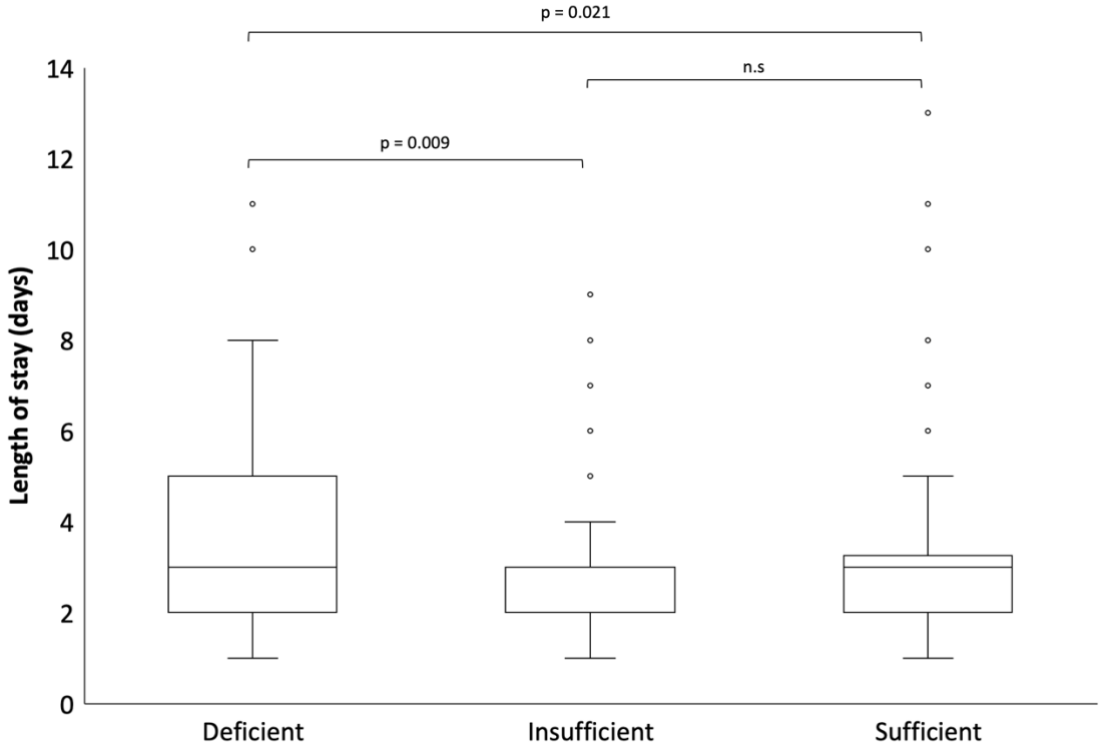


Figure 3.5: Box and whisker plot of median length of stay for each group *The outlying patient in the sufficient group with a length of stay of 74 days is excluded from the figure for clarity purposes. n.s = not significant.*

3.3.6 Medical Complication Data

The overall medical complication rate within 30 days following surgery was low, with no significant difference between groups (table 3.2). Ten patients required re-admission within

30 days of surgery. Three were as day-attendees to the ambulatory care unit (two with leg-swelling and one with oesophageal spasm), one required overnight admission for intravenous fluid for an acute kidney injury, and one required admission for melena secondary to a bleeding gastric ulcer. The remaining five patients were readmitted with oozing wounds, and of these three required a return to theatre for debridement. One patient had required a return to theatre for debridement of an oozing wound during the same admission as their index procedure.

Table 3.2: Post-operative readmissions and medical complications

	Deficient (<25nmol/L)	**Insufficient (25-49nmol/L)**	**Sufficient (≥50nmol/L)**	***p* value**
Readmission (n, %)	2 (3.2)	1 (0.6)	7 (2.7)	0.220
RTT same admission (n, %)	0	0	1 (0.4)	1.000
RTT new admission (n, %)	1 (1.6)	1 (0.6)	1 (0.4)	0.378
DVT (n, %)	0	0	0	n/a
PE (n, %)	0	0	0	n/a
CVA (n, %)	0	0	0	n/a
TIA (n, %)	0	0	0	n/a
AKI (n, %)	3 (4.8)	1 (0.6)	7 (2.7)	0.123
UTI (n, %)	1 (1.6)	2 (1.3)	1 (0.4)	0.450
MI (n, %)	0	0	0	n/a
Pneumonia (n, %)	0	0	2 (0.8)	0.644
Blood transfusion (n, %)	1 (1.6)	0	0	0.131
ICU Admission (n, %)	1 (1.6)	0	2 (0.4)	0.238

RTT = return to theatre; DVT = deep vein thrombosis; PE = pulmonary embolism; CVA = cerebrovascular accident; TIA = transient ischaemic attack; AKI = acute kidney injury; UTI = urinary tract infection; ICU = intensive care unit.

3.3.7 PROM scores when the threshold for defining deficiency is varied

Table 3.3: Effect on PROM scores with differing thresholds for defining deficiency.

	Deficiency = <25nmol/L			**Deficiency = <50nmol/L**			**Deficiency = <75nmol/L**		
	Deficient	*Sufficient*	*p-value*	*Deficient*	*Sufficient*	*p-value*	*Deficient*	*Sufficient*	*p-value*
n (%)	62 (13)	413 (87)	n/a	218 (46)	257 (54)	n/a	366 (77)	109 (23)	n/a
Oxford pre-op	15.5	19	0.001***	18	19	0.241	18	19	0.122
Oxford post-op	36.5	41	0.005**	40	41	0.211	40	42	0.006**
Oxford change	20	20	0.787	20	20	0.882	20	22	0.229
EQ-5D Index pre-op	0.159	0.516	0.034*	0.516	0.516	0.247	0.516	0.516	0.749
EQ-5D Index post-op	0.7435	0.796	0.086	0.796	0.796	0.362	0.796	0.796	0.185
EQ-5D Index change	0.4525	0.327	0.183	0.383	0.309	0.330	0.347	0.413	0.481
EQ-5D Scale pre-op	55	70	0.002**	65	70	0.042*	70	70	0.269
EQ-5D Scale post-op	70	80	0.004**	80	80	0.390	80	80	0.557
EQ-5D Scale change	5	10	0.898	10	10	0.575	10	10	0.893

=≤0.05; **=≤0.01; *=≤0.001*

Due to the lack of consensus on what defines a sufficient vitamin D level, different thresholds were chosen, according to those commonly cited. Significant differences in baseline and post-operative Oxford scores were noted when the definition of deficiency was 25nmol/L, but not at 50nmol/L. There was no difference in pre-operative scores when deficiency was defined as <75nmol/L, however there was a statistical difference in post-operative scores, although this was not an important difference in score response (40 versus 42, *p = 0.006*). The score change was equal across groups, suggesting all patients have the same perceived improvement following surgery. Similar patterns were seen for EQ-5D Index and VAS scores (table 3.3).

Baseline vitamin D level had very poor correlation to pre-operative, post-operative or change in PROM scores for both Oxford and EQ-5D measures ($R^2 < 0.01$).

3.4 Discussion

This is the first reported study to investigate the relationship between vitamin D level and the Oxford hip and knee scores following arthroplasty surgery. It is also the largest dataset reporting on the relationship between vitamin D and a patient reported outcome measure following THR or TKR, and has used the preferred technique of LC-MS/MS rather than immunoassay for the measurement of vitamin D levels. Vitamin D deficiency (<25nmol/L) was associated with poorer pre- and post-operative Oxford and EQ-5D-3L VAS scores, but no difference was noted when higher thresholds for defining deficiency were used.

Corroborating this, a significant difference in either pre- or post-operative outcome score in patients with deficiency has also been reported in seven studies using alternative outcome measures, and a summary of these is provided in table 3.4. Four used an outcome measure requiring completion by a health care professional (HHS, KSS)[174,178,179] whereas the other three utilised a patient-reported outcome measure (WOMAC).[172,175,221] However, there is no uniformity in the reported outcomes across the studies; some demonstrate differences in pre-operative but not post-operative scores, whilst others using the same outcome measure report differences in post-operative but not pre-operative scores, and some were only able to demonstrate a difference in a sub-set of a score. Furthermore, the definition of vitamin D deficiency between studies is inconsistent, (<30 to <75nmol/L), as too is the reported percentage of patients defined as deficient (14 to 65%), even where the same vitamin D level was used to define deficiency.

In contrast, other authors have reported no difference exists between patients with deficiency and sufficiency when evaluating outcome using WOMAC,[176,181,222,223] KSS,[223] EQ-5D,[176] VAS,[223] or SF36[181] scores.

The work presented in this chapter utilised both a joint specific measure and a general health measure, as per the PROMs programme. Disease-specific measures are more responsive than

Table 3.4: Summary of studies reporting a significant association between vitamin D deficiency and an outcome score.

Author	Joint	n	Definition and % deficiency	Outcome Score Used	Pre-operative finding	Post-operative finding
Lavernia *et al*.[179]	Hip	60	<75nmol/L (65%)	HHS	Worse (43 vs. 52; $p = 0.035$)	Worse (83 vs. 92; $p = 0.002$)
Nawabi *et al*.[178]	Hip	62	<40nmol/L (24%)	HHS	Worse (32 vs. 42; $p = 0.018$)	No difference (85 vs. 89; $p = 0.067$)
Shin *et al*.[174]	Knee	87	<30nmol/L (49%)	KSS* (functional component)	No difference (54 vs. 55; p = ns)	Worse (68 vs. 74; $p = 0.045$)
Allain *et al*.[172]	Knee	92	<30nmol/L (14%)	WOMAC* (stiffness component change)	N/A	Worse change score (0 vs. -1.6; $p = 0.03$)
Maniar *et al*.[175]	Knee	120	<75nmol/L (54%)	WOMAC	Worse (48 vs. 42; $p = 0.04$)	No difference (18 vs. 16; $p = 0.362$)
Jansen *et al*.[221]	Knee	138	<40nmol/L (24%)	WOMAC	No difference (77 vs. 75; $p = 0.73$)	Worse adjusted outcome (+5 points; $p = 0.028$)
Jansen & Haddad[173]	Knee	139	<40nmol/L (24%)	KSS	Worse (32 vs. 37; $p = 0.047$)	No difference (75 vs. 80; $p = 0.075$)

*HHS = Harris Hip Score; KSS = Knee Society Score; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; ns = not significant. * = only part of the score showed a significant difference.*

generic questionnaires in detecting clinically significant changes,[224] although one study has shown moderate correlation between the EQ-5D-3L and the Oxford hip and knee scores.[225] The EQ-5D has a short completion time and is responsive to changes following THR/TKR surgery.[158] The EQ-5D score has been shown to relate to vitamin D status,[226,227] and this was apparent in the present study, in particular the VAS component of the score.

The EQ-5D VAS question asks patients *"how good or bad your health is TODAY"* and therefore responses may reflect a patient's general health and wellbeing, rather than solely in relation to their joint symptoms and function. Given that vitamin D is reported to influence a wide range of health measures, including mood, then a generic score may be sensitive to the influence of sufficiency status on self-reported outcomes.

The population demographics in this study were representative of patients undergoing THR/TKR in the UK,[228] and the distribution in each vitamin D status category was as expected for the local population, when comparing data from chapter two. Except for a greater percentage of female sex in the deficient group, baseline characteristics were equal. The similar change in score following surgery suggests all patients benefitted equally from operative intervention, and so other factors may account for the variability in reported scores at baseline and post-operatively. It should be noted that the EQ-5D index score is calculated based on UK population data norms, and so the absolute results for this study may not be comparable if the study was to be repeated in other countries.

Significant differences in length of stay were noted between groups, being longest in those with deficiency. Similarly, a longer length of stay in those with deficiency was reported by Maier *et al.*[182] (15.6 versus 11.3 days, $p = 0.014$), and remained significant when baseline variables were accounted for in multivariate analysis. However, their definition of deficiency was <50nmol/L, and the length of stay even in those with sufficiency was significantly longer than that reported in this work. There are likely to be institutional and behavioural differences in practice affecting length of stay, for example the use of a fast-track protocol, and whilst it

is interesting to observe the same reported pattern with length of stay and vitamin D status, the results are not directly comparable.

3.4.1 Strengths and limitations

The strengths of this work are that with the inclusion of 475 patients, it is the largest study investigating vitamin D and its influence on a patient-reported outcome measure following THR/TKR. Vitamin D levels were measured in one laboratory by LC-MS/MS, which is considered to be the gold-standard method, and therefore avoided the limitations of using immunoassays which have been highlighted in chapter one. Finally, the study involved patients who underwent surgery in a single unit, which utilises streamlined protocols and processes, and therefore minimises potential sources of bias, such as implant choice or rehabilitation technique, which were not accounted for in the analysis.

However, the following limitations in this study should be considered. The baseline demographic database used for this study did not include if patients were taking vitamin D supplementation at the time of their operation. Therefore there is no way to determine if those with sufficiency had been supplemented or achieved this through natural means, and whether this influences the reported outcome. Ethnicity data was not available for the cohort which may have influenced the measured vitamin D levels. If the measured vitamin D levels were predominantly from patients of Black or Asian ethnicity, then the recorded vitamin D levels may be lower than average levels seen in White British patients. Furthermore, information on BMI was not available, and as was discussed in Chapter 2, it is known that lower vitamin D levels are seen in those with obesity, and that the North East has the highest prevalence of obesity in the UK.

Only one timepoint of vitamin D measurement was used, and so individual variability in measured levels to due season, diet or presence of inflammation cannot be accounted for.

Use of repeated measurement or calculation of an annualised average should be considered in further studies.

The plasma samples utilised in this study had been stored in the biobank freezer at -80°C, and therefore the levels of vitamin D may have deteriorated due to the delay between collection and analysis. However, vitamin D is not reported to be affected by storage,[42–46] and has been described *"as solid as a rock".*[47] The original LC-MS/MS technique described by the UEA group used serum for analysis, whereas plasma samples were utilised in this study. However, both plasma and serum can be used for analysis of vitamin D levels, and there has been no reported difference in measured levels when directly comparing the two.[42,46]

Although some of the reported results, both in this study and in the wider literature, were deemed to be statistically significant, this does not mean they are clinically important. Furthermore, due to the observational nature of this work, any correlation between vitamin D deficiency and outcomes does not prove causation, nor the direction of any association, as unknown variables which may have an influence cannot be accounted for. Higher baseline vitamin D levels tend to be associated with individuals with a higher baseline health status, who may be able to go out in the sun, exercise and consume a healthier diet, and may therefore report higher health-related outcome scores than somebody with a lower baseline health status.[12]

3.5 Conclusion

This work has added to the growing body reporting the association between vitamin D deficiency and poorer outcomes, either prior to or following THR/TKR. As a consequence of this association, some authors have concluded that vitamin D supplementation should be given to those undergoing THR/TKR to improve outcomes. However, due to the mixed outcomes reported in the literature, and that all studies to date, including this one, are observational in nature, this conclusion cannot be made. Additionally, it is not clear if supplementation would be of benefit to all patients or only those with deficiency. Future studies are therefore required to investigate the influence of supplementation on post-operative outcomes, ideally comparing to a control group who remain deficient. Attempts should also be made to achieve consensus on the definition of deficiency in relation to outcomes following THR/TKR.

Chapter 4: The effect of vitamin D supplementation on outcomes following total hip or knee arthroplasty surgery; a rapid systematic review of current evidence

4.1 Background

The relationship between vitamin D level and THR/TKR outcomes has been reported previously, with mixed conclusions on a range of outcomes including patient reported outcome scores, physical function, length of hospital stay, and the requirement for revision surgery. An overview of this literature, as well as its limitations was discussed in chapter one, with most authors concluding that vitamin D deficiency should be corrected pre-operatively with supplementation, or that randomised-controlled trials are required.

Previous systematic reviews[229–232] have described an association between deficiency and poorer outcomes following THR/TKR, although two meta-analyses[229,231] allude to the inclusion of retrospective and non-randomised studies with significant heterogeneity. However, no review has yet addressed whether offering vitamin D supplementation to correct deficiency peri-operatively improves outcomes following THR/TKR.

4.2 Aim

To perform a systematic rapid review of the literature to determine if peri-operative vitamin D supplementation has an effect on reported outcomes following total hip or knee arthroplasty surgery.

4.3 Methods

4.3.1 Registration

This review was conducted as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[233] It was prospectively registered on 1st March 2021 with the PROSPERO International Register of Systematic Reviews, hosted by the Centre for Reviews and Dissemination at the University of York (CRD42021238086).

4.3.2 Database Searches

A systematic search of the literature as was performed from inception to March 1st 2021. The following resources were searched:

MEDLINE

EMBASE

PubMed

Cochrane Library

ISRCTN Registry

ClinicalTrials.gov Database

International HTA Database

4.3.3 Search Terms

The search terms and Boolean operators used to search for relevant articles were:

"THR" <OR> "THA" <OR> "hip replacement" <OR> "hip arthroplasty" <OR> "TKR" <OR> "TKA" <OR> "knee replacement" <OR> "knee arthroplasty"

<AND>

"vitamin D" <OR> "cholecalciferol" <OR> "ergocalciferol" <OR> "25 hydroxy vitamin D".

These keywords were also used to search The Cochrane Library, ISRCTN Registry, ClinicalTrials.gov, and the International HTA Database for the same time period. Reference sections of retrieved articles were also reviewed to check for further relevant publications.

4.3.4 Inclusion and Exclusion Criteria

All randomised, cohort or case-controlled studies of adult patients undergoing hip or knee replacement where vitamin D supplementation was given in the peri-operative period and at least one post-operative outcome was reported, were eligible for inclusion. Post-operative outcomes were either clinical or patient reported (via either a procedure-specific or general-health outcome questionnaire).

Conference abstracts, studies reporting on vitamin D in relation to osteoporosis and hip fracture, those involving animal models, and publications not reported in English were excluded from review. Those studies reporting vitamin D levels and post-operative outcomes but had not offered peri-operative supplementation, were also excluded.

4.3.5 Data Extraction

Searches of the above databases using the described keywords were performed in March 2021, and the titles and abstracts of retrieved articles were reviewed for eligibility. I'm very grateful to Mr. William Fishley [WF], a fellow Trauma and Orthopaedic surgery registrar, Health Education England North East who independently duplicated the searches and

screening of articles for inclusion. Following discussion and consensus a final list of studies was agreed upon, and the full text of these records were retrieved and read independently. The reference sections of retrieved studies were also reviewed to capture any further relevant studies.

Data extraction from retrieved articles which met the inclusion and exclusion criteria included study design, number of patients, age, gender, vitamin D assay method, definition of vitamin D levels, supplementation dose, timing and route, as well as the timing of and study outcome used as the primary outcome measure. This information was recorded in a Microsoft Excel chart.

4.3.6 Bias Assessment

Risks of bias for all eligible studies were performed according to the Cochrane Handbook for Systematic Reviews of Interventions[234] using the RoB 2 tool for randomised studies,[235] and the ROBINS-I tool for non-randomised studies of interventions.[236] The decision made for each assessment of bias was then discussed with WF to check for agreement.

The RoB2 tool assesses five domains where bias may arise in randomised studies:

Arising from the randomisation process

Due to deviations from the intended interventions

Due to missing outcome data

In measurement of the outcome

In selection of the reported result

An overall judgement can then be made, as per the criteria shown in table 4.1.

Table 4.1: Overall risk of bias judgement in ROB2 *(taken from* [235]*)*

Overall risk-of-bias judgment	Criteria
Low risk of bias	The study is judged to be at low risk of bias for all domains for this result
Some concerns	The study is judged to raise some concerns in at least one domain for this result, but not to be at high risk of bias for any domain
High risk of bias	The study is judged to be at high risk of bias in at least one domain for this result, or the study is judged to have some concerns for multiple domains in a way that substantially lowers confidence in the result

The ROBINS-I tool[236] assesses seven domains where bias may arise in non-randomised studies of interventions:

1. *Due to confounding*
2. *In selection of participants into the study*
3. *In classification of interventions*
4. *Due to deviations from intended interventions*
5. *Due to missing data*
6. *In measurement of outcomes*
7. *In selection of the reported result*

An overall judgement can then be made, as per the criteria shown in table 4.2.

Table 4.2: Overall risk of bias judgement in ROBINS-I *(taken from* [236]*)*

Judgement	Within each domain	Across domains	Criterion
Low risk of bias	The study is comparable to a well performed randomised trial with regard to this domain	The study is comparable to a well performed randomised trial	The study is judged to be at **low risk of bias for all domains**
Moderate risk of bias	The study is sound for a non-randomised study with regard to this domain but cannot be considered comparable to a well performed randomised trial	The study provides sound evidence for a non-randomised study but cannot be considered comparable to a well performed randomised trial	The study is judged to be at **low or moderate risk of bias for all domains**
Serious risk of bias	The study has some important problems in this domain	The study has some important problems	The study is judged to be at **serious risk of bias** in at least one domain, but not at critical risk of bias in any domain
Critical risk of bias	The study is too problematic in this domain to provide any useful evidence on the effects of intervention	The study is too problematic to provide any useful evidence and should not be included in any synthesis	The study is judged to be at **critical risk of bias in at least one domain**
No information	No information on which to base a judgement about risk of bias for this domain	No information on which to base a judgement about risk of bias	There is no clear indication that the study is at serious or critical risk of bias *and* there is a lack of information in one or more key domains of bias (*a judgement is required for this*)

4.3.7 Synthesis of Data

A narrative synthesis of the extracted data is reported, with description of the key characteristics for each individual study. Due to the limited number of studies meeting the inclusion criteria and the heterogeneity in reported clinical outcomes, a meta-analysis or sub-group analysis was not possible.

4.4 Results

4.4.1 Study retrieval

Following identification and screening, three studies comprising 413 patients in total, fulfilled the criteria for offering supplementation of vitamin D in the peri-operative period, and reporting on at least one post-operative outcome (figure 4.1). One study was an RCT comparing a multivitamin tablet to placebo,[237] one was an RCT comparing two different strengths of vitamin D supplementation on two primary endpoints,[238] and the last was a non-randomised prospective cohort study.[175] All retrieved studies involved patients undergoing TKR, with no studies reporting the outcome following THR. A summary of key trial characteristics, patient demographics, intervention details and outcome measures for each study are included in table 4.3.

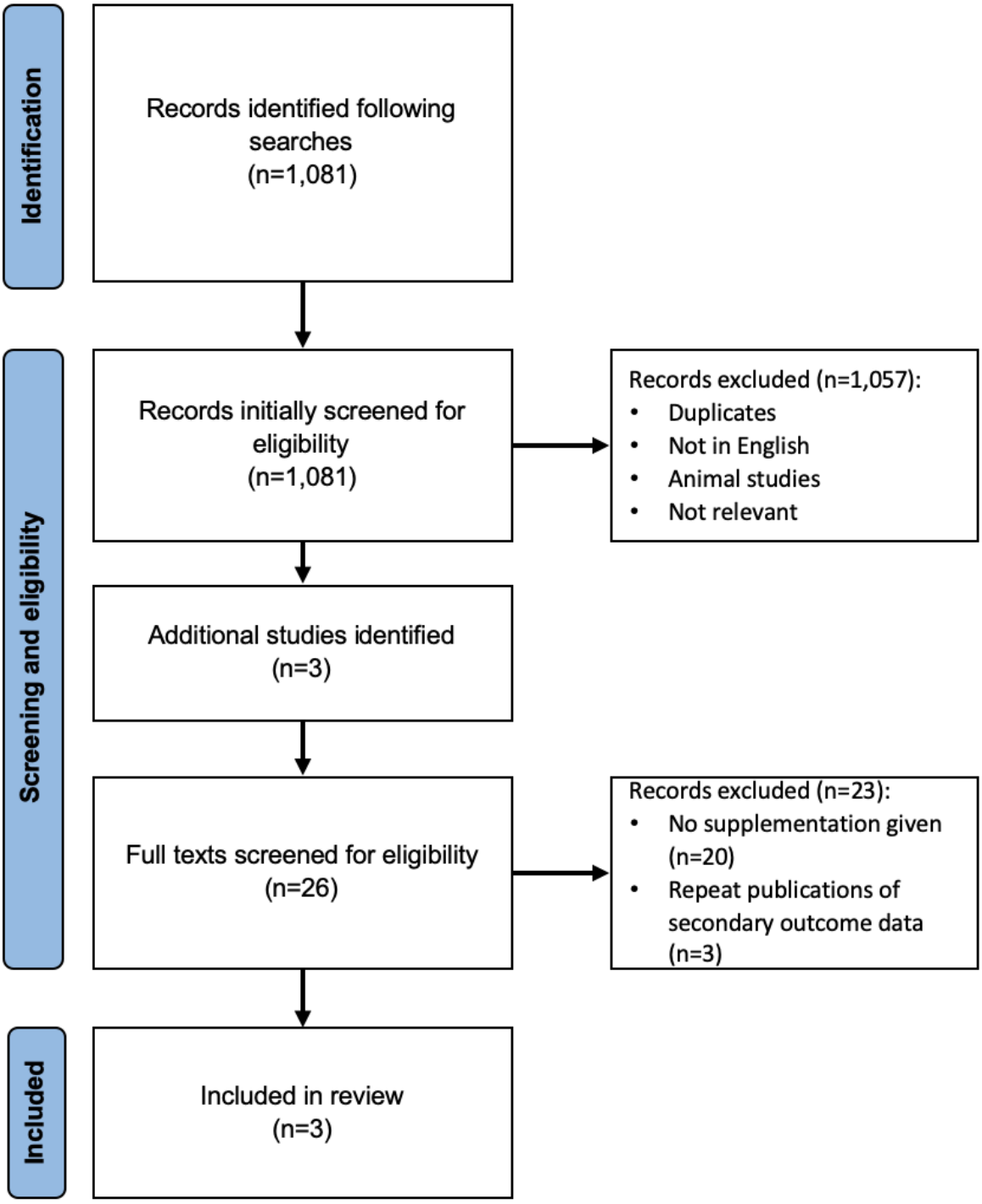


Figure 4.1: PRISMA Flow Diagram presenting the systematic review process used

Author	Design	n	Supplement dose	Supplement timing	Vitamin D status definition; mean per group	Age (years)	Gender (% female)	Vitamin D Assay	Outcome(s) Measured	Time of Measurement	Findings
Barker *et al*. (2021)	RCT	20 TKR	900IU /day as part of multivitamin tablet vs. placebo	Daily from 6 weeks pre-op	Not defined. MV: 70nmol/L P: 78nmol/L	MV:62 P: 63	MV: 50 P: 55	Not recorded	IL-6:IL-10 ratio	Baseline 24 and 48 hours post-op	Multivitamin reduces IL-6:IL-10 ratio post-op at 24 hrs (effect size 0.64) and 48 hrs (effect size 0.48)
Bischoff-Ferrari *et al.* (2018)	RCT	273 TKR	800 IU vs. 2000 IU	Daily from 6 weeks post-op	Not defined. 800IU: 68nmol/L 2000IU: 68nmol/L	800 IU: 71 2000 IU: 70	800 IU: 57 2000 IU: 50	HPLC-MS/MS	Rate of falls, WOMAC	Baseline (6-weeks post-op), and 6, 12, 18, 24 months post-op	No difference at any measured timepoint for primary or secondary outcomes between 800 IU and 2,000 IU. Power calculation was based on rate of falls.
Maniar *et al*. (2016)	Retrospective review of prospectively collected data	120 TKR	20 IU for all patients	Daily from 2 weeks post-op for 4 weeks	D: 53% <75nmol/L S: 47% ≥75nmol/L	D: 67 S: 69	D: 78 S: 84	Not recorded	WOMAC SF-12 KSS	Pre-op 3 months post-op	Deficient group had worse pre-op WOMAC scores (48.3 vs. 42.3, $p = 0.04$), but no difference post-op (17.6 vs. 15.8, $p = 0.362$). No difference in pre- or post-op KSS or SF-12 scores.

Table 4.3: Summary data of retrieved manuscripts

RCT = randomised controlled trial; TKR = total knee replacement; IU = international units; MV = multivitamin; P = placebo; D = deficient; S = sufficient; HPLC-MS/MS = high performance liquid chromatography tandem mass spectrometry; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index; KSS = Knee Society Score; SF-12 = 12-item Short Form Survey

4.4.2 Bias Assessment

Using the RoB-2 criteria to assess the two randomised trials, one was judged to be at 'high risk' of bias, and there were 'some concerns' of bias with the other (table 4.4). Using the ROBINS-I tool, the only non-randomised study included in this review was deemed to be at 'serious risk' of bias (table 4.5).

Table 4.4: RoB-2 bias assessment for each domain of randomised trials[237,238]

	D1	D2	D3	D4	D5	Overall
Barker *et al*. (2021)	+	+	!	+	-	-
Bischoff-Ferrari *et al.* (2018)	+	+	+	+	!	!

D1 = Randomisation process; D2 = Deviations from the intended interventions; D3 = Missing outcome data;
D4 = Measurement of the outcome; D5 = Selection of the reported result.
+ = Low concern of bias; ! = Some concern of bias; - = High risk of bias

Table 4.5: ROBINS-I bias assessment for the non-randomised trial[175]

Domain	Bias Assessment
Bias due to confounding	Serious
Bias in selection of participants into the study	Low
Bias in classification of interventions	Low
Bias due to deviations from intended interventions	Low
Bias due to missing data	Low
Bias in measurement of outcomes	Low
Bias in selection of the reported result	Low
Overall bias	**Serious**

4.4.3 The effect of supplementation on inflammatory response

A pilot study RCT from a single centre in the USA randomised 22 adult patients to receive either a daily multivitamin (containing 900 IU of vitamin D along with vitamins A, B, C, E, K and a number of salts and trace minerals) or placebo tablet from six weeks prior until six months following TKR surgery.[237] There was a non-significant increase in the measured vitamin D level in those receiving the multivitamin from a baseline average of 70-75nmol/L. Both groups were noted to have a lower vitamin D level at 48 hours following surgery compared to pre-operatively. A statistically significant reduction in the IL6:IL10 ratio was seen in those patients receiving the multivitamin compared to placebo at 24- and 48-hours following surgery, with a reported effect size of 0.64 and 0.48 respectively. The authors also measured serum levels of TNF-alpha, IL-6, IL-8, IL-10 and high-sensitivity CRP at the same timepoints but no significant change was seen in any of these levels.

4.4.4 The effect of supplementation on falls and WOMAC score outcomes

In a double-blinded RCT between January 2008 and March 2014, 273 patients 60 years and older undergoing TKR due to OA at a single-centre in Switzerland, were randomised to receive either 800 IU or 2,000 IU vitamin D supplement per day from 6-weeks following TKR.[238] The primary outcomes were the WOMAC scores for both operated and non-operated knees, and the rate of falls over 24 months. Secondary outcomes included sit-to-stand test, 4m normal gait speed, activity level and radiographic progression in the contralateral knee. Baseline serum vitamin D levels were equal in both groups at 68nmol/L. No difference was seen between the two groups at 24 months for any of the outcomes measured.

4.4.5 The effect of supplementation on patient reported outcome measures - WOMAC, KSS and SF-12 scores

Maniar *et al.*[175] report on 120 patients undergoing TKR by a single surgeon in India. All patients were given 20 IU of vitamin D daily for four weeks, beginning two weeks following surgery. Those patients with vitamin D deficiency at baseline (53% of patients had vitamin D levels

<75nmol/L) had worse pre-operative WOMAC scores compared to those with sufficiency (48.3 vs. 42.3, p = 0.04), however there was no difference in post-operative scores at three months (17.6 vs. 15.8, p = 0.362). Furthermore, no difference was noted in pre-operative or post-operative KSS and SF-12 scores between the two groups.

Due to differences in definitions of vitamin D sufficiency, supplement dose, timing and duration, as well as the variation in the reported outcomes measures used, meta-analysis of the retrieved studies was not possible.

4.5 Discussion

Between 2016 and 2021 three separate cohorts of patients undergoing TKR received vitamin D supplementation in the peri-operative period. No studies have reported on patients requiring THR.

Vitamin D was given prior to surgery in only one study, where 11 of 22 patients were randomised to receive a multivitamin tablet containing 900 IU of vitamin D.[237] The authors excluded one patient from the multivitamin group prior to data analysis due to an outlying BMI of 53 kg/m^2 but don't clarify why this was done. The other two studies started vitamin D supplementation for all patients post-operatively; one at two-weeks[175] and the other at six-weeks[238] following surgery. Neither used a placebo or compared to a control group who did not receive supplementation.

A fall in serum vitamin D level has previously been reported following both THR and TKR, and may remain lower than before surgery for up to three months.[69,70] Barker *et al*.[237] demonstrated a reduction at 48 hours following surgery in both treatment and placebo arms, but noted that the administration of a multivitamin significantly modulated the post-operative inflammatory response, as indicated by a reduction in the Il-6:Il-10 ratio. As a multivitamin was used in this study then the authors are unable to conclude that the impact is solely related to vitamin D, but it does suggest that if supplementation is to be effective then it should be given prior to surgery.

Bischoff-Ferrari *et al*.[238] present a well-conducted RCT with pre-published primary and secondary endpoints. They showed no difference in WOMAC scores or rate of falls at 24 months between patients receiving 800 IU or 2,000 IU, although the study was powered for falls rather than WOMAC score. As no placebo group was used, the authors acknowledge that the efficacy of vitamin D versus no treatment could not be assessed.

Not all of the 15 pre-defined secondary outcomes were reported in the main paper, and three subsequent publications were found which addressed glucose metabolism,[239] cognitive performance,[240] and total blood pressure (BP) reduction[241] at 24-months. There was no difference in any of the main outcomes, although there was a small but statistically significant difference reported in the reduction of systolic BP variability in those taking 2,000 IU compared to 800 IU (-0.37mmHg; 95%CI -0.70,-0.04; p = 0.04).[241]

These three subsequent papers appear to be 'salami-sliced' secondary outcome publications of the main trial and therefore were excluded from analysis. Furthermore, their focus appeared to be on the difference between 800 and 2,000 IU of vitamin D on the outcome of interest, rather than in relation to the role of vitamin D in influencing patients' post-surgical outcomes.

Maniar *et al.*[175] reported lower pre-operative WOMAC scores in those with deficiency, and although this was statistically significant, the clinical relevance is questioned as the baseline difference between groups was lower than the reported MCIDs for the WOMAC score.[242–244] A very low-dose of vitamin D (20 IU) was given to all patients after surgery, whether deficient or not, but there was no post-operative vitamin D level check to determine the influence supplementation had on serum levels. However, with such a low dose of supplementation a significant rise would not be expected. No difference in post-operative scores was noted between the two groups. Furthermore, the lack of a control group who did not receive supplementation prevents any conclusions on the role of vitamin D to be made.

The full manuscripts of 23 studies reporting the relationship between vitamin D level and a post-operative outcome were identified and read, although these were excluded from analysis as no peri-operative intervention with vitamin D supplementation was offered. Some studies have found no association between vitamin D level and post-operative outcomes,[180,181,222,223,245] with one study even concluding that higher vitamin D levels were associated with an increased risk of prosthetic joint infection (PJI).[246] In contrast, others have linked insufficiency with worse pain scores,[176] lower pre-operative[173,178,179,247] or post-

operative[172,174,179,221] functional scores, longer length of stay,[182] differences in gait kinematics and kinetics,[248] an increased risk of developing post-operative complications,[197,249] and a greater risk of PJI and need for revision surgery.[196]

In a mouse-model study of PJI, vitamin D deficiency was demonstrated to be associated with a greater bacterial load and neutrophil infiltration, although this could be reduced by the pre-operative administration of vitamin D.[250] The authors recommend that vitamin D deficiency may be a modifiable risk-factor to prevent PJI. In a nationwide population-based study using a Korean Health Insurance database, 142,147 patients undergoing TKR between 2009 and 2018 were identified.[251] The combined use of calcium and vitamin D for more than one year before surgery was associated with a reduction in the risks for revision surgery in both patients with PJI (RR = 0.63, 95% CI 0.42-0.95) and patients without infection (RR = 0.70, 95% CI 0.54-0.91). Cost-estimation modelling has predicted that non-selective vitamin D supplementation may be an effective option to help reduce the risk of joint infection following TKR, based on the low-cost of supplementation compared to the cost of performing a laboratory vitamin D test.[252]

This review has focussed on the impact of supplementation in the peri-operative period to determine the effect on surgical outcomes. Although there have been four systematic reviews on the relationship between vitamin D level and hip and knee arthroplasty outcomes, and one on outcomes following surgery in general, none have focussed on the role of peri-operative supplementation.

As the majority of natural vitamin D is obtained through sunlight, then deficiency may be a marker of the inability to get outdoors due to comorbidity or functional limitations from advanced joint disease. Worse pre-operative function has been shown to be related to poorer post-operative outcomes.[132,253–256] Whether altering vitamin D status with supplementation enables those with deficiency to achieve the same outcomes as those who have a 'naturally' sufficient status remains to be seen, and future appropriately powered studies are required to determine this.

4.5.1 Strengths and limitations

The strengths of this review are that it was prospectively registered, with a clearly defined novel search question. All types of interventional study, whether prospective or retrospective, were included, as were all types of post-operative outcome. Two reviewers independently searched for and screened articles for inclusion in the review. It focussed on the role of supplementation, rather than the association between serum vitamin D levels and a post-operative outcome which previous reviews have addressed.

Due to the time and resource limitations of this PhD then this was a 'rapid review', and so some literature sources, such as 'grey literature' or conference proceedings, were not searched and therefore some relevant publications may have been missed.

Systematic reviews are considered the 'gold standard' of knowledge synthesis, although the process does have some limitations. They are time and resource-heavy, with a consequential financial cost.[257] They may take up to two years for specialist teams to complete, with one study reporting the mean time for completion and publication of reviews registered on PROSPERO was 67.3 weeks.[258]

As a consequence, rapid reviews are becoming more popular and are often used to address urgent health issues or help answer high-priority questions. A rapid review has been defined as: *"…a form of knowledge synthesis that accelerates the process of conducting a traditional systematic review through streamlining or omitting specific methods to produce evidence for stakeholders in a resource-efficient manner".* [259]

Streamlining the systematic review process may risk a rapid review being susceptible to bias,[260] although it has been reported that when comparing rapid and traditional systematic reviews, the overall conclusions did not vary.[261,262] Posing a well-defined question in a specific context is important to ensure rapid reviews are valid to make informed decisions.[263] It has been suggested that rapid reviews can be enhanced by utilising a second author to check 20%

of titles and abstracts,[264] and the use of a second reviewer in this present study addressed this.

This review is limited by the quality of studies that were included and all were limited by biases. One study used a multivitamin in 22 patients, one study used a very low dose of vitamin D for all patients and the other compared two doses of vitamin D with no placebo group. The timing of supplementation in the peri-operative period was different between studies, and none specifically targeted those patients with deficiency. Only one study reported the method of vitamin D analysis used. The three studies were from different countries (India, Switzerland and the United States of America), and reported on different outcomes. The generalisability of the reported findings to different populations may therefore be limited.

4.6 Conclusion

This is the first systematic review to examine the role of peri-operative vitamin D supplementation on outcomes following THR/TKR. To date there are only three studies which have reported on the administration of vitamin D and an outcome following TKR and all were judged to be limited by bias. None have reported on patients undergoing THR. There is currently insufficient evidence to make a recommendation one way or the other on the role of peri-operative vitamin D supplementation, and therefore further adequately powered randomised-controlled trials are required to assess if deficiency is a modifiable risk factor to improve outcomes following THR/TKR.

Chapter 5: Vitamin D and Arthroplasty Surgery Outcomes - the VASO Feasibility Trial

5.1 Introduction

An increasing number of studies have reported a link between a low vitamin D level and adverse outcomes following THR/TKR surgery, and this has been discussed in Chapter 1. The results from Chapter 3 have shown that this association is also evident when using the Oxford hip or knee scores to determine outcome, as per the UK PROMs programme.

However, these studies only demonstrate a correlation between the two variables and do not prove causation. To establish if vitamin D insufficiency does predispose to adverse outcomes, and if it is a modifiable risk-factor, then pre-operative correction of vitamin D status with supplementation is required. The systematic review in Chapter 4 has shown that to date, no study has been published where a group of patients with insufficiency undergoing THR/TKR are given pre-operative supplementation and their post-operative outcome is compared to a control group who remained insufficient. The Vitamin D and Arthroplasty Surgery Outcomes (VASO) trial was therefore designed to address this. This was a feasibility study to help determine trial processes, and not a definitive, statistically powered trial.

5.2 Methods

5.2.1 Trial Sponsor and Chief Investigator

Northumbria Healthcare NHS Foundation Trust was designated the Sponsor for the trial, as defined in the UK policy framework for health and social care research.[265] The Research and Development (R&D) team at the trust were involved at an early stage whilst designing the trial, ensuring that appropriate support and expertise could be provided, including that of a trial statistician. Relevant links were also established with the pharmacy department and biochemistry laboratory which were required for the trial to run successfully.

The trust has four main hospital sites where arthroplasty surgery is performed, and in the year 2018-19 performed 1,359 TKR procedures and 1,316 THR procedures, compared to an average of 246 and 243 respectively per hospital site in England, Wales and Northern Ireland.[228]

Professor Mike Reed, Consultant Trauma and Orthopaedic Surgeon at Northumbria Healthcare NHS Foundation Trust, was designated the Chief Investigator. He is an experienced clinical academic and has run a number of successful orthopaedic trials through the trust, which have influenced clinical practice.[266–269]

5.2.2 Medicines and Healthcare products Regulatory Agency (MHRA) approval

The Humans Medicines Regulations 2012[270] defines a medicinal product as:

> *(a) any substance or combination of substances presented as having properties of preventing or treating disease in human beings; or*
>
> *(b) any substance or combination of substances that may be used by or administered to human beings with a view to—*

(i) restoring, correcting or modifying a physiological function by exerting a pharmacological, immunological or metabolic action, or

(ii) making a medical diagnosis.

Given the above, it was questioned during the development of the protocol whether the use of vitamin D supplements in the VASO trial would be classed as a 'Clinical Trial of Investigational Medicinal Products' (CTIMP). The algorithm *'Is it a clinical trial of a medicinal product'* provided by the MHRA on their website was consulted, and the proposed trial protocol was submitted to the MHRA Clinical Trial Helpline for review. Confirmation was subsequently received that the VASO trial was not classified as a CTIMP (Appendix D).

5.2.3 Patient and Public Involvement (PPI)

The Total Hip User Group (THUG) was set-up in 1984 by patients who had undergone joint replacement at North Tyneside general hospital in the North East of England, with the aim of providing pre-operative and post-operative advice, information and support to fellow patients undergoing surgery. The group also raises funds and equipment for the orthopaedic and physiotherapy departments at the hospital, and are happy to be consulted for a patient opinion on matters relating to joint replacement.

I contacted THUG to convene a focus-group and six members attended. The aim of the meeting was to outline the VASO trial, gauge the perceived reception from patients on recruiting to such a trial, as well as to review the patient information sheet and consent form for clarity and ease of reading, before these were submitted for ethical approval. The feedback and input from those attending the focus-group was gratefully received and relevant amendments to the documentation and protocol were made based on this.

5.2.4 Involvement of a second recruiting site

A second site was used in the VASO study to optimise patient recruitment and to test the feasibility of randomisation processes and the running of a trial remote to the Sponsor site. The South Tees Hospitals NHS Foundation Trust was chosen as this is a high-volume arthroplasty centre within the region, with research-ready surgeons and a history of involvement in clinical trials. The trust has two main hospital sites where arthroplasty surgery is performed, and in the year 2018-19 performed 590 TKR procedures and 642 THR procedures.[228] Furthermore, the local population which the trust serves has a greater ethnic diversity with 92% of patients registered as 'White British' compared to 97% in the population served by Northumbria Healthcare NHS Foundation Trust.[218]

The Principle Investigator for South Tees was Mr. Craig White, Consultant Trauma and Orthopaedic surgeon, with support and collaboration from Mr. Paul Baker and Professor Amar Rangan, along with research nurses and the R&D department.

5.2.5 Protocol and Study Document Development

Invitation letters (Appendix E) and a Patient Information Sheet (Appendix F) were created. The trial protocol was developed and written as per the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist,[271] and approved by all co-authors (Appendix G). The protocol was subsequently peer-reviewed and published prior to the completion of recruitment.[272] An overview of the trial is shown in figure 5.1.

5.2.6 Study Approval

An application for the necessary permissions and approvals for the trial was submitted via the Integrated Research Application System (IRAS) – reference 216934. A favourable ethical opinion was given by the Yorkshire & The Humber – Bradford Leeds Research Ethics Committee on 13th March 2017 (reference 17/YH/0067, Appendix H). Given this favourable

opinion, no further ethical review was required by Newcastle University (reference 13369/2016). Approval by the Health Research Authority to run the trial in NHS Hospital sites was obtained on 19th April 2017 (Appendix I), and the trial was granted National Institute for Health Research portfolio status (Central Portfolio Management System identifier 33969). The trial was registered with the International Standard Registered Clinical/soCial sTudy Number (ISRCTN) database prior to recruitment of the first patient (reference ISRCTN14533082).

A Material Transfer Agreement was made between each hospital trust and Newcastle University to enable additional blood samples donated from patients taking part in the trial to be taken to and stored anonymously in the university biobank for use in future research studies.

On being granted the above permissions, local site induction visits were then completed, and site approval and green light status to begin recruitment was given.

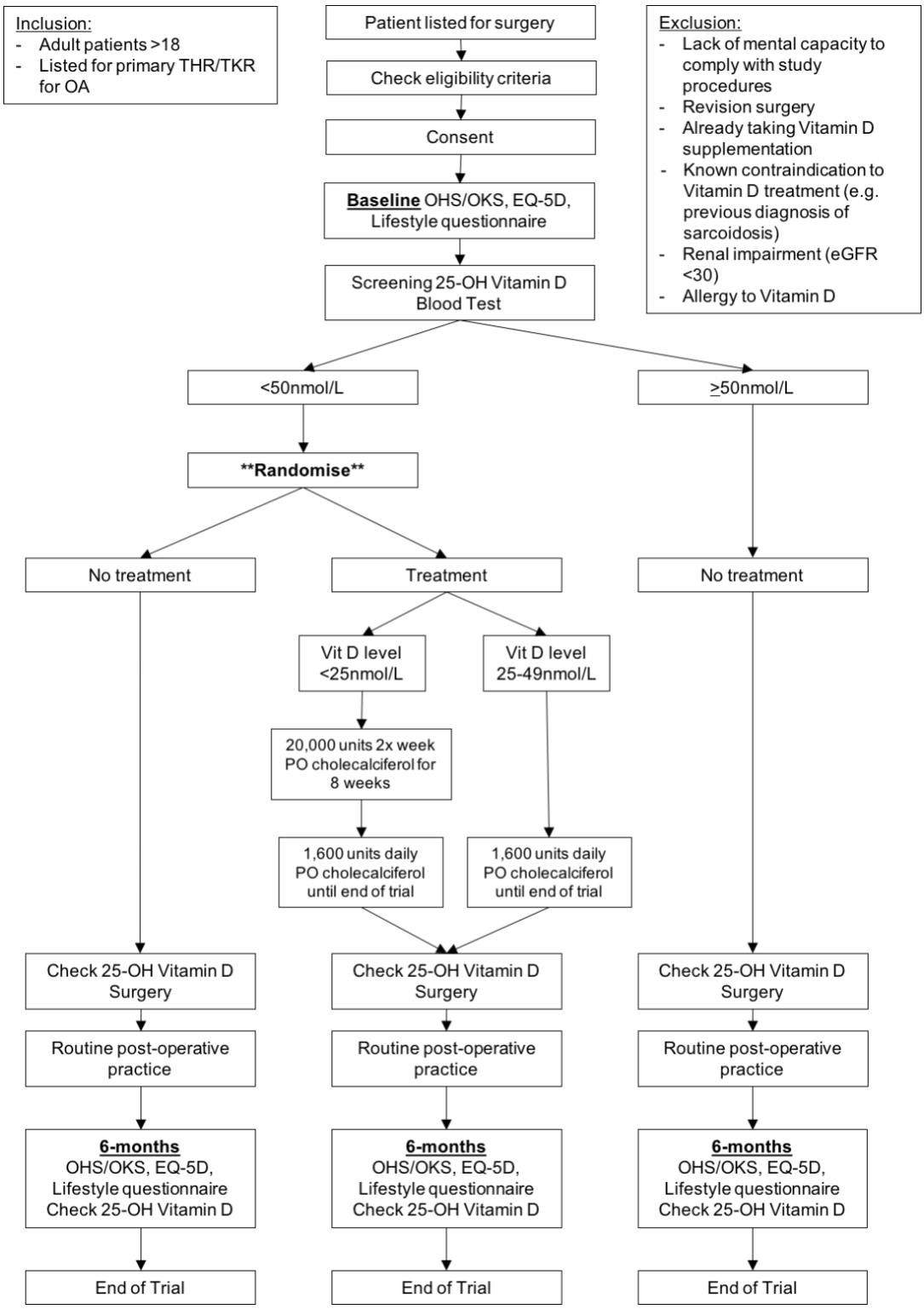


Figure 5.1: Participant flow for the VASO trial

5.2.7 Patient screening and recruitment

Clinic referral letters were screened to identify potential participants in advance of their clinic attendance. A patient information sheet along with an accompanying letter highlighting the trial was then sent by post to these potential participants.

When participants were seen in the clinic, if the treating surgeon added the patient to the waiting list for a primary hip or knee replacement and deemed the patient potentially suitable for inclusion in the trial, then the patient was reviewed by a research nurse in the clinic. A copy of the patient information sheet was again given to the patient, and they were offered the opportunity to ask any questions regarding the trial. A screening questionnaire to confirm inclusion and exclusion criteria was completed. A log of all patients who had been screened was recorded.

5.2.8 Inclusion and exclusion criteria

Patients were eligible for inclusion in the trial if they were aged over 18, and were presenting at a participating trial site to undergo primary THR or TKR.

Patients were excluded from participating if they lacked the mental capacity to understand or comply with the study procedures, if they were undergoing revision surgery, if they had a known contraindication to vitamin D treatment (including a previous diagnosis of sarcoidosis, primary hyperparathyroidism or other hypercalcaemic disorder), had a known allergy to vitamin D or if they were already taking a vitamin D supplement. If a patient was subsequently found to have renal impairment with an eGFR <30 mL/minute then they were also excluded.

5.2.9 Baseline procedures

Patients who were screened and met the initial inclusion and exclusion criteria were suitable for recruitment. If the patient was happy to consent for their inclusion into the trial on the same day, then this was permitted and informed written consent was obtained in the clinic by Good Clinical Practice trained research nurses (Appendix J). If patients wished to have the opportunity to discuss the trial with family or friends prior to giving their agreement to taking part, then a contact telephone number for the research team was provided and another consenting appointment was arranged.

Patients were recruited to the trial in May and June. They were asked to complete baseline CRF questions including confirmation of contact and demographic details, medication and drug history, and a lifestyle questionnaire which included questions on diet, sun exposure, smoking and alcohol (Appendix K). Patients then had their serum vitamin D level measured, along with their routine blood tests which are obtained when a patient is added to the waiting list to undergo THR/TKR.

5.2.10 Vitamin D testing

Routine blood tests included the collection of a sample in a serum separating tube, and using this a 25-OH vitamin D test could be requested. Permission and agreement had been obtained from each trust laboratory for the additional processing requirement of vitamin D tests for the purposes of the research trial.

In the biochemistry laboratory at Northumbria, the serum tubes were spun at 1300g for 10 minutes and stored in a fridge between 2-8°C. Vitamin D levels were measured using the cobas e 601 total 25-OH vitamin D immunochemiluminescence assay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland), with a detection range of 7.5–175 nmol/L and a laboratory-quoted coefficient of variation of 7.7%.

At South Tees, serum tubes were spun at 2000g for 10 minutes and stored in a fridge between 2-8°C. Measurement of vitamin D was performed daily using the IDS-iSYS 25-Hydroxy Vitamin D immunoassay (Immuno Diagnostic Systems [IDS], Boldon, UK), with a detection range of 18–313 nmol/L and a quoted coefficient of variation up to 11.6%. Both laboratories subscribed to the DEQAS scheme for the analysis and reporting of vitamin D tests.

For those patients who gave their consent for an additional sample of blood to be taken for storage for future studies, both trust laboratories were able to process and send this separate sample via hopper bus to Newcastle University for storage in the biobank. These samples were spun at 1300g for ten minutes and the serum samples subsequently stored in 2mL aliquots at -80°C.

The vitamin D results of patients at both sites were retrieved using the Sunquest Integrated Clinical Environment (ICE) system (Sunquest Information Systems (Europe) Ltd., Norwich, United Kingdom). Those with a 25-OH D level <50nmol/L were eligible for randomisation. Those patients with a 25-OH D level $\geq$50nmol/L were deemed to be 'sufficient' and were therefore not eligible for randomisation and acted as the 'normal' control group.

5.2.11 Randomisation process

A research nurse at each trust telephoned the R&D administrator at the Sponsor site to obtain the treatment arm allocation and trial number when randomising an eligible patient. The trial allocation sequence had been randomly generated using the website www.randomization.com, with each trust split into ten blocks of ten patients, with each block allocation evenly split 1:1 between 'treatment' and 'no treatment'. Additional written confirmation of each patient's allocation and their assigned trial number was then sent to the recruiting hospital's research team via secure NHS.net e-mail. The randomisation log was held on a password-protected database stored on a Northumbria Healthcare Trust computer, and was only accessible by the R&D administrator and myself.

5.2.12 Vitamin D supplementation

Approval was obtained from the Lead Clinical Pharmacists at each hospital trust regarding the requirement for additional vitamin D supplies, as well as the logistics of obtaining the supplements to send to patients as part of the trial. Vitamin D supplementation for the duration of the trial was prescribed to those patients randomised to receive treatment. The dose was based on the trust guidelines at the time,[220] which had the same treatment thresholds as those described in the 2010 review by Pearce and Cheetham.[93]

For those patients who were randomised to receive vitamin D supplementation, a prescription for this was written by one of the clinicians involved in the trial at each trust. In those with insufficiency (25-49nmol/L), a daily dose of 1,600 international units (IU) cholecalciferol was prescribed, to be taken from the day of randomisation until six months following surgery. For those with deficiency (<25nmol/L), a 'loading dose' of 20,000IU twice per week for eight weeks was prescribed, followed by a maintenance dose of 1,600 IU per day until six months following surgery.

Vitamin D supplements were delivered to patients via recorded post, to minimise the burden on patients having to reattend the hospital to collect their prescription. A follow-up telephone call was made by research nurses within five days to ensure the supplementation had been received. Patients were advised to continue their treatment until they completed their six-month follow-up visit, and to contact the research team if they required more supplements, for example if there had been a delay to their planned surgical date.

5.2.13 Day of surgery testing

On the day of surgery, a sample of blood was taken from patients prior to their operation to measure their vitamin D level. This sample was obtained and processed in the same way as discussed in sub-section 5.2.10. Patients then underwent their primary THR/TKR under the care of their consultant orthopaedic surgeon, and no specific guidance was given with regard

to the choice of implant, the surgical approach, method of wound closure, peri-operative care, rehabilitation or clinical follow-up; the approach was pragmatic and followed routine care for each unit.

5.2.14 Six month follow-up

Patients were contacted via telephone to remind them of their involvement in the trial, and to organise a research visit to obtain a final blood test and repeat the lifestyle questionnaire. If patients preferred a home visit, to minimise the burden on them attending the hospital for the purpose of a research appointment, then this was offered by one of the research nurses where available.

The six-month blood test was obtained and processed in the same way described previously, and the lifestyle questionnaire was repeated. Those patients who had been randomised to treatment were asked about compliance, and if they had been unable to take the supplements, the reasons for this.

Patients were also reminded to complete the post-operative PROM questionnaires that were sent to patients' homes from NHS Digital as part of the national PROMs programme, separate to the trial.

5.2.15 PROMS – patient level data at each trust

An outline of the PROMs programme, the method of collection of pre- and post-operative data and the use of results has been discussed in chapter 1. The feasibility of using PROMs data provided by the national programme was chosen as the outcome measure for the trial.

Hospital trusts are able to obtain their provider-level PROMs data each month from the Health and Social Care Information Centre (NHS Digital). Caldicott Approval was obtained in both trusts to access and use this data for the purposes of the trial. Individual patient episodes were identified by NHS number. Pre-and post-operative absolute scores, as well as the 'health-gain' for the Oxford hip or knee and EQ-5D Index and VAS questionnaires were recorded.

5.2.16 Trial completion and withdrawal

Once patients had attended their six-month follow-up visit, their involvement in the trial was complete. A letter (Appendix L) was sent to the patient, and their GP, confirming this, as well as notifying them of their final vitamin D level. If this was insufficient (<50nmol/L), then patients were advised this was below the normal level and to discuss with their GP whether starting supplements was required.

Patients were able to withdraw from the study at any timepoint without giving a reason, as per Good Clinical Practice. Those patients who did not answer the reminder phone call and/or attend their six-month appointment were deemed to have been 'lost to follow-up'. A letter was also sent to these patients and their GP informing them of this, as well as the most recent recorded vitamin D level.

5.2.17 Adverse event reporting

Any adverse events occurring during the trial which appeared to be related to an aspect of taking part in the study and was deemed to be an unexpected occurrence, were recorded on the relevant adverse event form. This was submitted to the Sponsor, whereby the Chief Investigator assessed causality – definitely, probably, possibly, unlikely, or unrelated.

Adverse events were defined as "*any untoward medical occurrence in a subject to whom the research treatment or procedure has been administered*". Serious adverse events were those which resulted in death, were a life-threatening event, required unplanned hospitalisation or prolonged the existing admission, resulted in persistent or significant disability or incapacity or was another important medical condition, such as a new diagnosis of cancer

5.2.18 Feasibility trial outcome assessment

Whilst health-gain as assessed by PROM scores was chosen as the main outcome measure, as this was a feasibility trial then the outcomes of interest were related to the conduct of the trial. Questions to determine from the trial included:

- *Were patients recruited to the study at both sites?*
- *How many patients were lost to follow-up?*
- *Did those patients allocated to receive treatment receive this prior to surgery?*
- *Did patients accept treatment and adhere to treatment for the trial duration?*
- *Did deficient patients complete their loading dose of vitamin D treatment prior to surgery?*
- *Were vitamin D levels measured on the day of surgery?*
- *Did patients attend to get their vitamin D level checked at 6 months following surgery?*
- *Were PROM scores a reliable tool to use to evaluate this outcome?*
- *Was a link between deficiency and poor outcome, and improved outcomes with a normal vitamin D level noted?*
- *What was the adverse event rate?*

5.2.19 Data management

Trial data was collated in CRFs, identified by a unique trial number. All study documents were stored in a secure, locked location for the duration of the trial, in accordance to GCP guidelines. Electronically held data were stored on password-protected NHS trust computers,

with permission for access as detailed in the delegation log. Data sent electronically to the Sponsor was through secure NHS.net email, accessed only by the study team who followed trust data protection policies. All essential trial documents will be retained for a minimum period of five years after study completion, and held according to the Data Protection Act.[273]

5.2.20 Statistical analysis

Statistical analysis was performed using IBM SPSS software (IBM, Armonk, NY, USA). Average pre-operative, post-operative and health gain PROM scores between groups were compared at baseline and at six months, with significance denoted at $p < 0.05$. The statistical test chosen to compare groups depended on the type of data and its distribution.

For comparing two groups with parametric data, the Student's *t* test for independent samples was used, and for non-parametric data the Mann-Whitney *U* test was used. For comparing more than two groups, the one-way ANOVA test for parametric data, and the Kruskal-Wallis test for non-parametric data were used. Where indicated, *post-hoc* Bonferroni correction was utilised to determine within-group differences. To compare data within groups, such as pre- and post-operative scores, either the paired *t* test or Wilcoxon signed-rank tests were used, depending on the distribution of data. The chi-square test was used for categorical data.

The main aim of the VASO trial was to inform a future fully powered study, rather than to draw direct inferences regarding outcomes. The observed recruitment rate and retention rates for the trial, along with the mean and standard deviation of the PROM scores collected, as well as the reported minimal clinically important difference for the Oxford hip and knee and EQ-5D-3L scores, will all be used to enable a sample size calculation for a larger, future trial to be performed.

5.3 Results

5.3.1 Recruitment

Both Trusts were given the green light to commence recruitment in May 2017, with Northumbria starting three weeks before South Tees. A total of 137 patients were screened, with 102 subsequently consenting to participate in the trial (74% recruitment rate). The actual rate of recruitment exceeded the anticipated rate (figure 5.2).

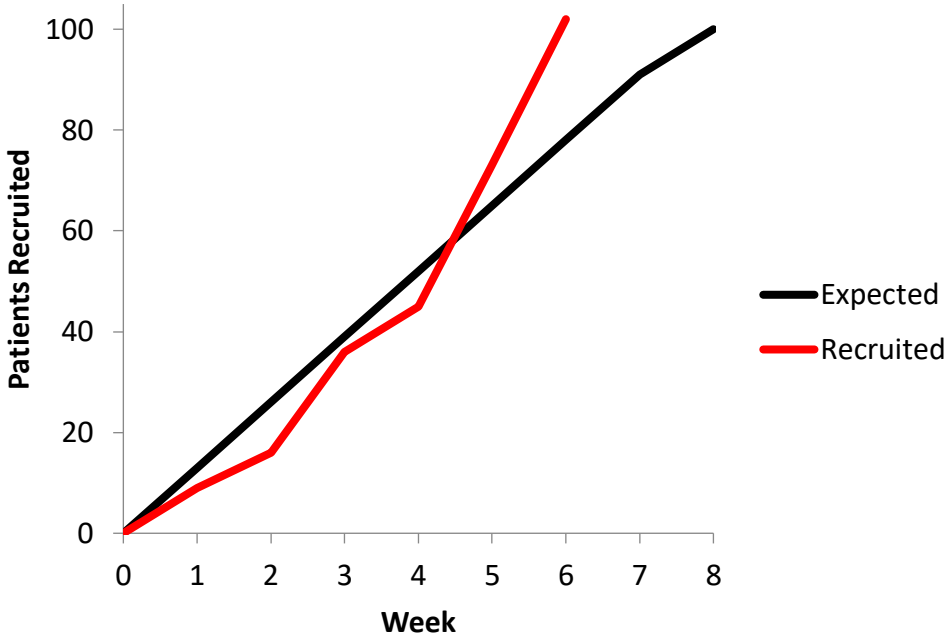


Figure 5.2: Expected versus actual recruitment rate timeline

Of the 137 patients who were screened, fifteen (11%) could not be recruited for a 'logistical reason' which was due to either no research nurse availability, or that patients were unable to commit the time required in the clinic to complete the baseline trial procedures. Thirteen (9%) were excluded as they were already taking a vitamin D supplement, four (3%) were not willing to participate in the trial, and three patients (2%) were not scheduled to undergo primary arthroplasty surgery. A summary Consort flow diagram for the trial is presented in figure 5.3.

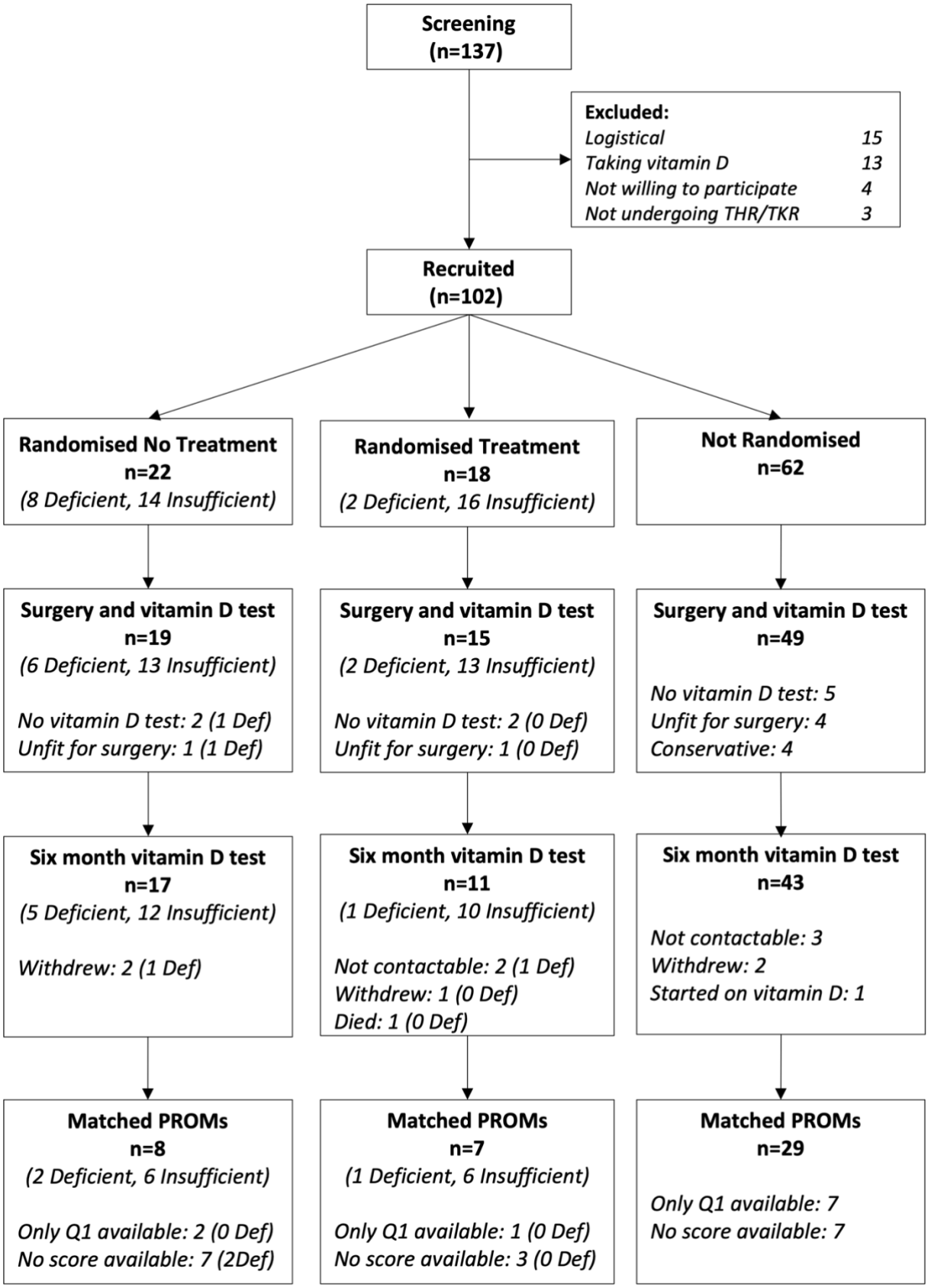


Figure 5.3: CONSORT diagram for the trial

Of the 102 patients recruited at baseline all had their vitamin D level measured and a valid result obtained. Of these, 92 (90%) went on to have surgery within eight months of the start of the trial; six were deemed to be unfit at their pre-assessment appointment and so their surgery was either postponed or cancelled, and four changed their mind about surgery and elected to continue with non-operative management. Only 44 patients (43%) had complete data available at the end of the trial period if they were to be analysed *per protocol* with a vitamin D test at every timepoint and both pre- and post-operative PROMs results available. In the following sub-sections, the results for each component of the feasibility trial will be presented.

5.3.2 Baseline demographics

Baseline demographic and comorbidity data collected for the 102 patients recruited to the trial are given in table 5.1.

Table 5.1: Baseline demographic and comorbidity data

	No Treatment n=22 (21.6%)	**Treatment n=18 (17.6%)**	**Not Randomised n=62 (60.8%)**
Median age (*years, IQR)*	66 (21)	66 (7)	67 (10)
Female (*n, %*)	14 (64)	10 (56)	29 (53)
TKR (*n, %*)	15 (68)	8 (44)	36 (42)
White ethnicity (*n, %*)	21 (95)	17 (94)	61 (98)
Baseline vitamin D, nmol/L (*median +IQR*)	30 (17)	43 (15)	67 (26)
Comorbidity:			
Hypertension (n, %)	12 (55)	8 (44)	21 (34)
Ischaemic heart disease (n, %)	5 (23)	1 (6)	7 (11)
Hypercholesterolaemia (n, %)	5 (23)	4 (22)	13 (21)
Respiratory disease (n, %)	5 (23)	5 (28)	7 (11)
Thyroid disease (n, %)	1 (5)	1 (6)	7 (11)
Diabetes (n, %)	6 (27)	5 (28)	3 (5)
Gastrointestinal disease (n, %)	10 (45)	4 (22)	22 (35)
Rheumatoid Arthritis (n, %)	1 (5)	0 (0)	4 (6)
Cancer (n, %)	1 (5)	6 (33)	7 (11)

5.3.3 Vitamin D testing

All recruited patients to the trial had their vitamin D level checked at baseline. Of the 92 who went on to have surgery, 83 patients (90% of those who had surgery / 81% of those who were randomised) had their vitamin D level checked on the day of surgery. Nine patients did not have their vitamin D level checked on the day of surgery and this was due to either no research nurse availability, or that the patient's date of surgery had been changed at short-notice and this was not recognised by the research team.

At the six month visit, 70 patients (76% of those who had surgery / 69% of those who were randomised) had their vitamin D level checked. Five patients wished to withdraw from the trial, five were not contactable, one was excluded as they had been started on vitamin D supplementation in the intervening period by their GP, and one patient had died of a cause unrelated to the trial or their joint replacement. Of note there was no significant difference in the measured vitamin D levels when performing analysis *per protocol* compared to analysing all results which were available.

5.3.4 Vitamin D levels

Three trial groups were created according to baseline vitamin D status; those with sufficiency ($\geq$50nmol/L) were not randomised, and those with insufficiency (<50nmol/L) were randomised to either 'treatment' or 'no treatment' arms. The box and whisker plot in figure 5.4 demonstrates the vitamin D levels in each group at the three measured timepoints. There was no significant change in measured vitamin D levels throughout the trial for those randomised to 'no treatment', or for those with baseline sufficiency.

Patients who were randomised to receive supplementation were treated according to local guidelines. Two patients with 'deficiency' (<25nmol/L) required eight weeks of high-dose cholecalciferol followed by a daily maintenance dose of 1,600 IU, and 16 patients with 'insufficiency' required the daily maintenance dose of 1,600 IU. Supplementation led to a

significant increase in the recorded median vitamin D levels from baseline to the day of surgery (43nmol/L vs. 73nmol/L, $p < 0.001$). There was a median interval length of 58 days (range 15 to 211) from baseline to day of surgery for those patients receiving supplementation, and this was similar to the whole group interval of 65 days (range 9 to 239 days). Vitamin D levels in the supplemented group continued to increase when checked at six months, and at this timepoint were also significantly higher than the 'sufficient' group (83nmol/L vs. 63.5nmol/L, $p = 0.036$).

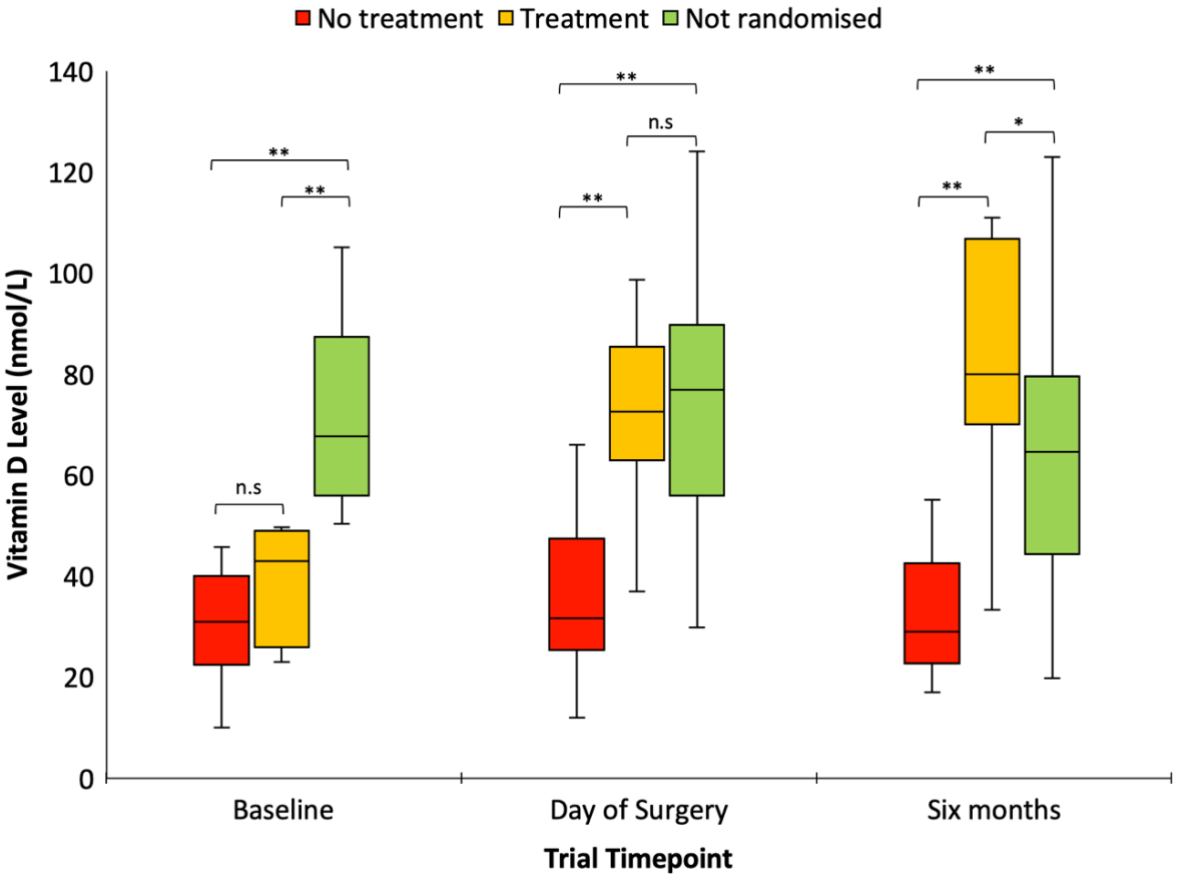


Figure 5.4: Box and whisker plot to demonstrate vitamin D levels per group at each trial timepoint *n.s = not significant; * = <0.005; ** = <0.001*

All baseline vitamin D levels were obtained in May and June. The wide interval range from baseline to day of surgery meant patients underwent their joint replacement in summer, autumn or winter. Consequentially the six month vitamin D checks occurred in winter, spring and summer. However, there was no significant difference in vitamin D levels on the day of surgery or at six months when accounting for season.

On further analysis of individual data, the vitamin D status for some patients changed despite not receiving supplementation. Three patients (14%) who were 'insufficient' at baseline and not randomised to treatment had a 'sufficient' level when checked on the day of surgery, and two (9%) were 'sufficient' at their six month check. In contrast, seven patients (11%) who were classed as 'sufficient' at baseline had an 'insufficient' level when their vitamin D level was checked on the day of surgery, and 16 (26%) were subsequently classed as insufficient at the six month check.

There was no difference in length of post-operative stay with a median of 2 days for each group (range 0 – 14 days).

5.3.5 Oxford scores

Provider-level PROMs databases were searched for up to 12 months following the last date of surgery, and the NHS number was used to identify and retrieve Oxford scores. There were 75 pre-operative scores (82%) and 52 matched post-operative scores (57%) available for those patients who underwent surgery, although this dropped to 43% if analysis was performed *per protocol* as discussed in sub-section 5.3.1. However, for analysis for the feasibility trial, all available Oxford data was included (figure 5.5).

A significant increase in post-operative Oxford score was seen within all groups following surgery (p <0.001). At baseline, reported median Oxford scores were 14 in those randomised to no treatment, 10.5 in those randomised to treatment, and 19 in those with baseline

sufficiency, with no statistical difference reported between groups (p = 0.069). Following surgery, median scores for the three groups were 33 [no treatment], 38.5 [treatment] and 40 [sufficient] (p = 0.111). The median change between pre- and post-operative scores was 18, 22 and 19 respectively (p = 0.516).

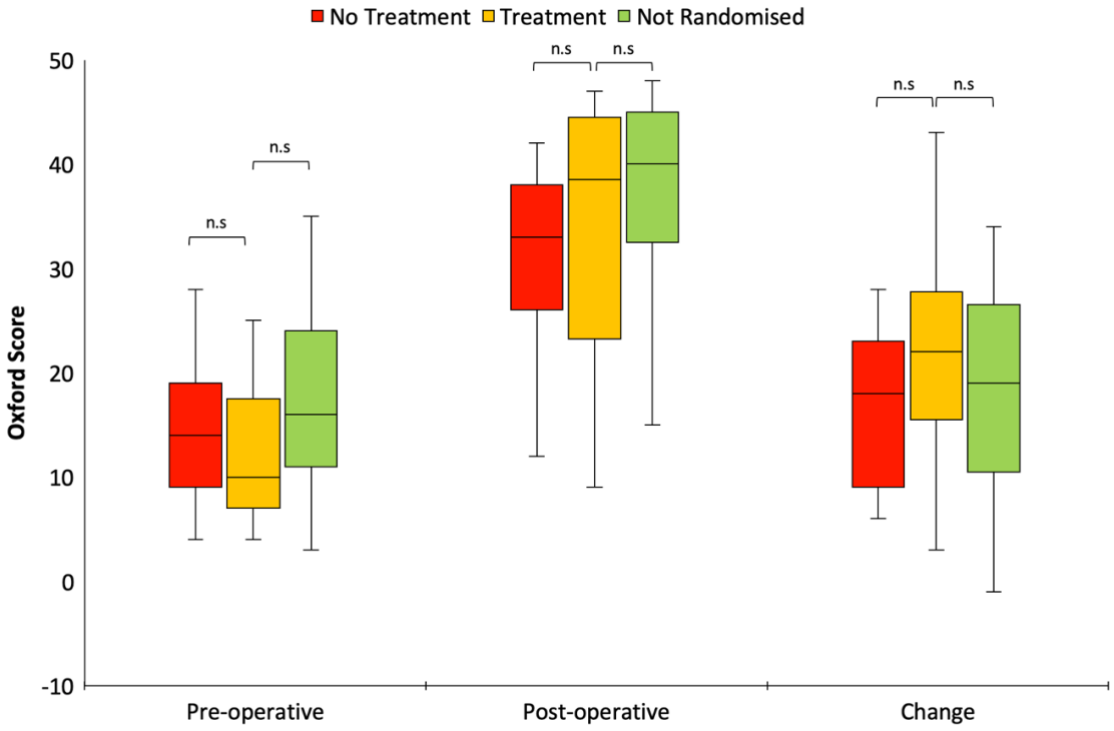


Figure 5.5: Box and whisker plot to demonstrate pre-operative, post-operative and change in Oxford scores *n.s = not significant*

5.3.6 EQ-5D-3L index scores

There were 68 pre-operative scores (74%) and 46 matched post-operative scores (50%) available for those patients who underwent surgery. A significant increase in post-operative EQ-5D index score was seen within all groups following surgery (p < 0.001) (figure 5.6).

At baseline, reported median EQ-5D index scores were 0.222 in those randomised to no treatment, -0.010 in those randomised to treatment, and 0.587 in those with baseline

sufficiency, the treatment group having a significantly lower score than those with sufficiency with *post-hoc* Bonferroni testing (p = 0.011). Following surgery, median scores for the three groups were 0.691 [no treatment], 0.604 [treatment] and 0.760 [sufficient] (p = 0.171). The median change between pre- and post-operative scores was 0.327, 0.401 and 0.309 respectively (p = 0.900).

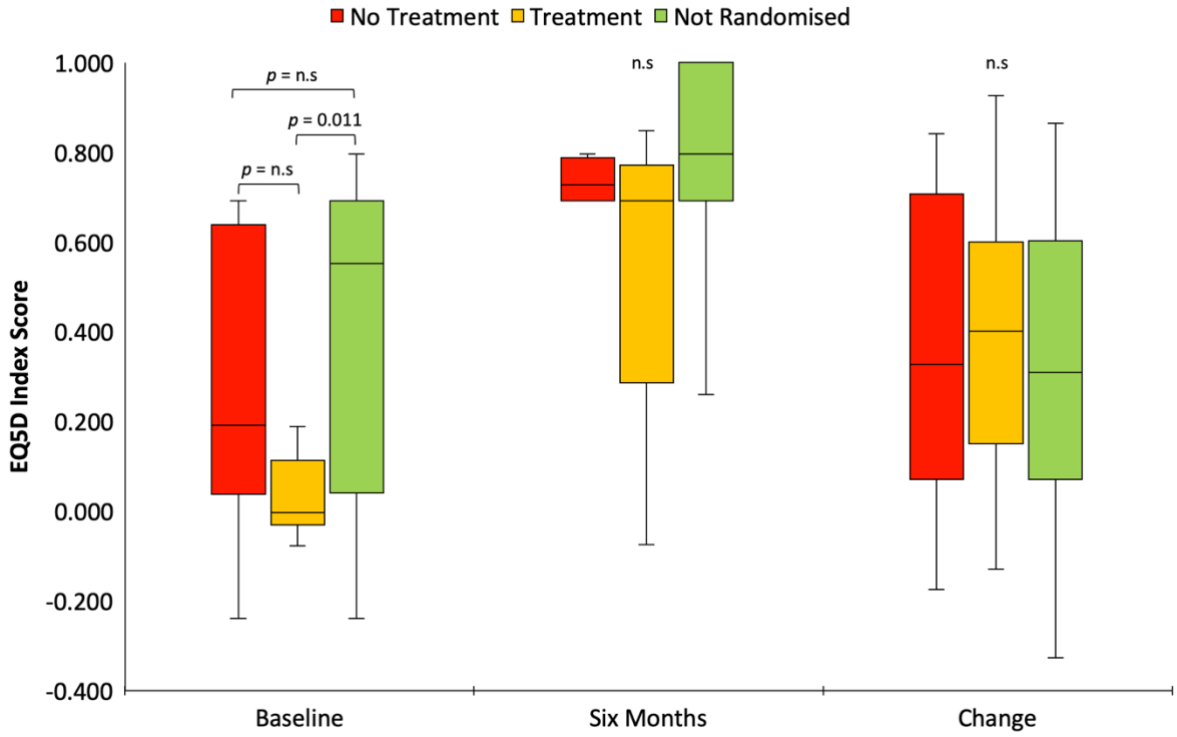


Figure 5.6: Box and whisker plot to demonstrate pre-operative, post-operative and change in EQ-5D-3L index scores *n.s = not significant*

5.3.7 EQ-5D-3L scale scores

There were 65 pre-operative scores (71%) and 46 matched post-operative scores (50%) available for those patients who underwent surgery. A significant increase in post-operative EQ-5D scale score was seen within all groups following surgery (p < 0.001) (figure 5.7).

At baseline, reported median EQ-5D scale scores were 50 in those randomised to no treatment, 40 in those randomised to treatment, and 75 in those with baseline sufficiency. Those with sufficiency had a statistically higher score compared to those randomised to no treatment (p = 0.039), and those randomised to treatment, although this latter difference was no longer significant when adjusted by the Bonferroni correction (p = 0.096). Following surgery, median scores for the three groups were 70 [no treatment], 73 [treatment] and 80 [sufficient] (p = 0.134). The median change between pre- and post-operative scores was 15, 24.5 and 9 respectively (p = 0.125).

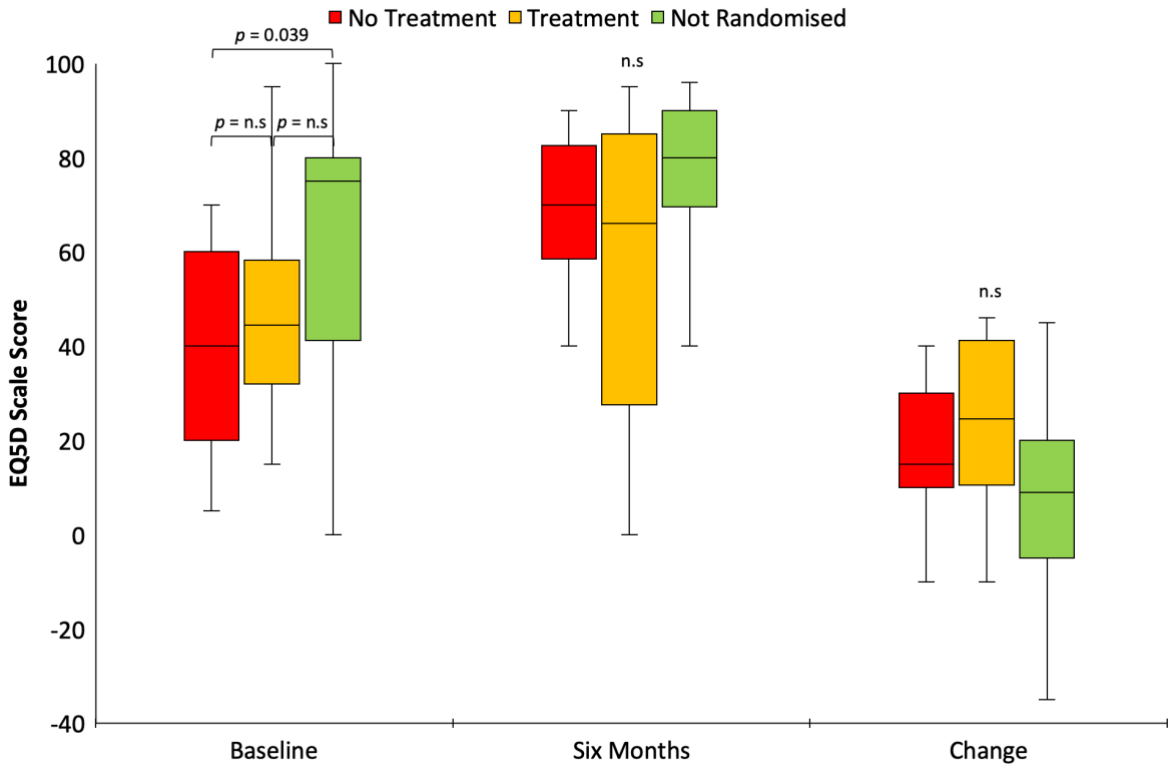


Figure 5.7: Box and whisker plot to demonstrate pre-operative, post-operative and change in EQ-5D-3L scale scores *n.s = not significant*

5.3.8 Lifestyle questionnaire results

For the purposes of analysis, answers to the lifestyle questionnaire at baseline were dichotomised into two responses – 'yes' and 'no'. Higher vitamin D levels at baseline were associated with consumption of alcohol, use of a sunbed, travel overseas or having had a suntan (table 5.2). In the six months prior to surgery, 40% of patients with baseline sufficiency reported having travelled overseas, in comparison to 8% of those with baseline insufficiency. Similarly, 54% of those with sufficiency considered themselves to have had a suntan in the previous six months, compared to 23% of those with insufficiency. The remaining variables were not predictive of vitamin D level, although consumption of oily fish did have a positive effect on vitamin D level (52 vs. 60nmol/L). Suntan and overseas travel were not predictive variables of pre-operative, post-operative or change in Oxford score (all $p > 0.05$).

Table 5.2: Lifestyle factors predictive of higher median vitamin D levels

	No	Yes	p-value
Overseas travel in last 6 months	51nmol/L	77 nmol/L	<0.001
Suntan in last 6 months	48 nmol/L	71 nmol/L	<0.001
Alcohol consumption	51 nmol/L	60 nmol/L	0.03
Use of a sunbed in last 6 months	57 nmol/L	80 nmol/L	0.05

The six month questionnaire, where completed, was used to help postulate why vitamin D levels may have increased in those not randomised to supplementation, e.g. if a patient had been abroad and considered themselves to have had a suntan.

In those randomised to treatment, compliance was recorded and deemed to be excellent. One patient had lost their vitamin D supplements when moving house and had not requested further supplies, and this was reflected in their six month vitamin D level being insufficient at 33nmol/L. The supplements were well tolerated with no reported side-effects.

5.3.9 Adverse events

There were a total of 12 adverse events reported during the trial period. Four were for wound infection, three of which resolved with oral antibiotics and one which required further surgery. One patient reported an allergy to the dressing which resolved once the dressing type was changed, one patient had a prolonged admission due to post-operative pneumonia and hyperkalaemia requiring treatment, and one patient suffered anaphylaxis during induction of anaesthesia. Whilst these adverse events were as a consequence of the patient having surgery, they were deemed unrelated to participation in the trial.

There were a further five reported adverse events which were deemed entirely unrelated to the trial:

- One patient had a new diagnosis of lung cancer and one had a new diagnosis of prostate cancer prior to surgery and so their planned operations were cancelled and they were excluded from the trial.
- One patient had a transient ischaemic attack (TIA) nearly six months following surgery.
- One patient was admitted with, and subsequently died from bowel obstruction, just over five months following surgery.
- One patient was readmitted to hospital with a proximal femoral fracture which required surgery.

There were no cases of hypercalcaemia in those patients randomised to receive vitamin D supplementation.

5.4 Discussion

The VASO feasibility trial has demonstrated that patients are willing to be recruited to a trial investigating the influence of vitamin D supplementation on outcomes following THR/TKR. Vitamin D levels can be measured pre-operatively, and patients can be successfully randomised to receive supplementation. Vitamin D is well tolerated and no adverse events related to its administration were recorded. Supplementation is able to correct insufficiency in a suitable timeframe prior to surgery, and this may improve a patient's 'health gain'.

5.4.1 Benefits of a feasibility study, the use of PPI and trial registration

The NIHR defines feasibility studies as "*pieces of research done before a main study. They are used to estimate important parameters that are needed to design the main study".*[274] These parameters may include willingness of clinicians to recruit patients, willingness of patients to be randomised, recruitment and follow-up rates, and assessment of trial processes. The standard deviation of the outcome measure, along with MCID data can then be used to perform a power calculation.[275,276] Valuable information on such parameters has been obtained from the VASO study, can answer the feasibility questions asked in the methods section 5.2.18. These will be considered in the design of a future study.

The involvement of patients and the public in the design of clinical trials is important, so that researchers are "*experimenting with*" instead of "*experimenting on*" patients.[277] Furthermore, their inclusion increases the rate of patient enrolment and may increase retention, as well as providing a different perspective to make the research more relevant and of better quality.[278,279] The suggestions from members of 'THUG' at the focus group meeting helped with the design and subsequent running of the VASO trial, in particular the wording and layout of the trial documents. Their contribution to this was extremely valuable and well-received.

The VASO trial was registered with ISRCTN, and the protocol was published in advance of the trial completing recruitment. Publishing a trial protocol helps minimise reporting bias by

signalling the study hypothesis and analysis intent in advance, and informs the scientific community of studies which are in progress and therefore minimising duplication.[280] Publishing the VASO protocol allowed for peer-review of the trial and provided external validation of the intended research. It has subsequently generated correspondence from national and international researchers, and the protocol has been cited by others.[222,230,252,281–283]

5.4.2 Trial recruitment and loss to follow-up

In eight weeks, 102 patients were recruited across the two trusts - a recruitment rate of six patients per week per trust. The demographic data of those recruited to the VASO trial was comparable to national data of those undergoing THR/TKR surgery.[127] Based on this recruitment rate, a future trial utilising multiple hospitals and over a longer time period should be able to successfully recruit the number of patients required for a fully-powered trial. Participation in the VASO trial required minimal additional input from a patient perspective and so was perhaps seen as an attractive study to join, which may reflect the successful recruitment phase.

Use of a second trust provided the ability to test the feasibility of randomisation processes, working remotely and with different teams. To ensure the data generated from the VASO trial was representative of real-world practice, it was important to ensure normal systems and processes were evaluated, without influence by me being keen for the study to succeed!

The nine patients who did not have their vitamin D level tested on the day of surgery were all Northumbria patients. This was due to the multi-site nature of the trust, and at times there were no research staff who were involved in the trial available to travel between sites. This logistical aspect should therefore be considered in future studies.

Ten patients (11%) withdrew at the six month mark – they were either not contactable or had asked to cease their involvement with the trial. At this stage following surgery, most patients reported they "*were fine*", had forgotten about their involvement in the trial, and did not wish to travel to the hospital solely for a blood test to be obtained for the purpose the of the trial. This loss-to follow-up reason is therefore an important consideration when planning a future trial.

5.4.3 Vitamin D testing, recorded levels and supplementation

Although LC-MS/MS has been highlighted as being the preferred technique to measure vitamin D levels, and was used for the study reported in chapter three, the two hospital laboratories involved in the VASO trial utilised immunoassays, as is common throughout the NHS. Pragmatically, this method was therefore used for the trial as this reflects 'real-life' practice. Whilst there may be reported differences in the measured vitamin D levels between immunoassays, patients had their repeated measurements at the three time points in the trial at the same hospital laboratory. Furthermore both laboratories have external validation of their analysis, and are deemed proficient through the DEQAS scheme.

Local guidelines were followed for the dose of supplementation given in the study. There were only two patients with deficiency who were randomised to receive the 'loading-dose' of 20,000 IU, and both patients were found to be replete when subsequently checked. A maintenance dose of 1,600 IU was chosen as vitamin D levels are known to drop following surgery, and a general criticisms of negative interventional studies have been that the dose used may have been too low. Encouragingly supplementation in the VASO trial increased vitamin D levels to a sufficient status prior to surgery, and maintained these at six months.

No placebo was used in this study and therefore those patients who received treatment were aware of this. However, those patients who were not randomised to treatment were not aware of their baseline vitamin D status. Additionally, research staff were not blinded to the

treatment arm, although they had no influence on either the surgical intervention or completion of the PROMs questionnaires.

There was no significant variation in average vitamin D levels across the trial at a group-level, in those randomised to no treatment, or with baseline sufficiency. However, at the individual level, some variability in recorded levels was noted, which was enough to cross thresholds for different status definitions, e.g. some were considered insufficient at baseline but sufficient on the day of surgery, and vice-versa. Higher vitamin D levels were seen in those who had travelled abroad or reported having had a suntan. As well as affecting vitamin D level, the ability to travel abroad may be an indicator of better mobility or baseline health status, and may therefore be a confounding variable on the relationship between vitamin D status and PROM score. This should be considered in future trials.

5.4.4 Use of PROMs data

When obtaining patient-level PROMS data through the national programme, the availability of all matched pre- and post-operative questionnaires for the trial was 57%, and this reduced to 43% if the trial was analysed *per protocol*. The completeness of matched data is unlikely to ever reach 100%, due to patients not returning questionnaires, deciding not to proceed with surgery, or their episodes not being linked due to the unavailability of adequate identifying information.[140] This suggests that obtaining adequate patient-level data through the PROMs programme to be used as a trial outcome measure is not feasible. Researchers should therefore consider administering their own questionnaires as part of their study. However, due to the way in which data from the PROMs programme is used, as well as its contribution to the award of best practice tariff payments, researchers cannot interfere with or influence data which contributes to the national programme at six months following surgery.

Only PROMs data which could be linked and identified by NHS number were used in this study, as the use of a unique identifier was felt to prevent potential mismatching of records. NHS

Digital report other ways to identify and match PROMs episodes when the NHS number is missing, including the use of date of birth, sex and postcode, and have provided a summary of matching ranks depending on which data fields are used (table 5.3). Use of these other identifiers, rather than only NHS number, may have increased the availability of PROMs data available.

Table 5.3: Matching ranks for identification and linkage of PROMs data *(from* [140]*)*

Patient matching ranks		
Match rank	**Description**	**Score**
1	Exact match of DOB, SEX, NHSNO and POSTCODE	15
2	Exact match of DOB, SEX, NHSNO	14
3	Partial match of DOB and exact match of SEX, NHSNO and POSTCODE	13
4	Partial match of DOB, and exact match of SEX, NHSNO	12
5	Exact match of POSTCODE and NHSNO	11
6	Exact match of DOB, SEX and POSTCODE (where NHSNO does not contradict the match and DOB is not 1 January and the POSTCODE is not in the 'ignore' list)	10
7	Exact match of DOB, SEX and POSTCODE (where NHSNO does not contradict the match and DOB is not 1 January)	9
8	Exact match of DOB, SEX and POSTCODE (where DOB is not 1 January)	8

When assessing the Oxford score results for the trial, each group had a significant improvement in score following surgery. Although no statistically significant differences were seen between the groups, this feasibility study was not powered to detect a change in score, and so statistical inferences should be minimised. Following surgery, those patients randomised to treatment had a score which was higher than those randomised to no treatment, and this difference was 5.5 points, which is deemed an important difference for the score.[151,284] Furthermore, this group had higher change scores following surgery. A similar pattern was seen for VAS score. These patterns are of interest and therefore require further investigation with adequately powered studies.

The EQ-5D-3L index score for the treatment group (figure 5.6) was lower at baseline compared to the 'no-treatment' group, and significantly lower than the 'not-randomised' groups. This pattern was different that seen in baseline index scores in the retrospective cohort reported in chapter three (figure 3.3). The data were rechecked and no outlying values to account for the difference could be found, and it is likely related to the low number of scores available (n=7). A future trial would be adequately powered to address if this was a real difference.

5.5 Conclusion

A randomised trial to investigate the role of vitamin D supplementation to improve outcomes following THR/TKR is feasible. If a PROM is chosen as an outcome measure, then to improve the response rate this should be collected specifically as part of the trial and not collected through the national PROMs programme. This, alongside other considerations for the design of a future large trial to be run in the NHS are discussed in more detail in chapter six.

Chapter 6: Delivery of a fully-powered vitamin D trial in UK elective orthopaedic THR/TKR practice; points to consider

A recent Government policy paper highlights clinical research to be "*the single most important way in which we improve our healthcare*".[285] To address this, the authors suggest "*embedding clinical research at the heart of patient care across the NHS, making participation as easy as possible and ensuring all health and care staff feel empowered to support research*".

To help identify relevant areas for research, The James Lind Alliance (JLA) brings carers, patients and clinicians together on an equal footing in a 'Priority Setting Partnership' (PSP) with the aim of identifying areas of uncertainty for a specific health problem. All PSP members work to prioritise any identified uncertainties to create a 'top ten' list of areas for research which are important to all groups.

One of the top ten questions in 'hip and knee replacement for osteoarthritis', raised by a JLA PSP was "*In people with osteoarthritis, what are the pre-operative predictors of post-operative success (and risk factors of poor outcomes)?"* Another prioritised question, although not ranked in the top ten, was "*What is the optimum pre-operative management for the best outcome for knee/hip replacement for people with osteoarthritis?"* [286]

There is an expected exponential increase in the need for joint replacement surgery due to population growth, ageing and rising obesity rates. Predictions suggest that by 2035 more than 400,000 THR and more than 1,000,000 TKR procedures will be performed per year in the UK, depending on the model of prediction used,[287] and that the incidence of revision surgery is set to rise by more than 300%.[288] Firstly, this highlights the need for research which seeks to optimise patients prior to surgery, to help reduce the risk of complications for both patient benefit and to reduce the financial implications for, and service demands of, the NHS. Secondly, it highlights that there will be a growing pool of patients who can be approached to participate in research trials as part of their NHS care.

As has been written in the preceding chapters, optimising a patients' vitamin D status prior to surgery may improve post-operative outcomes, but further adequately powered randomised trials are required to determine this. The key points for delivery of such a trial in the NHS, using the results of the VASO feasibility trial in chapter five, are considered below.

6.1 Identifying a research trial population

In orthopaedic trauma surgery, a UK-based multi-centre cohort study was successfully set-up to evaluate outcomes in those patients undergoing surgery for a hip fracture.[289] This 'World Hip Trauma Evaluation (WHiTE) framework allows for the delivery of embedded trials to evaluate different interventions to improve outcomes, with such trials either having successfully completed[266,290] or still in progress.[291,292] Acceptance of the orthopaedic community to participate in and recruit to such multi-centre trials is growing, and has contributed to the success of similar pragmatic trials.

Using a comparable model in the elective surgery setting, forty NHS hospital trusts in England enrolled in a trial to assess the impact of screening for and treating pre-operative anaemia and decolonisation for Methicillin Sensitive *Staphylococcus aureus* (MSSA) prior to THR/TKR surgery.[293] This new network of orthopaedic units have established communication pathways and are 'research-ready' and could therefore be approached to participate in an analogous trial to determine if there is benefit in screening for and treating vitamin D insufficiency prior to THR/TKR.

The VASO trial recruited patients ahead of the anticipated timeline, and may reflect the simple study design which required little additional involvement from patients. This recruitment rate is encouraging for future trials, particularly as it has been shown that the majority of RCTs have difficulty in recruiting sufficient numbers of patients.[294]

6.2 Trial sample size

The VASO trial sought to assess the feasibility of using the OHS and OKS as the primary measure, with the EQ-5D-3L as a secondary outcome. Using the data from section 5.3.5, the group randomised to treatment with vitamin D had an average post-operative Oxford score of 38, with a standard deviation of 13. Assuming that pre-operative supplementation does confer a benefit in relation to post-operative outcome, and using the minimal reported difference in Oxford score of five points to show a significant difference when comparing two groups,[151] then 146 patients per group would be required for a trial with 90% power and a statistical significance of p =0.05, based on the feasibility data. Anticipating a loss to follow-up rate of 20%, then 365 patients would need to be recruited to obtain this. However, as the results from chapter three indicate a poorer outcome was only associated with vitamin D levels <25nmol/L, then the trial should recruit 365 patients with baseline deficiency. The local population data, reported in chapter two, indicates the proportion of patients with deficiency ranged from 13 to 32%, with an average of 20% across the year. Assuming this, 1,825 patients in total would need to be recruited to obtain the required sample size powered on patients with deficiency. As seventy five per cent of patients who were invited to participate in the VASO trial were actually recruited, then 2,433 patients undergoing THR/TKR would need to be screened. This represents 1.2% of the average number of these procedures performed each year in England and Wales, and therefore suggests this would be a realistically obtainable study size.

If the EQ-5D-3L index scale was instead chosen as the primary outcome score because of its general assessment of quality of life, then 4,000 patients would need to be recruited, based on an MCID of 0.074 and an estimated standard deviation of 0.30, considering the screening and loss to follow-up data percentages above.

6.3: Online recruitment and trial document completion

In response to increasing patient demands and the COVID-19 pandemic, healthcare providers now provide online consultations, offering convenience to both patients and professionals.

Harnessing such digital methods for a clinical trial may improve patient engagement and recruitment rates, as patients may be able to complete trial processes from their own home. In the VASO trial, 15 of 137 patients who were screened were not recruited due to either a lack of research nurse availability in clinic, or that patients were limited by time and could not stay to complete baseline questionnaires. Similarly, there was a loss to follow-up of 11% at six months as patients did not want to attend the hospital solely for a research appointment.

Online recruitment and the electronic completion of required trial paperwork by patients has now been successfully utilised in studies running in the UK.[295,296] Offering the option for remote or online study 'visits' in a future vitamin D trial may help reduce the losses observed in the VASO trial.

6.4 Blood testing

To reflect clinical practice, vitamin D samples in the feasibility trial were analysed at each hospital trust rather than centrally, despite both using different immunoassay methods. Rolling out a trial at multiple sites across the UK using the same approach would therefore, whilst remaining pragmatic, increase the variability in measured vitamin D levels due to the different analytical methods used – either the more common immunoassay or the less-common but more accurate mass spectrometry technique. It may therefore be scientifically more rigorous in the setting of a clinical trial if all patients had their vitamin D level analysed using the same method.

Black Country Pathology Services offers a postal vitamin D testing service for the public from four centres in the West Midlands, as part of Sandwell and West Birmingham NHS Trust.[297] A blood spot sample kit is posted to a patient's house and they obtain a small sample of blood from the finger, similar to a diabetic patient measuring their own blood glucose level. The sample is then returned to the laboratory via post and using LC-MS/MS techniques, total as well as vitamin D2 and D3 levels are reported within 10-days. The service has been in place

since 2011, has certified proficiency as part of the DEQAS validation scheme, and also offers testing for university and research teams.

The benefits of using such a service for the definitive trial include that a single laboratory and single analysis method is used, and as mass spectrometry is considered the gold standard technique, avoids the need for immunoassay testing common to most NHS laboratories. Furthermore it means that patients do not have to travel to hospital for additional testing procedures for the trial, nor are research nurse staff required to obtain blood samples at each hospital site. This means that, along with online recruitment and electronic questionnaire completion, a trial could be run remotely and reduces the burden on patients travelling to hospital solely for the purposes of research. This approach may prevent the loss to follow-up of 11% which was seen at the six month timepoint in the VASO trial.

It would be scientifically more rigorous for patients to have their vitamin D level checked at each key trial point to determine its relationship to the outcome variable chosen, as per the VASO study. However, repeat testing causes additional costs and was a source of loss to follow-up. Furthermore, the VASO trial demonstrated supplementation did increase measured vitamin D levels in patients, which reflects the wider literature. Therefore, baseline testing may be all that is required for a definitive study, with predictive models used to estimate vitamin D status at different timepoints if needed.[88]

6.5 Supplementation

Public Health England advice is that all adults should consider taking vitamin D supplements during winter months, and all year for those groups deemed at high-risk of deficiency.[213] This advice is now more widely acknowledged since its introduction in 2016, and the role of vitamin D supplementation is very topical since a possible association between vitamin D and COVID-19 was raised.[298,299] At present, vitamin D is not routinely checked pre-operatively in our unit, and there is no current evidence that normalising this pre-operatively is of benefit. However,

whilst the ethics committee who reviewed the VASO trial protocol in 2017 were happy that patients could be randomised to receive either supplementation or no supplementation at that time, it is now questionable whether having a 'no supplementation arm' would be ethical, particularly during winter months, and therefore ways to address this could be considered.

All patients recruited to the trial could be given a baseline dose of 400 units which would satisfy the ethical concerns of randomising to 'no treatment'. Those who were randomised to the treatment arm could then be given a higher 'treatment' dose, for example 1,600 units as per the VASO trial, or even higher up to the safe tolerable upper limit of 4,000 units per day. The current evidence for 400 units per day is for the maintenance of bone health in the general population, not for extra-skeletal benefits, nor for those undergoing surgery where vitamin D levels are known to drop post-operatively. Furthermore, during a patients' recovery following THR/TKR surgery, they may spend less time outdoors and therefore cutaneous synthesis will be limited. Although a recent study in older patients has not shown any benefit to higher doses when using bone mineral density change as the outcome,[300] it may be that in a group of surgical patients who are at risk of post-operative insufficiency, a higher dose than 400 units is required to have an effect.

The use of a different trial design could also be considered to overcome the ethical issue of having a 'no treatment' arm, such as a 'trial within cohort' study.

6.6 Trials within Cohorts (TwiC) Study Design

'Trials within Cohorts' is a relatively new approach in which to run randomised clinical trials.[301] Patients with a characteristic of interest, e.g. those undergoing THR/TKR surgery at centres across the UK are recruited to form a cohort. From this, patients who fulfil the eligibility criteria for a potential trial are identified and randomised to either receive the trial intervention or to continue as part of the cohort. Those patients randomised to participate in the trial are contacted to provide their consent for the trial intervention, whilst those who are

randomised to not receive the trial intervention are unaware of this and continue with routine follow-up as part of the overall cohort study. They form the 'control group' to which the intervention group are compared (figure 6.1).

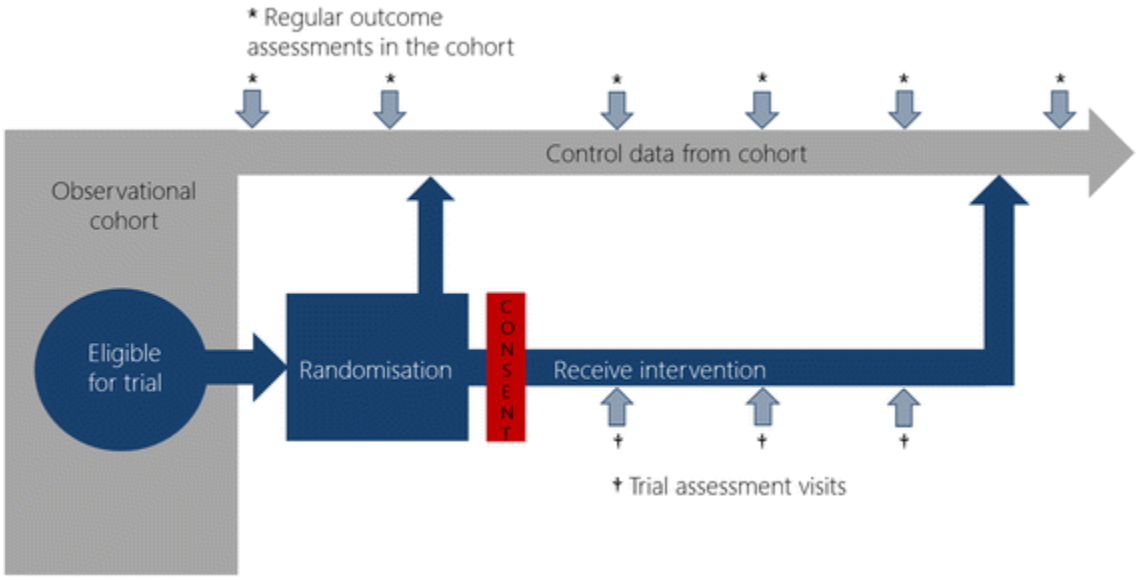


Figure 6.1: Diagrammatic representation of a Trials within Cohorts (TwiC) Study Design *(from figure 1 in* [301]*)*

A TWiCs approach could therefore be used within a national cohort of THR/TKR patients. All patients within the cohort would complete baseline demographic data, pre- and post-operative PROM scores, as well as lifestyle questionnaires, with particular focus on activity and sun-exposure which were deemed to significantly influence vitamin D levels in the VASO trial. Half of the patients in the cohort would be randomised to the trial intervention which would be to have their vitamin D level checked, and supplementation offered accordingly. The other half remain as the control group who were not randomised to have their vitamin D level checked, but continue to provide follow-up data as part of the cohort.

6.7 Treat all or treat only insufficient?

It has been reported that vitamin D levels drop following surgery[69,70] and, as was shown in chapter five, some patients who were sufficient at baseline became insufficient on the day of surgery. Therefore, if optimising vitamin D levels prior to THR/TKR is found to be significant, then it would be important to know if it is only those with baseline deficiency who benefit from supplementation, or whether all patients irrespective of baseline status do. Using a TWiCs model, half of the cohort could be randomised to receive supplementation and the other half of the cohort remain as the control group.

This approach would help guide clinical pathways for an orthopaedic surgeon in the clinic when listing patients for THR/TKR surgery to decide how to proceed. If supplementation only benefits those with baseline deficiency, then patients will need a baseline blood test to determine their vitamin D status. However, if pre-operative treatment is shown to benefit all who take it, then the surgeon could advise patients to start this and avoid the need for testing. Future trials should therefore consider this approach, although must ensure it is powered based on the number of patients with deficiency.

6.8 PROMS questionnaire delivery

The VASO trial had a response rate of 57% when obtaining patient-level PROM scores through the national PROMS programme. This data reflects those patients who had completed and returned both the pre-operative questionnaires (Q1) and the post-operative questionnaires (Q2) and that these two response events could be matched by NHS number. Most recent data from NHS Digital for the financial year 2019-2020 indicates matched response rates of 79.6% for Northumbria Trust, and 62.8% for South Tees, compared to an England-average of 64.4%.[302] A recent systematic review has concluded that there is an ongoing downward trend in patient response rates as part of cohort or registry-based studies and that this needs to be addressed.[303] Therefore using outcome data obtained through the national PROMs programme may not be the most reliable method to use as an outcome measure for a clinical trial.

In a trial comparing the type of bandage used following TKR surgery at 26 NHS hospital sites, with a patient demographic similar to those in the VASO study, the authors report a response rate of >85% for the completion of post-operative questionnaires at 12 months.[304,305] These questionnaires were posted directly by the study team, and were not part of the national PROMs programme.

Two SWATs (Study Within A Trial) have explored the impact of including a pen with the questionnaire,[304] or sending personalised text messages[306] in an attempt to improve response rates. Inclusion of a pen increased the response rate to 89%, as well as increasing the completion rate and response time significantly ($p < 0.01$).[304] There was no difference in the response rate if a text message reminder sent to patients was personalised or not. Similar SWATs in other trials to explore ways to boost recruitment or minimise drop-out rates have noted handwriting the name of the trial participant on an invitation letter[307] or offering a small financial incentive,[308] has no effect on overall recruitment rates. The inclusion of a pen in postal questionnaires sent by the trial team should therefore be considered to maximise response rates in a future trial, if physical copies of questionnaires are to be completed rather than digital versions.

6.9: The impact of COVID-19 on research provision

During the first-wave of the COVID-19 pandemic, in line with the NHS response to pause routine clinical services, research nurses were either redeployed to provide clinical support, or were allocated to deliver prioritised COVID-19 related studies. However, previously paused non-COVID-19 studies have now started to resume, and the NIHR have developed a 'Restart Framework' to help guide this.[309]

The Framework includes twelve guiding principles, and the following is taken from this:

"COVID-19 related research will form a significant and high-profile component of the NIHR portfolio for the foreseeable future and COVID-19 Urgent Public Health Research studies will continue to be prioritised. Any further surges in COVID-19 could affect the research system again and the potential for a 're-pause' must be considered by sponsors, funders, Chief Investigators and local sites in the plans to restart individual studies".

Therefore, non-COVID-19 studies, such as a trial investigating the influence of vitamin D on outcome following arthroplasty, may not be able to rely on NIHR research nurse support, and so alternative sources for trial delivery should be explored.

6.10: Surgical trainees and trainee research collaboratives to help deliver research studies

Surgical trainees are required to "*demonstrate evidence of appropriate knowledge of research principles and concepts and the translation of research into practice*", as per the General Medical Council's Generic Professional Capabilities Framework,[310] and are encouraged to participate in collaborative trials.[311]

The West Midlands Research Network has spearheaded collaborative trainee research since 2007, and in 2013 the Collaborative Orthopaedic Research Network (CORNET) was the first to be set up in orthopaedic surgery. There are now a number of other regional Trauma and Orthopaedic trainee led research collaboratives emerging throughout the UK. Regional networks allow trainees to run multi-centre projects within their training programme with the benefit of not losing continuity due to placement rotation, whilst engaging with the local research infrastructure such as the Clinical Research Network. Recognition of participation in collaborative projects often includes authorship on any published or presented work, typically under a collaborative name, and guidance has been published suggesting ways to recognise different levels of contribution.[312] In addition to the participation in trainee research collaboratives, the Certificate of Completion of Training (CCT) requirements in T&O encourage

trainees to recruit patients to trials. Furthermore, publication as a collaborative author is now a recognised achievement which contributes towards the completion of surgical training. Inclusion of trainees in a future multicentre trial should therefore be encouraged to aid recruitment and to help future surgeons develop research skills.

6.11: Associate PI Scheme – developing research leaders of the future

The Associate Principle Investigator (API) scheme is endorsed by the National Institute for Health Research (NIHR) and a number of medical Royal colleges. Its aim is to develop 'research leaders' of the future by recruiting trainee surgeons (as well as other medical, nursing and allied health professionals) to work alongside the principle investigator (PI) in running a clinical trial at a local level. As trainee-surgeons may be the first health professional to interact with a patient in a clinical setting, then this could be the best opportunity to develop rapport with an eligible patient to discuss a clinical trial and encourage their participation in this. Furthermore, this reduces the workload or requirements of research nursing staff.

The benefit of the scheme to the API is that they have the opportunity for more in-depth involvement in how a clinical trial is run at a local level, without the responsibility for these delegated tasks which remains with the PI. The API takes on roles such as identifying suitable patients and leading recruitment, obtaining consent, ensuring local study training requirements are in place, and assisting with completion of trial documentation. This is a more involved role than trainees who 'only' recruit patients to obtain collaborative authorship, and so to recognise this the NIHR provides a certificate to acknowledge the trainee's role. This can then be used as evidence for them having participated in research, as well as demonstrating leadership and management skills.

A large-scale vitamin D trial similar to VASO would be simple to run and is not resource- or time-intensive. It may therefore be a suitable trial to encourage more trainees to take part in the API programme, helping to develop future research leads.

Chapter 7: Conclusion

Vitamin D continues to receive extensive interest, and its influence on a diverse array of reported endpoints, beyond skeletal health, is now widely published. Its potential benefits have fuelled a dedicated lifestyle industry with the offering of vitamin D lamps, enhanced foods, and supplementation. Nevertheless, opinion varies in the scientific community on the true influence of the vitamin on reported outcomes, what constitutes a normal level, or what dose or route of supplementation should be offered. Results of trials where supplementation is given to determine the influence on a range of outcome variables are mixed, although negative trials have been criticised for not including enough patients with deficiency. Further research to address these issues is required, when considering vitamin D in general.

Similarly, there have been mixed conclusions in studies specific to arthroplasty surgery, although a recurring theme is that vitamin D insufficiency has been linked to longer length of stay, increased complication rates, lower functional scores and poorer patient reported measures following arthroplasty surgery. Given the prevalence of vitamin D insufficiency, the ease in which it can be corrected, and the anticipated increase in the number of joint replacements performed, its association with the reported adverse outcomes following THR/TKR merits further interest.

The work which has contributed to this PhD is multi-faceted, and adds to the current evidence base regarding the relationship between vitamin D status and post-operative outcomes. In the largest study to date, and the first to use the Oxford hip and knee scores as an outcome measure, lower PROMs scores were seen at baseline and following surgery in those with deficiency. As this is an observational finding, causation cannot be proved, and so information from prospective interventional studies is required to confirm this. However, the systematic review has shown that current evidence for the peri-operative use of vitamin D supplements in THR/TKR patients is limited, with the publication of only three inadequate studies. This was the first review of whether offering supplementation to correct deficiency peri-operatively

improves outcomes following THR/TKR, as previous reviews have focussed only on the association between a patient's measured vitamin D status and their reported outcome. Well-designed randomised trials are therefore recommended to address this.

The trends reported in local population vitamin D levels and testing is not a novel finding, but this was important to enable comparison to published data and to validate the use of the population served by Northumbria Healthcare to run a clinical trial. The VASO trial is the first to investigate the feasibility of randomising patients to receive supplementation for insufficiency, prior to THR/TKR. Whilst this was designed to analyse trial processes and so was not statistically powered, the pattern of higher scores seen in those who were given supplementation compared to those randomised to no treatment is promising, and adequately powered trials should be utilised to confirm this in the future. Authors of such trials should consider the discussion points and suggestions offered in light of the VASO feasibility results, particularly given the current challenges faced in the NHS.

Appendix

Appendix A: Oxford Hip Score[147]

Problems with your hip

During the past 4 weeks..

✓tick one box for every question.

1. ***During the past 4 weeks*****.....**

How would you describe the pain you usually had from your hip?

None	Very mild	Mild	Moderate	Severe
❑	❑	❑	❑	❑

2. ***During the past 4 weeks*****.....**

Have you had any trouble with washing and drying yourself (all over) because of your hip?

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

3. ***During the past 4 weeks*****.....**

Have you had any trouble getting in and out of a car or using public transport because of your hip? *(whichever you tend to use)*

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

4. ***During the past 4 weeks*****.....**

Have you been able to put on a pair of socks, stockings or tights?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

5. ***During the past 4 weeks*****.....**

Could you do the household shopping on your own?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

6. ***During the past 4 weeks*****.....**

For how long have you been able to walk before pain from your hip becomes severe? *(with or without a stick)*

No pain/ More than 30 minutes	16 to 30 minutes	5 to 15 minutes	Around the house only	Not at all -pain severe on walking
❑	❑	❑	❑	❑

P.T.O./

During the past 4 weeks...

✓tick one box for every question

7 ***During the past 4 weeks*.....**

Have you been able to climb a flight of stairs?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

8 ***During the past 4 weeks*.....**

After a meal (sat at a table), how painful has it been for you to stand up from a chair because of your hip?

Not at all painful	Slightly painful	Moderately painful	Very painful	Unbearable
❑	❑	❑	❑	❑

9 ***During the past 4 weeks*.....**

Have you been limping when walking, because of your hip?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
❑	❑	❑	❑	❑

10 ***During the past 4 weeks*.....**

Have you had any sudden, severe pain - 'shooting', 'stabbing' or 'spasms' - from the affected hip?

No days	Only 1 or 2 days	Some days	Most days	Every day
❑	❑	❑	❑	❑

11 ***During the past 4 weeks*.....**

How much has pain from your hip interfered with your usual work *(including housework)*?

Not at all	A little bit	Moderately	Greatly	Totally
❑	❑	❑	❑	❑

12 ***During the past 4 weeks*.....**

Have you been troubled by pain from your hip in bed at night?

No nights	Only 1 or 2 nights	Some nights	Most nights	Every night
❑	❑	❑	❑	❑

Appendix B: Oxford Knee Score[148]

PROBLEMS WITH YOUR KNEE

During the past 4 weeks..

✓tick one box for every question

1 *During the past 4 weeks.....*

How would you describe the pain you usually have from your knee?

None	Very mild	Mild	Moderate	Severe
❑	❑	❑	❑	❑

2 *During the past 4 weeks.....*

Have you had any trouble with washing and drying yourself (all over) because of your knee?

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

3 *During the past 4 weeks.....*

Have you had any trouble getting in and out of a car or using public transport because of your knee? (whichever you would tend to use)

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

4 *During the past 4 weeks.....*

For how long have you been able to walk before pain from your knee becomes **severe**? (*with or without a stick)*

No pain/ More than 30 minutes	16 to 30 minutes	5 to 15 minutes	Around the house only	Not at all - pain severe when walking
❑	❑	❑	❑	❑

5 *During the past 4 weeks.....*

After a meal (sat at a table), how painful has it been for you to stand up from a chair because of your knee?

Not at all painful	Slightly painful	Moderately painful	Very painful	Unbearable
❑	❑	❑	❑	❑

6 *During the past 4 weeks.....*

Have you been limping when walking, because of your knee?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
❑	❑	❑	❑	❑

/P.T.O

During the past 4 weeks...

✓tick one box for every question

7 *During the past 4 weeks.....*

Could you kneel down and get up again afterwards?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

8 *During the past 4 weeks.....*

Have you been troubled by pain from your knee in bed at night?

No nights	Only 1 or 2 nights	Some nights	Most nights	Every night
❑	❑	❑	❑	❑

9 *During the past 4 weeks.....*

How much has pain from your knee interfered with your usual work *(including housework)*?

Not at all	A little bit	Moderately	Greatly	Totally
❑	❑	❑	❑	❑

10 *During the past 4 weeks.....*

Have you felt that your knee might suddenly 'give way' or let you down?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
❑	❑	❑	❑	❑

11 *During the past 4 weeks.....*

Could you do the household shopping on your own?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

12 *During the past 4 weeks.....*

Could you walk down one flight of stairs?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

Appendix C: EQ-5D-3L Score[154]

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑

I have some problems in walking about ❑

I am confined to bed ❑

SELF-CARE

I have no problems with self-care ❑

I have some problems washing or dressing myself ❑

I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems with performing my usual activities ❑

I have some problems with performing my usual activities ❑

I am unable to perform my usual activities ❑

PAIN / DISCOMFORT

I have no pain or discomfort ❑

I have moderate pain or discomfort ❑

I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

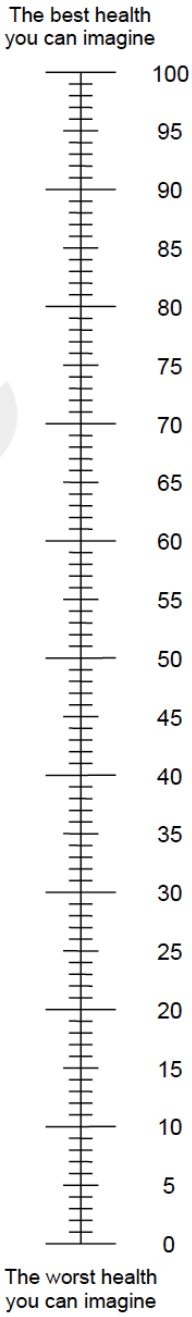
I am not anxious or depressed ❑

I am moderately anxious or depressed ❑

I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Please mark an X on the scale to indicate how your health is TODAY.
- Now, write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Appendix D: Confirmation of trial status from MHRA

From: **Clinical Trial Helpline** ctdhelpline@mhra.gsi.gov.uk
Subject: RE: for Scope review - Vitamin D and Arthroplasty Surgery Outcomes
Date: 22 December 2016 at 09:44
To: Rory Morrison rorymorrison@doctors.org.uk, Clinical Trial Helpline ctdhelpline@mhra.gsi.gov.uk

CH

Notification that a Clinical Trial Authorisation (CTA) is not required

Dear Mr Morrison,

Thank you for your email dated 12th December 2016.
I can confirm that your proposal is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC and no submission to the Clinical Trials Unit at the MHRA is required.
Kind regards

Clinical Trial Helpline

MHRA

Medicines & Healthcare products
Regulatory Agency

From: Rory Morrison [mailto:rorymorrison@doctors.org.uk]
Sent: 12 December 2016 08:50
To: Clinical Trial Helpline
Subject: for Scope review - Vitamin D and Arthroplasty Surgery Outcomes

Hello,

Please find attached our protocol for review and clarification of status. We believe that the use of Vitamin D in our trial is based on current clinical practice. We'd be grateful if you could clarify whether this is classified as a non-CTIMP study.

Many thanks,
Rory Morrison

Appendix E: Pre-clinic invitation letter

Northumbria Healthcare NHS
NHS Foundation Trust

Pre-clinic Information Letter Regarding VASO Trial

Address:

Date:

Hello,

Your GP has referred you to the orthopaedic clinic, and from the referral letter, it may be that you are a potential candidate for joint replacement surgery. This can only be clarified when you are seen face-to-face in the clinic.

However, we wanted to inform you, before you attend the clinic, about a trial which is currently running in the Trust. This trial, called the VASO trial (Vitamin D and Arthroplasty Surgery Outcomes) is to investigate the influence of Vitamin D on hip or knee replacement surgery. We have included a Patient Information Sheet so you can read more about this.

If joint replacement surgery is discussed at your appointment, then this trial may be relevant to you. We would discuss this in more detail at the time of your appointment.

We look forward to seeing you soon.

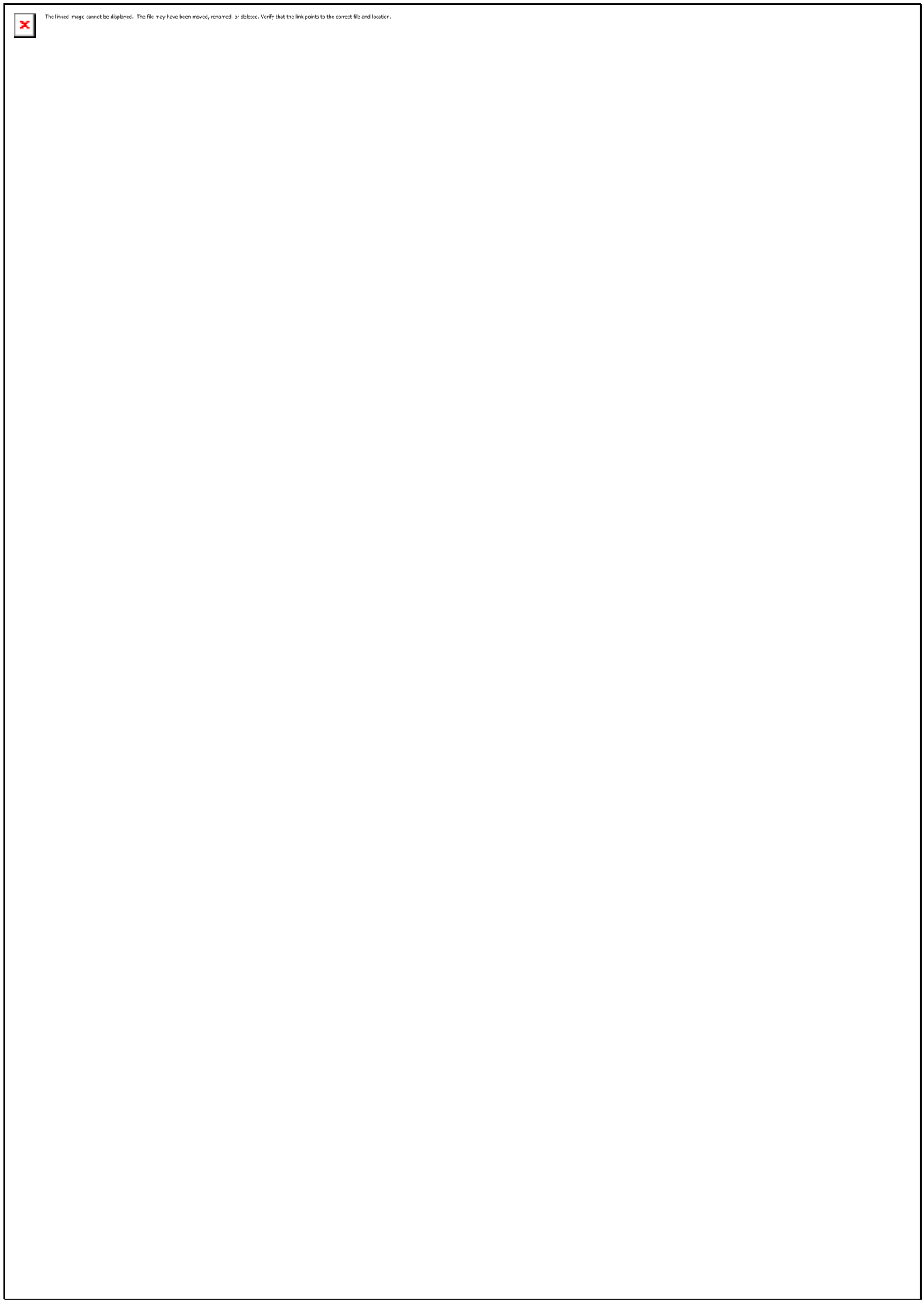
Yours sincerely,

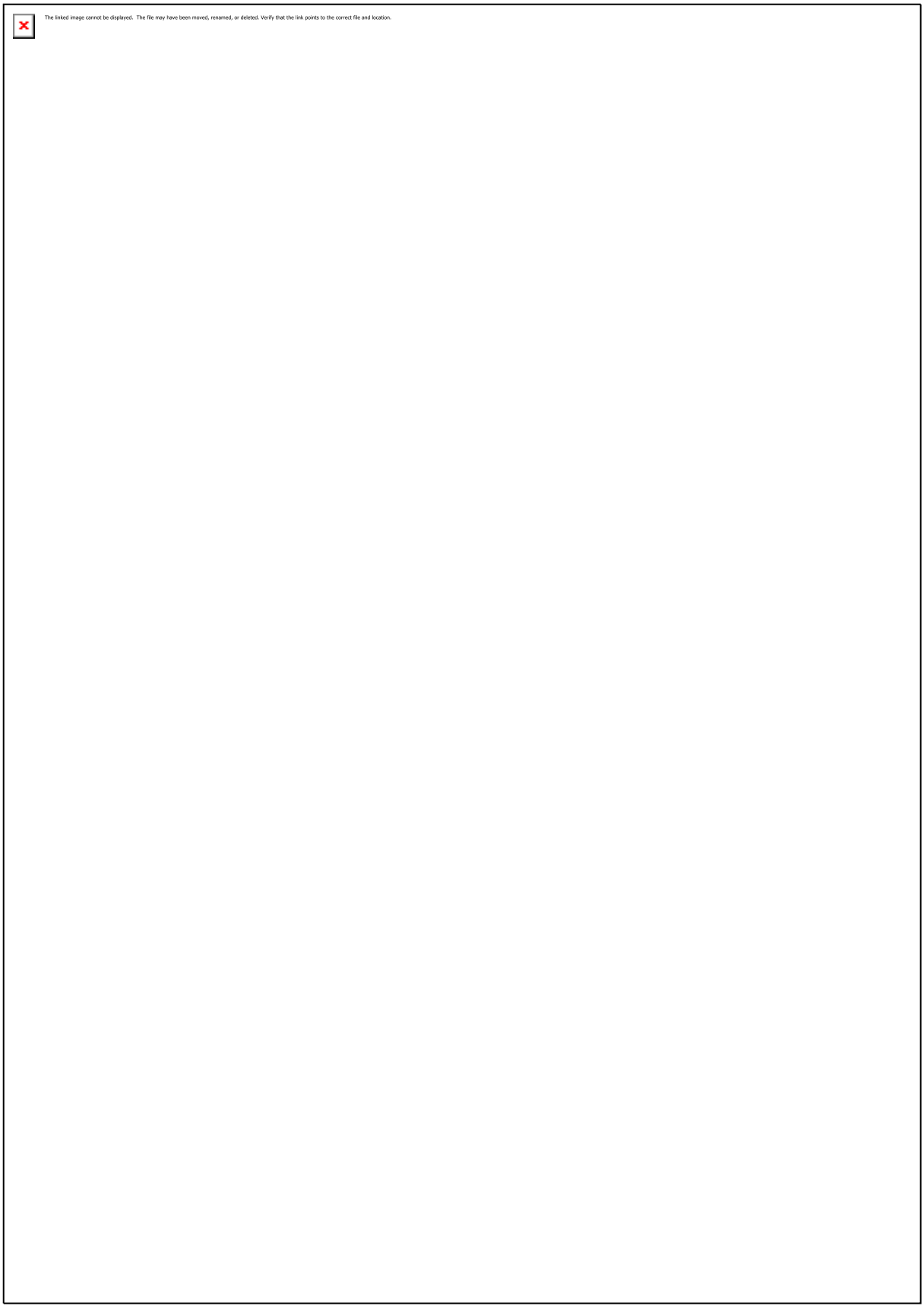
Mr Mike Reed
Chief Investigator,
Consultant Orthopaedic Surgeon, Honorary Senior Lecturer Newcastle University

Mr Rory Morrison
Co-investigator, Orthopaedic Registrar

VASO Trial
Research and Development Department, North Tyneside General Hospital, Rake Lane, North Shields, Tyne and Wear, NE29 8NH. 0191 2934087

Appendix F: VASO trial patient information sheet

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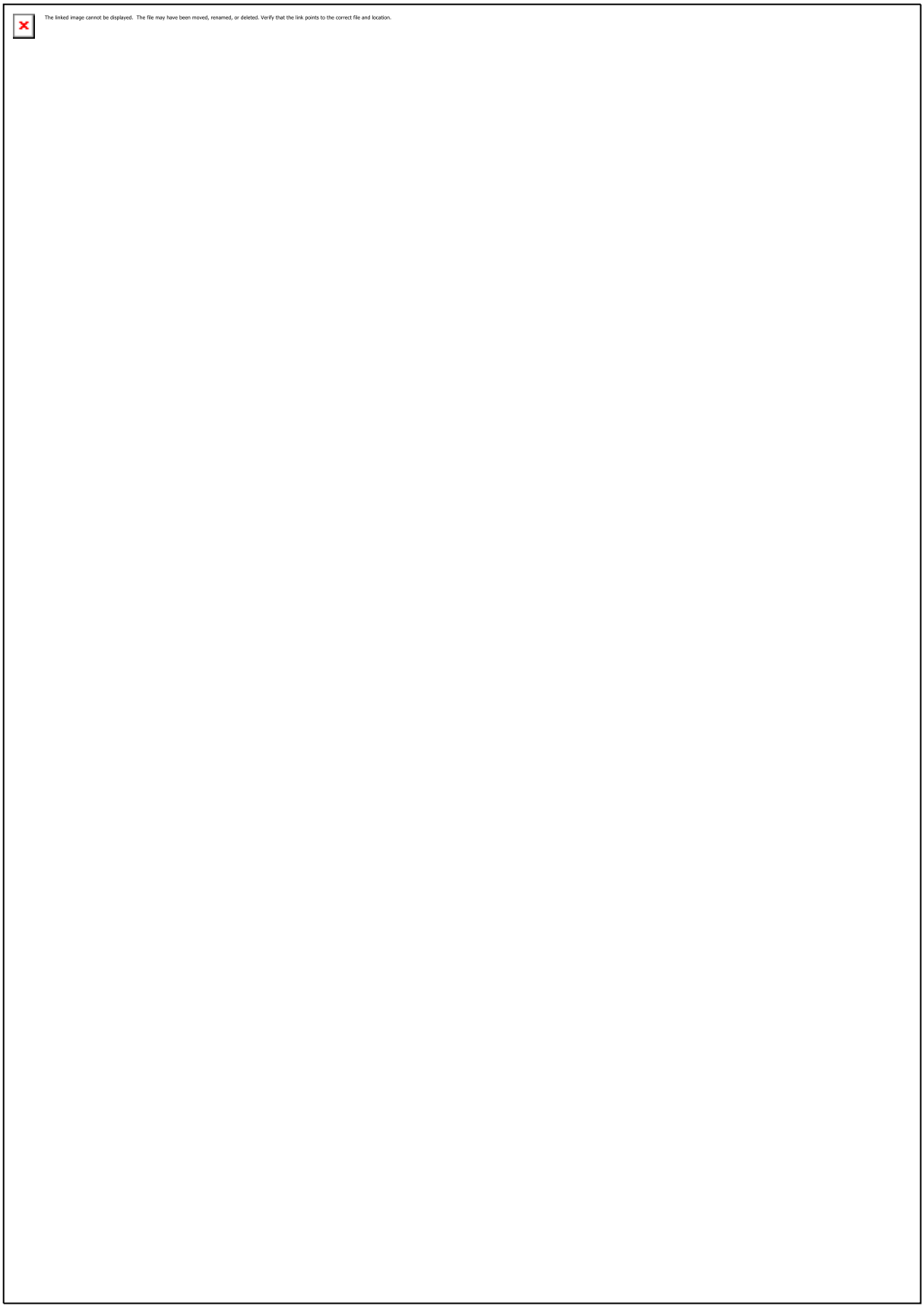
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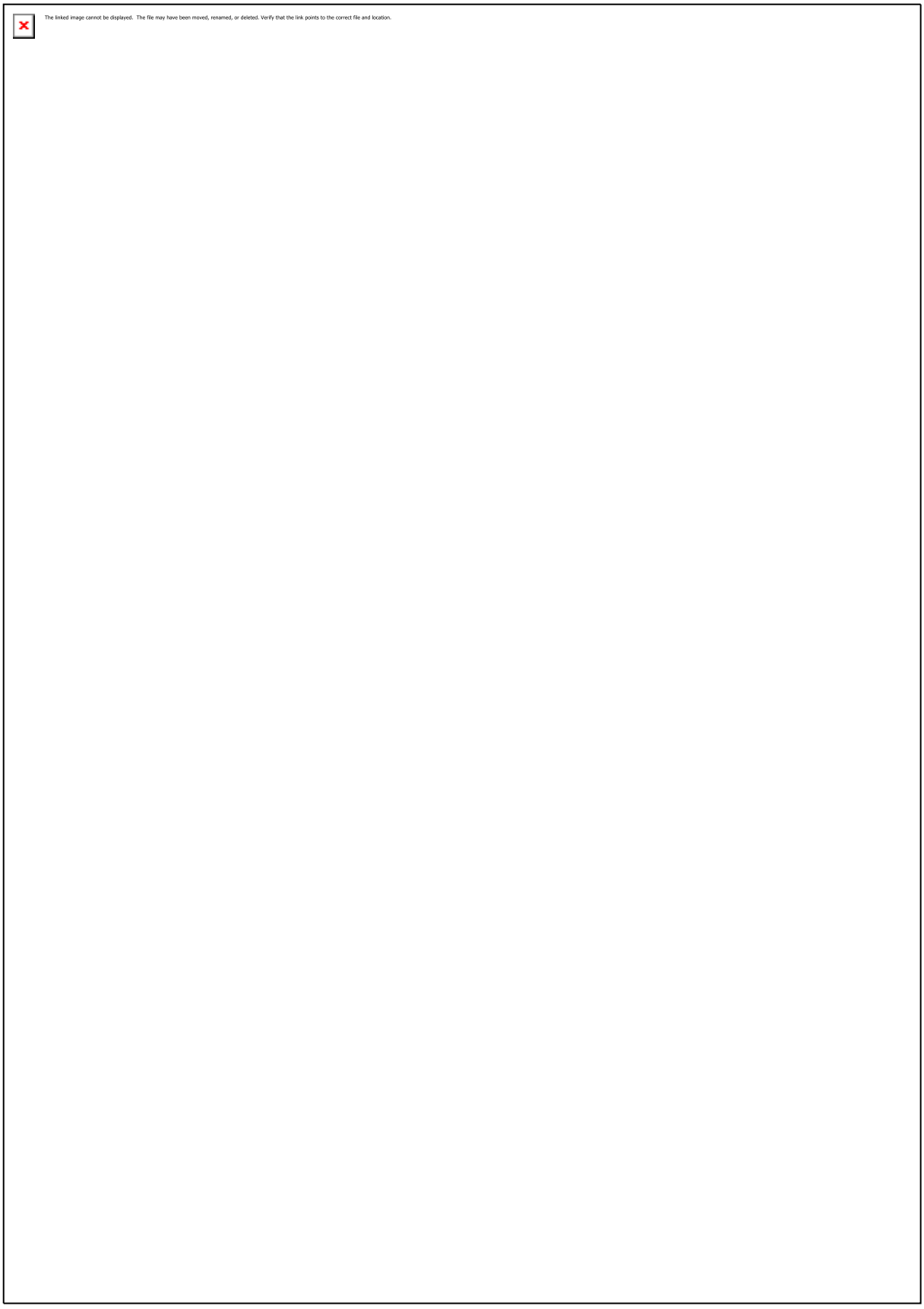
What are the possible risks of taking part?

There are no additional risks to us screening your blood to check the Vitamin D level. If your Vitamin D level were found to be low, then you may be offered treatment. Vitamin D replacement for deficiency is an accepted treatment in the NHS, and there are guidelines to support this.

Treatment of Vitamin D deficiency with tablets at a prescribed dose is considered safe. Taking too much Vitamin D can lead to a high calcium level in your blood, but this would only happen if you were to take very high doses of Vitamin D for a prolonged period, and is very rare. There are some medical conditions (such as Sarcoidosis) where Vitamin D levels may go too high with supplements. This is why we will go through a questionnaire with you to check for these medical conditions before starting treatment.

If you experience any complications or have any concerns during your treatment, please contact the study team directly on 0191 2934087.

If you are considering taking part in the study, please read the additional information in Part 2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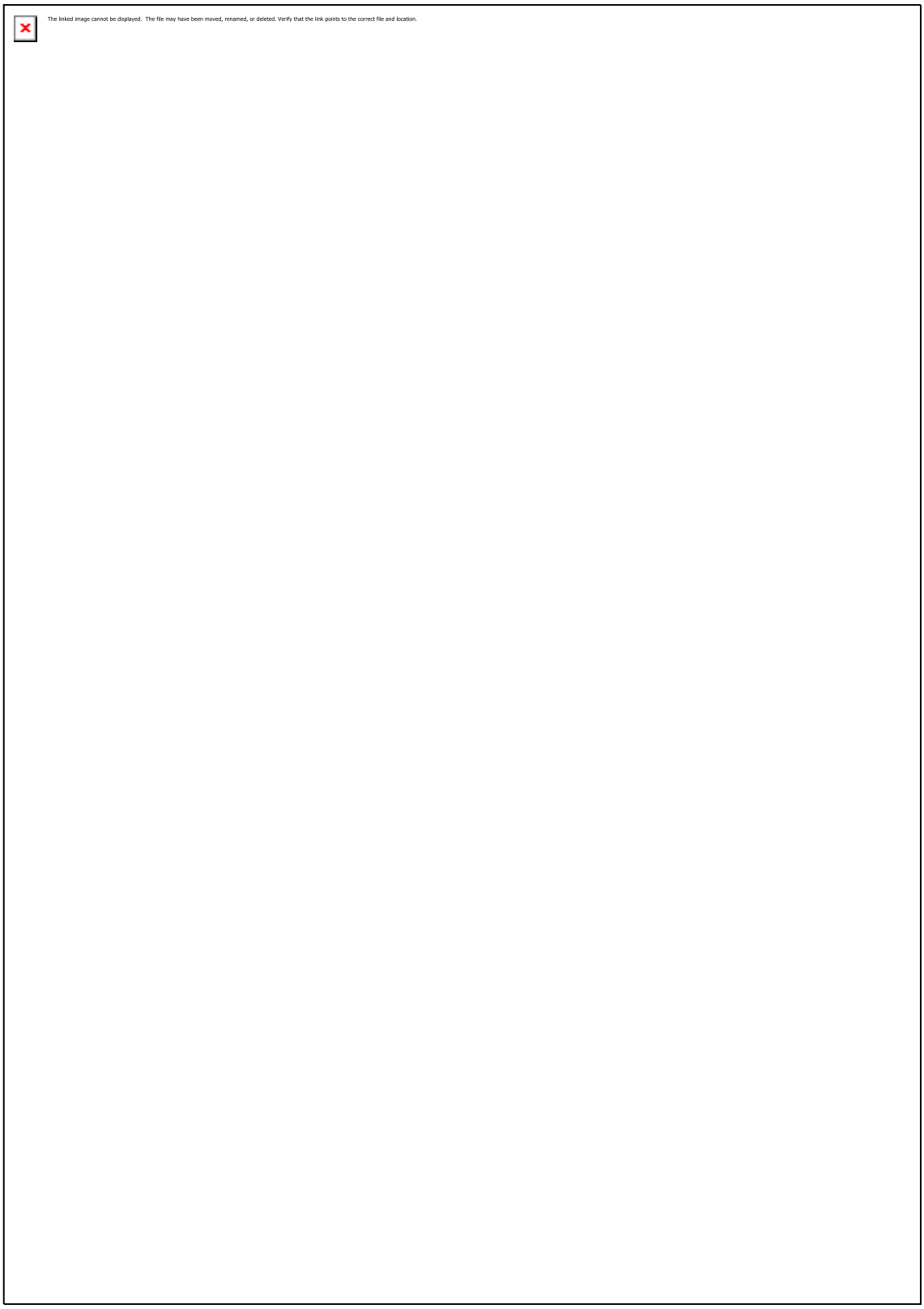
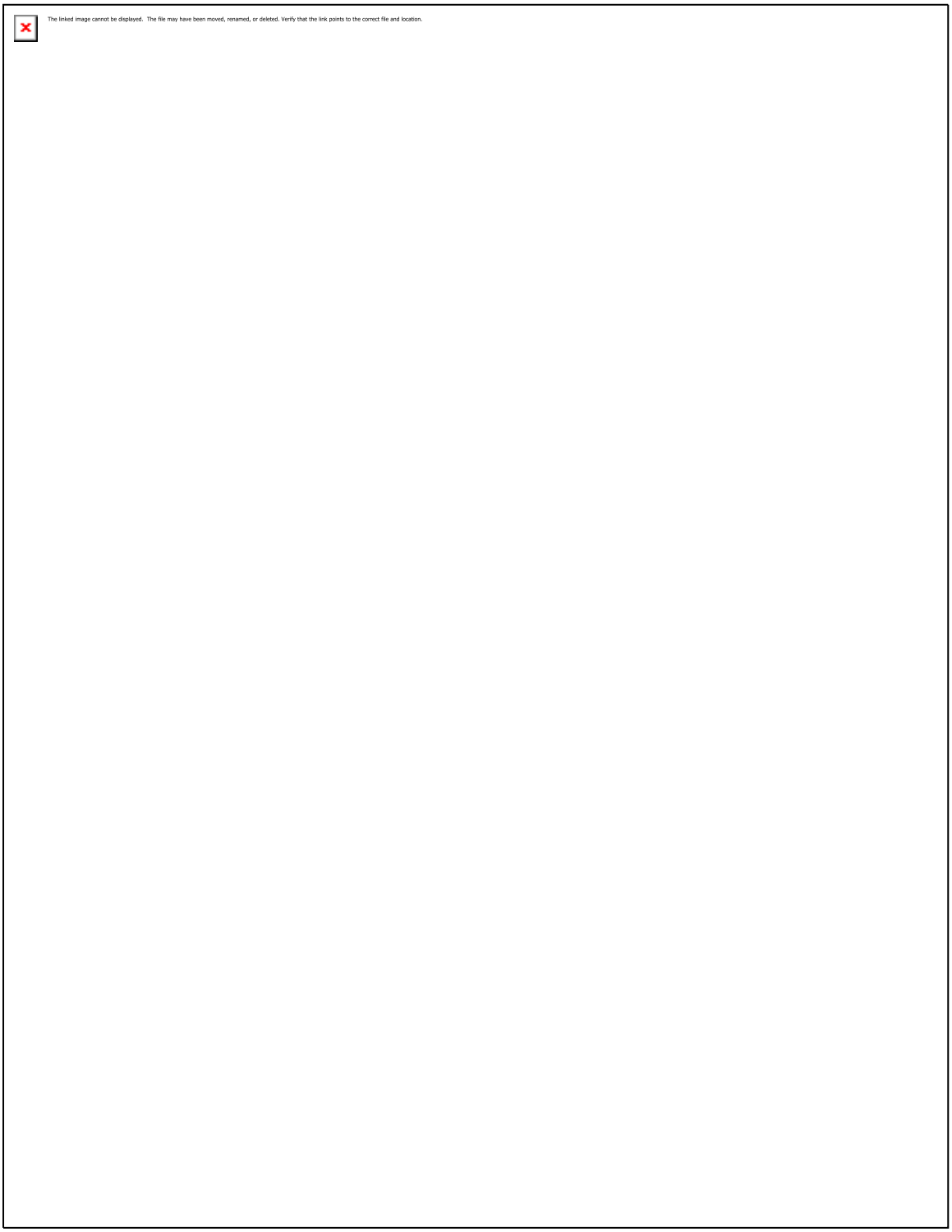
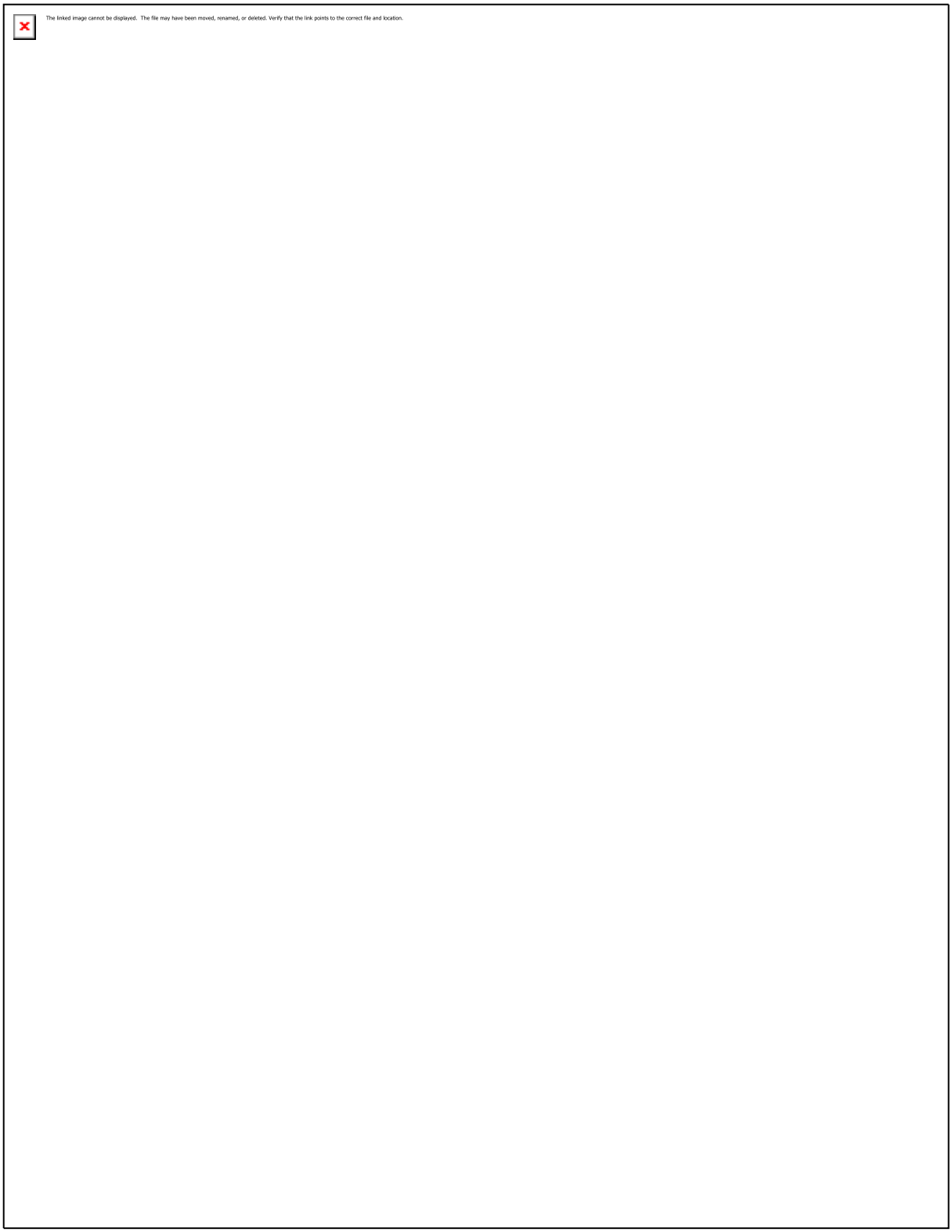
Appendix G: Cover page of trial protocol



Appendix H: Favourable ethics opinion

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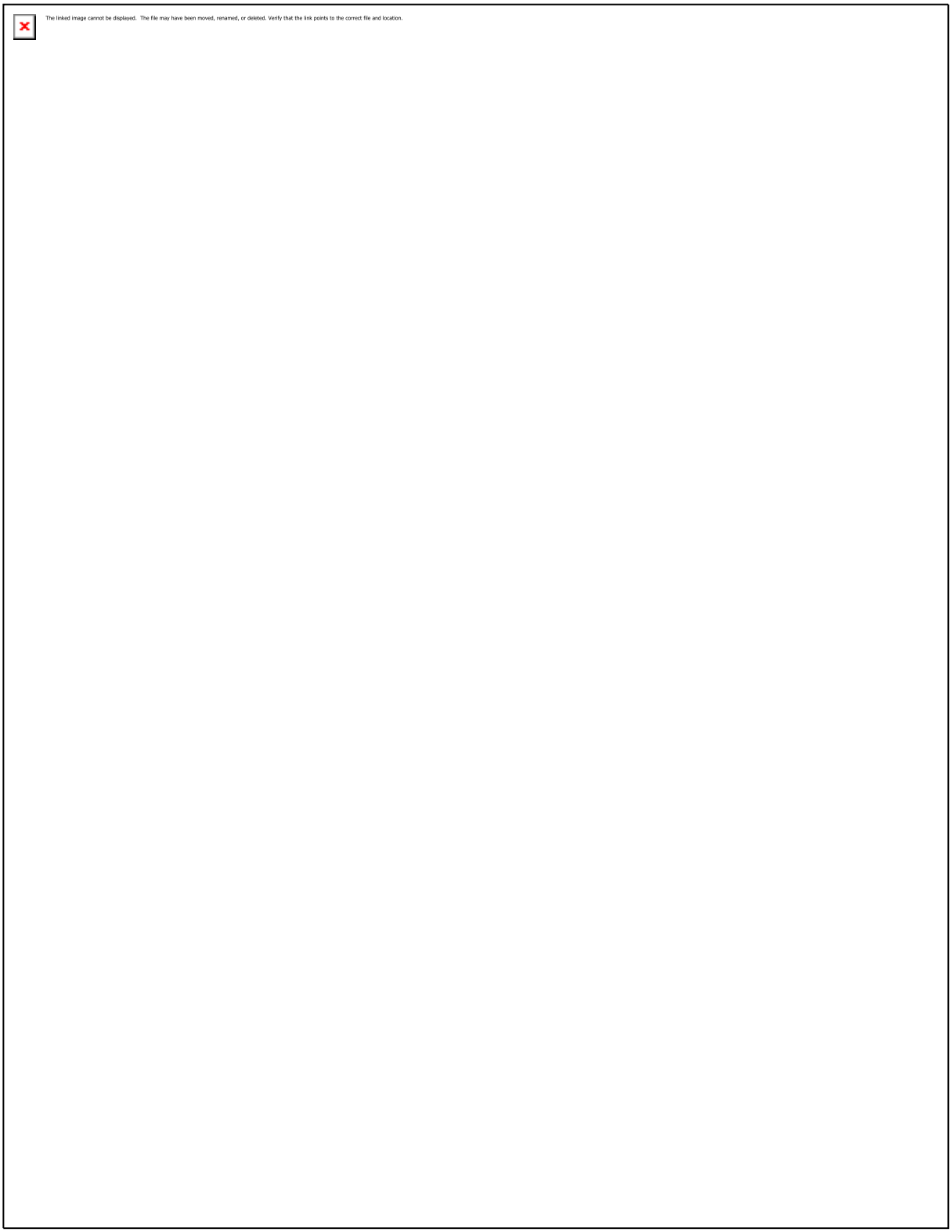
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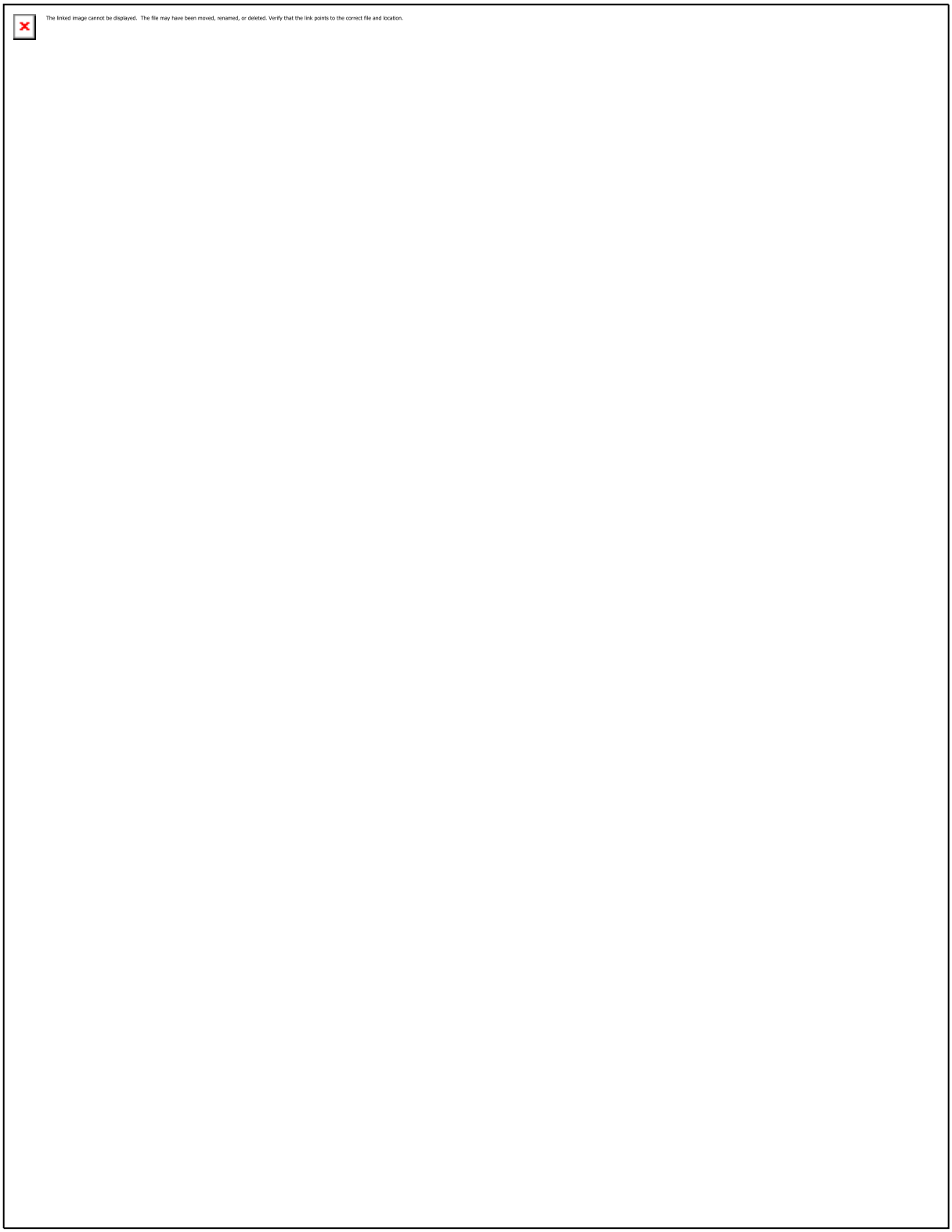
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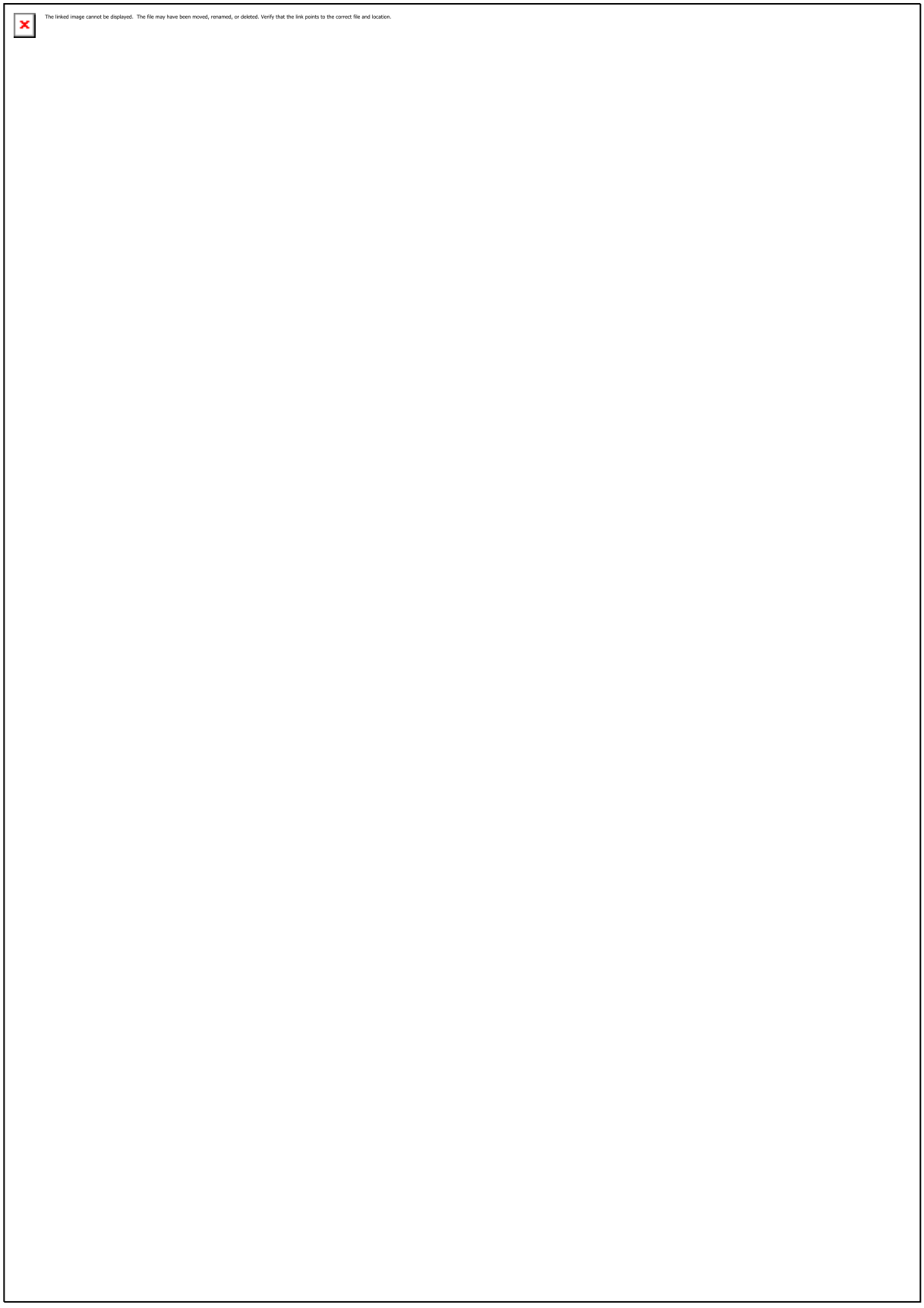
Appendix I: Confirmation of HRA approval

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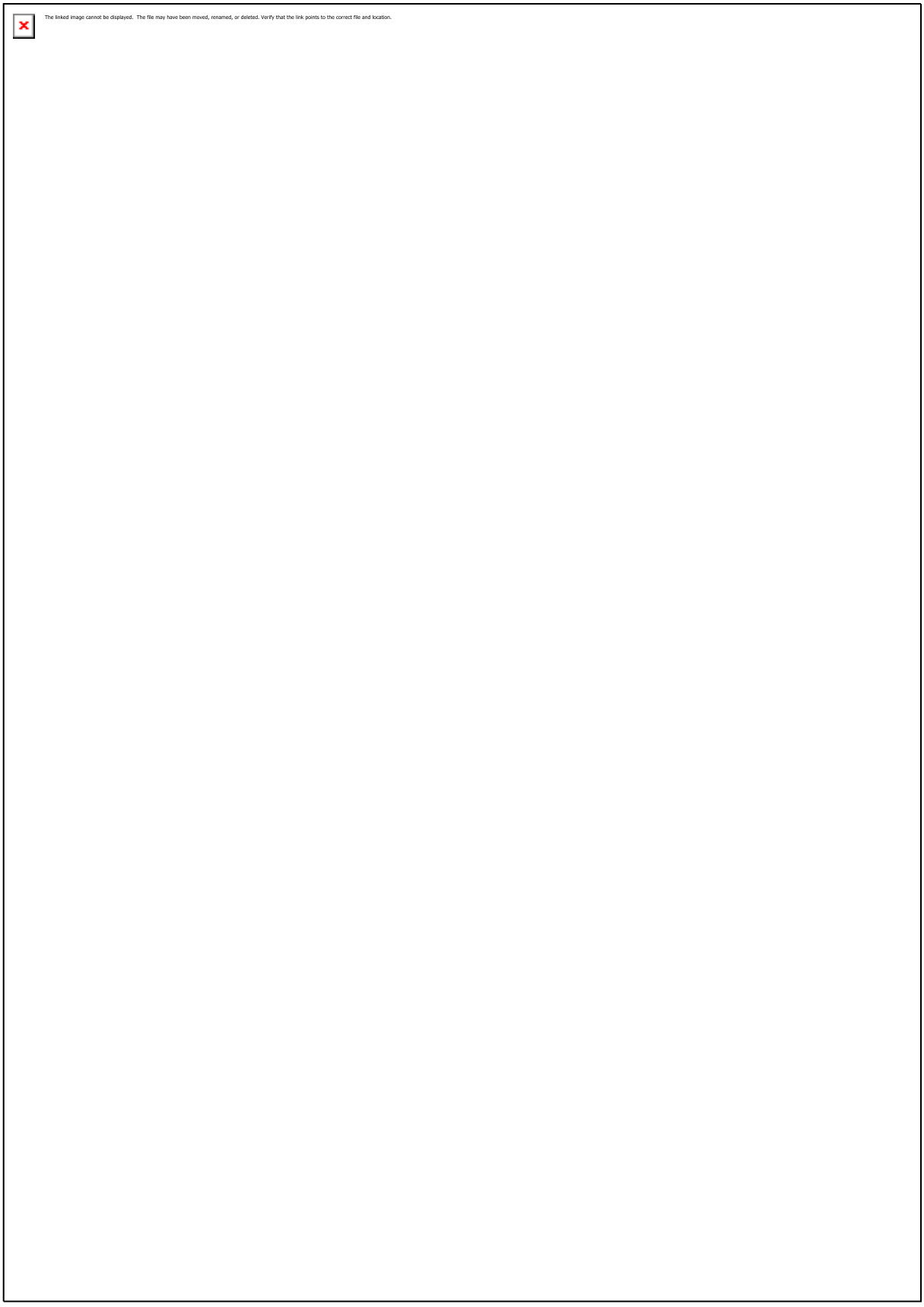
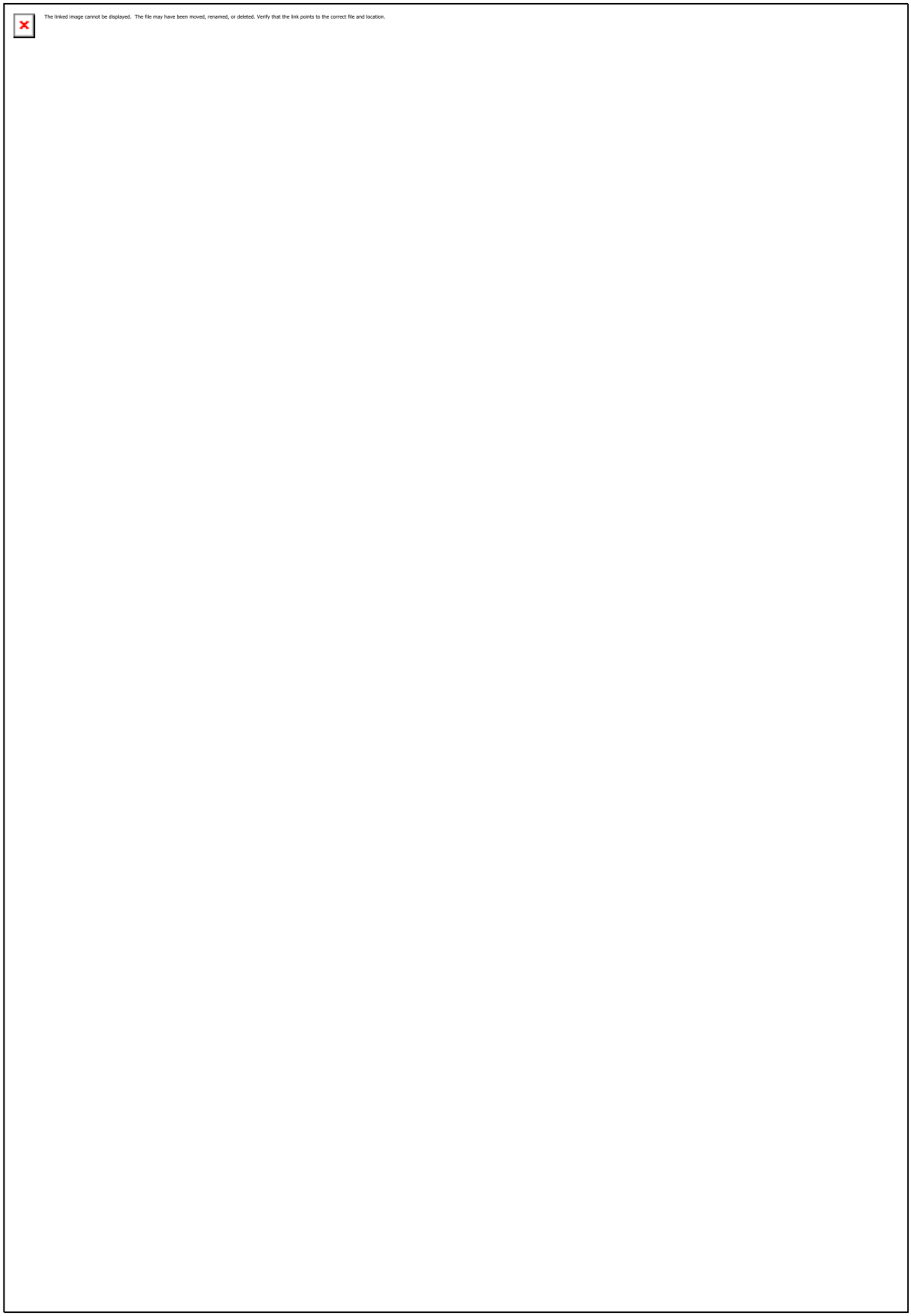
Appendix J: Informed consent form for the VASO trial



Appendix K: Baseline lifestyle questionnaire

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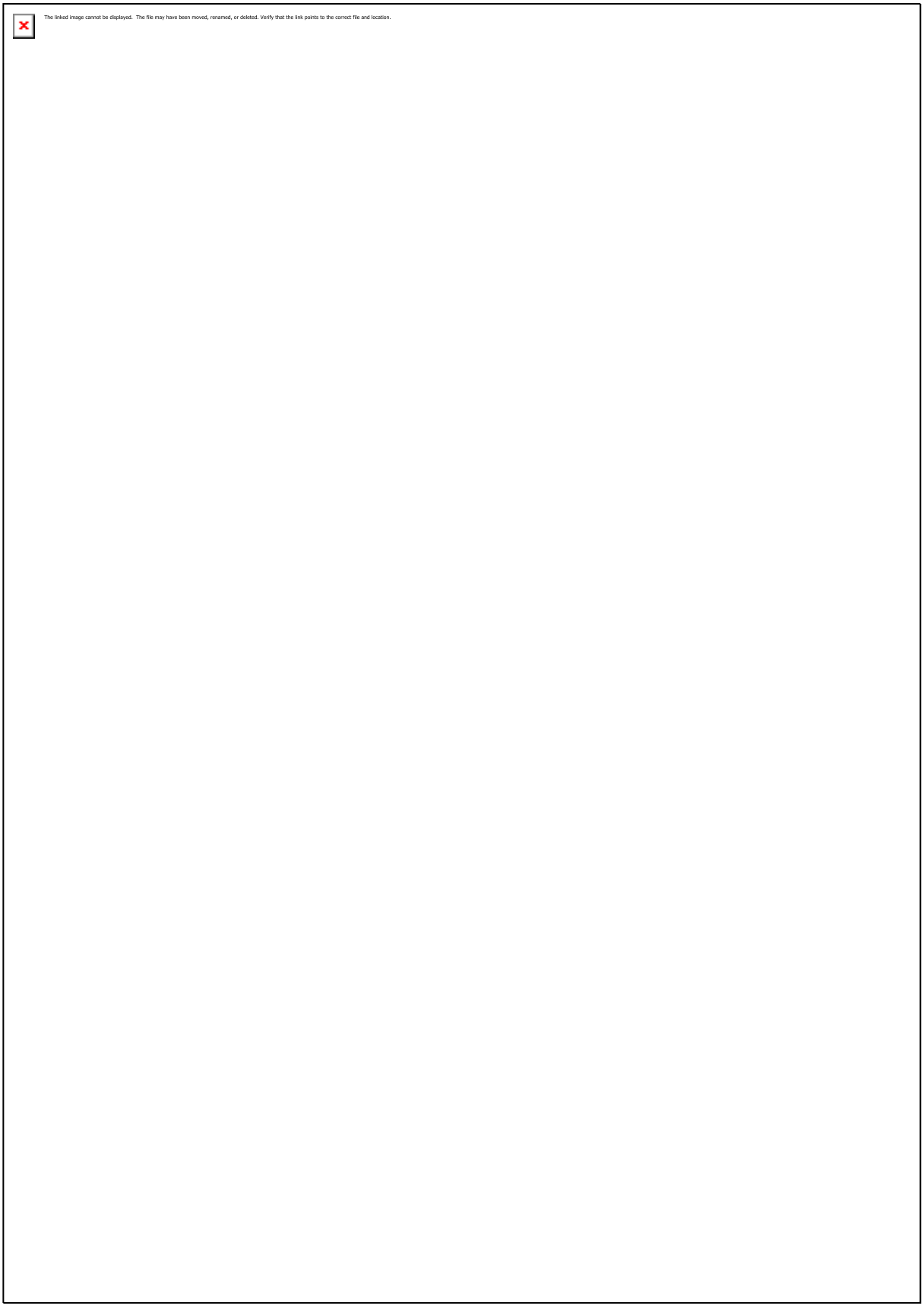
Appendix L: End of trial letter to patients



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