THE INFLUENCE OF VITAMIN D3 SUPPLEMENTATION ON THE COMPONENTS OF THE METABOLIC SYNDROME.

by

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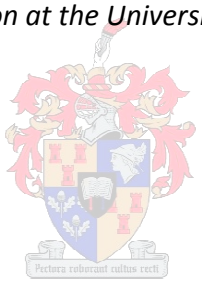

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DECLARATION OF AUTHENTICITY

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ABSTRACT

Background:

During the past decade, there has been increased interest in vitamin D and its effect on disease states beyond bone and skeletal health. One of the conditions vitamin D has been associated with is the metabolic syndrome which consists out of four components namely obesity, hypertension, dyslipidaemia and glucose intolerance. The relationship between vitamin D and the components of the Metabolic Syndrome were investigated in this systematic review.

Objectives:

To assess the effects of vitamin D administration on components of the metabolic syndrome in various population groups.

Search methods:

The following electronic databases were searched: The Cochrane Central Register of controlled trials, Medline (accessed via Pubmed), Science Direct, ISI Web of knowledge and Scopus. The searches were performed in October 2010 and repeated in May 2012. Only trials of which full text articles were available were included.

Selection criteria:

Trials were included if they have a randomised controlled trial design; adult participants of any ethnicity with a insufficient or deficient vitamin D status and who were diagnosed with at least one of the components of the metabolic syndrome; and where the effect of vitamin D at any dose, duration and route of administration, administered as monotherapy or in combination therapy, was compared to placebo or no intervention.

Data collection and analysis:

Two authors independently selected studies for inclusion, extracted data and assessed the risk of bias using the Cochrane Collaboration risk of bias tool. Where data allowed, mean differences for continuous outcomes were calculated and presented with 95% confidence intervals. Medians and ranges or P-values were otherwise reported.

Results:

Four randomized controlled trials were included in this narrative review (N=373). A meta-analysis could not be performed due to heterogeneity in study design and outcomes measured.

The first trial (N=81) examined the effect of 4 000IU vitamin D3 supplementation versus placebo daily on insulin resistance in vitamin D deficient woman for six months. The authors found that by improving vitamin D status in insulin resistant women, both insulin resistance ($p=0.02$) and insulin sensitivity ($p=0.003$) improved, but there was no change in insulin secretion.

The second trial (N=28) looked at the effect of high doses of vitamin D supplementation (300 000IU vitamin D3 intramuscularly) on glucose tolerance in type 2 diabetics. There were no difference between the vitamin D3 and distilled water groups, outcomes were unchanged by vitamin D supplementation in this study.

The third trial (N=200) compared the effects of vitamin D supplementation (332IU cholecalciferol daily) with placebo for one year on weight loss and cardiovascular disease risk markers in overweight adults. Weight loss between the two groups did not differ. Triglycerides significantly improved ($p<0.001$) in the vitamin D treated group, as well as tumor necrosis factor ($p=0.049$) LDL-Cholesterol was however increased ($p<0.00l$) in the vitamin D treated group.

The fourth study (N=61) looked at varying doses of vitamin D3 on markers of vascular health in type 2 diabetics. No difference in endothelial function, insulin resistance or HbAIc was found between the groups. Systolic blood pressure ($p=0.03$) in the vitamin D groups improved compared to the control group.

Authors' conclusions:

The results of the four randomized controlled trials evaluated in this systematic review are variable and do not provide sufficient evidence to guide clinicians as to the effects of using vitamin D supplementation in patients presenting with components of the metabolic syndrome.

KEYWORDS:

Vitamin D; Vitamin D supplementation; metabolic syndrome; systematic review

PLAIN LANGUAGE SUMMARY

The possible advantages of vitamin D supplementation on various cardiometabolic conditions have been examined over the past few years. Vitamin D supplementation has possibly shown effects on each of the individual components of the metabolic syndrome i.e.: obesity, hypertension, dyslipidaemia and glucose intolerance. The aim of this systematic review was to ascertain whether or not vitamin D supplementation has any effect on any of the components of the metabolic syndrome. We searched the (Cochrane Central Register of Controlled Trails (Central), Medline, Science direct, ISI Web of knowledge and Scopus during 2010 (repeated search in 2012). We found four randomized controlled trials that met our inclusion and exclusion criteria. Three hundred and seventy three patients were included in these four randomized controlled trails comparing vitamin D supplementation with placebo. Duration of treatment was a minimum of 4 weeks, through to a maximum of on-year. The different trials looked at various components of the metabolic syndrome as outcomes. The results were not consistent amongst the trials and the results could not be combined in a meta-analysis due to heterogeneity in study design and outcomes measured. The current systematic review highlights the shortcomings in the published data and we recommend further trials be undertaken before vitamin D supplementation can be recommended as beneficial for patients with the metabolic syndrome.

OPSOMMING

Agtergrond:

Gedurende die afgelope dekade was daar toenemende belangstelling in vitamien D en die uitwerking daarvan op siektetoestande bo en behalwe been- en skelet-gesondheid. Een van die toestande waarmee vitamien D verbind word, is die metaboliese sindroom. Vitamien D is met elk van die individuele komponente van die sindroom verbind m.a.w. vetsug, hipertensie, dislipidemie en glukose-intoleransie. Die bestaan van afdoende bewyse wat sodanige verband steun, is in hierdie sistematiese oorsig ondersoek.

Doelstellings:

Om die invloed van vitamien D-toediening op komponente van die metaboliese sindroom by verskillende bevolkingsgroepe te bepaal.

Soekmetodes:

Die volgende elektroniese databasisse is nagevors: die Cochrane sentrale register van gekontroleerde proewe, Medline (toegang via Pubmed verkry), Science Direct, ISI Web of Knowledge en Scopus.

Die soektog is in Oktober 2010 onderneem en in Mei 2012 herhaal. Slegs proewe waarvan volteksartikels beskikbaar was, is ingesluit.

Kriteria vir seleksie:

Proewe was ingesluit as hulle verewekansigde gekontroleerde proewe was. Dellneemers moes volwassens wees van enige rassegroep. Die pasiënte moes gediagnoseer wees met ten minste een van die komponente van die metaboliese sindroom. Proewe is vir insluiting by hierdie

sistematiese oorsig oorweeg indien hulle die uitwerking van vitamien D teen enige dosis, roete van toediening en vir enige tydsduur, as monoterapie of in kombinasieterapie toegedien, teenoor plasebo of geen intervensie vergelyk het.

Data-insameling en -analise:

Twee outeurs het onafhanklik van mekaar studies vir insluiting gekies, data onttrek en die risiko van sydigheid bepaal met behulp van die Cochrane Collaboration-instrument vir die bepaling van die risiko van sydigheid. Indien data toegelaat het is gemiddelde verskille vir kontinue uitkomste bereken en die resultate is aangebied met 95% geloofwaardigheidsintervalle. Mediane en reikwydtes op p-waardes is gerapporteer**.**

Resultate:

Vier verewekansigde gekontroleerde proewe is by hierdie narratiewe oorsig (N=373) ingesluit. ’n Meta-analise kon nie uitgevoer word nie weens heterogeniteit in die ontwerp van die onderskeie studies en die gemete uitkomste.

Die eerste proef (N=81) het die uitwerking van vitamien D-aanvulling 4 000IE D3 per dag op insulienweerstand by vroue met vitamien D-tekort of plasebo vir ses maande ondersoek.

Die uitkomste van hierdie proef het aangetoon dat, met verbetering van vitamien D-status by insulienweerstandige vroue, sowel insulienweerstand (p=0.02) as insuliensensitiwiteit (p=0.003) verbeter het, maar dat daar geen verandering in insuliensekresie was nie.

Die tweede proef (N=28) het gekyk na die uitwerking van hoë dosisse vitamien D-aanvulling (met ander woorde 300 000IE vitamien D3 binnespiers) op glukosetoleransie by tipe 2-diabete. Die uitkomste was onveranderd ná vitamien D-aanvulling in hierdie studie.

Die derde proef (N=200) het die uitwerkings van vitamien D-aanvulling 332IE cholekalsiferol daagliks of plasebo vir een jaar op gewigsverlies ondersoek en die risikomerkers by kardiovaskulêre siekte tydens gewigsverlies is nie beduidend deur vitamien D-aanvulling geraak

nie. Risikomerkers by kardiovaskulêre siekte soos trigliseriede is meer beduidend ($p<0.001$) by die vitamien D-behandelde groep verlaag en tumornekrosefaktor ($p=0.049$) by die vitamien D-behandelde groep is ook beduidend geraak in vergelyking met die kontrolegevalle. LDL-cholesterol het egter ($p<0.00l$) by die vitamien D-behandelde groep verhoog.

Die vierde studie (N=61) het gekyk na wisselende dosisse vitamien D3 op merkers van vaskulêre gesondheid by tipe 2 diabete. Vitamien D-aanvulling het nie endoteelfunksie, insulienweerstand of HbAIc geraak nie. Sistoliese bloeddruk ($p=0.03$) en B-tipe natriuretiese peptiedvlakke ($p=0.02$) is verbeter.

Outeurs se gevolgtrekkings:

Die resultate van die vier verewekansigde gekontroleerde proewe wat in hierdie sistematiese oorsig geëvalueer is, is uiteenlopend en verskaf nie voldoende bewys om klinici te lei ten opsigte van die effek van die gebruik van vitamien D-aanvulling by pasiënte wat met komponente van die metaboliese sindroom presenteer nie.

Opsomming in eenvoudige taal

Vitamien D-aanvullings se uitwerking op komponente van die metaboliese sindroom

Die moontlike voordele van vitamien D-aanvullings op verskillende kardiometaboliese toestande is oor die afgelope paar jaar ondersoek. Daar is aangetoon dat vitamien D-aanvullings uitwerkings het op elk van die individuele komponente van die metaboliese sindroom naamlik vetsug, hipertensie, dislipidemie en glukose-intoleransie. Die doel van hierdie sistematiese oorsig was om vas te stel of vitamien D-aanvullings enige uitwerking het op enige van die komponente van die metaboliese sindroom of nie. Ons het gedurende 2010 soektogte uitgevoer op die Cochrane Sentrale register van gekontroleerde proewe (Central), Medline, Science Direct, ISI Web of Knowledge en Scopus (soektog is in 2012 herhaal). Ons het vier verewekansigde gekontroleerde proewe wat aan ons insluiting- en uitsluitingskriteria voldoen het, opgespoor. Driehonderd drie en sewentig pasiënte is by die vier proewe ingesluit. Al vier proewe het vitamien D-aanvullings met plasebo vergelyk. Die duur van behandeling het van 4 weke tot een jaar gestrek. Die verskillende proewe het gekyk na verskillende komponente van die metaboliese sindroom as uitkomste. Die resultate van die onderskeie proewe was nie konsekwent nie. Die huidige sistematiese oorsig belig die tekortkominge in die gepubliseerde data en ons beveel aan dat verdere proewe onderneem word om vas te stel of dit nuttig is om vitamien D aanvullings vir pasiënte met die metaboliese sindroom aan te beveel, en of dit dalk skadelik kan wees.

CONTRIBUTIONS OF AUTHORS

CONTRIBUTIONS BY PRINCIPAL RESEARCHER AND FELLOW RESEARCHERS

The principal researcher, Dr Charlene Wolberg developed the protocol, screened the results of electronic searches to select potentially relevant studies, retrieved relevant full text articles, extracted data from relevant articles and assessed risk of bias of included studies, wrote up results, discussion and conclusion.

Mrs. Maryke Rabe - Second reviewer, screened the results of electronic searches to select potentially relevant studies, retrieved relevant full articles, extracted data on relevant articles and assessed risk bias of included studies.

Prof MG Herselman: Third reviewer and supervisor, contributed to developing the protocol and provided input in the whole thesis.

Prof FS Hough: Co-supervisor, contributed to the protocol.

Mr. A Musekiwa – Statistician and assisted with results section.

The principal researcher (Charlene Wolberg) developed the idea and the protocol. The principal researcher planned the study, undertook data collection with Maryke Rabe, captured the data for analyses, analysed the data with the assistance of a statistician (Mr A. Musekiwa), interpreted the data and drafted the thesis. Prof, MG Herselman and Prof. FS Hough (Supervisors) provided input at all stages and revised the protocol and thesis.

DECLARATIONS OF INTEREST

No conflict of Interest.

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LIST OF ABBREVIATIONS

UVB	Ultraviolet B
25 (OH) D	25-Hydroxyvitamin D
1,25 $(OH)_2D$	1,25 Dihydroxyvitamin D
DBP	Vitamin D–binding protein
VDR	Vitamin D-receptor
VDRES	Vitamin D responsive elements
IOM	Institute of Medicine
RDA	Recommended Dietary Allowance
DRI	Dietary Reference Intake
NOAEL	No observed adverse effect level
PTH	Parathyroid Hormone
HRT	Hormone Replacement Therapy
ATPIII	American Treatment Panel

LIST OF DEFINITIONS

Calcifediol: a physiologic form of vitamin D.[1]

Calciferol: a fat soluble, crystalline, unsaturated alcohol produced by ultraviolet irradiation of ergosterol and used as a dietary supplement in the prophylaxis and treatment of rickets, osteomalacia, and other hypocalcemic disorders. It occurs naturally in milk and fish-liver oils. Also called ergocalciferol, oleovitamin D2, vitamin D2.[1]

Immunoassay: a competitive-binding assay in which the binding protein is an antibody.[1]

Chemiluminescence assay: chemiluminescence is the name given to light emission produced during a chemical reaction.[2]

Liquid chromatography mass-spectrometric detection: Identifying a substance by sorting a stream of charged particles according to their mass.[1]

CHAPTER 1:
BACKGROUND AND MOTIVATION FOR THE STUDY

1.1 INTRODUCTION

For more than twenty years we have witnessed an increased interest and research in the role of vitamin D. There have been numerous articles published in both the scientific journals and popular media concerning a wide range of possible health effects. Of particular interest is the possible non-skeletal effects of vitamin D such as in cancer, autoimmune diseases and the various components of the metabolic syndrome.

The list of diseases for which vitamin D has been claimed to have an effect has grown so extensively it would appear that this vitamin is the panacea for all ills.[3]

Over the past few decades, the incidence of the metabolic syndrome has increased and affects as much as 25% of the population. A possible role of vitamin D in the various components of the metabolic syndrome has been postulated.[4] Metabolic syndrome is known under a number of names such as Syndrome X, Deadly Quartet and Reaven's Syndrome.

1.2 BACKGROUND TO VITAMIN D

1.2.1 Biochemistry of Vitamin D

Vitamin D is part of a group of secosteroid molecules that are traditionally associated with bone and calcium metabolism, rather than cardiometabolic physiology for example. Five forms of vitamin D are known, namely D1 to D5 (See Table 1.1). Vitamins D2 and D3 are the most studied forms.[5]

Vitamin D and its metabolites can be divided into two families of secosteroids, the cholecalciferols and ergocalciferols.

Cholecalciferol (vitamin D3), the parent compound of the form naturally occurring in humans, is produced in the skin on exposure to sunlight. The ultraviolet B (UVB) [290-315nm] portion of sunlight converts 7-dehydrocholesterol to previtamin D3. Previtamin D3 undergoes thermal isomerisation from vitamin D3.[6]

Vitamin D2 (ergocalciferol) is derived from ergosterol. Ergosterol is a sterol produced by some phytoplankton, invertebrates, and fungi. These organisms use ergosterol and sunlight to produce

vitamin D2. Because they lack ergosterol, land plants or vertebrates cannot produce vitamin D2. The action of light causes ring cleavage to yield an intermediate pre-ergocalciferol (plus lumisterol and other related congeners), which rearranges under thermal conditions to ergocalciferol.2

Vitamin D2 is different from D3 in that D2 has a double bond between carbon 22 and carbon 23 and a methyl group on carbon 24.[2] Both vitamin D2 and D3 can also be consumed in supplements or fortified foods.

Unless otherwise indicated, when vitamin D and its metabolites are written without a subscript, both families of vitamin D, ergocalciferols and cholecalciferols, are being included.6

Table 1.1 Vitamin D and its metabolites

NAME	ABBREVIATION	GENERIC NAME	SERUM CONCENTRATION Mean ± SD
VITAMIN D (total for Vit D2 and D3)	D	CALCIFEROL	1.6 ± 0.4ng/ml 4.1 +- 1.0nm/l
VITAMIN D2	D2	ERGOCALCIFEROL	-
VITAMIN D3	D3	CHOLECALCIFEROL	-
25-Hydroxyvitamin D	25(OH)D	CALCIFEDIOL	26.5 ± 5.3ng/ml 66.25 +- 13.25nm/l
1,25-Dihydroxyvitamin D	$1,25(OH)_2D$	CALCITRIOL	34.1 ± 0.4pg/ml 81.84 +- 0.96nm/l
24,25-Dihydroxyvitamin D	$24,25(OH)_2D$	-	1.3 ± 0.4ng/ml 3.12 +- .96nm/l
25,26-Dihydroxyvitamin D	$25,26(OH)_2D$	-	0.5 ± 0.1ng/ml 1.2 +- 0.24nm/l

Table modified from Basic and Clinical Endocrinology.[7]

1.2.2 Vitamin D Metabolism

Vitamin D metabolites are transported in the blood bound mainly to vitamin D–binding protein (DBP) 85% and albumin 15%. The conversion of vitamin D to 25-hydroxyvitamin D occurs mainly in the liver with the help of the enzyme 25-hydroxylase. The kidney mainly controls vitamin D metabolism. In a rate limiting step the kidneys convert 25-hydroxyvitamin D to its active form $1,25(OH)_2D3$ by 1 alpha-hydroxylase. Production of $1,25(OH)_2D3$ in the kidney is stimulated by parathyroid hormone (PTH) and is inhibited by high blood levels of calcium and phosphate.[2]

$1,25(OH)_2$ vitamin D is the active form of vitamin D. This binds to vitamin D specific receptors. This receptor is referred to as VDR and is part of the class 2 steroid hormones. Similar receptors are the thyroid hormone, retinoid X and retinoic acid receptors. There are certain important domains on this receptor, similar to these other receptors. There is the C-domain, which binds DNA, the E-domain which binds ligands and the F-domain, which is an activating domain. In humans, the receptor is a peptide, which has 427 amino acids. It acts by means of VDRES, the vitamin D responsive elements. These are sequences of nucleotides supported by 3 bases. They are found very close to the target gene. The retinoic acid X receptor binds to the 5'arm and the VDR to the 3'arm.[viii]

CYP 24, known as 24 hydroxylase enzyme, causes degradation of vitamin D. This gene is very strongly regulated and its own destruction is pre-programmed. This aspect is a vital one in the endocrine system.

The VDR and the retinoid x receptor form a heterodimer at the VDR element. It also binds other proteins necessary for the transcription complex. Essentially an activator is acquired. Three co-activators are known: SARC1, SARC2 and DRIP 205. There could be other co-activators and the co-activators could be selective as to gene expression.

After formation of the complex, the DNA bends and there is phosphorylation on serine-205. The initiation or suppression of transcription takes place and this relates to which gene is involved.[8]

The vitamin D receptor is widely distributed in enterocytes, osteoblasts, parathyroid gland cells and distal renal tubule cells.[4] They have recently been found in the liver, immune system, prostate, breast, lungs, pancreas, skeletal and cardiac muscle.[4]

1.2.3 Sources of Vitamin D

The main sources of vitamin D for individuals are from sunlight exposure, dietary sources and supplementation. Exposure to the sunlight and cutaneous synthesis of vitamin D provides 80-90% of vitamin D to humans. Solar UVB radiation (wavelength 290-315nm) penetrates the skin and converts 7-dehydrocholestrol to pre-vitamin D3, this is then converted to vitamin D3. Vitamin D3 intoxication is unlikely to be caused by over exposure to sunlight, as any excess previtamin D3 or vitamin D3 is destroyed by sunlight.[5] If an individual wears minimal clothing and their whole body is exposed to the sun, 1 minimal erythermal dose will provide equivalent vitamin D to 250-500ug (10,000-20,000) vitamin D.[4] Vitamin D is not widely found in foods and mainly is present in oily fish (e.g. salmon, mackerel, sardines and herring) and some fortified foods for example milk and juices in the United States of America (USA) and Canada where fortification provides >100IU per 8oz serving. Daily vitamin D obtained from standard dietary sources is 2.5ug (100IU), and fortified foods provided up to 5-10ug (200-400IU) of vitamin D per day.[4] In South Africa mainly margarine is fortified.

1.2.4 Recommended Vitamin D Intake

Over the past several years, the increased interest in vitamin D and its proposed health benefits has led to an increasing frequency of vitamin D testing and increase in the use of vitamin D supplements.

The USA and Canadian governments felt that much of this practice was not based on scientific evidence.[9] In response the assembled a committee of experts to assess the growing body of research on vitamin D and give guidelines regarding vitamin D intake.[9]

The IOM committee increased the recommended dietary allowance (RDA) from the 1997 IOM report, which lacked sufficient evidence for development of the RDA and had to instead estimate the adequate intake (AI).[13]

The IOM based its recommendations for vitamin D intake using bone health as an indicator.[14] Extra skeletal indicators were not used for Dietary Reference Intake (DRI) development because of insufficient evidence of causality, inconsistency in the evidence, or inability to develop a dose response relationship.

The IOM concluded that 50nmol/L is optimal for bone health.**Error! Bookmark not defined.** This serum level orresponds to an intake of 25-hydroxyvitamin D level corresponding is 600IU/d. The adequate intake (AI)

was set at 400IU/d for infants and the RDA was set at 600IU/d for children and adults and 800IU/d for those over 70 years of age. This intake in the elderly is predicted to result in a serum 25-hydroxvitamin D level of 73nmol/L. The "no observed adverse event level "NOAEL" was proposed to be 10,000IU/d. However, the upper limit (UL) was set lower at 4 000IU/d, corresponding to a serum vitamin D level of 125nmol/L.

As mentioned earlier, the IOM committee followed an evidence-based approach and set a RDA-intake value of 50nmol/L based on the literature for skeletal health. However, The Endocrine Society and other experts are of the opinion that the cut off point for serum vitamin D adequacy is over 75nmol/L.**Error! Bookmark not defined.** By accepting the higher cut off points, a so-called "epidemic" of vitamin D inadequacy has been created.

To further add to the controversy when the IOM recommendations were published and stated that using the 50nmol/L as a cut-off most Americans were receiving enough vitamin D, the most recent data from the National Health and Nutrition Examination survey (NHANES; 2005 to 2006) had not been published yet.**Error! Bookmark not defined.** In this survey it was found that the mean 25(OH)D vitamin D levels were 49.7nmol/L. Thirty one percent of non-Hispanic whites were vitamin D deficient using the 50 nmol/L and below cut off, whereas 63% of Hispanics and 82% of non-Hispanic blacks were vitamin D deficient.

The Endocrine Society also assembled recently to provide clinicians with guidelines for the evaluation, treatment and prevention of vitamin D deficiency.[15] Their emphasis was on the care of patients who are at risk for deficiency.

Some confusion has also arisen for clinicians and patients as a result of the RDA being used as different goals. The IOM report regards the RDA as the target for vitamin D intake. By contrast the Endocrine Society recommends the RDA values as the minimum. A reason for this may be that the patients to whom the Endocrine Society is addressing their recommendations are at risk for deficiency, making a higher dose of vitamin D necessary. The recommendations published in the guidelines are contrasted with those in the IOM report and identified as suggestions for patient care rather than recommended actions for the healthy population.[9,]**Error! Bookmark not defined.,Error! Bookmark not defined.**

It is uncommon to see hypervitaminosis D. It can be caused by over supplementation. The symptoms of hypervitaminosis D are related to hypercalcaemia and present as loss of appetite, nausea and vomiting, polyuria, polydipsia, weakness, nervousness, pruritis and kidney failure.[5]

1.2.5 Serum Levels of Vitamin D

The major circulating metabolite of vitamin D is serum 25(OH) vitamin D. Its levels reflect sunlight exposure and dietary intake. Serum $1,25(OH)_2$ vitamin D levels can be normal or even elevated in patients with vitamin D deficiency.[5] There was previously no consensus on the optimal levels of 25(OH) vitamin D as measured in serum. The IOM has established guidelines for clinicians regarding vitamin D levels, vitamin D deficiency is defined by the IOM as a 25-hydroxyvitamin D level of less than 30nmol/L. Vitamin D inadequacy as between 30-50nmol/L and vitamin D greater than 125nmol/L as the upper limit of normal.[9]

The Endocrine Society clinical practice guidelines define vitamin D deficiency as 25 hydroxyvitamin D below 20ng/ml (50nmol/L) and vitamin D insufficiency as a 25 hydroxyvitamin D of 21-29 ng/ml (52,5-72,5 nmol/L).[15]

Vitamin D deficiency or insufficiency affects approximately 1 billion people worldwide.[6] Several factors increase the risk of vitamin D deficiency. These include the elderly, increased skin pigmentation, institutionalized or homebound status, increased distance from the equator, winter , clothing (which decreases the amount of skin surface exposed), the use of sunscreen, air pollution, cigarette smoking, obesity, malabsorption, renal disease, liver disease and medications.5

Statistics from several studies show how winter affects serum levels of vitamin D. In one study in the USA, 36% of young healthy free living adults aged 18-29 years had vitamin D deficiency at the end of winter.[5] In other studies in the USA 42% of 15-49 year old black girls plus woman had vitamin D deficiency i.e. serum vitamin D levels below 20ng/ml.[6] A study in a Boston hospital reported that 32% of healthy students, physicians and residents presented with a vitamin D deficiency, despite drinking a glass of milk, taking a multivitamin containing vitamin D daily and eating salmon at least once a week.[6]

According to several studies, 40-100% of USA and European elderly men and women still living in the community (not in nursing homes) are deficient in vitamin D.[6] Studies in Victoria, New South Wales and Western Australia revealed that older people who are institutionalized or housebound are particularly at risk of vitamin deficiency. Eighty percent of women and 70% of men living in hostels or nursing homes were frankly deficient in vitamin D, and overall 97% of the surveyed people had a 25 (OH) vitamin D level below the median value of the healthy reference range.[ix] Data from the National Nutritional Survey of New

Zealand showed that 1.6% of males over 65 years and 5.8% of females had blood levels below 17.5nmol/L for serum 25(OH)D and that 20.5% of men and 39.6% of woman had levels below 37.5nmol/L. This survey did not include institutionalized people. Plasma 25(OH) vitamin D levels are inversely related to menopausal status. More than 50% of post menopausal women taking medication for osteoporosis had suboptimal levels of 25 (OH) vitamin D, with levels below 30ng/ml (75nmol/L). Studies in the United Kingdom and South Africa reported that 13% to 33% of patients with hip fractures had histological evidence of osteomalacia that may have been caused by chronic vitamin D deficiency.[10] People living in sunny climates and who are exposed to sunlight tend to have higher levels of vitamin D. However, even countries such as New Zealand and Australia, which have plenty of sunshine, as well as latitudes with sufficiently moderate climates to allow some endogenous synthesis throughout the year, are diagnosing cases of hypovitaminosis D.[12] In sunny climates where most of the skin is shielded from the sun, such as Saudi Arabia, the United Arab Emirates, Australia, Turkey, India and Lebanon, 30-50% of children and adults had 25(OH) vitamin D levels under 20ng/ml.[6]

1.2.6 Assays for Vitamin D Measurement

There are several different methods to measure vitamin D levels in blood. Most laboratories measure 25(OH) vitamin D levels. Amongst the private laboratories in South Africa, Lancet Laboratories use the chemiluminescence assay.[16] Ampath Laboratories use a immunoassay.[17] Several types of assays are available for measurement as serum 25(0H)D, but the two most common are antibody type methods and liquid chromatography-based methods. There is concern about the performance of these assays. It is generally thought that the "gold standard" is liquid chromatography mass-spectrometric detection. However, these methods both measure physiologically relevant parameters, i.e. the total serum 25(OH)D. There is concern about the accuracy of serum 25(OH)D measurements in individual laboratories.**Error! Bookmark not defined.** An International organization, Vitamin D eternal quality assessment scheme (DEQAS) runs a quality assurance programme to identify issues of quality control in individual laboratories and helps with their corrections.[14]

1.3 DESCRIPTION OF THE METABOLIC SYNDROME AND THE ROLE OF VITAMIN D

1.3.1 Vitamin D and Cardiometabolic Disease

Vitamin D has long been known to be vital to bone health. More recently, vitamin D has been postulated to play a role in the risk for malignancy and infections as well as normal immune function and cardiovascular health.[18]

There is mounting interest in the role of vitamin D in the constellation of metabolic abnormalities grouped under the term "metabolic syndrome", which includes hypertension, dyslipidaemia, abdominal obesity, glucose intolerance and type II diabetes.**Error! Bookmark not defined.**

Several systematic reviews have been published looking at the relationship between levels of vitamin D and cardiometabolic disorders. In a review by Parker et al.[19] they included cross-sectional studies, case-control, cohort and randomised controlled trials. A meta-analysis was performed to assess the risk of developing cardiometabolic disorders comparing the highest and lowest groups of serum 25(OH)D. They found that the highest levels of serum 25(OH)D were associated with 43% reduction in cardiometabolic disorders. This finding applied to all outcomes reported: cardiovascular disease, diabetes mellitus or metabolic syndrome.

In another review by Pittas et al.[20] they included longitudinal observational studies and randomised controlled trials. They examined the association between vitamin D status, including the effect of vitamin D supplementation on cardiometabolic outcomes. They concluded that the association between vitamin D status and cardiometabolic outcomes is uncertain, and that the trials showed no clinically significant effect of vitamin D supplementation at the dosages given. In a review by Wang et al.[21] prospective studies and randomised trials were included. They felt that vitamin D supplements at moderate to high doses may reduce cardiovascular disease.**Error! Bookmark not defined.**

The authors of all the studies concluded by echoing the same sentiment. In order to make recommendations regarding vitamin D supplementation and cardiovascular risk factors adequate randomised trials are needed. These trials need to be conducted in well-defined populations (e.g. pre diabetics, pre hypertension or white versus non white persons). The dosages of vitamin D need to be optimal and the vitamin D status of the population needs to be specified i.e. deficient versus optimal.

Low serum 25(OH)D concentrations have been shown to correlate with impaired glucose tolerance and an increased risk of type 2 diabetes, while a correlation between hypovitaminosis D and insulin resistance has been identified in pregnant women and obese adolescents.[11] A 10 year prospective study identified an inverse relationship between baseline serum 25(OH)D concentrations and later risk of insulin resistance.**Error! Bookmark not defined.**

Studies have shown variable results regarding the association between vitamin D levels and the metabolic syndrome. Several studies implicated low vitamin D levels, specifically 25(OH) vitamin D with an increased prevalence of the metabolic syndrome. Other studies show no association between 25 hydroxyvitamin D levels and the metabolic syndrome.**Error! Bookmark not defined.**[22,23]

The mechanism whereby the vitamin D endocrine system is thought to have an effect on the metabolic syndrome is via insulin secretion and insulin resistance. Studies have shown that vitamin D and calcium are both important in the regulation of insulin secretion from the beta-islet cells of the pancreas.**Error! Bookmark not defined.** Pancreatic beta cells have both receptors for vitamin D and vitamin D dependent calcium – binding proteins. This provides evidence for the role of Vitamin D in the regulation of beta cell insulin secretion. Deficiency of both calcium and vitamin D leads to impaired insulin secretion.

This hypothesis has been documented in both rat and human studies.[24,25] One of the possible explanations is that vitamin D maximizes glucose-induced insulin release by stimulation of islet cell calcium uptake. The vitamin D endocrine system is also thought to be important for insulin synthesis. Vitamin D deficiency is accompanied by decreased pancreatic preproinsulin mRNA which is reversible by vitamin D treatment. Vitamin D has also been linked to insulin sensitivity. Both studies in vitro and in rats have shown an effect on insulin sensitivity.**Error! Bookmark not defined.**

1.3.2 The Contribution of Vitamin D Deficiency to Disease States

Since the individual components of glucose intolerance, hypertriglyceridaemia, obesity and hypertension are associated with low 25-hydroxyvitamin D levels, it is not surprising that 25-hydroxyvitamin D deficiency is also associated with the metabolic syndrome in several studies.[6]

1.3.2.1 Vitamin D and Obesity

Different theories are advanced as to the mechanism of the association of low 25(OH) vitamin D serum levels with the diverse parts of the metabolic syndrome.

Foss [26] suggests that obesity as well as the metabolic syndrome have evolved in order to deal with the winter environment, as described below.

The concept of an adipostat, by which weight is centrally controlled and maintained, is commonly proposed. This ensures that body weight is maintained at a fixed level by balancing energy intake and output by means

of several homeostatic mechanisms. Consequently if body size increases, surface area to volume ratio decreases. This result in less heat loss. More fat can obviously be stored in a bigger body when food is scarce. All of this means that a bigger body improves survival chances in winter. This can be taken further to explain each of the parts of the metabolic syndrome. Shivering needs additional fuel in the form of carbohydrates, lipids or proteins. Glucose, lipids and triglycerides are all increased in the metabolic syndrome.

There are two types of shivering namely low and high intensity shivering. These rely on type I and type II fibres respectively. Glucose transport into type II fibres is not as dependent on insulin as type I. Insulin resistance would favour preferential delivery of glucose to the type II fibres. This would enable the shivering response. The cause of this winter response might be a fall in Vitamin D levels. Vitamin D in turn is synthesised as a result of UVB radiation. At higher latitudes, UVB decreases in autumn and almost disappears in winter.

There is a theory that vitamin D evolved in primitive organisms as a receptor to signal changes in sunlight levels. The hypothalamus can sense the level of vitamin D and consequently raise the set point of the body mass.[26]

Another mechanism whereby vitamin D levels are decreased in overweight individuals is through sequestration of vitamin D in the adipose tissue.[6]

Sneve et al.[27] enrolled four hundred and forty five healthy, overweight and obese participants. They were randomized into three groups: Those given 20 000IU cholecalciferol twice a week, or 20 000IU once a week plus placebo, or placebo twice a week for 12 months. All participants were given 500mg calcium. At the end of the 12 months period it was established that cholecalciferol supplementation did not lead to more weight reduction in overweight and obese people compared to placebo.**Error! Bookmark not defined.**

There are varying results when examining the studies looking at the effects of vitamin D supplementation on weight. Sneve et al.**Error! Bookmark not defined.** referred to a study by Lijunghall et al. on 65 men aged 61-65 years, in which there was a significant weight loss of 1.1 kg in the group given 0.75ug alphacalcidol when compared to the placebo group over a 12 week period. Lind et al.[28] noted a small but significant weight loss of 0,9 kg over 18 months in a group of 14 middle aged men treated with 2 micrograms of alphacalcidol daily over 18 months.

In contrast a study by Nilas and Christiansen, cited in Sneve et al.**Error! Bookmark not defined.**, that included

In contrast a study by Nilas and Christiansen, cited in Sneve et al.**Error! Bookmark not defined.**, that included 238 post menopausal women, treated for 1 year with either 2 000IU cholecalciferol, 0.25ug alphacalcidol or 0.25 – 0.50ug calcitriol, reported no effect on the body weight when compared with placebo.

Zittermann et al.[29] observed the association between low 25-hydroxyvitamin D (<43nmol/L) and obesity. It has been observed that 1.25$(OH)_2$D3 levels are also low in obese individuals. Higher calcitriol levels have been shown to be beneficial to cardiac muscle cells and to vessels. In those patients who are at a higher risk of cardiovascular mortality, calcitriol concentrations less than 60pmol/L are independently associated with poor clinical outcomes.

Zitterman et al.**Error! Bookmark not defined.** looked at vitamin D supplementations' beneficial effects of weight loss on cardiovascular disease risk markers. The study showed that a daily vitamin D supplement of 3332IU cholecalciferol beneficially influenced some traditional and non-traditional cardiovascular disease risk factors. These included: blood pressure, cholesterol, C-reactive peptide, tumour necrosis factor – alpha, interleukin-6, glucose, HbA1c and pro-insulin, but had no effect on weight loss in overweight plus obese subjects. The beneficial biochemical effects were independent of the losses in body weight, fat mass and fat mass in the abdominal region.

Boon et al.[30] examined the effects of a seven day oral supplementation with 2 000IU cholecalciferol per day on energy and substrate metabolism and adipose tissue gene expression of proteins related to energy and metabolism. No differences in resting metabolic rate or fat oxidation were observed. There were also no differences in gene expression of five of the genes involved in the lipogenic and lipolytic pathways.

Another pathway whereby supplementation with vitamin D could affect weight is dependent on calcium and PTH as key determining factors. The benefits of a high calcium diet have been observed, and increase in the loss of fat mass and increased energy expenditure have been noted in higher calcium diets.**Error! Bookmark not defined.** In vitro plus in cultures of human adipocytes,1,25-(OH)-D3 has been shown to cause increases in *intracellular* calcium. This increased intracellular calcium in the adipocytes increases lipogenesis and inhibits lipolysis. Serum 1,25-(OH-D3 responds to changes in dietary calcium intake. Therefore by decreasing dietary calcium intake an accompanying increase in serum 1,25-(OH)-D3 will occur resulting in an increase in intracellular calcium and fat accumulation.**Error! Bookmark not defined.**

On looking at in vitro studies PTH has also been found to increase intracellular calcium levels, which in turn may induce fat accumulation. If Vitamin D is administered this would result in a decrease in PTH levels, the

ultimate effect on adipocytes following Vitamin D supplementation would depend on the relative importance of reducing the PTH level versus increasing the 1,25-(OH)D3 level.**Error! Bookmark not defined.**

1.4.2.2 Vitamin D and impaired glucose tolerance or Type II Diabetes

The second component of the metabolic syndrome associated with hypovitaminosis D is impaired glucose tolerance or Type II diabetes.

When considering what role vitamin D plays in diabetes mellitus a number of mechanisms need to be considered. These include chronic inflammation, dysfunction of pancreatic B-cells and resistance to insulin peripherally.[5]

In experiments, vitamin D receptors have been shown to exist in the pancreas islets. Further, vitamin D dosing in Vitamin D deficient animals raises the secretion of insulin.[31]

In vitamin D receptor knockout mice the rate of insulin secretion after a glucose challenge was impaired despite the basal insulin rate not being changed. Pancreatic intracellular calcium is an important stimulus for the secretion of insulin. This is affected by vitamin D deficiency.

The expression of insulin receptors as well as the response of insulin for glucose transport has both been shown to be dependent on vitamin D. Thus, the role of vitamin D in secretion and sensitivity of insulin is well demonstrated.**Error! Bookmark not defined.**

Pancreatic B-cell dysfunction, peripheral tissue resistance to insulin and chronic inflammation appear to be possible mechanisms for the role of vitamin D in diabetes mellitus.[3] Experimentally, vitamin D receptors have been identified in pancreatic islets and administration of vitamin D increases insulin secretion in vitamin D-deficient animals.[28] Basal insulin rate was not altered in vitamin D receptor-knockout mice, but insulin secretion rate after a challenge with glucose diet was impaired in vitamin D deficiency. Vitamin D may affect intracellular calcium levels in pancreatic cells, which is an important stimulus for insulin secretion. Vitamin D has also been shown to control insulin receptor-expression and insulin responsiveness for glucose transport, establishing its role in insulin secretion and sensitivity.[5]

There are a significant number of studies looking at vitamin D supplementation and its effects on fasting blood glucose, glucose tolerance and insulin sensitivity.[5] The results are variable. This variability has been postulated to be due to ethnic differences or vitamin D receptor gene polymorphisms.[5]

Parekh et al.**Error! Bookmark not defined.** echoed this sentiment in his review paper, citing several studies conducted in glucose tolerant as well as glucose intolerant human subjects to evaluate the role of vitamin D on insulin sensitivity, insulin secretion and glycaemic status. The outcomes have been inconsistent in these investigations. Among publications that evaluated the role of vitamin D supplementation in patients with glucose intolerance and Type 2 diabetes, only one report including women with gestational diabetes mellitus showed a significant decrease in blood glucose levels.**Error! Bookmark not defined.**

In a study by Patel et al.[32] in which conventional vitamin D treatment improved, but did not optimise serum 25(OH)D no improvements in glycaemic, insulin sensitivity or the lipid profile were seen.**Error! Bookmark not defined.**

Parekh et al.**Error! Bookmark not defined.** are in agreement with the paper by Vanga et al.5 in which they stated that vitamin D receptor gene polymorphisms may affect the glycaemic response to vitamin D and ethnic variation in vitamin D receptor polymorphisms (a well described entity) is responsible for the varied responses seen.

A small study involving 10 women with Type 2 diabetes treated with cholecalciferol 1332 IU daily for one month showed increased first-phase insulin secretion. Fasting and post-change (75g glucose) insulin levels were also decreased with increased 25(OH) vitamin D concentrations in 142 elderly non-diabetic.**Error! Bookmark not defined.**

In a trial by Witham et al.[33] looking at a group of Type 2 diabetics, who were randomized into 3 groups of single dose 100,000, 200,000 IU of vitamin D3 and placebo, the effects of raising 25(OH)D levels on endothelial function, blood pressure and markers of glycaemic control were examined. High dose vitamin D3 had no significant effect on HbA1c or insulin resistance. Systolic blood pressure was improved and B-type natriuretic peptide levels improved.

This study highlighted the fact that the positive effects of vitamin D on insulin resistance have been documented in a few studies where the patients have been pre diabetic. In contrast, in patients with Type 2

diabetes administration of vitamin D has not had a positive effect on insulin resistance or glycaemic control.[35]

In another trial by Nagpal et al.[34] 100 healthy, middle-aged centrally obese men were given short-term vitamin D supplementation (3 doses of vitamin D3 fortnightly, 120,000IU each) to determine its effect on insulin sensitivity. The authors concluded that vitamin D supplementation increased the oral glucose insulin sensitivity index in centrally obese men. The response was better in subjects with lower serum 25(OH)D concentrations and in those with greater central adiposity. Nagpal and his colleagues felt there was a paucity of intervention trials on the effect of vitamin D supplementation on insulin resistance or glucose metabolism. In addition the available trials were conducted using small sample sizes and in different clinical settings, using different agents, regimens and outcome parameters therefore results documented were inconsistent.[34]

In the Women's Health initiative trial calcium and vitamin D3 were randomly assigned to postmenopausal women. Among 33,951 participants without self-reported diabetes at baseline, new diagnoses of diabetes treated with oral hypoglycaemic agents or insulin were ascertained by treatment assignment. The effects of the intervention on fasting measurements of glucose, insulin and insulin resistance were examined among a subset of participants. The Women's Health Initiative was a good opportunity to address the effect of calcium plus vitamin D supplementation on the development of diabetes because of its large size, placebo control and relatively long duration of treatment and observation. In conclusion, the study found that calcium plus vitamin D3 supplementation did not reduce the risk of developing diabetes.[35]

To date the Women's Health initiative is the largest trial on vitamin D supplementation. This trial reported no statistically significant effects for all cardiometabolic outcomes examined. Notable problems with this trial included: a relatively small dose of vitamin D was used (400IU/d), and participants in both intervention groups were permitted to receive supplemental vitamin D. There were difficulties with adherence over a 7 year period.**Error! Bookmark not defined.**

The vitamin D dosage of 400IU used increased median plasma 25(OH) vitamin D levels from 42.3 nmol/L to on 54.1 nmol/L. Extrapolating these data to achieve 25(OH) vitamin D levels about 75nmol/L, the recommended level for several health outcomes, would require supplementation of at least 1000IU/d to determine whether improvements in vitamin D status may prevent cardiovascular disease.[22] A protective

effect of vitamin D supplementation on cardiovascular disease is possible, but a moderate to high dose may be needed.

The evidence for the role of hypovitaminosis D in impaired glucose tolerance and Type II diabetes is limited. There is a lack of large randomized controlled trials. The trials that have been done show inconsistent results. Type II diabetes is a condition which is increasing in epidemic proportions.[7]

If vitamin D has a preventative role to play in its aetiology and course, this would have significant implications. The need for large well-conducted randomised controlled trials is clearly evident.

1.4.2.3 Vitamin D and blood pressure

An inverse relationship between vitamin D and hypertension has been documented. In vitro studies showed that 1,25-dihydroxyvitamin D inhibits renin expression in the juxtaglomerular apparatus and blocks the proliferation of vascular smooth muscle cells, which could result in changes in systemic blood pressure.[36] This hypothesis was reinforced by Li et al. (in Forman et al)[36] who showed that 1,25-hydroxyvitamin D inhibits renin expression in mice and inhibits the growth of cultured vascular smooth muscle cells. The association between vitamin D and hypertension may be mediated via the renin-angiotensin system and the vasculature.[36] In vitamin D deficient subjects replacement with vitamin D significantly improved flow-mediated dilation of the brachial artery, suggesting a role of vitamin D in the sensitivity of vascular smooth muscle cells.[5]

Geographical location plus latitude seem to play a role in the relationship between vitamin D and blood pressure. In the INTERSALT study, which studied more than 10 000 participants worldwide, both systolic and diastolic blood pressure was significantly and positively associated with distance from the Equator.[36] In keeping with this study, it has been noted among Africans that those residing in northern regions have higher blood pressure than those residing closer to the Equator. Seasonal variation of blood pressure within the same population has also been documented.[36]

In a smaller study, 18 hypertensive patients participated in a randomized controlled trial. One group received repeated exposure to artificial UVA radiation (which cannot produce Vitamin D3) and the second

group received UVB radiation (which leads to cutaneous Vitamin D3 synthesis). The UVB treatment group showed an average of 6mm Hg decrease in both systolic and diastolic blood pressure and a 180% increase in serum 25(OH)D levels versus the group exposed to UVA, who showed no change in serum 25(OH)D levels or blood pressure.[37]

Pfeifer et al.[38] conducted a randomised, placebo-controlled, double blind trial of 148 elderly women in which one group was given 1200mg calcium plus 800IU vitamin D3 and the second group 1200mg calcium daily. The trial took place over an 8 week period. At the end of the trial the intervention group showed a decrease in systolic blood pressure of 9.3% (p = 0.0). Sixty participants in the vitamin D3 and calcium group compared to 35 participants in the calcium group, showed a decrease in systolic blood pressure of 5mm Hg or more (p=0,04). There was no change in diastolic blood pressure. The authors concluded that supplementation with vitamin D3 and calcium is more effective in reducing systolic blood pressure than calcium alone.[38]

In a large prospective study in which more than 200 000 men and women from the two Nurses Health studies and the Health Professionals follow up study were studied, it was concluded that higher intake of vitamin D was not associated with a lower risk of incident hypertension.[39]

In the Nurses Health Study I, 27 084 participants reported developing hypertension. Vitamin D intake was not associated with a lower risk of hypertension. [39] In the Nurses Health Study II, 7 372 participants reported developing hypertension. In this study there was also no association between vitamin D intake and the risk of hypertension. Women whose intake of vitamin D was <200IU/d had no increased risk compared to those whose intake was higher than 1000IU/d (multivariable RR, 1.03; 95% CI 0.85 to 1.25).[39] In the Health Professionals follow up study 8 834 men developed hypertension, and no association between vitamin D and hypertension was documented when men who consumed >100IU/d were compared with men who consumed <200IU/d (multivariable RR, 1.06; 95% CI 0.90 to 1.22).[39]

Pfeifer et al.[38] hypothesized that using calcium plus vitamin D3 for a short while may improve blood pressure as well as secondary hyperparathyroidism more effectively than calcium alone.

Forman et al.[36] postulated that the difference in outcomes between the different studies may be explained by the fact that some studies recruited participants from the general population,[39] whereas others recruited only vitamin D deficient subjects.[38] To give us an estimation of the plasma 25-hydroxyvitamin D levels in the adult population we can look at the levels from the National Health and Nutrition Examination Survey III

data. The mean 25-hydroxyvitamin D levels in white adults over the age of 30 years were 28ng/ml, which might be an approximation of the mean level in the observational study.[39] In the interventional study,[38] the mean 25-hydroxymvitamin D level increased from 10.2 ng/ml at baseline to 25.9 ng/ml after supplementation.

These findings suggest that a threshold may exist, and vitamin D deficiency may have detrimental effects on blood pressure, whereas higher levels of vitamin D may not have such a marked effect on blood pressure.[36]

In a systematic review and meta-analysis by Witham et al.[40] a small reduction in blood pressure using vitamin D compounds was seen in hypertensive patients but no effect was seen in normotensive patients. The authors of the review concluded that the evidence was not sufficient to make recommendations for the use of vitamin D in clinical practice as a tool for lowering blood pressure.[40]

1.3.2.4 Vitamin D and lipid profile

The final component of the metabolic syndrome to be examined is the serum lipid profile. There are very few studies looking at the relationship between vitamin D3 and lipid parameters. Most of the studies have either been done on animal models, or have used dosages of vitamin D3 that have been higher than that of the normal therapeutic range. The results have also been inconclusive.

One study by Heikkinen et al. [41] looked at the long term effects of hormone replacement therapy (HRT) and vitamin D3 on the concentration of serum lipids in a population based prospective three year study. Four hundred and sixty four women were randomized into four groups:

- Group 1 received HRT
- Group 2 received 300IU/d Vitamin D3
- Group 3 received HRT plus vitamin D3 (dose as above)
- Group 4 received placebo

The outcomes after three years showed positive effects of HRT on lipid parameters, vitamin D3 was thought to have unfavourable effects on lipids in this group of postmenopausal women as vitamin D3 caused an increase in serum LDL cholesterol levels. The positive effects of HRT were diminished when combined with vitamin D3.[41]

Lind et al.[42] in a cross-sectional population based study looked at the relationship between vitamin D and various cardiovascular risk factors in 34 middle aged men. An inverse relationship was found between vitamin D, blood pressure and markers of lipid plus insulin metabolism.[42] The authors put forward a hypothesis that although the possibility exist that active vitamin D might have yet unknown direct effects on glucose and lipid metabolism, low levels of active vitamin D may be involved in the aetiology of the metabolic syndrome as a whole.

Low levels of vitamin D may be only a marker for a yet undefined abnormality resulting in abnormal blood pressure, lipid, glucose and mineral metabolism, and in active vitamin D production. One theory postulated is that of altered cell membrane fluidity.[42]

Vitamin D levels may be increased by the use of statins. This could explain the multiple non-cholesterol effects of these drugs. The possible mechanisms are as follows:

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-Co-A) reductase is inhibited by the statins. This causes increased 7-dehydroxycholesterol. This in turn is converted by sunlight to 25-hydroxycholecalciferol.[5]

Clearly, all of the above provides evidence both for and against the role of vitamin D in the metabolic syndrome**.**

1.4 HEALTH CLAIMS

Over the past few years there has been a marked interest in both the scientific and lay media of the health effects of vitamin D.

The proposed health benefits attained when taking vitamin D supplementation come in an era when similar claims regarding antioxidant vitamins and folic acid supplementation were unsubstantial. The use of beta-carotene and vitamin E supplements (two antioxidant vitamins that were believed to be safe), showed an increase in mortality in randomised controlled clinical trials.[43]

When using vitamin supplements such as vitamin D in areas beyond bone and skeletal health, For example; cardio-metabolic cases, well-conducted studies documenting both benefits and harms are needed.

In one systematic review done by Pittas et al.[21] on observational studies, the association between serum levels of vitamin D and cardiometabolic outcomes was uncertain. Three of six analyses reported a lower incident diabetes risk in the higher versus the lowest vitamin D status groups. Eight randomised controlled trials found no effect of vitamin D supplementation on glycaemia or incident diabetes. In meta-analysis of 3 cohort studies, lower 25-hydroxyvitamin D concentration was associated with incident hypertension. In a meta-analysis of 10 randomised controlled trials vitamin D supplementation non-significantly reduced systolic blood pressure and did not affect diastolic blood pressure.[21] The range of dosages used in these trials ranged from 400 to 8571IU/d and the study participants were heterogeneous.

In a second systemic review done by Wang et al.[21] also on 17 observational studies, the association between vitamin D and calcium supplementation and the prevention of cardiovascular events was examined. The results showed consistent reductions in cardiovascular disease mortality among adults who received vitamin D supplements. Five of these six studies were conducted among patients receiving dialysis and the generalisability of the results to healthy adults is therefore uncertain.[21]

The effect of vitamin D supplements on cardiovascular disease, diabetes, dyslipidaemia and hypertension remain uncertain. However, the available evidence in favour of vitamin D supplementation is far more promising than for other vitamin or mineral supplements.[43]

1.5 WHY IT IS IMPORTANT TO DO THIS REVIEW

Evidence-based decision-making should been important concept in the practice of all healthcare professionals. Sacket and Haynes et al.[44] defined evidence-based medicine as a "conscientious, explicit and judicious use of current best evidence in making decisions" where conscientious means evidence is relevant and is applied consistently, judicious means evidence is combined with clinical expertise to obtain a balance of risk and benefit for the patient, and current best evidence means that the best type of evidence for the specific type of question (e.g. randomized controlled trials for questions on prevention and treatment), within the context of the totality of the body of evidence (i.e. drawing on systematic review methods), is used..

According to the American Heart Association, almost 25% of Americans are affected by the metabolic syndrome. There are currently no statistics available for the prevalence of the metabolic syndrome in South Africa. A single paper was published looking at the prevalence of the syndrome in a small group of

corporate executives in 2007. Using the ATPIII criteria, 31% of the group was diagnosed as having the metabolic syndrome.[45] The limitations of this study include population bias as there was no inclusion of any segment of the rural population of South Africa.

This review would be useful for any patient suffering from the Metabolic Syndrome or any of the individual components of the Metabolic Syndrome and practitioners treating such patients. Currently there are no systematic reviews looking at the influence of Vitamin D deficiency on the metabolic syndrome. This review will therefore provide some evidence – based material as to the use of Vitamin D in the metabolic syndrome.

1.6 CONCEPTUALISATION

The influence of vitamin D supplementation on components of the metabolic syndrome was examined in this systematic review, as illustrated in Fig. 1.1:

- Etiological causes of vitamin D deficiency (background literature review)
- Etiological factors associated with the metabolic syndrome are indicated as genetic predisposition and unhealthy lifestyle (background literature review)
- The individual components that make up the metabolic syndrome are obesity, hypertension, dislipidaemia and impaired glucose tolerance (background literature review)
- The effect of vitamin D deficiency on the components of the metabolic syndrome measured by waist circumference, BMI, blood pressure, lipid profile, glucose profile, pro-inflammatory markers and prothrombotic markers (systematic review of randomised controlled trials)

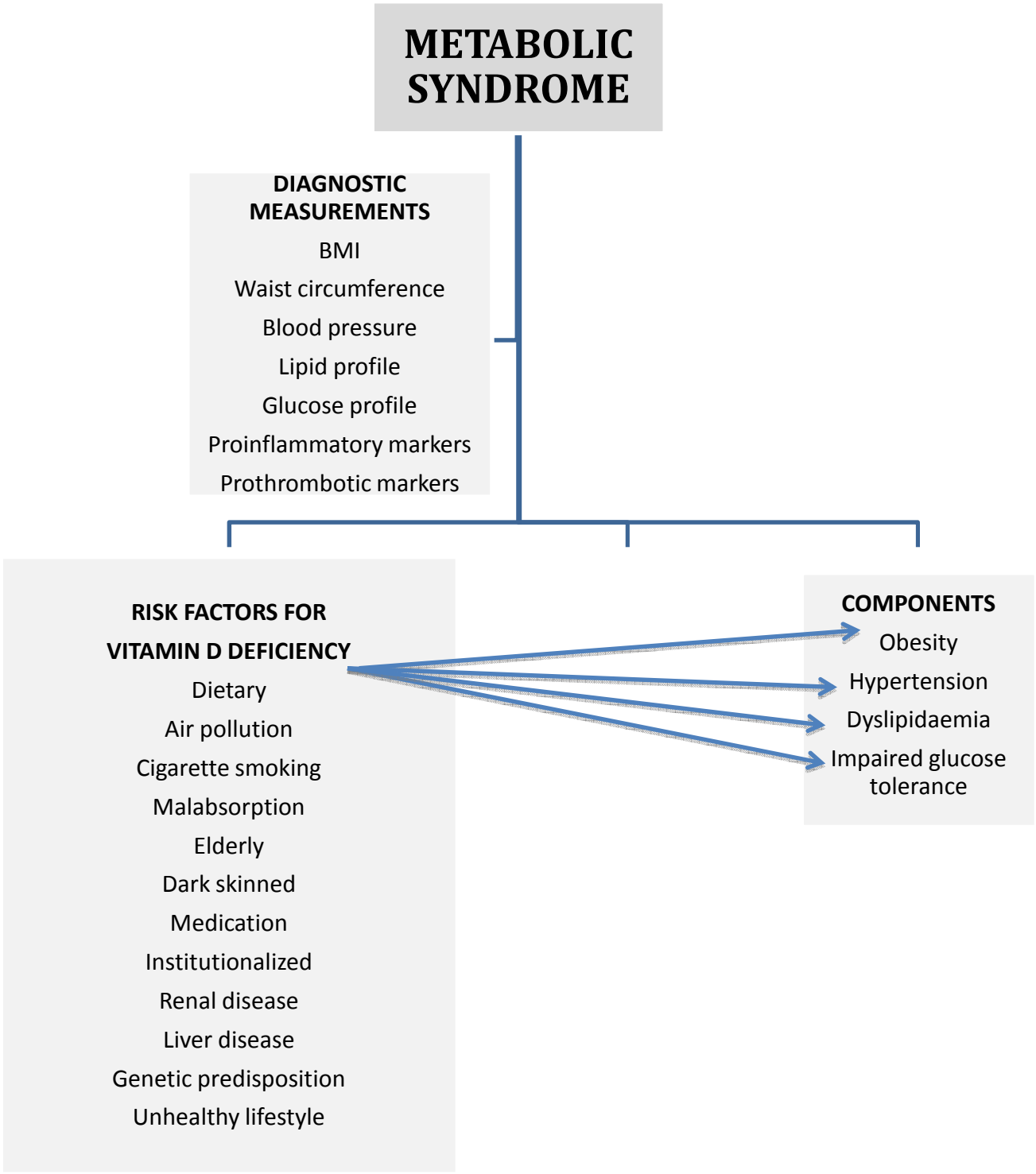

Figure 1.1 Conceptual Diagram of Vitamin D and the Metabolic syndrome

1.7 DESCRIPTION OF THE INTERVENTION

Trials were considered for inclusion in this systematic review if they compared the effect of vitamin D at any dose, duration and route of administration versus placebo or no intervention. In addition, vitamin D may have been administered as monotherapy or in combination therapy. Studies were excluded if they used fortified foods as intervention.

1.8 HOW THE INTERVENTION MIGHT WORK

Vitamin D supplementation may affect each of the individual components of the metabolic syndrome:

1. Obesity - increased weight and loss
2. Blood pressure – decrease systolic, diastolic blood pressure
3. Cholesterol – improve profile
4. Glucose – decreased serum glucose

    - decreased insulin resistance

CHAPTER 2: METHODOLOGY

2.1 RESEARCH QUESTION

What is the influence of Vitamin D_2 and D_3 supplementation, compared to placebo or no intervention, on the components of the metabolic syndrome (raised BMI, hypertension, dislipidaema, glucose intolerance) in vitamin D deficient/ insufficient adults?

2.2 OBJECTIVES

To assess the influence of vitamin D_2 and D_3 administration in treating and preventing components of the metabolic syndrome in various population groups.

2.3 CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW

2.3.1 Types of Studies

Randomised controlled trials investigating the effect of vitamin D supplementation on components of the metabolic syndrome.

2.3.2 Types of Participants

Participants who met the following selection criteria were included:

2.3.2.1 Inclusion criteria

- Adult participants (aged 18 years and older) including the elderly
- All race groups
- Patients diagnosed with any one or more of the components of the metabolic syndrome (Table 2.1). Drug treatment for elevated serum triglycerides, reduced HDL-cholesterol levels, hypertension or elevated blood glucose levels was considered an alternate indicator of the relevant diagnostic criteria of the metabolic syndrome. In addition, studies that used population and country specific definitions for cut-off points that differed from those in Table 2.1 were also considered for inclusion.

- Subjects were considered for inclusion in the study if there 25(OH) vitamin D levels were less than 80nmol/L.

- Only studies for which full text articles could be obtained, were included.

Table 2.1: Diagnostic criteria of the metabolic syndrome.[61]

IDF	**NCEP**	**WHO**	**AACE**
Diagnosed if glycaemia is abnormal and 2 additional criteria are present	Diagnosed if 3 out of 5 criteria are present	Diagnosed if glycaemia is abnormal and 2 additional criteria are present	Indicates risk factors
Fasting glycaemia 100-125 mg/dl or DM2	Glycaemia 110-125 mg/dl	Glucose intolerance DM2 or insulin-resistance due to HOMA-IR	Fasting glycaemia 110-125 mg/dl > 140 mg/dl 2 hours after oral GTT
WC > 94cm M WC > 80cm W	WC > 102 cm M WC > 88 cm W	BMI > 30 and HWR > 0.9 M and > 0.85 W	BMI > 25 and WC > 102 cm M and WC > 88 cm W
TG > 150 mg/dl or HDL < 40 M and < 50 W	TG > 150mg/dl HDL < 40 M and < 50 W	TG > 150mg/dl or HDL < 35 M and < 39 W	TG > 150mg/dl or HDL < 40 M and < 50W
On treatment for SAH or BP > 130/85 mmHg	BP > 130/85 mmHg	On treatment for SAH or BP > 160/90 mmHg Microalbuminuria > 20 mcg/min	BP > 130/85 mmHg
HOMA IR > 1.93 = insulin resistance			

AACE = American College of Endocrinology/American Association of Clinical Endocrinologists; BMI = body mass index; BP = arterial blood pressure; DM2 = diabetes mellitus type 2; GTT = oral glucose tolerance test; HDL= high density lipoprotein ; HOMA-IR = homeostasis model assessment; HWR = hip: waist ratio; IDF = International Diabetes Federation; M = men; NCEP = US National Cholesterol Education Program; SAH = systemic arterial hypertension; TG = triglycerides; W = women; WC = waist circumference; WHO = World Health Organisation.

2.3.2.2 Exclusion criteria

- Pregnant/lactating women
- Kidney and liver disease

2.3.3 Types of Interventions

Trials were considered for inclusion if they compare the effect of vitamin D at any dose, duration and route of administration versus placebo or no intervention. Vitamin D may have been administered as monotherapy or in combination therapy.

2.3.4 Types of Outcome Measures

Studies reporting on the following outcomes were considered for this systematic review.

2.3.4.1 Primary outcomes

- BMI
- Waist circumference
- Blood pressure
- Lipid profiles LDL, HDL, triglyceride levels.
- Blood glucose, insulin profiles, HbA1c, HOMA IR
- Serum 25(OH)D_2 and/or 25-(OH)D_3

2.3.4.2 Secondary outcomes

- Proinflammatory states fibrinogen, plasminogen, C-reactive protein (CRP) interleukins
- Prothrombotic state
- Adverse effects observed

2.4 SEARCH METHODS FOR IDENTIFICATION OF STUDIES

2.4.1 Electronic Searches

2.4.1.1 Search criteria / string

Search terms were formulated for the condition, the intervention, the use of controls and outcomes. The search was performed by a qualified librarian looking for randomised controlled trials using the following search string, adapted for individual databases:

Topic=((metabolic syndrome OR insulin resistance OR abdominal obesity OR cardiometabolic OR visceral obesity)) AND Topic= ((vitamin D OR calcitriol OR 25 ohd OR 25 hydroxyvitamin D OR 25-ohd* OR 25-hydroxyvitamin D OR 25 (oh)d)) AND Topic=((placebo OR no intervention OR without)) AND Topic=((waist circumference OR blood pressure OR hdl OR tg Or lipoprotein OR triglyceride OR glucose OR sugar OR insulin OR bmi OR proinflammatory OR prothrombotic OR fibrinogen OR plasminogen OR CRP OR C reactive protein OR IL OR interleukin))

The librarian performed a literature review using different MeSH Terms including “metabolic syndrome”, “insulin resistance”, and “abdominal obesity” in combination with “vitamin D”, “calcitriol”, “25-hydroxyvitamin D” and “blood pressure”, “waist circumference”, “HDL”, “lipoprotein”, “triglyceride”, “glucose”, “sugar”, “insulin”, “BMI”, “proinflammatory”, “prothrombotic”, “fibrinogen”, “plasminogen”, “CRP”, “C reactive protein”, “interleukin” and “IL”.

This search string was adapted for individual databases by the librarian.

A search filter in Pubmed restricted the search to randomised controlled trials.

2.4.1.2 Databases searched

The electronic search was done in October 2010 at Stellenbosch University Library by the investigator and a qualified Librarian. The search was repeated under the same circumstances in May 2012.

The following databases were used for identification of trials from inception to May 2012:

- The Cochrane Central Register of Controlled Trials (Central)
- Medline (accessed via Pubmed)
- Science Direct
- ISI Web Of Knowledge
- Scopus

2.4.1.3 Searching other resources

- Vitamin D manufacturers were contacted for unpublished trials

Electronic searches focused on studies published in English databases, but a record was kept of publications in other languages so that they could be traced if necessary.

2.5 DATA COLLECTION AND ANALYSIS

2.5.1 Selection of Studies

Two review authors independently screened the titles and abstracts of the electronic search results and selected potentially relevant studies using an eligibility form (ADDENDUM A: Part of Data Extraction Form). The search was repeated in the same way in May 2012. Corresponding full-text articles of potentially relevant studies were retrieved and used in applying the eligibility criteria. Each of the articles was scrutinized to ensure that multiple publications from the same study were included only once. Where eligibility was unclear, clarification was sought from the trial authors and the corresponding articles were re-assessed. Differences between the reviewers regarding the eligibility results were resolved through discussion with other review authors. Studies that did not meet the inclusion criteria were excluded and the reasons for exclusion were recorded.

2.5.2 Data Extraction and Management

Two review authors independently extracted data on methods, participants, interventions, comparisons, study designs, and outcomes from each included study using a pre-designed data extraction form (ADDENDUM A) designed specifically for this review. One review author (statistician) also independently extracted data relevant for analysis of the effects of intervention. There were no dichotomous outcomes in this review. For each continuous outcome, the number of participants randomized, the number of participants analysed, the mean and standard deviation (or information to estimate the standard deviation) in each treatment group, were extracted. Where necessary, medians and ranges were extracted instead of means and standard deviations. Other relevant data extracted were study authors, titles, country and setting, source of publication, participant baseline characteristics, ethical clearance, funding source, and inclusion and exclusion criteria of participants. The study authors were contacted to request missing data and any disagreements were resolved by discussion with other review authors.

2.5.3 Assessment of Risk of Bias in Included Studies

Two review authors independently assessed the risk of bias of the included studies across the following six components of the Cochrane Collaboration Risk of Bias tool[60] (Table 2.2):

- sequence generation (selection bias)
- allocation concealment (selection bias)
- blinding (of participants, personnel, and outcome assessors) (performance and detection bias)
- incomplete outcome data (attrition bias)
- selective outcome reporting (reporting bias)
- other sources of bias

Table 2.2: Criteria for assessing risk of bias in included studies*

DOMAIN	DESCRIPTION	Risk of bias
SEQUENCE GENERATION	Comment: 1. Was information provided? 2. Were patients randomly allocated? 3. Was randomization properly carried out?	**Was the allocation sequence adequately generated?** UNCLEAR (unclear or unknown risk of bias) YES (low risk of bias): • Random number table • Coin tossing • Computer random number generator • Shuffling cards or envelopes • Throwing dice • Drawing of lots • Minimization NO (high risk of bias) • Odd or even date of birth • Date/day of admission • Hospital or clinic record number • Allocation by judgement of physician • Allocation by preference of participant • Allocation based on the result of a laboratory test • Allocation by availability of intervention
ALLOCATION CONCEALMENT	Describe allocation sequence method used	**Was the allocation sequence method used sufficient to conceal the intervention allocations?** UNCLEAR: • Insufficient information YES (low risk of bias): • Central allocation - telephone, web-based, pharmacy • Controlled randomization • Sequentially numbered drug containers of identical appearance • Sequentially numbers opaque, sealed envelopes NO (high risk of bias): • Using an open random allocation schedule e.g. list of random numbers. • Assignment envelopes were used without appropriate safeguarding e.g.: unsealed, an opaque, non-sequentially numbered

DOMAIN	DESCRIPTION	Risk of bias
BLINDING OF PARTICIPANTS AND OUTCOME ASSESSORS	Describe all measures	**Was knowledge of the allocated intervention adequately prevented during the study?** UNCLEAR (unclear or unknown risk of bias) YES (low risk of bias): • No blinding - but the outcome and the outcome measurement are not likely to be influenced by lack of binding • Blinding of participants and key study personnel ensured and unlikely that the blinding could have been broken • Either participants or some key study personnel were not blinded, but outcome assessment was blinded and the non-blinding of others unlikely to introduce bias NO (high risk of bias): • No blinding/incomplete blinding and the outcome or outcome measurement is likely to be influenced by lack of blinding • Blinding attempted but likely could have been broken • Participants/key study personnel were not blinded and the non-blinding of others likely to introduce bias
INCOMPLETE OUTCOME DATA	Describe attrition Plus exclusions - reasons Plus any re-inclusion analysis performed	**Were incomplete outcome data adequately addressed?** UNCLEAR: • Insufficient information • Study did not address this outcome YES (low risk of bias): • No missing outcome data - no dropouts • Reasons for missing outcome data unlikely to be related to true outcome • Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups • Missing data have been imputed using appropriate methods NO (high risk of bias): • Reasons for missing outcome data likely to be related to true with either imbalance in numbers or reasons for missing data across intervention groups • Potentially inappropriate application of simple imputation

DOMAIN	DESCRIPTION	Risk of bias
SELECTIVE OUTCOME REPORTING	How was selective outcome reporting examined and findings?	**Are reports of the study free of suggestion of selective outcome reporting?** UNCLEAR: • Insufficient information • Study did not address the outcome. YES (low risk of bias): • Protocol available, primary and secondary outcomes reported in the pre-specified way • Protocol NOT available but it is clear that the published reports include all expected outcomes, including those that were pre-specified NO (high risk of bias): • Not all pre-specified primary outcomes have been reported • One or more primary outcome is reported using measurements, analysis methods or subsets of data that were not pre-specified • One or more reported primary outcomes were not pre-specified (unless justification is provided) • One or more outcomes of interest are reported incompletely • Study fails to include results for a key outcome that would be expected to have been reported
OTHER	Other sources of bias not addressed by other domains	**Was the study free of other sources of bias?** UNCLEAR YES NO

*Table adapted from the 2008 Cochrane Handbook for Systematic Reviews of Interventions.[60]

Judgments were categorised to indicating a low, high, or unclear risk of bias respectively. The results were summarised using the *'Risk of bias summary'* and the *'Risk of bias graph'* in addition to the *'Risk of bias table'* for each included study. Where necessary, study authors were contacted for clarification and disagreements were resolved by consensus with other review authors.

2.5.4 Measures of Treatment Effect

Review Manager Version 5 (RevMan 2008) was used to conduct the analyses. Mean differences (MD) for continuous outcomes were calculated and the results were presented with 95% confidence intervals (CI). Medians and ranges for continuous data were reported in table format. There were no dichotomous outcomes in this review.

2.5.5 Unit of Analysis Issues

There were no cross-over and cluster-randomized trials included in this review. For the one study with two active treatment arms for different doses of vitamin D versus one placebo arm, results were reported separately using pair-wise comparisons to avoid unit-of-analysis error as a result of double-counting participants.

2.5.6 Dealing with Missing Data

Where data were missing, authors of studies were contacted to obtain relevant missing information. Intention to treat (ITT) analyses were planned where there was no missing data. In the case of missing data, an available case analysis was planned.

2.5.7 Assessment of Heterogeneity

Although it had been planned to assess heterogeneity using both the Chi^2 and I^2 tests, this was not possible because there was no meta-analysis in this review.

2.5.8 Assessment of Reporting Biases

Funnel plots to assess evidence of publication bias were not constructed because less than ten trials were included.

2.5.9 Data Synthesis

Separate analyses were carried out for different medical conditions. Since there were few studies per condition and also because some outcomes were reported as different units of measurement, only effect sizes of individual studies could be calculated.

2.5.10 Subgroup Analysis and Investigation of Heterogeneity

No subgroup analysis or investigation of heterogeneity could be done because there was no meta-analysis in this review. If sufficient studies were available possible sub-group analyses would have been performed using different age groups, different genders, different forms of supplementation, different quality studies, different durations of intervention and different study sizes.

2.5.11 Sensitivity Analysis

No sensitivity analyses to assess the influence of potential factors on the effect size could be done because there was no meta-analysis in this review. A sensitivity analysis would have been performed to explore the influence of the following factors on effect size: repeating the analysis taking account of trial quality; repeating the analysis excluding trials using the following filters- diagnostic criteria, language of publication, source of funding; repeating the anaylsis excluding various study designs.

CHAPTER 3: RESULTS

3.1 *RESULTS OF THE SEARCH*

The results of the literature search yielded 338 studies (Fig.3.1). The database Science Direct identified 329 publications but many were not directly related to the topic being investigated. A librarian performed the searches in the Scopus (5 publications with one duplicate), PUBMED (7 publications), Cochrane library (18 publications with three duplicates) and Web of knowledge (69 publications with five duplications) (Table 3.1). The local manufacturers of vitamin D supplementation, Georen pharmaceuticals and Arctic pharmaceuticals were contacted by personal interviews, but no additional records were indentified. After many of the irrelevant articles from Science Direct were excluded and duplicates excluded, 31 records were screened. Fourteen full text articles were assessed for eligibility, 10 were excluded. Four papers were included but data did not allow meta-analyses.

TABLE 3.1: Results of electronic searches

DATABASE	**PERIOD SEARCHED**	**SEARCH STRING**	**NUMBER OF DUPLICATES**	**TOTAL NUMBER RETRIEVED TO BE SCREENED**
SCIENCE DIRECT	Inception to October 2010, repeated in May 2012	Topic=((metabolic syndrome OR insulin resistance OR abdominal obesity OR cardiometabolic OR visceral obesity)) AND Topic= ((vitamin D OR calcitriol OR 25 ohd OR 25 hydroxyvitamin D OR 25-ohd* OR 25-hydroxyvitamin D OR 25 (oh)d)) AND Topic=((placebo OR no intervention OR without)) AND Topic=((waist circumference OR blood pressure OR hdl OR tg Or lipoprotein OR triglyceride OR glucose OR sugar OR insulin OR bmi OR proinflammatory OR prothrombotic OR fibrinogen OR plasminogen OR CRP OR C reactive protein OR IL OR interleukin))	-	329
SCOPUS			1	5
PUBMED			-	7
COCHRANE LIBRARY			3	18
WEB OF KNOWLEDGE			5	69

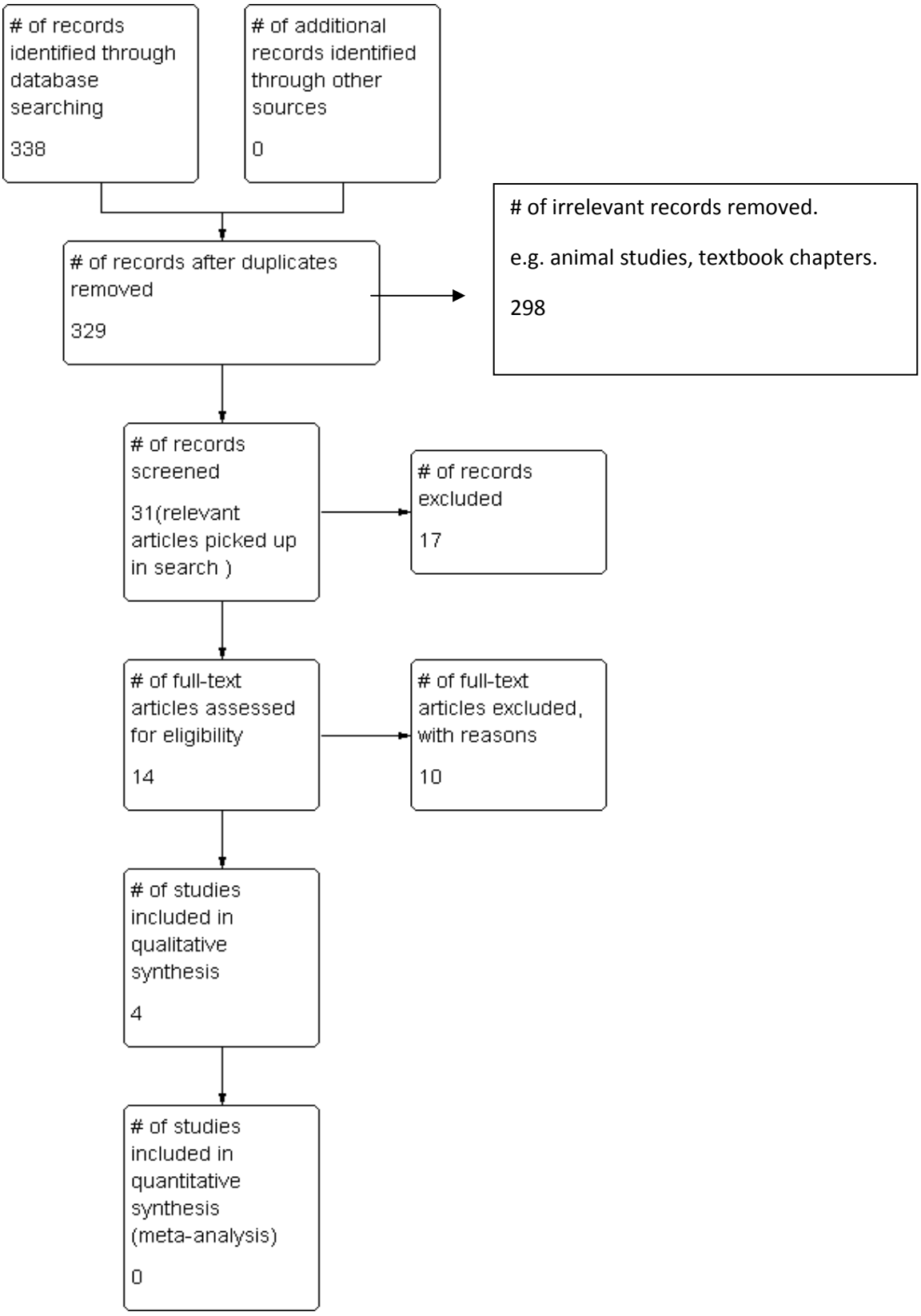

Fig 3.1 Flow diagram demonstrating the searching and selection process

3.2 DESCRIPTION OF STUDIES

3.*2.1 Characteristics of Included Studies*

Four studies with a parallel-group design were included in the systematic review (Table 3.2). The first study by Parekh et al.**Error! Bookmark not defined.** was a randomized, double-blind, placebo-controlled study in India, where twenty eight diabetic Asian Indian patients were randomly assigned to a vitamin D treated group (N=14) or a placebo group (N=14). The vitamin D treated group received 300,000IU D3 intramuscularly, once off. The patients were followed up for 4 weeks. The outcomes examined included: Serum 25-hydroxyvitamin D, haemoglobin AIc , HOMA-IR, serum fructosamine, serum insulin and oral glucose tolerance test was performed.

The second study by Witham et al.[33] in the United Kingdom, was a randomized, blinded, placebo-controlled trial. Sixty one diabetic participants were randomized into three groups. One group received 100 000IU vitamin D3 (N=19) a second group 200 000IU vitamin D3 (N=20) and the third group was given a placebo (N=22). The participants were followed over a sixteen week period. The outcomes examined included serum 25 hydroxyvitamin D, blood pressure, insulin resistance, haemoglobin AIc, endothelial function and serum cholesterol.

The third study by Zittermann et al.[29] was a randomized blinded placebo-controlled trial in Germany. Two hundred over-weight participants were given either 3332 IU cholecalciferol daily (N=100) or placebo for one 1 year (N=100). The outcomes examined included: Serum 25-hydroxyvitamin D, fasting plasma glucose, blood pressure, serum cholesterol, triglycerides, serum LDL, tumour necrosis factor and body weight.

The final study by Von Hurst et al.[11] in New Zealand, was a randomized, placebo-controlled, double-blind trial. This trial included insulin resistant South Asian Women (N=81). The vitamin D group (N=42) was given 4000IU D3 per day for six months. The outcomes examined included: Fasting plasma glucose, fasting serum insulin, HOMA 2%, CRP, serum, 25-hydroxyvitamin D.

TABLE 3.2: Characteristics of included studies

STUDY DESIGN	**STUDY 1** Parekh et al.[31] (India)		**STUDY 2** Witham et al.[33] (U.K.)			**STUDY 3** Zittermann et al.[29](Germany)		**STUDY 4** Von Hurst et al.[11] (New Zealand)	
	Controls	**Intervention**	**Controls**	**Intervention 1**	**Intervention 2**	**Controls**	**Intervention**	**Controls**	**Intervention**
TREATMENT	1 ml distilled water im serving as placebo	Vitamin D3 300000 iu im for 4 weeks	Placebo: Miglyol oil	Vitamin D3 100000 iu once off	Vitamin D3 200000 iu once off	5 drops vitamin d free oil (placebo)	83.3 ug (3332iu) cholecalciferol daily for 12 months	4 oral placebo capsules	Vitamin D3 4000 iu per day for 6 months
NUMBER OF PATIENTS ALLOCATED TO EACH GROUP	14	14	22	19	20	100	100	39	42
NUMBER OF PATIENTS WHO COMPLETED TRIAL	13	14	21	19	18	83	82	Not mentioned	Not mentioned
REASONS GIVEN FOR NON-COMPLIANCE OF TRIAL	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
DURATION OF FOLLOW-UP	4 Weeks	4 Weeks	16 Weeks	16 Weeks	16 weeks	1 Year	1 Year	6 Months	6 Months
GENDER	Male + Female	Male + Female	Male + Female	Male + Female	Male + Female	Male + Female	Male + Female	Female	Female
AGE RANGE	35-50 Years	35-50 Years	18 Years And Older	18 Years And Older	18 Years and Older	18-70 Years	18-70 Years	20+ Years	20+ Years
RACE	Asian Indian	Asian Indian	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	South Asian	South Asian

STUDY DESIGN	**STUDY 1** Parekh et al.[31] (India)		**STUDY 2** Witham et al.[33] (U.K.)			**STUDY 3** Zittermann et al.[29](Germany)		**STUDY 4** Von Hurst et al.[11] (New Zealand)	
	Controls	**Intervention**	**Controls**	**Intervention 1**	**Intervention 2**	**Controls**	**Intervention**	**Controls**	**Intervention**
BODY MASS INDEX (kg/m^2) (Mean)	20-26	20-26	33.3 (7.1)	31.1 (6.7)/29.7 (4.2)		>27	>27	27.4	27.5
DIAGNOSIS OF DIABETES MELLITUS	YES	YES	YES	YES	YES	NO	NO	NO	NO
Diagnoses of impaired glucose tolerance	YES	YES	YES	YES	YES	NO	NO	NO	NO
Vitamin D deficiency <25nmol/L (Mean)	16.74(6.69)	14.91(6.76)	-	-	-	-	-	19	21
Vitamin D insufficiency 40-80 nmol/L (Mean)	-	-	45(17)	41(14)	48(21)	30.3(20.1)	30(17.5)	-	-
Component of MS present in subjects	Elevated blood glucose	Elevated blood glucose	Elevated blood glucose	Elevated blood glucose	Elevated blood glucose	Elevated waist circumference	Elevated waist circumference	Impaired glucose tolerance	Impaired glucose tolerance

STUDY DESIGN	**STUDY 1** Parekh et al.[31] (India)		**STUDY 2** Witham et al.[33] (U.K.)			**STUDY 3** Zittermann et al.[29](Germany)		**STUDY 4** Von Hurst et al.[11] (New Zealand)	
	Controls	**Intervention**	**Controls**	**Intervention 1**	**Intervention 2**	**Controls**	**Intervention**	**Controls**	**Intervention**
Outcomes measured	Serum 25(OH)D HbAIc HOMA-IR Serum Fructosamine Serum Insulin OGTT		Serum 25 (OH)D BP Insulin Resistance HbAIc Endothelial Function Serum Cholesterol			Serum 25 (OH)D FPG BP Serum Cholesterol Triglycerides Serum LDL TNF Body Weight		Serum 25 (OH)D FPG Serum Insulin HOMA 2% CRP	

Serum 25(OH)D: Serum 25 – Hydroxyvitamin D, HbAIc: Haemoglobin AIc, HOMA-IR: Homeostasis model assessment for insulin sensitivity, OGTT: Oral Glucose Tolerance Test, BP: Blood Pressure, TNF: Tumour Necrosis Factor, FPG: Fasting Plasma Glucose, CRP: C Reactive Protein, HOMA 2%: Insulin Resistance

The total number of subjects measured for each outcome is shown in Table 3.3.

There were different outcomes measured in each study. Each of the components of the metabolic syndrome were examined across the included studies. However, none of the studies assessed all of the components.

Table 3.3: Total number of subjects examined for each outcome

25-hydroxyvitamin D	373
Haemoglobin AIc	173
HOMA IR	89
HOMA 2%	84
Serum fructosamine	28
Serum insulin	112
Plasma glucose	112
Blood pressure	261
Endothelial function	61
Serum cholesterol	261
Triglycerides	200
Serum LDL	200
Tumour necrosis factor	200
Body weight	200
CRP	84

3.2.2 Characteristics of Excluded Studies (Relevant records only)

Reasons for the exclusion of trials from the systematic review are described in Table 3.4. Ten trials were excluded because they were not conducted as randomized controlled trials, one paper was only the protocol (the whole article was subsequently published and included in the review (excluding the protocol) and one paper was an Iranian publication that could not be accessed. Five papers had problems with the diagnosis of the components of the metabolic syndrome and in four of these neither vitamin D deficiency or insufficiency was present. A

further 10 full-text articles were excluded. Characteristics of excluded randomised controlled studies are described in Table 3.5.

The trial by De Boer et al.[55] examined the effect of calcium and vitamin D supplementation on the incidence of diabetes in post menopausal women. It was a randomized controlled trial, participants were given 1000mg calcium and 400IU of vitamin D3 daily or placebo. This trial included 33,951 participants. The participants were followed up for a seven year period. Results indicated that supplementation did not reduce the risk of developing diabetes. The study was excluded from the systematic review because the patients did not have at least one component of the metabolic syndrome at diagnosis. In addition vitamin D measurements were not available for all patients; they were only available for a select group of patients.

The trial by Pittas et al.[56] compared the effects of combined calcium and vitamin D supplementation versus placebo on blood glucose and markers of inflammation in non-diabetic adults. The study was designed as a randomized double blind, controlled trial, where participants received either 500mg calcium and 700IU vitamin D3 or placebo daily for 3 years. The findings of this trial were that supplementation may attenuate increases in glycaemia and insulin resistance in patients with impaired fasting glucose. This study was excluded from the systematic review because only some of the patients were diagnosed with one of the components of the metabolic syndrome and not all the patients were vitamin D deficient or insufficient.

The trial by Nagpal et al.[34] looked at the effects of vitamin D3 supplementation on insulin sensitivity in healthy, centrally obese men. This was a double-blind randomized controlled trial, 100 patients were given 3 doses of vitamin D3 (120 000IU each) fortnightly or placebo. The group was followed up for six weeks. The conclusion of the trial was that vitamin D3 supplementation improved postprandial insulin sensitivity. The study was excluded from the systematic review because although the criterium of waist circumference was used as the component of the metabolic syndrome, not all the patients would always have a significantly raised waist circumference.

The study by Jorde et al.[22] examined the effect of vitamin D on cardiovascular risk factors. The trial was a double blind placebo-controlled intervention trial. A total of 438 patients were randomized to receive either 40 000IU vitamin D per week, 20 000IU vitamin D per week or placebo, all patients were given 500mg calcium daily. The follow up period was one year. The outcomes did not find a positive effect of vitamin D supplementation on glucose tolerance, blood pressure or serum lipids. The study was excluded as it met none of the inclusion criteria.

Avenell et al.[53] conducted a randomized controlled trial. The primary outcome was to look at the effects of vitamin D3 on the development of diabetes. The 5292 patients were randomized to take 800IU vitamin D3 daily, 1000mg calcium daily, both or placebo. This trial did not find vitamin D3 had a protective effect against the development of type 2 diabetes. The study was excluded as it did not meet any of the inclusion criteria.

Table 3.4: Excluded studies with reasons (Relevant records only)

Title	Reason For Exclusion
Science Direct:	
Vitamin D metabolism and cardiovascular risk factors in postmenopausal women[46]	Not a RCT
Serum 25-hydroxyvitamin D is independently associated with high-density lipoprotein cholesterol and the metabolic syndrome in men and women[47]	Not a RCT
Poor vitamin D status may contribute to high risk for insulin resistance, obesity and cardiovascular disease in Asian Indians[48]	Not a RCT
Vitamin D deficiency is the cause of common obesity[266]	Not a RCT
Cardiovascular risk in menopausal women and prevalent related coma-bid conditions: facing - the post-women's health initiative era[49]	Not a RCT
Influence of obesity on vitamin D-binding protein and 25 hydroxyvitamin D levels in African American and white women [57]	Not a RCT
Vitamin D and type 2 diabetes: Is there a link?[51]	Not a RCT
Interaction of 25-hydroxyvitamin D levels with metabolic characteristics in polycystic ovary syndrome[52]	Not a RCT
Pubmed:	
Vitamin D supplementation and type 2 diabetics: a sub study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438)[53]	No vitamin D deficiency/ insufficiency, no metabolic syndrome
Study protocol - Metabolic syndrome, vitamin D and bone status in South Asian women living in Auckland, New Zealand: A randomized, placebo-controlled, double-blind vitamin D intervention.[54]	Only a protocol

Title	Reason For Exclusion
The Cochrane Library:	
A double-blind, randomized, placebo controlled trial of the short-term effect of vitamin D3 supplementation an insulin sensitivity in apparently healthy, middle-aged centrally obese men[34]	Sent to third reviewer Exclude- not all the patients would always have a sufficiently raised waist circumference to meet inclusion criteria
Calcium and vitamin D supplementation and the risk of incident diabetes in the women's health initiative[55]	1. Excluded- Patients did not have components of metabolic syndrome at diagnosis 2. Vitamin D concentrations were only available for a select group of patients
The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in non-diabetic adults[56]	Sent to third reviewer Consensus on paper sent to third reviewer: Exclude Not all the patients will be vitamin D insufficient which is one of the inclusion criteria.
ISI Web of Knowledge:	
Vitamin D intake is associated with insulin sensitivity in African American, but not European American women[57]	Exclude - not RCT
Hypovitaminosis D in Chinese type 2 diabetes: Lack of impact on clinical metabolic status and biomarkers of cellular inflammation[58]	Exclude - not RCT
Assessment of vitamin D supplementation effect on insulin resistance among type 2 diabetic patients	IRANIAN PUBLICATION - not accessible
No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D3 for one year[22]	Exclude - none of the metabolic components i.e.: blood pressure, plasma glucose, serum LDL, serum HDL met the diagnostic criteria for the metabolic syndrome There was no Vitamin D deficiency or insufficiency

*RCT: Randomised Controlled Trial

Table 3.5: Characteristics of excluded randomised controlled studies

Study	Intervention	Duration of follow up	Study subjects	Age range (years)	BMI (kg/m^2)	Diagnosis of diabetes mellitus	Diagnosis of impaired glucose tolerance	Diagnosis with one of the components of the metabolic syndrome	Vitamin D deficiency <25 nmol/L	Vitamin d insufficiency 40-80- nmol/L
De Boere et al.[51]	1000mg calcium 400iu vitamin D daily	7yrs	Postmenopausal women (N= 2,291)	50-79	-	Not all subjects	Not all subjects	Patients did not have components of the metabolic syndrome at diagnosis	Vitamin d concentrations were only available for a select group of patients	Vitamin d concentrations were only available for a select group of patients
Pittas et al.[52]	500mg calcium 700iu vitamin D Daily	3yrs	Caucasian adults (N=314)	>=65	27.8 +- .6	No	Yes	Yes (some of the patients)	Not all patients	Not all patients
Nagpal et al.[31]	120 000iu vitamin D 3 doses fortnightly	6 weeks	Male (N=100)	>=35	No	No	Not all subjects	Not all the patients would always have a significantly raised waist circumference	Yes	Yes
Jorde et al.[19]	Vitamin D 40 000/20 000 per week calcium 500mg daily	1yr	Male and female (N=438)	21-70	28-47	No	No	No	No	No
Avenell et al.[49]	800iu vitamin D daily and or 1000 mg calcium	24-62 months	Male and female (N= 5292)	>=70	-	No	No	No	No	No

3.3 RISK OF BIAS IN INCLUDED STUDIES

The risk of bias in individual included studies are summarised in Tables 3.6 – 3.9.

Selection Bias:

Parekh[31], Zittermann[29] and Von Hurst's[11] studies did not provide sufficient information regarding allocation concealment. There was no selection bias in Witham's[33] study.

Performance Bias:

All the trials were adequately blinded and free of performance bias.

Detection Bias:

All the trials were free of detection bias.

Attrition Bias:

The studies of Parekh[31] and Zittermann[29] did not have attrition bias, but bias was present in Witham[33] and Von Hurst's[11] papers.

Reporting Bias:

All the studies were free of reporting bias.

Other Bias:

No other sources of bias were present.

The protocols were not searched for.

Table 3.6: Risk of bias table 1 (Parekh et al.)[31]

PILOT STUDY TO EVALUATE THE EFFECT OF SHORT-TERM IMPROVEMENT IN VITAMIN D STATUS ON GLUCOSE TOLERANCE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS		
ENTRY	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Unclear	Insufficient information Quote: *"randomization was performed with use of simple randomization"*
Allocation concealment?	Unclear	Not reported
Blinding?	Yes	"Double blind, placebo-controlled pilot study". Quote: *"Placebo or cholecalciferol injections were administered by a person unaware of the randomization assignment"* Probably done
Incomplete outcome data addressed?	Yes	1 missing from control group versus none from the treatment group Quote: *"withdrew from the study after baseline investigations"* No reasons provided.
Free of selective reporting?	Yes	Protocol not available but the published reports included all data
Free of other bias?	Yes	

Table 3.7: Risk of bias table 2 (Witham et al.)[33]

THE EFFECT OF DIFFERENT DOSES OF VITAMIN D3 ON MARKERS OF VASCULAR HEALTH IN PATIENTS WITH TYPE 2 DIABETES: A RCT		
ENTRY	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	Quote: *"Tayside Pharmaceuticals prepared a computer-generated randomization code stored in sealed envelopes until the end of the study"* Probably done
Allocation concealment?	Yes	Quote: *"Each participant was given the next numbered medication bottle in sequence to preserve allocation concealment".* Probably done
Blinding?	Yes	Quote: *"The participants and the research team remained blinded to treatment allocation until after the main analyses were completed".* Probably done
Incomplete outcome data addressed?	No	8 week visit: 3 missing from intervention group 2; 16 week visit: 1 missing from control group; 2 missing from intervention group 2. Reasons differ across groups
Free of selective reporting?	Yes	Protocol not available but the published reports include all data.
Free of other bias?	Yes	

Table 3.8: Risk of bias table 3 (Zittermann et al.)[29]

VITAMIN D SUPPLEMENTATION ENHANCES THE BENEFICIAL EFFECTS OF WEIGHT LOSS ON CVD RISK MARKERS		
ENTRY	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	Quote: "all participants were randomly assigned in a double-blind manner from computer generated random number list." Probably done
Allocation concealment?	Unclear	Insufficient information
Blinding?	Yes	Quote: *"At study entry all participants were randomly assigned in a double-blind manner"* Probably done
Incomplete outcome data addressed?	Yes	Quote: *"the number of dropouts did not differ between study groups (P > 0,05)"* Intervention group: Started 100 Lost to follow up: 18 Non-compliant : 15 Disease : 1 Pregnant : 2 Final no. of drop-outs: 36 Placebo group 100 : Started 100 Lost to follow up: 17 Non-compliance: 15 Disease : 1 Pregnancy: : 1 Final no. of drop-outs: 34
Free of selective reporting?	Yes	Protocol not available but the published reports include all data
Free of other bias?	Yes	

Table 3.9: Risk of bias table 4 (Von Hurst et al.)[11]

VITAMIN D SUPPLEMENTATION REDUCES INSULIN RESISTANCE IN SOUTH ASIAN WOMEN LIVING IN NEW ZEALAND WHO ARE INSULIN-RESISTANT PLUS VITAMIN D DEFICIENT - A RANDOMIZED, PLACEBO-CONTROLLED TRIAL		
ENTRY	**JUDGEMENT**	**DESCRIPTION**
Adequate sequence generation?	Yes	Quote*: "Randomisation of the Vitamin D/placebo capsules and allocation to the members of each pair were performed by Blackmores Ltd using a query Advisor, version 6.0 (statistical solutions) "* Probably done
Allocation concealment?	Unclear	Quote: *"randomisation + allocation were fully concealed from the researchers until after statistical analysis of data"* Insufficient info for patients
Blinding?	Yes	Quote: *"randomized, placebo-controlled, double-blind trial"*
Incomplete outcome data addressed?	No	There was an imbalance in numbers between groups.
Free of selective reporting?	Yes	Protocol not available but the published reports include all data
Free of other bias?	Yes	

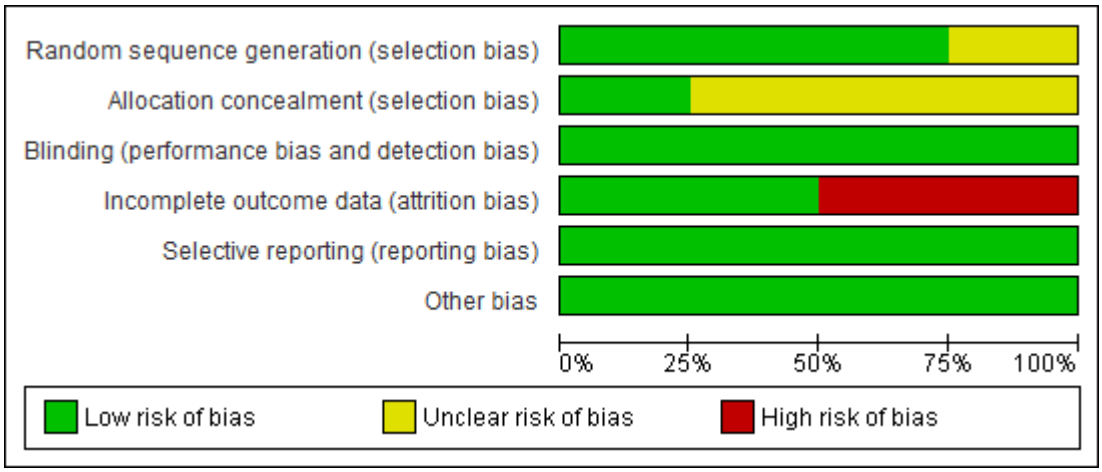

Figure 3.2: Risk of bias graph

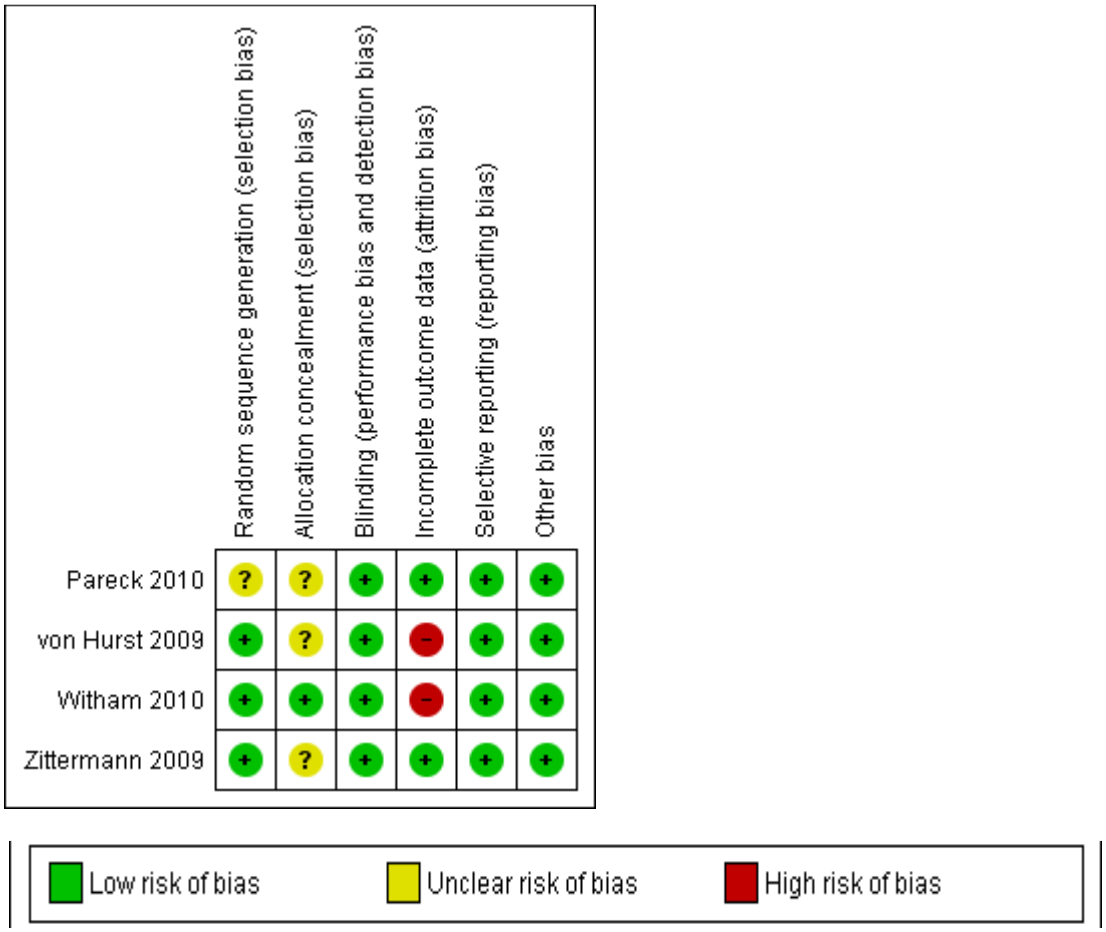

Figure 3.3: Risk of bias summary for individual trials

3.4 EFFECTS OF INTERVENTIONS

3.4.1 The Influence of Vitamin D Administration on Components of the Metabolic Syndrome

3.4.1.1 Comparison 1: The effect of vitamin D versus placebo in patients with type 2 diabetes mellitus who were also vitamin D insufficient.

Two studies (Parekh 2010 [31] and Witham 2010[33]) assessed the effect of vitamin D versus placebo in patients with Type 2 diabetes mellitus. The results from Witham 2010 [33] are shown in Table 3.10 and the results from Parekh 2010 [31] are shown in Table 3.11.

Table 3.10: The effect of vitamin D versus placebo in patients with type 2 diabetes mellitus – Witham 2010 study[33]

Characteristic	**Treatment group**			100,000IU vitamin D versus Placebo	200,000IU vitamin D versus Placebo
	100,000IU vitamin D (n=19)	200,000IU vitamin D (n=20)	Placebo (n=22)		
Unit of information	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean Difference (95% CI)**	**Mean Difference (95% CI)**
Systolic BP (mmHg)					
Baseline					
8 Weeks	149.6 (24)	145.1 (25)	143.9 (24.4)		
16 Weeks	141.4 (16.6)	136.8 (12.9)	146.4 (19.5)	-5.00 (-16.05 to 6.05)	-9.00 (-19.76 to 0.56)
	144.6 (20.4)	139.5 (15.4)	143.4 (16.4)	1.20 (-10.35 to 12.75)	-3.90 (-13.89 to 6.09)
Diastolic BP (mmHg)					
Baseline					
8 Weeks	81.7 (12.4)	80.7 (14.3)	80.3 (9.7)		
16 Weeks	77.1 (11.7)	74.4 (9.8)	78.9 (9.2)	-1.80 (-8.32 to 4.72)	-4.50 (-10.54 to 1.54)
	79.6 (11.9)	77.6 (11.7)	78.4 (10.3)	1.20 (-5.73 to 8.13)	-0.80 (-7.77 to 6.17)
Cholesterol (total) (mmol/L)					
Baseline	4.09 (1.14)	4.07 (0.97)	3.66 (0.71)		
8 Weeks	3.90 (1.00)	3.69 (0.71)	3.77 (0.75)	0.13 (-0.42 to 0.68)	-0.08 (-0.52 to 0.36)
16 Weeks	3.88 (0.92)	3.94 (0.77)	3.83 (0.89)	0.05 (-0.51 to 0.61)	0.11 (-0.39 to 0.61)
HbA1c (%)					
Baseline	7.0 (1.6)	6.9 (0.8)	7.8 (1.3)		
8 Weeks	7.1 (2.0)	7.0 (0.7)	7.6 (1.3)	-0.50 (-1.55 to 0.55)	-0.60 (-1.22 to 0.02)
16 Weeks	6.9 (1.5)	6.7 (0.9)	7.5 (1.4)	-0.60 (-1.49 to 0.29)	-0.80 (-1.51 to -0.09)
HOMA IR					
Baseline	11.7 (12.7)	12.0 (14.5)	13.0 (9.7)		
8 Weeks	13.5 (12.8)	11.9 (10.4)	25.3 (33.6)	-11.80 (-26.97 to 3.37)	-13.40 (-28.29 to 1.49)
16 Weeks	15.9 (14.3)	10.5 (7.2)	17.2 (19.3)	-1.30 (-11.76 to 9.16)	-6.70 (-15.60 to 2.20)
Serum 25(OH)D (nmol/L)					
Baseline	41 (14)	48 (21)	45 (17)		
8 Weeks	63 (20)	79 (31)	54 (20)	9.00 (-3.28 to 21.28)	25.00 (8.06 to 41.94)
16 Weeks	59 (18)	76 (30)	53 (20)	6.00 (-5.78 to 17.78)	23.00 (6.71 to 39.29)

FMD (%)					
Baseline	5.1 (3.0)	6.4 (3.2)	5.4 (2.7)		
8 Weeks	4.3 (2.3)	4.9 (3.2)	5.2 (3.1)	-0.90 (-2.56 to 0.76)	-0.30 (-2.21 to 1.61)
16 Weeks	5.2 (2.1)	6.5 (2.6)	5.1 (1.8)	0.10 (-1.11 to 1.31)	1.40 (0.03 to 2.77)

SD=Standard Deviation, CI=Confidence Interval, BP=Blood Pressure, HbA1c =Glycated Haemoglobin, HOMA IR=Homeostasis Model Assessment for Insulin Sensitivity, 25(OH)D=25 hydroxyvitamin D, FMD=Flow-Mediated Dilation

Table 3.11: The effect of vitamin D versus placebo in patients with type 2 diabetes mellitus – Parekh 2010[31]

Characteristic	Treatment group		Vitamin D versus Placebo
	Vitamin D (n=14)	Placebo (n=14)	
Unit of information	Mean (SD)	Mean (SD)	Mean Difference (95% CI)
Fasting plasma glucose –OGTT at 120min (mg/dl)			
Baseline	225.36 (69.78)	229.71 (42.81)	
Final (4 Weeks)	228.36 (52.18)	215.54 (44.94)	12.82 (-23.84 to 49.48)
Final – Baseline	3.0 (63.03)	-14.23 (60.07)	17.23 (-29.21 to 63.27)
Serum Insulin –OGTT at 120min (uIU/ml)			
Baseline	78.98 (43.42)	64.91 (17.83)	
Final (4 Weeks)	63.25 (31.31)	79.75 (40.63)	-16.50 (-44.01 to 11.01)
Final – Baseline	-15.72 (37.88)	13.87 (44.61)	-29.59 (-60.92 to 1.74)
Serum Fructosamine (umol/L)			
Baseline	306.21 (29.94)	322.14 (26.07)	
Final (4 Weeks)	316.36 (30.13)	313.15 (24.99)	3.21 (-17.61 to 24.03)
Final – Baseline	10.14 (35.32)	-8.54 (37.00)	18.68 (-8.65 to 46.01)
HbA1c (%)			
Baseline	7.58 (0.57)	7.83 (0.63)	
Final (4 Weeks)	7.67 (0.61)	7.63 (0.60)	0.04 (-0.42 to 0.50)
Final – Baseline	0.09 (0.69)	-0.18 (0.87)	0.27 (-0.33 to 0.87)
HOMA IR			
Baseline	13.69 (7.48)	13.78 (5.28)	
Final (4 Weeks)	13.01 (6.16)	14.51 (6.92)	-1.50 (-6.46 to 3.46)
Final – Baseline	-0.71 (5.31)	0.81 (7.17)	-1.52 (-6.31 to 3.27)
Serum 25(OH)D (nmol/L)			
Baseline	14.91 (6.76)	16.74 (6.69)	
Final (4 Weeks)	41.59 (12.21)	17.98 (4.36)	23.61 (16.79 to 30.43)
Final – Baseline	26.69 (14.33)	0.73 (4.22)	25.96 (18.11 to 33.81)

SD=Standard Deviation, CI=Confidence Interval, OGTT=Oral Glucose Tolerance Test, HbA1c =Glycated Haemoglobin, HOMA IR=Homeostasis Model Assessment for Insulin Sensitivity, 25(OH)D=25 hydroxyvitamin D

Primary outcomes

- *BMI*

This outcome was not evaluated by either of the two studies.

- *Blood pressure*

The Witham 2010 study [33] measured systolic and diastolic BP (mmHg) at baseline, 8 weeks, and at 16 weeks (Table 3.10). There were two active treatment groups with differing dosages, that is, 100,000 IU and 200,000IU once off. At 8 weeks, there were no significant differences observed in systolic BP both in the 100,000IU group (MD -5.00, 95%CI: -16.05 to 6.05, 41 participants) and the 200,000IU group (MD -9.00, 95%CI: -19.76 to 0.56, 39 participants) compared to the placebo. Also at 8 weeks, there were no significant differences observed for diastolic blood pressure both in the 100,000IU group (MD -1.80, 95%CI: -8.32 to 4.72, 41 participants) and 200, 000IU group (MD -4.50, 95%CI: -10.54 to 1.54, 39 participants) compared to the placebo. At 16 weeks, the systolic BP levels were not significantly different from placebo for both the 100, 000IU group (MD 1.20, 95%CI: -10.35 to 12.75, 40 participants) and the 200, 000IU group (MD -3.90, 95%CI: -13.89 to 6.09, 39 participants). Similarly at 16 weeks, the values for diastolic BP were not significantly different from placebo for both the 100, 000IU group (MD 1.20, 95%CI: -5.73 to 8.13, 40 participants) and the 200, 000IU group (MD -0.80, 95%CI: -7.77 to 6.17, 39 participants).

The treatment effects for change in BP from baseline to either 8 weeks or 16 weeks could not be calculated because the standard deviations of change were not reported.

The Parekh 2010 study[31] did not report on BP.

- *Lipid profiles LDL, HDL, triglyceride levels*

Total cholesterol

The Witham 2010 study [33] measured total cholesterol (mmol/L) and the results in Table 3.10 shows no significant differences in cholesterol levels between vitamin D and placebo at both 8 weeks (100,000IU vitamin D: MD 0.13, 95%CI: -0.42 to 0.68, 41 participants; 200,000IU vitamin D: MD -0.08, 95%CI: -0.52 to 0.36, 39 participants) and 16 weeks (100,000IU vitamin D: MD 0.05, 95%CI: -0.51 to 0.61, 41 participants; 200,000IU vitamin D: MD 0.11, 95%CI: -0.39 to 0.61, 39 participants). The treatment effects for change in cholesterol from baseline to either 8 weeks or 16 weeks could not be calculated because the standard deviations of change were not reported.

The Parekh 2010 study [31] did not report on total cholesterol.

- *Blood glucose, insulin profiles, HOMA IR*

Blood glucose

The Witham 2010 study [33] did not report on blood glucose.

The Parekh 2010 study [31] reported the plasma glucose (mg/dL) and serum insulin (µIM/ml) levels during an oral glucose tolerance test (OGTT) at 0, 30, 60, 90, and 120 minutes (Table 3.11). Treatment effects were calculated using values at 120 minutes. There were no significant differences in the final values at 4 weeks for both glucose (MD 12.82, 95%CI: -23.84 to 49.48, 27 participants) and insulin (MD -16.50, 95%CI: -44.01 to 11.01, 27 participants) between the vitamin D and placebo groups.

The results also showed that no significant differences were observed in the change (from baseline to 4 weeks) in both glucose (MD 17.23, 95%CI: -29.21 to 63.67, 27 participants) and insulin levels (MD -29.59, 95%CI: -60.92 to 1.74, 27 participants) between the vitamin D and placebo group.

HbA1c

The Witham 2010 study[33] reported the glycolated haemoglobin (HbA1c%) levels at baseline, 8 weeks, and at 16 weeks (Table 3.10). At 8 weeks, there were no significant differences between the placebo and both 100,000IU vitamin D group (MD -0.50, 95%CI: -1.55 to 0.55, 41 participants) and the 200,000IU vitamin D group (MD -0.60, 95%CI: -1.22 to 0.02, 39 participants). However at 16 weeks, although there were no significant differences between the placebo and the 100,000IU vitamin D group (MD -0.60, 95%CI: -1.49 to 0.29, 40 participants), there were significantly fewer HbA1c levels in the 200,000IU vitamin D group compared to placebo (MD -0.80, 95%CI: -1.51 to -0.09, 39 participants). The treatment effects for change in HbA1c levels from baseline to either 8 weeks or 16 weeks could not be calculated because the standard deviations of change were not reported.

The Parekh 2010 study[31] also reported HbA1c (%) levels at baseline, 4 weeks, and for the change from baseline to 4 weeks (Table 3.11). There were no significant differences in the HbA1c final values at 4 weeks (MD 0.04, 95%CI: -0.42 to 0.50, 27 participants). The results also showed no significant difference in the change in HbA1c levels from baseline to 4 weeks between the vitamin D group compared to the placebo group (MD 0.27, 95%CI: -0.33 to 0.87, 27 participants).

The results for HbA1c levels from the two studies could not be pooled in a meta-analysis because they reported results at different time periods.

Serum fructosamine

The Witham 2010 study [33] did not report on serum fructosamine.

The Parekh 2010 study[31] reported on serum fructosamine (umol/l) levels at baseline, 4 weeks, and for the change from baseline to 4 weeks (Table 3.11). There were no significant differences in the serum fructosamine final values at 4 weeks (MD 3.21, 95%CI: -17.61 to 24.03, 27 participants). The results also showed no significant difference in the change in serum

fructosamine levels from baseline to 4 weeks between the vitamin D group and the placebo group (MD 18.68, 95%CI: -8.65 to 46.01, 27 participants).

HOMA IR

The Witham 2010 study [33] reported the HOMA IR levels at baseline, 8 weeks, and at 16 weeks (Table 3.10). At 8 weeks, there were no significant differences between the placebo and both 100,000IU vitamin D group (MD -11.80, 95%CI: -26.97 to 3.37, 41 participants) and the 200,000IU vitamin D group (MD -13.40, 95%CI: -28.29 to 1.49, 39 participants). Similarly at 16 weeks, no significant differences were evident between the placebo and both 100,000IU vitamin D group (MD -1.30, 95%CI: -11.76 to 9.16, 40 participants) and the 200,000IU vitamin D group (MD -6.70, 95%CI: -15.60 to 2.20, 39 participants). The treatment effects for change in HOMA IR from baseline to either 8 weeks or 16 weeks could not be calculated because the standard deviations of change were not reported.

The Parekh 2010 study[31] reported HOMA IR levels at baseline, 4 weeks, and for the change from baseline to 4 weeks (Table 3.11). There were no significant differences in the HOMA IR final values at 4 weeks (MD -1.50, 95%CI: -6.46 to 3.46, 27 participants). The results also showed no significant difference in the change in HOMA IR levels from baseline to 4 weeks between the vitamin D group and the placebo groups (MD -1.52, 95%CI: -6.31 to 3.27, 27 participants).

The results for HOMA IR levels from the two studies could not be pooled in a meta-analysis because they reported results at different time periods.

- ### *Serum 25(OH)D*

The Witham 2010 study[33] reported the serum 25 hydroxyvitamin D (25(OH)D) levels (ng/mL) at baseline, 8 weeks, and at 16 weeks (Table 3.10). The 200,000IU Vitamin D group had significantly higher levels of 25(OH)D than the placebo group at both 8 weeks (MD 25.00, 95%CI: 8.06 to 41.94, 39 participants) and 16 weeks (MD 23.00, 95%CI: 6.71 to 39.29, 39 participants) although there were no significant differences observed between the 100,000IU vitamin D and placebo groups at both 8 weeks (MD 9.00, 95%CI: -3.28 to 21.28, 41 participants)

and 16 weeks (MD 6.00, 95%CI: -5.78 to 17.78, 40 participants). The treatment effects for change in serum 25(OH)D levels from baseline to either 8 weeks or 16 weeks could not be calculated because the standard deviations of change were not reported.

The Parekh 2010 study[31] also reported on serum 25(OH)D levels at baseline, 4 weeks, and for the change from baseline to 4 weeks (Table 3.11). The final serum 25(OH)D levels at 4 weeks were significantly higher in the vitamin D group than the placebo group (MD 23.61, 95%CI: 16.79 to 30.43, 27 participants). The results also showed a significantly greater increase in serum 25(OH)D levels in the vitamin D group than the placebo group from baseline to 4 weeks (MD 25.96, 95%CI: 18.11 to 33.81, 27 participants).

The results for serum 25(OH)D levels from the two studies could not be pooled in a meta-analysis because they reported results at different time periods.

Secondary outcomes

- *Proinflammatory states fibrinogen, plasminogen, C-reactive protein (CRP) interleukins.*

Flow-Mediated Dilation (FMD)

The Witham 2010 study [33] reported the FMD (%) levels at baseline, 8 weeks, and at 16 weeks (Table 3.10). At 8 weeks, there were no significant differences between the placebo and both 100,000IU vitamin D group (MD -0.90, 95%CI: -2.56 to 0.76, 41 participants) and the 200,000IU vitamin D group (MD -0.30, 95%CI: -2.21 to 1.61, 39 participants). However at 16 weeks, although there were no significant differences between the placebo and the 100,000IU vitamin D group (MD 0.10, 95%CI: -1.11 to 1.31, 40 participants), there were significantly higher FMD levels in the 200,000IU vitamin D group compared to placebo (MD 1.40, 95%CI: 0.03 to 2.77, 39 participants). The treatment effects for change in FMD levels from baseline to either 8 weeks or 16 weeks could not be calculated because the standard deviations of change were not reported.

- *Prothrombotic state*

This outcome was not evaluated by the two studies assessing comparison 1.

3.4.1.2 Comparison 2: The effect of vitamin D versus placebo in patients with vitamin D deficiency and insulin resistance

One study (von Hurst 2010) [11] assessed this comparison for patients having both vitamin D deficiency and insulin resistance (Table 3.12).

Primary outcomes

- *BMI*

The study reported values for BMI at baseline only. It is reported that there were no significant changes in BMI during the study in either the vitamin D or placebo groups.

- *Blood pressure*

Systolic and diastolic blood pressures were only reported at baseline and at 16 weeks in the one study assessing this comparison. Both systolic and diastolic blood pressures were slightly increased at 16 weeks, compared to 8 weeks.

- *Lipid profiles LDL, HDL, triglyceride levels*

The values for LDL-, HDL-cholesterols, and TAG were only reported at baseline. It is reported that changes in HDL-cholesterol and TAG from baseline were not significant either within or between the vitamin D and placebo groups.

- *Blood glucose, insulin profiles, HOMA IR*

Changes in fasting serum glucose (FSG) between baseline and 6 months (end of study) did not significantly differ both within and between the vitamin D and placebo groups (Table 3.12).

However, significant improvements were observed in fasting serum insulin (FSI) with vitamin-D supplementation compared with placebo (p=0.02 as reported by study authors, Table 3.12).

It is reported that a significant difference was observed in the change in HOMA1-IR between the vitamin D and placebo study groups (p=0.03 as reported by study authors, Table 3.12). The authors reported a median (IQR) decrease of -0.25 (0.24, -0.81) in the vitamin D group and an increase of 0.36 (1.16, -0.41) in the placebo group.

- *Serum 25(OH) vitamin D*

A significant increase in serum 25(OH) vitamin D concentrations was observed both within and between the vitamin D-supplemented and placebo groups from baseline to 6 months of study (p<0.001 as reported by study authors, Table 3.12).

Table 3.12: The effect of vitamin D versus placebo in patients with insulin resistance –von Hurst 2010 study[11]

Characteristic	Treatment group				Vitamin D versus Placebo
	Vitamin D (n=42)		Placebo (n=39)		
Unit of information	Median (IQR)	P-value (Difference within group)	Median (IQR)	P-value (Difference within group)	P-value (Difference between groups)
FSG (mmol/L) Baseline Final (6 months) Final - Baseline	 4.7 (4.5 to 5.1) 4.8 (4.6 to 5.2) 0.1 (0.4 to -0.1)	 0.154	 4.9 (4.5 to 5.2) 5.0 (4.7 to 5.4) 0.1 (0.4 to -0.2)	 0.07	 0.82
FSI (mU/L) Baseline Final (6 months) Final - Baseline	 13.2 (10.1 to 16.8) 11.2 (7.9 to 11.9) -1.3 (-3.6 to 1.0)	 0.02	 11.9 (9.9 to 15.4) 13.1 (10.2 to 17.3) 1.1 (-2.5 to 4.2)	 0.27	 0.02
HOMA1- IR Baseline Final (6 months) Final - Baseline	 2.70 (2.13 to 3.61) NR -0.25 (0.24 to -0.81)	 NR	 2.53 (2.11 to 3.47) NR 0.36 (1.16 to -0.41)	 NR	 0.03
25(OH)D (nmol/L) Baseline Final (6 months) Final - Baseline	 21 (11 to 40) 80 (67 to 94) 49 (21 to 66)	 <0.001	 19 (13 to 29) 29 (23 to 36) 8 (-1 to 16)	 < 0.001	 < 0.001
hs-CRP (mg/L) Baseline Final (6 months) Final - Baseline	 2.50 (1.0 to 4.5) 2.15 (1.25 to 3.4) 0.00 (-1.05 to 0.4)	 0.19	 2.4 (1.0 to 4.6) 2.9 (1.5 to 4.6) 0.2 (-0.1 to 0.7)	 0.38	 0.05

IQR=Interquartile Range (25th to 75th percentile), NR=Not Reported, FSG=Fasting Serum Glucose, FSI=Fasting Serum Insulin, HOMA1-IR=Homeostasis Model Assessment for Insulin Sensitivity (Model 1), 25(OH)D=25 hydroxyvitamin D, hs-CRP=high sensitivity C-reactive Protein.

Secondary outcomes

- *Pro-inflammatory states fibrinogen, plasminogen, C-reactive protein (CRP) interleukins*

The study reported no significant differences in changes in high sensitivity C-reactive protein (CRP) both within and between the vitamin D and placebo groups during the study (Table 3.12).

- *Prothrombotic state*

This outcome was not evaluated by the two studies assessing comparison 2.

3.4.1.3 Comparison 3: The effect of vitamin D *versus* placebo on weight loss in overweight vitamin D deficient patients

One study (Zittermann 2009) [29] assessed this comparison for overweight patients (Table 3.13).

Primary outcomes

- BMI

The Zittermann 2009 study[29] reported on BMI (kg/m^2) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the BMI final values at 12 months between the vitamin D and placebo groups (MD 0.90, 95%CI: -0.46 to 2.26, 165 participants). The results also showed no significant difference in the change in BMI from baseline to 12 months between the vitamin D and the placebo groups (MD 0.20, 95%CI: -0.40 to 0.80, 165 participants).

- Blood pressure

Systolic BP

The Zittermann 2009 study[29] reported on systolic BP (mmHg) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the systolic BP final values at 12 months between the vitamin D and placebo groups (MD -1.0, 95%CI: -5.59 to 3.59, 165 participants). The results also showed no significant difference in the change in systolic BP from baseline to 12 months between the vitamin D and the placebo groups (MD -1.0, 95%CI: -5.88 to 3.88, 165 participants).

Table 3.13: The effect of vitamin D versus placebo in overweight patients–Zittermann 2009 study[29]

Characteristic	Treatment group		Vitamin D versus Placebo
	Vitamin D (n=82)	Placebo (n=83)	
Unit of information	Mean (SD)	Mean (SD)	Mean Difference (95% CI)
BMI (kg/m^2)			
Baseline	33.7 (4.1)	33.0 (4.3)	
Final (12 months)	31.8 (4.3)	30.9 (4.6)	0.90 (-0.46 to 2.26)
Final – Baseline	-2.0 (2.0)	-2.2 (1.9)	0.20 (-0.40 to 0.80)
Systolic BP (mmHg)			
Baseline	128 (15)	128 (14)	
Final (12 months)	124 (14)	125 (16)	-1.0 (-5.59 to 3.59)
Final – Baseline	-4 (16)	-3 (16)	-1.0 (-5.88 to 3.88)
Diastolic BP (mmHg)			
Baseline	86 (8)	86 (9)	
Final (12 months)	83 (8)	83 (9)	0.0 (-2.60 to 2.60)
Final – Baseline	-3 (9)	-3 (10)	0.0 (-2.90 to 2.90)
LDL cholesterol (mmol/l)			
Baseline	3.52 (0.93)	3.65 (0.78)	
Final (12 months)	3.70 (1.04)	3.57 (0.91)	0.13 (-0.17 to 0.43)
Final – Baseline	0.19 (1.03)	-0.09 (0.64)	0.28 (0.02 to 0.54)
HDL cholesterol (mmol/L)			
Baseline	1.46 (0.38)	1.51 (0.37)	
Final (12 months)	1.44 (0.36)	1.46 (0.40)	-0.02 (-0.14 to 0.10)
Final – Baseline	-0.02 (0.22)	-0.04 (0.20)	0.02 (-0.04 to 0.08)
Triglycerides (mmol/L)			
Baseline	1.43 (0.68)	1.31 (0.57)	
Final (12 months)	1.23 (0.50)	1.35 (0.74)	-0.12 (-0.31 to 0.07)
Final – Baseline	-0.19 (0.54)	0.03 (0.50)	-0.22 (-0.38 to -0.06)
Glucose (mmol/L)			
Baseline	5.67 (0.78)	5.67 (1.17)	
Final (12 months)	5.44 (0.61)	5.39 (0.72)	0.05 (-0.15 to 0.25)
Final – Baseline	-0.21 (0.51)	-0.27 (0.82)	0.06 (-0.15 to 0.27)
HbA1c (%)			
Baseline	5.62 (0.40)	5.66 (0.57)	
Final (12 months)	5.37 (0.30)	5.42 (0.44)	-0.05 9-0.16 t 0.06)
Final – Baseline	-0.25 (0.23)	-0.25 (0.23)	0.00 (-0.07 to 0.07)
25(OH)D (nmol/L)			
Baseline	30.0 (17.5)	30.3 (20.1)	
Final (12 months)	85.5 (57.5)	42.0 (35.0)	43.50 (28.95 to 58.05)
Final – Baseline	55.5 (55.8)	11.8 (36.3)	43.70 (29.32 to 58.08)
Interleukin-6 (pg/ml)			
Baseline	8.9 (15.2)	7.8 (12.3)	
Final (12 months)	5.4 (4.5)	6.0 (5.4)	-0.60 (-2.12 to 0.92)
Final – Baseline	-3.5 (14.0)	-1.9 (10.9)	-1.60 (-5.43 to 2.23)
Tumour necrosis factor-alpha (pg/ml)			
Baseline	7.84 (3.15)	8.12 (3.43)	
Final (12 months)	7.04 (2.25)	7.90 (2.80)	-0.86 (-1.63 to -0.09)
Final – Baseline	-0.80 (2.5)	-0.26 (2.9)	-0.54 (-1.37 to 0.29)

SD=Standard Deviation, CI=Confidence Interval, BMI=Body Mass Index, BP=Blood Pressure, LDL-Low Density Lipoprotein, HDL=High Density Lipoprotein, HbA1c =Glycated Haemoglobin, HOMA IR=Homeostasis Model Assessment for Insulin Sensitivity, 25(OH)D=25 hydroxyvitamin D.

Diastolic BP

The Zittermann 2009 study[29] reported on diastolic BP (mmHg) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the diastolic BP final values at 12 months between the vitamin D and placebo groups (MD 0.0, 95%CI: -2.60 to 2.60, 165 participants). The results also showed no significant difference in the change in diastolic BP from baseline to 12 months between the vitamin D and the placebo groups (MD 0.0, 95%CI: -2.90 to 2.90, 165 participants).

- *Lipid profiles LDL, HDL, triglyceride levels*

LDL-cholesterol

The Zittermann 2009 study[29] reported on LDL-cholesterol (mmol/l) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the final values at 12 months between the vitamin D and placebo groups (MD 0.13, 95%CI: -0.17 to 0.43, 165 participants). However, vitamin D supplementation significantly increased LDL cholesterol levels from baseline to 12 months compared to the placebo group (MD 0.28, 95%CI: 0.02 to 0.54, 165 participants).

HDL-cholesterol

The Zittermann 2009 study[29] reported on HDL-cholesterol levels (mmol/l) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the final values at 12 months between the vitamin D and placebo groups (MD -0.02, 95%CI: -0.14 to 0.10, 165 participants). The results also showed no significant difference in the change from baseline to 12 months between the vitamin D and the placebo groups (MD 0.02, 95%CI: -0.04 to 0.08, 165 participants).

Triglycerides

The Zittermann 2009 study[29] reported on triglyceride levels (mmol/l) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the triglyceride final values at 12 months between the vitamin D and placebo groups (MD -0.12, 95%CI: -0.31 to 0.07, 165 participants). The results also showed that vitamin D supplementation significantly decreased triglyceride levels from baseline to 12 months compared to the placebo groups (MD -0.22, 95%CI: -0.38 to -0.06, 165 participants).

- *Blood glucose, insulin profiles, HOMA IR*

Glucose

The Zittermann 2009 study[29] reported on glucose (mmol/l) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the glucose final values at 12 months between the vitamin D and placebo groups (MD 0.05, 95%CI: -0.15 to 0.25, 165 participants). The results also showed no significant difference in the change from baseline to 12 months between the vitamin D and the placebo groups (MD 0.06, 95%CI: -0.15 to 0.27, 165 participants).

HbA1c

The Zittermann 2009 study[29] reported on HbA1c (%) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the values at 12 months between the vitamin D and placebo groups (MD -0.05, 95%CI: -0.16 to 0.06, 165 participants). The results also showed no significant difference in the change from baseline to 12 months between the vitamin D and the placebo groups (MD 0.00, 95%CI: -0.07 to 0.07, 165 participants).

- *Serum 25(OH)D*

The Zittermann 2009 study[29] reported on 25(OH)D at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). The final values at 12 months were significantly higher in the vitamin D group compared to the placebo group (MD 43.50, 95%CI: 28.95 to 58.05, 165 participants). The results also showed that vitamin D supplementation significantly increased the 25(OH)D levels from baseline to 12 months compared to the placebo group (MD 43.70, 95%CI: 29.32 to 58.08, 165 participants).

Secondary outcomes

- *Proinflammatory states fibrinogen, plasminogen, C-reactive protein (CRP) interleukins*

Tumour-Necrosis Factor (TNF)

The Zittermann 2009 study[29] reported on TNF (pg/ml) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). The final values at 12 months were significantly lower in the vitamin D group compared to the placebo group (MD -0.86, 95%CI: -1.63 to -0.09, 165 participants). The results showed no significant difference in the change from baseline to 12 months between the vitamin D and the placebo groups (MD -0.54, 95%CI: -1.37 to 0.29, 165 participants).

Interleukin-6

The Zittermann 2009 study[29] reported on interleukin-6 levels (pg/ml) at baseline, 12 months, and for the change from baseline to 12 months (Table 3.13). There were no significant differences in the final values at 12 months between the vitamin D and placebo groups (MD -0.60, 95%CI: -2.12 to 0.92, 165 participants). The results also showed no significant difference in the change from baseline to 12 months between the vitamin D and the placebo groups (MD -1.60, 95%CI: -5.43 to 2.23, 165 participants).

- *Prothrombotic state*

This outcome was not evaluated by the study assessing comparison 3.

3.4.2 Adverse Effects Associated with Vitamin D Administration

In the trial by Zittermann et al.[29] it was found that LDL-cholesterol was significantly increased in the vitamin D treated group. No other adverse events were reported in any of the trials.

3.4.3 Dose-response Effect of Vitamin D Supplementation

The Witham[33] study investigated two different doses of vitamin D 100000IU and 200000IU. The 200000IU group did show a slightly more significant improvement in flow mediated dilatation (Table 3.10) and a decrease in B-type natriutretic peptide levels.

CHAPTER 4: DISCUSSION

4.1 SUMMARY OF MAIN RESULTS

The primary objective of this systematic review was to examine the effect of vitamin D supplementation on the components of the metabolic syndrome. Four trials were included in this review.

Several outcomes were examined. These included blood pressure, where both Zittermann[29] and Witham's[33] studies looked at blood pressure as an outcome and showed no significant differences in both systolic and diastolic blood pressure in either trials.

A second outcome investigated was lipid profiles, including total cholesterol, LDL, HDL and triglyceride levels. The Witham[33] study measured total cholesterol. The results showed no significant differences in cholesterol levels between the vitamin D and placebo groups. The Zittermann[29] study showed no significant difference in the final value of LDL between groups, however vitamin D supplementation significantly increased LDL cholesterol levels from baseline to 12 months in the vitamin D treated group.

Zittermann[29] also examined HDL and triglycerides. These showed no significant differences between the two groups.

Plasma glucose and serum insulin were measured in Parekh's trial.[31] There was no significant difference between the vitamin D and placebo group for both blood glucose and insulin at 4 weeks. Zittermann's study[29] also reported on blood glucose and found no significant changes between the groups.

Three studies, Witham's[33], Parekh's[31] and Zittermann's[29] investigated HbA1c.

Witham's trial[33] showed no difference between the groups at 8 weeks, But at 16 weeks the HbA1c levels were lower in the 200 000 iu vitamin D treated group compared to placebo.

Parekh[31] and Zittermann's[29] trials found no significant differences between the groups for HbA1c.

Neither Witham[33] nor Parekh[31] found any significant difference in Homa IR levels between intervention and control group. Von Hurst's study[11] found no significant changed in fasting

serum glucose between the groups, however significant improvements were observed in fasting serum insulin in the vitamin D versus the placebo group. A significant difference was also seen in Homa IR between vitamin D and placebo group.

BMI was examined in Zittermann's[29] study and no significant differences were found between the two groups.

The studies all showed an increase in 25(OH)D levels in the vitamin D groups compared to the placebo.

The studies all looked at various outcomes or end points. With the results presented in this systematic review, one could not be confident to make a positive recommendation on the use of vitamin D in any of the components of the metabolic syndrome.

4.2. QUALITY AND APPLICABILITY OF THE EVIDENCE

The random allocation was adequate in three studies, insufficient information concealment was provided in Parekh's study[31]. The allocation concealment was adequate in Zittermann[29] and Witham's studies[33], but unclear in Parekh[31] and Von Hurst's[11] studies.

The reasons for withdrawals were given in all the studies except Parekh's[31], and the number of dropouts was clear in all studies except Von Hurst's.[11]

The ideal systematic review would have been made up of trials that used all of the diagnostic criteria of the metabolic syndrome as their end-point, however no such trials were available. The four studies included in this systematic review differed in their outcomes and no single study focused on all the diagnostic criteria of the metabolic syndrome. The trials were heterogeneous in terms of measured outcomes looking at different components of the metabolic syndrome.

The included trials used varying doses of vitamin D, ranging from 3332IU daily, 4000IU daily, 100 000IU, 200 000IU to 300 000IU once off. The duration of follow up was also widespread, spanning 4 weeks to 12 months. The chances of a positive effect are more likely to be seen when using higher doses of vitamin D supplementation for a longer period of time.

The studies included were all of small sample size. Sample size is an important factor in determining the accuracy and repeatability of the results. If the sample size is too small there is a greater chance that the power of the study is too low to identify real and significant differences.

Attrition bias was addressed in half the studies. Attrition bias should have been taken into account in all the studies. The exclusion of the subjects from the analysis could lead to an overestimate of the effectiveness of the intervention.

4.3 POTENTIAL BIASES IN THE REVIEW PROCESS

Only randomized controlled trials were included in this systematic review. Only studies for which the investigator could obtain full text articles were included. It is possible that some published trials may have been missed in this review since the researcher did not have access to all possible databases. The literature search was performed in October 2010 and was repeated in 2012. A potential for bias may have occurred due to the exclusion of one non-English study – an Iranian publication. The review had several limitations including comprehensiveness of the literature search and hand searches of article references were not done. The search was however comprehensive in terms of the variety of electronic data bases searched. Another limitation is that additional outcomes were addressed by the investigator that were not initially specified in the protocol.

4.4 AGREEMENTS AND DISAGREEMENTS WITH OTHER STUDIES OR REVIEWS

In recent editorial in the British Medical Journal[59] the author called for some perspective regarding vitamin D supplementation. The high profile news coverage and the promotion of the results of observational studies as if they proved causality have resulted in unsubstantiated claims of benefits of vitamin D.

Several factors in the studies looking at vitamin D and its impact on diseases can make interpretation complex. The first of these factors is the definition of normality regarding vitamin D status. Studies have reported the serum 25 hydroxyvitamin D concentration at which PTH reaches a plateau to be between 25nmol/L and 75nmol/L, making it difficult to deduce a functional definition of vitamin D deficiency.[59]

Most evidence linking low vitamin D status to non-bone outcomes has been derived from observation. All studies are observational and can be limited by several factors. These include: reverse causality (disease may cause reduced exposure to sun), confounders (low physical activity may cause low vitamin D, obesity and increased risk of diabetics), classification bias (vitamin D status defined in terms of diet, not blood concentrations which correlate poorly with nutritional intake) and differences in assay methods.[59]

The safety of supplementing large segments of the population with vitamin D over a prolonged period of time is unknown. The editorial in the British medical journal[59] called for large, well designed randomized controlled trials with long-term follow up to prove the benefit, lack of benefit or even harm of vitamin D supplementation.

CHAPTER 5: AUTHORS' CONCLUSIONS

5.1 IMPLICATIONS FOR PRACTICE

Vitamin D is promoted to have beneficial effects for treating and preventing different components of the metabolic syndrome which affects large segments of populations worldwide. In our systematic review we, however, found that currently available evidence is not sufficient to support the use of vitamin D supplementation for this purpose. The available evidence also provides no assurance regarding the safety of long-term vitamin D supplementation.

The metabolic syndrome and its various components present a daily challenge for the clinical practitioner. It would be of enormous clinical benefit if it could be documented by evidence based practice that supplementation with vitamin D could in some way have a positive effect on any of the components of the metabolic syndrome. This would provide a relatively cheap option when used in adequate dosages and would provide benefits outweighing any harm.

The current evidence available as summarised in this review is limited as it is not definitive and gives no clear guidelines.

The primary papers examined in this review were relatively free of bias. There was no publication, detection or reporting bias in any of the papers. Half of the papers were free of attrition bias and one was free of selection bias.

5.2 IMPLICATIONS FOR RESEARCH

This review highlights the need for further large, well-designed randomized controlled trials in this area. Ideally, it would be favourable to assess vitamin D supplementation in patients with as many components of the metabolic syndrome as possible. It has been noted in several of the studies that the stage of the disease i.e. insulin resistance versus type II diabetes, and the severity of hypertension had an impact on how significant the effect vitamin D supplementation was.[5] Future trials need to include all age groups, including the elderly as they are vulnerable to vitamin D deficiency. Both male and female patients must be included, as well as various

ethnic groups as darker skinned individuals have lower vitamin D levels[5] and effects of supplementation and relationship to disease on all ethnic groups has to be examined. Obesity influences vitamin D status, so various ranges of BMI's need to be accounted for. Confounders such as seasonal variation also need to be taken into account.

It will be important to standardize vitamin D dosages used, and to include information on the use of alternative supplementation and dietary information. It is very important that an adequate dosage of vitamin D is supplemented, that baseline vitamin D status must be assessed, and to ensure that deficient patients reach sufficient levels for an effect to be seen.

The trials should also be of sufficient duration to allow for positive or negative outcomes to be reached.

With a sufficient number of high quality RCTs a systematic review and meta-analysis can then be performed to provide clinicians with evidence based data on which to base their clinical practice. It will improve our understanding of the impact of vitamin D supplementation on clinical conditions that practitioners treat on a daily basis.

5.3 DECLARATION OF INTEREST

No declaration of interest.

There were no conflicts of interest in this review nor were any stated in the included studies.

5.4 DEVIATIONS FROM PROTOCOL

In the original protocol trials with all the components of the metabolic syndrome were to be included, however no such studies were found with our literature search. The protocol was then amended to include trials with at least one component of the metabolic syndrome.

5.5 SOURCES OF SUPPORT

None.

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ADDENDUM A

Data Extraction Form

PROJECT IDENTIFICATION

1) STUDY TITLE

2) REVIEWER INITIALS

3) INVESTIGATORS

4) NAME OF STUDY

5) YEAR OF PUBLICATION

6) LOCATION: CITY

COUNTRY

INSTITUTION

SELECTION CRITERIA

Table 1

		Include	Exclude
STUDY DESIGN (STUDY TYPE)			
CONTROLS			
DURATION OF FOLLOW UP MONTHS: DAYS:			
PATIENT CHARACTERISTICS			
HUMAN SUBJECTS			
RACE GROUPS: CAUCASIAN NUMBERS % AFRICAN NUMBERS % INDIAN NUMBERS %			
AGE OF PARTICIPANTS MEAN SD			
COMPONENTS OF THE METABOLIC SYNDROME: FOR STUDIES TO BE INCLUDED AT LEAST ONE COMPONENT OF THE METABOLIC SYNDROME MUST BE PRESENT Elevated waist circumference Elevated triglycerides ≥ 1.7 mmol/L or drug treatment for increased triglycerides is an alternate indicator Reduced HDL-C (≤ 1.0mmol/L in males females≤ 1.3mmol/L or drug treatment) is an alternate indicator Elevated blood pressure (Systolic ≥ 130 diastolic ≥ 85mmHg and/or			

antihypertensive drug treatment in a patient with a history of hypertension) is an alternate indicator Elevated fasting glucose (≥ 5.6mmol/L or drug treatment of elevated glucose is an alternative indicator) *SEE TABLE AT END			
VITAMIN D CRITERIA			
VITAMIN D DEFICIENCY <25NMOL /L			
VITAMIN D INSUFFICIENCY 40-80 NMOL/L			
EXCLUSION CRITERIA			
PREGNANT OR LACTATING WOMEN			
CO-MORBID DISEASE STATES: a. KIDNEY DISEASE/ FAILURE b. LIVER DISEASE/ FAILURE			
TYPES OF INTERVENTION			
VITAMIN D2 OR D3 ADMINISTRATION			
DOSAGE			
DURATION			
ROUTE OF ADMINISTRATION			
MONOTHERAPY OR COMBINATION THERAPY			
COMPARISON GROUP: PLACEBO			

NO INTERVENTION			
CONCOMITANT THERAPY			
DIETARY RECOMMENDATIONS			
PHYSICAL ACTIVITY RECOMMENDATIONS			
COMPLIANCE			

Table 2

TOTAL NUMBER OF SUBJECTS ENROLLED I N TRIAL: INTERVENTION GROUP CONTROL GROUP TOTAL NUMBER OF SUBJECTS WHO COMPLETED TRIAL: INTERVENTION GROUP CONTROL GROUP FINAL GROUP SIZE		
REASONS GIVEN FOR NON COMPLIANCE TRIAL	YES	NO
PERCEIVED SIDE EFFECTS PREGNANCY MOVING LOST TO FOLLOW UP WITHDREW FROM STUDY OTHER		
ALL DROPOUTS ACCOUNTED FOR		
ADVERSE EFFECTS: HYPERCALCAEMIA HYPERVITAMINOSIS **D** HYPERPHOSPHATAEMIA OTHER		
INTENTION TO TREAT PER PROTOCOL ACTUAL CASE ANALYSIS		
STATISTICS		
1. ARE THE GROUPS COMPARABLE AND IF NECESSARY ADJUSTED FOR BASELINE DIFFERENCES? 2. HAVE RECOGNIZED STATISTICS TESTS BEEN USED?		

3. WERE PAIRED TESTS USED IN PAIRED DATA? 4. WAS A 2 TAILED TEST PERFORMED WHENEVER THE EFFECT OF AN INTERVENTION COULD BE A NEGATIVE ONE? 5. WERE OUTLIERS ANALYSED APPROPRIATELY WITH CORRECT STATISTICAL ADJUSTMENTS? 6. HAS CORRELATION BEEN DISTINGUISHED FROM REGRESSION HAS THE CORRELATION CO-EFFICIENT VALUE BEEN CALCULATED AND INTERPRETED CORRECTLY?		
ETHICAL APPROVAL SOURCE OF FUNDING		

PRIMARY OUTCOMES

OUTCOME DATA FOR INTERVENTION AND CONTROL GROUPS										
		Baseline						**End**		
		Mean	**SD**	**CI**	**P-Value**			**Mean**	**SD**	**CI**
BMI (KG/M^2)	Intervention									
	Control									
WC (cm)	Intervention									
	Control									
BP (mmHg)	Intervention									
	Control									
LDLmmol/L	Intervention									
	Control									
HDL	Intervention									

	Control									
Cholesterol	Intervention									
	Control									
HOMA 2% TG	Intervention									
	Control									
FSG mmol/L BG	Intervention									
	Control									
FSI mmol/L Insulin	Intervention									
Serum 25 HydroxyVit. D. nmol/L	Intervention									
	Control									
HOMA 2-IR	Intervention									
	Control									
HOMA –IR	Intervention									
	Control									

HOMA 2 – IR-insulin Resistance

FSI - Fasting serum insulin

FSG – Fasting serum glucose

SECONDARY OUTCOMES

		Mean	SD	CI	P-Value	Mean	SD	CI	P-Value
Plasminogen mg/dl	Intervention								
	Control								
C-Reactive Protein mg/L	Intervention								
	Control								
Interleukins	Intervention								

Table 1: Diagnostic Criteria of Metabolic Syndrome

IDF	NCEP	WHO	AACE
Diagnosed if glycaemia is abnormal and 2 further criteria are present	Diagnosed if 3 out of 5 criteria are present	Diagnosed if glycaemia is abnormal and further 2 criteria are present	Indicates risk factors
Fasting glycaemia 100-125 mg/dl or DM2	Glycaemia 110-125 mg/dl	Glucose intolerance DM2 or insulin-resistance due to HOMA-IR	Fasting glycaemia 110-125 Mg/dl ≥ 140 mg/dl 2 hours after oral GTT
WC ≥ 94cm MWC	WC > 102 cm MWC > 88 cm	BMI > 30 and HWR >	BMI ≥ 25 and WC > 102 cm

≥ 80cm W	W	0.9M and > 0.85 W	M and WC > 88 cm W
Tg ≥ 150 mg/dl or HDL < 40M and < 50W	TG ≥ 150mg/dl or HDL < 40 M and < 50W	TG ≥ 150mg/dl or HDL < 35 M and < 39 W	TG ≥ 150mg/dl or HDL < 40 M and < 50W
On treatment for SAH or BP ≥ 130x85 mmHg	BP ≥ 130x85 mmHg	On treatment for SAH or BP ≥ 160x90 mmHg Microalbuminuria≥ 20 mcg/min	BP ≥ 130/85 mmHg

AACE = American College of Endocrinology/American Association of Clinical Endocrinologists; BMI = body mass index; BP = arterial blood pressure; DM2 = diabetes mellitus type 2; GTT = oral glucose tolerance test; HOMA = homeostasis model assessment; HWR = hip: waist ratio; IDF = International Diabetes Federation; M = men' NCEP = US National Cholesterol Education Program; SAH = systemic arterial hypertension; Tg = triglycerides; W = women; WC = waist circumference; WHO = World Health Organisation.

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