Vitamin D status and relationship between vitamin D and risk factors of metabolic syndrome: A study in Taiyuan City in China

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Abstract

Background

Vitamin D deficiency is widespread, and the residents in Taiyuan City in China seem to be at high risk of vitamin D deficiency. The situation might be because the city is located in north China and air pollution in the city is heavy. Meanwhile, emerging evidence suggests that vitamin D deficiency may be associated with prevalence of metabolic syndrome (MetS), which usually progress to diabetes and increases the risk of cardiovascular disease. MetS has been becoming much more common in China, and even affects younger people.

Objectives

This study investigated the vitamin D status of non-manual workers living in Taiyuan City; and explored the relationship between vitamin D status and markers of MetS in 200 participants attending the Health 100 Check-up Center in Taiyuan City for their usual health check.

Methods

In this cross-sectional study; 200 non-manual workers aged 20-80 years old, living in Taiyuan City were recruited. The participants had their serum vitamin D levels measured and were asked questions about their lifestyle, including daily exercise, alcohol use and smoking. The Check-up Center provided data relating to MetS of the participants. These data included anthropometrics (height, weight and body circumferences), biochemical data (lipid profiles and fasting glucose from blood samples taken for the check-up) and blood pressure.

Results

Seventy eight percent of participants had vitamin D values less than 50 nmol/L. The women's serum 25-hydroxyvitamin D (25(OH)D) status (median; 32.70 nmol/L (upper and lower quartile; 25.80, 43.80)) was significantly lower than that of the men (44.00 nmol/L (32.30, 55.40)) (p<0.01). In females aged younger than 40 years vitamin D status (29.25 nmol/L (24.05, 40.85)) was significantly lower than older

participants (age>65). In the present study, multiple linear regressions showed the determinants of the vitamin D status were female gender, smoking, and increased fasting glucose (p<0.05). The prevalence of MetS, or abdominal obesity between the groups with and without vitamin D insufficiency were not significantly different (p=0.08; p=0.07). Multiple logistic regression analysis showed that vitamin D status was not associated with MetS.

Conclusions

Vitamin D insufficiency was highly prevalent in non-manual workers in Taiyuan City in China during the winter season. Vitamin D status in the women was lower than the men. Among the females, younger women had worse vitamin D status than the older women. So, in the present study, female gender, increased fasting glucose, and smoking were significant determinants for vitamin D insufficiency. Vitamin D insufficiency was not associated with the risk factors for MetS in the present study. However, female gender, increased waist circumference (WC), and raised serum triglycerides were associated with higher risk of MetS.

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List of abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
7-DHC	7-Dehydrocholesterol
25(OH)D	25-hydroxyvitamin D
25(OH)-epi-D	Epimer of 25-hydroxyvitamin D
ATP	National Cholesterol Education Program
BMI	Body mass index
BP	Blood pressure
CDS	Chinese Diabetes Society
CPBA	Competitive protein binding assay
CVD	Cardiovascular disease
DBP	Vitamin D binding protein
DBS	Dried blood spot
DEQAS	Vitamin D External Quality Assessment Scheme
DM	Diabetes mellitus
EIA	Enzyme immunoassay
FINS	Fasting insulin
FM	Fat mass
FPG	Fasting plasma glucose
Glu	Glucose
HC	Hip circumference
HDL-C	High density lipoprotein cholesterol
HOMA	Homeostasis model assessment
HPLC	High performance liquid chromatography
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin resistance
IS	Insulin sensitivity
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDL-C	Low density lipoprotein cholesterol

MetS	Metabolic syndrome
MUHEC	Massey University Human Ethics Committee
mVDR	Membrane vitamin D receptor
NCEP	National Cholesterol Education Program
NA-CLPBA	Nichols advantage-automated protein binding assay
nVDR	Nuclear vitamin D receptor
PD	Peritoneal dialysis
PTAD	4-phenyl-1,2,4-triazoline-3,5-dione
PTH	Parathyroid hormone
OR	Odds ratio
RAS	Renin-angiotensin system
RCT	Randomized controlled trial
RMP	Reference measurement procedure
SMD	Standardized mean difference
SPF	Sun protect factor
RIA	Radioimmunoassay
T2DM	Type 2 Diabetes mellitus
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor- α
UV	Ultraviolet
VDR	Vitamin D receptor
WC	Waist circumference
WHO	World Health Organization
WHR	Waist circumference/hip circumference ratio

Chapter 1. Introduction

1.1 Background

Vitamin D is a fat-soluble vitamin that is produced when ultraviolet rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamin D is also naturally present in some foods like oily fish and sun-dried mushroom (Holick, 2008b). The vitamin D from skin synthesis or digestive tract enters the blood circulation and is hydroxylated by a specific enzyme forming 25-hydroxyvitamin D (25(OH)D) in the liver. 25(OH)D is the main circulating form of vitamin D in the body. It is not bio-active but its serum concentration reflects the nutritional status of vitamin D. So, vitamin D status is assessed by the serum concentration of 25(OH)D. The 25(OH)D is formed in the liver then reaches the kidney via the circulation. In the kidney, 25(OH)D is further hydroxylated to form dihydroxyvitamin D ($1,25(OH)_2D$), which is the active metabolite in the body. $1,25(OH)_2D$ binds to the vitamin D receptor (VDR) in target cells to form a vitamin-receptor complex, which has its biological effect via gene expression.

It is well known that the characteristic biologic roles of vitamin D are to affect the bone, kidney and small intestine to regulate the metabolism of calcium and phosphorus (DeLuca & Zierold, 1998). However, the finding that many tissues synthesize $1,25(OH)_2D$, and that VDR is extensively expressed throughout the body, led to the concept that adequate vitamin D levels are important for many non-skeletal functions in the body, including the cardiovascular and immune systems (Holick, 2008a; Brandenburg et al., 2012). Emerging data indicated that $1,25(OH)_2D$ might play a role in immunoregulation, cellular proliferation, differentiation, and inducing apoptosis procedures by direct or indirect gene regulation (Holick, 2008a; Agmon-Levin et al., 2013; Luong & Nguyen, 2013). So, vitamin D deficiency could be a pathological condition for many diseases, including cardiovascular disease (Abu el Maaty & Gad, 2013), cancer (Sun et al., 2011; Hollis et al., 2013; Yousef et al., 2013) and autoimmune diseases (Agmon-Levin et al., 2013). Inversely, sufficient vitamin D status could be beneficial to the cardiovascular system. Both animal and human studies provided support for $1,25(OH)_2D$ restraining renin synthesis,

improving insulin sensitivity and strengthening cardiac contractility (Y. C. Li et al., 2004; Xiang et al., 2005; Kayaniyil et al., 2010; Matias et al., 2010; Vaidya & Williams, 2012; A. M. Belenchia et al., 2013). So, optimizing vitamin D status was suggested to be protective to reduce cardiovascular disease (CVD) risks, including obesity, insulin resistance, hyperglycemia, dyslipidemia, and hypertension. These risk factors are the well-known components of metabolic syndrome (MetS). That means vitamin D deficiency could be a potential risk factor of CVD by promoting progression of MetS, or could be a confounder for current CVD risk assessment. So, in that sense, it is necessary to identify the vitamin D status and its associations with the components of MetS in adults.

Metabolic syndrome (MetS) is a cluster of risk factors for cardiovascular events, including abdominal obesity, insulin resistance, hyperglycemia, hypertension, lowered high density lipoprotein-cholesterol (HDL-C), raised low density lipoprotein-cholesterol (LDL-C) and triglycerides (TGs) (Alberti et al., 2005). The diagnostic criteria for MetS are slightly different depending on the different international medical organizations. However, abdominal obesity and insulin resistance or impaired glucose tolerance or diabetes are agreed to be the central components in all of the diagnostic criteria. The causes of MetS are various; long-term unhealthy lifestyle is one of most important promoting factors (Kwasniewska et al., 2009). Many studies, especially observational studies, have identified that hypovitaminosis D is associated with MetS. However, most of the randomized, controlled trials (RCT) have been inconsistent. So the relationship between vitamin D and MetS is obscure.

In China, there have been very few specialized surveys which have investigated Chinese people's vitamin D status. However, from the limited studies carried out so far, the status of serum vitamin D in different groups of Chinese people was alarming (Zhang et al., 2013). Meanwhile, MetS is also becoming much more common in China, even affecting younger people (Fu & Prasad, 2014; Ming et al., 2014).

1.2 Significance of the study

China is vast in territory and its topography is varied and complicated. Different regions have different quantity of sunlight. Taiyuan city is a typical industrial city, which is located in Loess Plateau in north China (Figure 1) and 38 degrees north latitude. Its climate is typical mainland monsoon and has four distinct seasons. In winter, the maximum and the minimum temperatures are 5 degrees above zero and minus 10 degrees below zero, respectively. The average daily sunshine duration in winter is about 5.5 hours. The city also lacks special locations and facilities for the large population to do outdoor exercises. Together with the heavy air pollution and haze in winter caused by industry and burning coal for heating, the city surroundings are not suitable for outdoor physical activity. Therefore, it is of interest to investigate the vitamin D status of residents living in Taiyuan City. However, as far as the current author knows, there have been no studies evaluating their vitamin D status. The present study determined the serum vitamin D concentrations of 200 adult volunteers, to provide an initial evaluation of the vitamin D status of people living in Taiyuan.

Figure 1.1 Map of China, retrieved from: www.china.mike.com

MetS is a social health problem of concern in China. The relationship between vitamin D status and MetS has been studied elsewhere and is still controversial worldwide. However, there were a limited number of studies that explored the health consequences of low vitamin D status and the relationship between vitamin D status and MetS in Chinese people. The present study explored the relationship in the adults in Taiyuan city in China. The regular parameters of MetS components, including blood lipid profiles, fasting blood glucose, blood pressure, waist circumference, body mass index were collected. Also, daily smoking, alcohol drinking and physical exercise were collected and analyzed in the study. So, the study will provide background to the further studies in this area and possible policy modification regarding the improvement of vitamin D status.

The aims of the present thesis are to determine the vitamin D status of the adult group in Taiyuan city in China and to examine whether there is a relationship between the vitamin D status and MetS in these participants.

1.3 Hypotheses

The major null hypotheses (H0) for the present study were:
H01: General population living in Taiyuan City will have sufficient serum vitamin D concentrations.

H02: Insufficient vitamin D serum concentration is not associated with the risk of metabolic syndrome.

Chapter 2. Literature review

The objective of this review is to thoroughly examine the published literature on the relationship between vitamin D and the components of metabolic syndrome. The topics of the review include physiology of vitamin D in humans, relationships between vitamin D deficiency and metabolic syndrome, and measurement of vitamin D. The focus of the discussion is evidence from observational and interventional studies.

2.1 Overview to vitamin D

2.1.1 Sources and synthesis of vitamin D

The primary source of Vitamin D is synthesis of vitamin D_3 in the skin on exposure to sunlight. One study showed that the average individual synthesises 80% of their total vitamin D from sunlight (Nowson & Margerison, 2002). However, many factors would affect vitamin D_3 synthesis in people. Specifically, the intensity of ultraviolet B (UV-B) is a critical factor affecting synthesis of vitamin D_3. It was said that 20 mJ per cm^2 was the threshold of UV-B exposure for synthesis of pre-vitamin D in the skin (Bender, 2003). Low intensity radiation, for example, below 20 mJ per cm^2, did not produce significant pre-vitamin D_3. Thus, some regions above 40^o latitude in winter are not likely to receive sufficient UV-B intensity which will result in insufficient vitamin D status of the population. In northern China for example Taiyuan City, spring and summer are sunny and rainless. However in winter, there is low daily intensity of UV-B radiation, together with heavy air pollution, which hinders some UV-B radiation, so it is estimated that a significant number of local residents are likely to have vitamin D insufficiency and deficiency.

There are multiple external factors affecting vitamin D synthesis in the skin. UV-B dose is a critical one. A randomized controlled trial (Bogh et al., 2011) compared the plasma 25(OH)D in different groups who had similar baseline 25(OH)D levels before and after UV-B exposure of different dose and duration. The results showed that the increase in $25(OH)D_3$ production was positively related to UV-B dose rather

than dose rate. In other words, optional vitamin D_3 synthesis might be generated by the appropriate UV-B dose. Longer exposure does not increase production of pre-vitamin D_3 and increases the risk of DNA damage to the skin (Lehmann & Meurer, 2010). Ozone and cloud cover also decrease the intensity of UV-B radiation by absorbing and scattering photons (Kimlin, 2008). However, it has been suggested that sunlight is not absolutely essential for vitamin D synthesis in the skin, because UV-B can penetrate clouds. About 50% of UV-B radiation penetrates complete cloud cover and reaches the earth surface (Bender, 2003). Other factors, which affect the amount of UV-B received include time of day, different seasons, latitude and altitude, (Kimlin, 2008). Furthermore, in some countries, health information emphasizing sun safe practices and advocating sunscreen to protect from melanoma could result in decreased vitamin D_3 synthesis. Publicity like this enhances fear about exposure to sunlight in some people, which could lead to them having compromised vitamin D status (Houghton et al., 2010). In fact, Kennedy (2003) found that lifetime sun exposure was not associated with developing malignant melanomas. So, although excessive sun exposure without using sunscreen should be avoided, always using sunscreen and avoiding any sun exposure is not encouraged, because one study demonstrated that a sunscreen of 8 SPF could absorb most of the UV spectrum involved in pre-vitamin D_3 synthesis, therefore significantly compromising the production of vitamin D (Matsuoka et al., 1987). In addition, dark skin or veiled dressing in some cultures hinders them from receiving enough solar radiation despite plentiful ambient sunlight therefore leading to a lower vitamin D status.

The second source of vitamin D is diet. However, there are few foods containing noteworthy amounts of vitamin D, except for some oily fish like salmon, mackerel and fish oil, etc. (Holick, 2008b) and a few foods of animal origin, like dairy fat, egg and lean meat (Shrapnel & Truswell, 2006). In addition, some plants contain natural vitamin D_2, such as sun-dried mushroom (Holick, 2008b). However the content in plants is small, so it is not possible to meet vitamin D requirements from dietary sources. Vitamin D fortified foods and vitamin D supplements are another source. However, at present, there are no vitamin D fortified foods available in China except for infant formula milk powder, but these vitamin D fortified foods may be available in other countries. With regard to vitamin D supplements, only large-dose vitamin D

preparations are available in some medical and health institutions and these are only used to deal with severe pathological conditions in China. For example, dihydroxyvitamin D was often used for the patients with chronic kidney disease to improve their vitamin D status and suppress secondary hyperparathyroidism (Cheng, 2014). Furthermore, there are some calcium supplements and multivitamin supplements containing low doses of vitamin D.

2.1.2 Vitamin D hydroxylation

7-Dehydrocholesterol (7-DHC, provitamin D_3) is an intermediate in the synthesis of cholesterol, which is abundant in the basal and suprabasal layers of the skin. When the skin receives UV-B light irradiation, 7-DHC undergoes isomerization and transforms into precalciferol (pre-vitamin D_3). Then, the pre-vitamin D_3 undergoes a structural rearrangement and becomes cholecalciferol (calciol, vitamin D_3). The whole process is fast; about 50% of pre-vitamin D_3 transforms vitamin D_3 within 2.5 hours in the skin (Lehmann & Meurer, 2010). Once formed, vitamin D_3 immediately enters extracellular space and diffuses from epidermis into dermal capillary bed. In the circulation, vitamin D_3 quickly combines with vitamin D binding protein (DBP) and is transported to the liver for further hydroxylation (Lehmann & Meurer, 2010; Henry, 2011).

In the liver, vitamin D_3 is hydroxylated at the C25-position of the side chain to form 25-hydroxyvitamin D_3 (calcidiol, 25(OH)D_3), which is the main circulating form. The hydroxylation process is catalyzed by a microsomal cytochrome P_{450} enzyme *CYP2R1* and/or the mitochondrial cytochrome P_{450} *CYP27A1*. In addition, other P_{450}-dependent hydroxylases were recently found to exert vitamin D 25-hyydroxylase activities, including *CYP2C11, CYP3A4, CYP2D25* and *CYP2J3*. However, these enzymes might have different biological activities in different populations or species (Prosser & Jones, 2004). Once formed in human body, 25(OH)D_3 enters the circulation and binds to DBP which is transported to the kidneys (Lehmann & Meurer, 2010; Henry, 2011).

In the kidneys, 25-hydroxyvitamin D_3 is further hydroxylated to 1,25-dihydroxyvitamin D_3 (calcitriol, 1,25$(OH)_2D_3$) by several hydroxylases. This

form is the active metabolite of vitamin D in the body. *CYP27A, CYP27* in mitochondria and 1α-hydroxylase in microsomes are three cytochrome P_{450}-dependent enzymes in the kidneys that catalyze the 1-hydroxylation of 25-hydroxyvitamin D_3. Of these, the microsomal enzyme is synthesized in response to parathyroid hormone so as to improve the production of $1,25(OH)_2D_3$. On the contrary, its synthesis is suppressed by increased concentration of $1,25(OH)_2D_3$. So, it seems that the microsomal 1α-hydroxylase is the most important enzyme in controlling production of $1,25(OH)_2D_3$ (Bender, 2003).

Another enzyme in the kidney is calcidiol 24-hydroxylase, which hydroxylates both calcidiol and calcitriol. This enzyme is synthesized in response to calcitriol, and its function counters the 1α-hydroxylase. The two kinds of the calcidiol hydroxylase jointly regulate the synthesis of calcitriol so as to increase the 24-hydroxycalcidiol production when the calcitriol production is more than physiological requirement. The formation of 24-hydroxycalcidiol is thought to be a pathway for inactivation of calcitriol (Bender, 2003). This finding needs more evidence to support it.

Most vitamin D is excreted by the bile. Only a minor part is catalyzed to other metabolites and excreted in urine (Bender, 2003).

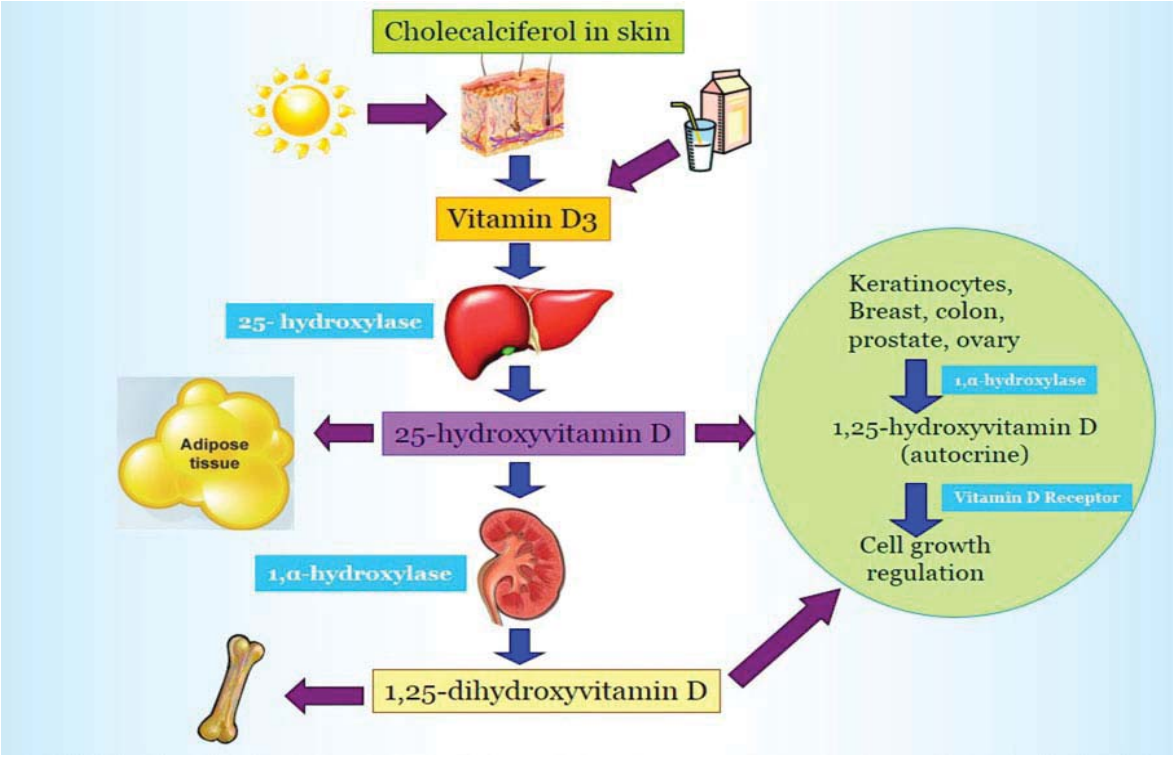

Figure 2.1 Synthesis of vitamin D_3 and the skeletal and extra-skeletal effects of vitamin D, derived from (Kannan & Lim, 2014) with permission from John Wiley and Sons.

2.1.3 Vitamin D receptor

Vitamin D receptor (VDR) mediates the effects of $1,25(OH)_2D$ moderating its biological functions. There are two types of VDRs: the membrane vitamin D receptor (mVDR) and the nuclear vitamin D receptor (nVDR) (Haussler et al., 2011). The former mainly takes part in the regulation of calcium and phosphorus and the latter acts on target genes after combining with $1,25(OH)_2D$ and affecting the expression of the structural genes which affect biological functions. However, many polymorphisms have been found in VDR genes in humans, which affect the biological function of vitamin D. These might be associated with incidence of some diseases, such as osteoarthritis, cancer, cardiovascular diseases, diabetes, renal diseases, and autoimmune disorders (Valdivielso & Fernandez, 2006).

2.1.4 Vitamin D binding protein

The vitamin D binding protein (DBP; Gc-globulin) is a member of the albumin family and it is predominantly produced in the liver. It is responsible for transporting

the majority (85-90%) of vitamin D and its metabolites in the circulation, including cholecalciferol, calcidiol, calcitriol and 24-hydroxycalcidiol (Daiger et al., 1975). Among them, calcidiol and 24-hydroxycalcidiol have higher affinity for the DBP than the other two. Because of the differences in primary structure, there are three types of Gc-globulin: Gc-2, Gc-1 slow and Gc-1 fast. Gc-globulin has unusually high circulating concentrations compared to other hormone binding proteins (Haddad, 1995). Its concentration reaches up to 6mmol/L while most of the others are below 1mmol/L, for instance, the concentration of thyroxine binding protein is 300 μmol/L and that of cortisol binding protein is 800μmol/L (Bender, 2003). Also, saturation of Gc-globulin with vitamin D derivatives is very low. Only about 2% to 5% of the binding sites are occupied under physiological conditions. This situation is important in protection against vitamin D intoxication (White & Cooke, 2000). It is thought that Gc-globulin might exert other physiological functions; this still needs more exploration. Furthermore, the concentration of Gc-globulin might impact on vitamin D levels. Under some conditions, Gc-globulin would change in quantity, affinity with its ligands, and its biological functions (Yousefzadeh et al., 2014). For example, Gc-globulin gene polymorphisms are extensively distributed in different groups (Malik et al., 2013). The polymorphisms produce Gc-globulin with different structures, which are not so effective in transporting vitamin D in the blood stream and so could lead to lowered vitamin D levels. This is thought to be related to some diseases, such as some cancer, inflammation, and autoimmune diseases (Chun, 2012; Yousefzadeh et al., 2014).

2.1.5 Functions of vitamin D

$1,25(OH)_2D$ is the bio-active form of vitamin D and its classic function is involved in bone metabolism. $1,25(OH)_2D$ combines VDR in the intestine to stimulate the formation of epithelial calcium channels, which significantly promote calcium absorption (Christakos et al., 2011). When the serum calcium concentrations decrease to below the normal level, $1,25\text{-}(OH)_2D$ is stimulated by parathyroid hormone to promote the maturation of osteoclasts, which release calcium ions into the blood stream from bone tissue (DeLuca & Zierold, 1998). Also,$1,25\text{-}(OH)_2D$ promotes the absorption of phosphorus which assists in the formation of calcium hydroxyapatite in the bone tissue (DeLuca & Zierold, 1998). Therefore, vitamin D

deficiency is associated with skeletal diseases, such as rickets in infants, osteoporosis in adults, bone pain, even osteomalacia and increased risk of fracture (Holick, 2008b). In conclusion, vitamin D is crucial to bone health from infancy to old age.

Meanwhile, vitamin D has multiple extra-skeletal functions. The discovery of extensive expression of vitamin D receptor (VDR), not only in the kidneys, but also in the breast, colon, brain, prostate, macrophages and other tissues, indicates the complex and diverse biological roles of vitamin D in the human body (Holick, 2008a). Accumulating evidence shows that vitamin D might be involved in numbers of biological processes, such as cellular proliferation, differentiation, anti-angiogenesis and inducing apoptosis, regulating immunity, improving glucose metabolism and other roles (Holick, 2008a). Accordingly, vitamin D deficiency might be associated with many extra-skeletal diseases, including infections, allergies, cancer, obesity, insulin resistance (IR), diabetes mellitus (DM) and cardiovascular diseases (Zittermann & Gummert, 2010). Among these diseases, obesity and IR are the critical pathological links to metabolic syndrome (MetS) (Fujita, 2007; Gallagher et al., 2010). So, with the increasing incidence of MetS, the relationship between vitamin D and MetS has become a topic of concern worldwide.

2.1.6 Definitions of vitamin D status

The definitions of vitamin D status are still controversial. The different cutoff values of 25(OH)D sufficiency and deficiency are presented in Table 2.1.

Table 2.1 Definitions of vitamin D status*

	Nowson, et al. (2012)[1] Munns, et al. (2006)[2]	Ross, et al. (2009)[3]	Arundel, et al. (2012)[4]	Holick, et al. (2011)[5]
Sufficiency	≥50nmol/L	>20ng/mL (≈50 nmol/L)	>50 nmol/L	30ng/mL (≈75 nmol/L)
Insufficiency	30-49 nmol/L, mild 12.5-29 nmol/L, moderate	12-20ng/mL (≈30-50 nmol/L)	25-50 nmol/L	21-29 ng/mL(≈50-75 nmol/L)
Deficiency	<12.5 nmol/L	<12ng/mL (≈30 nmol/L)	<25 nmol/L	<20ng/mL(≈50 nmol/L)

*To convert 25(OH)D concentration from ng/mL to nmol/L multiply by 2.459.
1. Working Group of the Australia and New Zealand Bone Mineral Society, Endocrine Society of Australia and New Zealand. 2. Working Group of the Australasian Paediatric Endocrine Group, Paediatric Bone Australasia. 3. Institute of Medicine. 4. British Paediatric and Adolescent Bone Group. 5. The Endocrine Society

Besides the cutoff values in Table 2.1, there are other recommended definitions of vitamin D sufficiency. The Warsaw conference, held in Warsaw, Poland, 2012 (http://www.witaminad.waw.pl/), reached a consensus on the optimal vitamin D concentrations, which were considered to be 30-50ng/mL (70-125nmol/L) in the Central European population (Pludowski et al., 2014). This range of vitamin D suficiency was determined according to evidence of 30-50ng/mL vitamin D concentrations being significantly associated with reduced risk of non-skeletal disease. However, this definition is used in a limited scope currently.

The rationales of the different opinions on 25(OH)D sufficiency or deficiency were mainly based on the effects of vitamin D on mineral homeostasis, bone health, and muscle functions (Holick et al., 2011; Nowson et al., 2012). Also, the factors affecting the vitamin D status and the evidence associating vitamin D levels with non-skeletal disease were considered. A suggestion of setting the threshold values specifically for age groups was also put forward. A study reported that for healthy postmenopausal women, when the average concentration of vitamin D was increased from 50.1 to 86.5nmol/L, calcium absorption correspondingly increased from 45% to 65% (Heaney et al., 2003). By contrast, another study found that a decrease of average vitamin D concentrations from 122 to 74 nmol/L did not lead to apparent changes in calcium absorption (Barger-Lux & Heaney, 2002). There was also evidence to suggest that bone density would be increased with serum vitamin D concentrations above 80nmol/L in elderly women (Devine et al., 2002). So, it was queried how much vitamin D was sufficient to exert both skeletal and non-skeletal functions, at least for postmenopausal women.

Similarly, another study found that 60nmol/L might be a better cutoff of vitamin D sufficiency for young children, because vitamin D concentration minimizing the parathyroid level was around 61nmol/L (Houghton et al., 2010). It was also asserted that parathyroid hormone concentration plateaus when vitamin D is within 75-100nmol/L (Holick, 2006). It is unclear whether there is any difference in the satisfactory level of vitamin D between children and adults. It is also unclear what the optimal values of 25(OH)D for the musculoskeletal system are, and the obscure relationship between vitamin D and diseases have led to inconsistent classifications

of vitamin D status.

The inaccuracy of the vitamin D assay methods was another reason for the inconsistency in classifications of vitamin D sufficiency or deficiency. The study conducted by Looker *et al.* (2002) defined vitamin D deficiency and insufficiency as <17.5 nmol/L and <62.5 nmol/L, respectively to account for potential impact of the measurement error of radioimmunoassay (RIA) assay adopted in the study.

So, it seems necessary to gather more evidence to standardize the cutoff values of 25(OH)D status, especially those studies exploring non-skeletal functions of vitamin D above 50nmol/L. Increasing accuracy and precision of vitamin D assays is also essential. Moreover, reference serum values for a population should take more factors into account, possibly including age, living location, season and race, which are the main determinants of vitamin D status (Mithal et al., 2009).

2.1.7 Vitamin D status in people worldwide

Vitamin D deficiency is estimated to affect approximately 30-50% of people worldwide (Lee et al., 2008). Possibly, more than one billion people have vitamin D deficiency or insufficiency worldwide (Holick, 2008a). A survey from USA (NHANES Ⅲ) reported that 25% to 57% of American adults in lower latitude areas in winter and 21% to 58% in higher latitude areas in summer had vitamin D insufficiency, which was defined as less than 62.5 nmol/L (Looker et al., 2002). The study also reported that more severe deficiency, defined as 17.5nmol/L, occurred in African Americans compared with other races (Looker et al., 2002). In Europe, it was estimated that most residents living in Central and Western Europe had vitamin D levels below 30-50 ng/mL of optimal values, which were consensus on the Warsaw conference (Pludowski et al., 2014). However, this value range for optimal vitamin D status was higher than that of other guidelines. In New Zealand, Rockell *et al.* (2006) conducted a survey of 2,946 participants aged 15 and older and reported 48% were lower than 50 nmol/L and 84% were lower than 80 nmol/L. Particularly, Pacific and Māori people had lower vitamin D status. Their mean 25(OH)D concentrations

were 37 nmol/L and 42 nmol/L, respectively. For South Asian women living in Auckland, up to 84% of participants had insufficient vitamin D levels (<50 nmol/L) (P. R. von Hurst et al., 2010). For pregnant women and children, the situation might be more severe. A study (Judkins & Eagleton, 2006) of pregnant women in Wellington reported that vitamin D deficiency was very prevalent in the group, affecting women of all ethnicities in New Zealand. Sixty one percent of those surveyed had 25(OH)D concentrations less than 25 nmol/L. Rockell *et al.* (2005) investigated children aged 5-14 years and reported that 41%, 59% and 25% of Maori, Pacific, and New Zealand European and Others had vitamin D insufficiency (<37.5 nmol/L). Children living in Dunedin also had high prevalence of vitamin D insufficiency (<50 nmol/L) and 10% of those living in Auckland had vitamin D deficiency (< 27.5 nmol/L) (Grant et al., 2009; Houghton et al., 2010). Surveys from other countries, such as Korea, Japan, India, Iran, Canada, Australia, and Brazil presented similar situations, with high prevalence of insufficiency of serum 25(OH)D in different groups and ethnicities (Newhook et al., 2009; Hirani et al., 2013; Maeda et al., 2013; Yoshimura et al., 2013; Faghih et al., 2014; G & Gupta, 2014). So, vitamin D deficiency or insufficiency has become common in all ages and ethnicities worldwide, even in sunny areas like Singapore (Hawkins, 2009). It must be noted that the results of the above studies indicated the prevalence of vitamin D insufficiency to some extent; however, the results were not comparable, because the definitions of vitamin D sufficiency and deficiency used were different.

In China, there was also no consensus on the definitions of vitamin D sufficiency or deficiency. Most studies in China defined vitamin D sufficiency in adults or elderly as >50 nmol/L, and defined vitamin D insufficiency and deficiency as <50 nmol/L and <25 nmol/L, respectively. However, for children and adolescents, the definitions of vitamin D sufficiency were inconsistent. Some adopted >75 nmol/L, others adopted >50 nmol/L. Nonetheless, vitamin D insufficiency is prevalent in most areas. The vitamin D status in elderly was poor, surveys from northern cities, such as Beijing and Shenyang (40°N and 42°N, respectively), showed that the women over 60 years had 98.8% and 84.2% vitamin D insufficiency in the Spring season (Zhou et al., 2003; Wang et al., 2009). In Shanghai (31°N), the situation was also grim and 84% and 89% of older males and females had vitamin D insufficiency (Lu et al.,

2009). Even in Hong Kong (22°N), the reported prevalence of vitamin D insufficiency and deficiency in the elderly was 40.3% and 22.5%, respectively (Wat et al., 2007). For adults, the vitamin D status in south China seemed better than that in north China. One study compared young women living in Beijing (40°N) with those living in Hong Kong (22°N), reporting that the mean serum vitamin D value of Beijing women was much lower than that of Hong Kong women ($p<0.0001$). In addition, 40% and 18% of young women in Beijing and Hong Kong, respectively, had vitamin D levels less than 25 nmol/L (Woo et al., 2008). Furthermore, middle-aged women in a study in Shenyang (42°N) had serum vitamin D concentrations between 27.2-38.3nmol/L in winter (Zhou et al., 2010).

Children and adolescents in different areas also had high proportions of vitamin D insufficiency. In Hangzhou (30°N), Zhu *et al.* (2012) found that the prevalence of vitamin D insufficiency and deficiency was high in primary and secondary school students; 89.6% and 46.4% of the group had vitamin D levels less than 75nmol/L and 50nmol/L, respectively. Another study from Beijing reported 89% of adolescent girls had serum vitamin D values lower than 50nmol/L (Foo et al., 2009). In summary, vitamin D insufficiency is prevalent in Chinese. However, a large survey on a national scale, especially with good design and appropriate standardized methodology is needed to provide a clear picture of the situation in China.

It has been known that vitamin D deficiency is associated with some diseases, not only in the skeletal system, but also in the extra-skeletal system. Figure 2.2 briefly presents the potential relationships between vitamin D and cardiovascular risk factors, all of which are involved in the development of metabolic syndrome (MetS). In addition, the present review will focus on the relationship between vitamin D status and MetS in more depth.

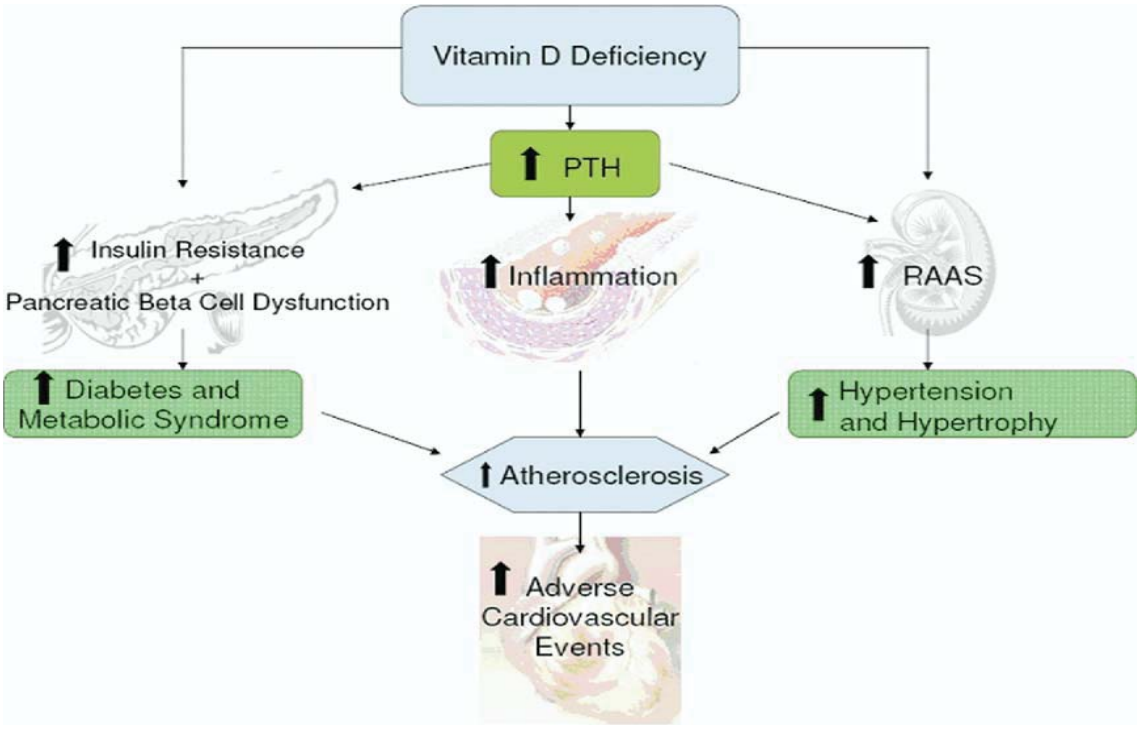

Figure 2.2 Potential mechanisms for cardiovascular risk factors and vitamin D deficiency, derived from (Lee et al., 2008), with the permission from Elsevier.

2.2 Overview of metabolic syndrome (MetS)

MetS refers to a cluster of cardiovascular risk factors, mainly central obesity, impaired glucose regulation and diabetes, hypertension, and dyslipidemia, which appear gradually following the accumulation of excess abdominal fat (Fujita, 2007). It also could be accompanied by a pathological state of chronic inflammation, oxidative stress and hypercoagulability, which gradually lead to endothelial damage, and eventually cardiovascular disease (Khoshdel et al., 2012). However, in the pathological state, obesity, especially intra-abdominal fat distribution, and the associated insulin resistance (IR) are thought to play critical roles in the progression of the components of MetS (Fujita, 2007). Essentially, MetS is the aggregate state of multiple risk factors for cardiovascular disease and individuals with MetS have a much higher risk of progressing to diabetes mellitus and cardiovascular disease. However, the diagnostic criteria for MetS are inconsistent. Different international expert groups use different diagnostic criteria. The summary of these diagnostic criteria for MetS is presented in Table 2.2:

Table 2.2 Definition of MetS by WHO, ATP Ⅲ, IDF and CDS

WHO (Alberti, 1998)	ATP Ⅲ (Alberti, 2009)	IDF (Alberti, 2005)	CDS (Chinese Diabetes Society)
Required DM, IFG, IGT, or IR & at least two of the following:	Any three of the following:	Required high WC by the cutoffs* & at least two of the following:	Any three of the following:
• WHR≥0.9 in men, ≥0.85 in women; • TG≥1.7 mmol/L or specific treatment for the lipid abnormality; • BP≥140/90 mmHg or treatment of previously diagnosed hypertension; • Urinary album excretion rate≥20 μg/minor, albumin-to-creatinineratio≥30 mg/g.	• WC≥102cm in men, ≥88cm in women; • TG≥1.7 mmol/L; • BP≥130/85 mmHg or taking anti-hypertensive medications • HDL<1.03 mmol/L in men <1.29 mmol/L in women; • Fasting Glu. ≥5.6 mmol/L.	• TG≥1.7 mmol/L or specific treatment for the lipid abnormality; • BP≥130/85 mmHg or treatment of previously diagnosed hypertension; • HDL<1.03 mmol/L in men, • <1.29 mmol/L in women, or specific treatment for the lipid abnormality; • Fasting Glu. ≥5.6 mmol/L, or previously diagnosed type 2 diabetes.	• BMI≥25kg/m^2; • TG≥1.7 mmol/L; • BP≥140/90 mmHg or taking anti-hypertensive medications; • Fasting Glu.≥6.1 mmol/L; • HDL≤0.9 mmol/L in men, ≤1.0 mmol/L in women.

*Ethnic-specific cutoffs for waist circumference (WC): Europeans: WC≥94cm in men and ≥80cm in women; South Asians: WC≥90cm in men and ≥80cm in women; Chinese: WC≥90cm in men and ≥80cm in women; Japanese: WC≥85cm in men and ≥90cm in women.
MetS: metabolic syndrome; WHO: World Health Organization; ATP Ⅲ: National Cholesterol Education Program---Third Adult Treatment Panel; IDF: International Diabetes Federation; CDS: Chinese Diabetes Society; DM: diabetes mellitus; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; IR: insulin resistance; WHR: the ratio of waist circumference/hip circumference; WC: waist circumference; BMI: body mass index; TG: triglycerides; HDL: high density lipoprotein cholesterol; BP: blood pressure; Glu: glucose.

Some studies on adipocytes identified that visceral fat, rather than subcutaneous fat, produces angiotensinogen and proinflammatory cytokines, such as TNF-α, IL-6, which are associated with the progression of insulin resistance and are positively related with adipocyte size (Qi & Pekala, 2000; Rahmouni et al., 2004). de Jongh *et al.* (2004) also demonstrated that obesity was associated with microvascular dysfunction, which might further relate to insulin resistance. On the other hand, a case-control study showed that insulin sensitivity, endothelial function and inflammatory response, which were evaluated 4.2±0.8 months after bariatric surgery, were significantly improved in morbidly obese patients compared with the control group (Vázquez et al., 2005). By contrast, liposuction on abdominal subcutaneous adipose tissue did not significantly affect insulin action or other obesity-related

metabolic abnormalities in patients, as reported by Klein *et al.* (2004). Carr *et al*. (2004) undertook a cross-sectional study of healthy adults and concluded that intra-abdominal fat (IAF) could be regarded as an independent indicator of MetS, because the results of their study showed that the IAF of participants with MetS was significantly higher than those without MetS (166.3 vs. 79.1 cm, $p<0.001$) and IAF also was associated with the components of MetS, including the insulin sensitivity index. The above studies imply that excessive ectopic fat accumulation is the principle cause of insulin resistance.

Insulin resistance (IR) is broadly defined as the reduced biological effect of insulin. IR has been generally thought to be an underlying pathophysiology that promotes the development of MetS and can be regarded as a predictor of MetS. Some studies confirmed these hypotheses (Carr et al., 2004; Reaven, 2011; Vonbank et al., 2011). However, a recent study suggested an alternative hypothesis. Karnchanasorn *et al.* (2013) showed that a statistically significant number of participants in the MetS group did not have IR; meanwhile, a statistically significant number of participants in the IR group were not diagnosed with MetS. Based on these results, Karnchanasorn *et al.* suggested IR is neither necessary nor required for MetS, although it is a risk factor for MetS. The study used homeostasis model (HOMA) to assess IR, which was often used for assessing individual's IR, IS, and insulin β-cell function worldwide currently. HOMA-IR is from log-transformed value of (FINS×FPG)/22.5 (Matthews et al., 1985). However, the method seems to be more applicable for Caucasian original younger adults and might not be reliable for older adults and other races (Matthews et al., 1985). Together with other bias, such as participant selection bias, the conclusions of the study need to be further investigated before being accepted.

In summary, obesity, especially visceral obesity, and IR are considered to be critical aetiological factors that contribute to the genesis and development of MetS. Here, it is necessary to stress that no one factor can promote the disease solely and any disease must be the result of the combined action of multiple risk factors. So, MetS is definitely caused by multiple risk factors through multiple pathways and not just

obesity and IR.

The underlying treatment for MetS is altering lifestyle and diet; including adjusting dietary composition, exercising, losing weight, giving up smoking and drinking so as to reduce abdominal fat and improve insulin resistance (Duclos, 2007; Sun et al., 2012; Wakabayashi, 2014). For those individuals with hyperlipidemia, hyperglycemia and hypertension, treatment by effective medication is essential besides adjusting life style. Successful treatment mostly depends on whether the patient compliance of therapeutic schedule is good.

2.3 Vitamin D and metabolic syndrome

Recent epidemiological investigations have identified that the vitamin D level is reversely associated with the incidence of MetS.

2.3.1 Vitamin D and obesity

The prevalence of obesity has continued to rise for the past 30 years worldwide, which makes obesity a global public health problem. Obesity increases the morbidity and mortality of cardiovascular and cerebrovascular diseases. It also produces lots of social issues, such as increasing health care expenses, lowered quality of life, detriment to physical and mental health as well as discrimination (O'Hara & Gregg, 2006). However, the aetiology of obesity is a very complicated process, which could be affected by endocrine disorder, heredity, psychological pressure, living conditions and surroundings, and eating habits (Jebb, 1997; Racette et al., 2003). In recent years, researchers have found that some micronutrients, such as vitamin D, could play certain roles in the occurrence and development of obesity. Meanwhile, fat accumulation also might influence serum vitamin D concentrations.

2.3.1.1 Effects of obesity on vitamin D levels

As previously mentioned, abdominal obesity is the principal cause of MetS and affects body metabolism. Currently, most cross-sectional studies on different

populations have similar findings, which are that serum vitamin D levels in obese populations were significantly lower than those in non-obese populations (Arunabh et al., 2003; Parikh et al., 2004; Lagunova et al., 2009; Josefson et al., 2013). In a cohort study, the percentage of participants with vitamin D less than 75nmol/L in obese population was 80%, which was significantly higher than the proportion in the non-obese population (p<0.0001) (Hyppönen & Power, 2006). It was also reported that BMI and fat content even in non-obese people was negatively correlated with vitamin D levels (Arunabh et al., 2003; Blum et al., 2008). Besides, McGill et al. (2008) studied 250 overweight adults and the results showed a decrease of 0.74 nmol/L (p=0.002) in vitamin D_3 per 1 kg/m^2 increase in BMI and a decrease of 0.29 nmol/L (p=0.01) per 1cm increase in waist circumference. A large bi-directional Mendelian randomization analysis of 21 adult-cohorts, totalling 42,024 participants, concluded that higher BMI led to lower 25(OH)D, but vitamin D had only a small effect on BMI after removing confounders' influences (Vimaleswaran et al., 2013). Based on these studies, it was concluded that vitamin D status is lower in obese population compared to people with normal weight. However, most of the studies related to the topic seem to be observational studies. More interventional trials are needed to see whether fat accumulation or decrease, affects vitamin D status.

2.3.1.2 The potential mechanism of fat effecting serum vitamin D level

The mechanisms for obesity affecting vitamin D level are controversial; but commonly accepted notions include the following: Firstly, vitamin D is fat soluble and easily trapped by adipose tissue which leads to a decrease in serum vitamin D in obese individuals. As early as 1971, Rosenstreich *et al.* (1971) found 80% of radioactive ^{14}C labelling vitamin D was deposited quickly into adipose tissue in an animal experiment. In a clinical study (Wortsman et al., 2000), the obese group had a 57% lower increase in serum vitamin D compared to the non-obese, age-matched control group after they received equal ultraviolet irradiation. A study reported that the synthesis of provitamin D_3 was not significantly different between the obese group and the non-obese group (Wortsman et al., 2000). That means that more subcutaneous fat in obese individuals may retain more 25(OH)D synthesized by the

skin and prevent it from being released into the blood stream, despite these obese individuals having more skin area exposed to ultraviolet irradiation. These studies suggest that fat tissue is a storage site of 25(OH)D and it seems that mobilizing 25(OH)D from fat tissue is very slow compared with vitamin D deposition which leads to lowered serum vitamin D in obese individuals.

Secondly, there is a much simpler explanation of volumetric dilution. Drincic *et al*. (2012) explored the relationship between 25(OH)D concentration and body size, reporting no difference in serum 25(OH)D concentrations between the obese group and the non-obese group after the results were adjusted for body size. That means a larger fat mass could have a dilution effect on vitamin D levels in obese individuals. Both proposed mechanisms would result in decreased serum vitamin D concentrations in obese people and reduced bioavailability on 25(OH)D. Based on the above studies, fat loss could result in increased serum vitamin D levels. A study conducted by Rock *et al.* (2012) in which 383 over-weight or obese women took part in a 2-year clinical study verified this point. The study reported that the participants with no weight loss during the study period had increased their mean serum vitamin D concentration by 1.9 ng/mL, while those with 5%-10% and more than 10% loss of baseline weight increased vitamin D concentrations by 2.7 ng/mL and 5.0 ng/mL, respectively. The results provided evidence to support the effect of fat on serum vitamin D level.

2.3.1.3 Effects of vitamin D on obesity

The proposed effects of vitamin D on obesity are even more controversial. Vitamin D was suggested to be a modulator of body weight and decreased vitamin D level in winter might stimulate the “winter response” of fat accumulation and abnormal metabolism, such as increased IR and blood pressure (Foss, 2009). Some observational studies provided evidence for this opinion (Brock et al., 2010; González-Molero et al., 2013). However, not all researchers thought so. A cross-sectional study implemented by Creo *et al.* (2013) reported no relationship between vitamin D levels and BMI. Further, several RCTs were conducted to see if vitamin D supplementation affects BMI and other obesity markers. The summary of

the RCTs in recent years is shown in Table 2.3. Collectively, most of the RCTs did not show an effect of vitamin D supplementation on obesity. Only two RCTs (Anthony M Belenchia et al., 2013; Wamberg et al., 2013) showed the study groups had received a little benefit from vitamin D supplementation. Both studies indirectly reflected that vitamin D supplements improved obesity based on vitamin D effect on the ratio of leptin to adiponectin and PTH concentration, respectively. Noticeably, the RCT conducted by Belenchia *et al.* (2013) was the only study specifically designed to investigate the effects of vitamin D on markers of obesity, although it did not find significant changes in BMI and WC in adolescent subjects after 4,000 IU daily vitamin D_3 was supplemented for six months. Furthermore, a meta-analysis based on 12 RCTs did not find that vitamin D supplementation affected the standardized mean difference (SMD) of BMI, fat mass (FM) and percentage fat mass (%FM) (Pathak et al., 2014). However, the efficacy of vitamin D supplementation on obese might only be evident if vitamin D is depleted in the body (Grimnes et al., 2011), while in most RCTs, the range of baseline vitamin D concentrations were broad. In addition, factors such as small sample size, inadequate dosage, short study duration, low compliance, not optimizing other nutrients limited accuracy of study result.

In summary, current research studies on the effects of vitamin D on obesity are inconclusive. However, the potential roles of vitamin D in the occurrence and development of obesity cannot be ignored, for some studies at the genetic level found that low plasma vitamin D levels might be a modest mediator between genetic variants associated with obesity and increased risk of diabetes (Afzal et al., 2014), which is strong rationale for more studies being done in future.

Table 2.3 Randomized controlled trials on the influence of vitamin D on obesity markers.

Reference	n*	Study population（intervention group）	Duration	Dose of VD_3 for intervention	Baseline 25(OH)D in intervention group	Final 25(OH)D in intervention group	Outcomes in study group
Wood *et al.*, (2014)	n_1=102/101 for two doses group n_2=102	Healthy postmenopausal women aged 60-70 yrs old.	1 year	400 IU and 1,000 IU for two study groups	The groups with normal, overweight and obese BMI were 34.3±14.7, 33.9±14.3 and 32.4±16.3 nmol/L, respectively.	400 IU: 33.3, 33.4, 31.5 nmol/L were increases in subjects with normal, overweight and obese BMI; 1,000 IU: 48.1, 38.8 and 43.0 nmol/L were increases in subjects with normal, overweight and obese BMI.	No significant changes were reported in obese markers within and between groups.
Petchey *et al.*, (2013)	n_1=11 n_2=13	Patients with chronic kidney disease	6 months	2,000 IU/d	95±37 nmol/L	146±25nmol/L	No significant differences on BMI and percentage of body fat before and after treatment between the two groups.
Belenchia *et al.*, (2013)	n_1=18 n_2=17	Obese adolescents	6 months	4,000 IU/d	Not given	Not given, but significant increase was told	No significant changes on BMI and WC.
Wamberg *et al.*, (2013)	n_1=21 n_2=22	Healthy adults aged 18-50 yrs with BMI>30 kg/m^2	26 weeks	7,000 IU/d	33 nmol/L (mean value)	110 nmol/L (mean value)	No significant changes on body fat and BMI. PTH significantly decreased from 5.3 pmol/L to 4.5 pmol/L in study group (p=0.03).
Salehpour *et al.*, (2012)	n_1=39 n_2=38	Healthy premenopausal over weight and obese women	12 weeks	25 μg/d	75±22 nmol/L	113.2±32 nmol/L	No significant changes on body weight, BMI, and WC before and after treatment between the two groups.

Reference	n*	Study population（intervention group）	Duration	Dose of VD_3 for intervention	Baseline 25(OH)D in intervention group	Final 25(OH)D in intervention group	Outcomes in study group
							A significant decrease in fat mass was resulted in the study group compare with the placebo group (p<0.001).
Ardabili *et al.*, (2012)	n_1=24 n_2=26	Women with polycystic ovary syndrome and vitamin D deficiency	2 months	50,000 IU, once every 20days.	6.9±2.8 ng/ml	23.4±6.1 ng/ml	No significant changes and differences on body weight and BMI in and between the two groups.
Gallagher *et al.*, (2012)	N_{total}= 163, to 7 groups with different doses from low to high and a placebo group.	healthy, white, postmenopausal women aged 57 to 90 years	1 year	Different doses of 400, 800, 1,600, 2,400, 3,200, 4,000, 4,800 IU per day.	38.2±9.4 nmol/L	Not given, but the scatter diagram showed significant, gradual increases from low to high doses groups.	There was no significant interaction between BMI and dose of vitamin D_3.
von Hurst *et al.*, (2010)	n_1=42 n_2=39	South Asian women aged 23-68 years, with IR and vitamin D insufficiency.	6 months	4,000 IU per day	Median value =21 nmol/L	Median value =80 nmol/L	BMI did not change significantly in either group during the study. There was no relationship between change in vitamin D concentration and BMI.

* n_1 is sample size of study group; n_2 is sample size of placebo group.

2.3.2 Vitamin D and insulin resistance

Insulin resistance (IR) refers the decreased biological effects of insulin on the target organs or tissues (Savage et al., 2007). It is the common underlying pathophysiology of obesity, type 2 diabetes mellitus (T2DM), dyslipidemia, and hypertension. So it can be said that IR is the central link in the occurrence and development of MetS (Fujita, 2007). When IR happens, the biological effects of insulin on target cells decrease. Meanwhile, serum glucose concentration rises and islet β cells have to secrete more insulin to maintain normal glucose metabolism. However, once the compensatory secretion of insulin from islet β cells is no longer able to sustain the situation, hyperglycemia occurs. During the progression of IR, increased cytokines from lipid tissue, such as leptin, tumor necrosis factor α (TNF-α), and decrease of adiponectin, have been found to be involved (Yadav et al., 2013) and gradually become a cause for concern.

2.3.2.1 Evidence for the effects of vitamin D on insulin resistance

Vitamin D is thought to play a role in the occurrence of IR and the development of type 2 diabetes although the exact mechanism is still unclear. Currently, the hyperglycemic-clamp method is the accepted gold standard worldwide for evaluating insulin sensitivity (Gordillo-Moscoso et al., 2004). A number of cross-sectional studies have explored the relationship of vitamin D and IR using hyperglycemia clamp method and reported that serum vitamin D level was inversely related with the prevalence of IR and diabetes mellitus (Chiu et al., 2004; Ford et al., 2005; Kayaniyil et al., 2010; Zhao et al., 2010; De Pergola et al., 2013).

In a prospective study, 524 non-diabetic middle-aged people were recruited. Their glycemic status, insulin resistance, blood pressure and anthropometry were traced for 10 years (Forouhi et al., 2008). The results showed that the baseline vitamin D levels were inversely associated with the 10-year risk of IR and hyperglycemia (p=0.005 and p=0.006, respectively) after adjustment for multiple confounding factors. However, the study's quality was limited by the high rate of loss to follow up, which was close to 50% of the initial cohort. Another even larger prospective study

followed 4,097 middle aged healthy participants over 17 years (Mattila et al., 2007). During the 17 years follow-up period, 187 cases of type 2 diabetes were diagnosed. A statistically significant inverse relationship was found between baseline vitamin D status and incidence of type 2 diabetes, although the relationship was attenuated after adjustments for BMI, exercise and smoking. In addition, the relative risk between the highest quartile and the lowest quartile of serum vitamin D was 0.60 (95% CI: 0.36-0.98, p=0.01). However, the correlation coefficients between vitamin D and risk factor of type 2 diabetes were low (from -0.13 to 0.21), which had almost no significance. Walsh *et al.* (2013) enrolled 60 pregnant women, and collected data about their serum vitamin D concentrations and insulin concentrations early in pregnancy and at 28 weeks. The results showed that those pregnant women with lower serum vitamin D concentration in early pregnancy had a higher HOMA index. This suggested that lower vitamin D levels were associated with higher risk of increased IR in pregnancy. Noticeably however, the findings were not adjusted for potential confounders, such as weight, stage of pregnancy and diet habits. Moreover, for the pregnant women, it should not be ignored that the marked hormonal changes play a role in weight gain, insulin secretion and sensitivity (McIntyre et al., 2010).. To sum up these prospective studies, their conclusions were conflicting and more studies with better designs and implementation are needed.

The effects of vitamin D supplementation on IR and other markers of type 2 diabetes have been investigated in several RCTs. In one study, in which 44 obese adolescents were randomly supplemented with 4,000 IU vitamin D_3 daily or a placebo for 6 months, significant improvements were found in HOMA IR index and quantitative insulin sensitivity in the supplemented group (Belenchia et al., 2012). The study's advantage is the use of random distribution and double-blind methods. The major shortcoming of the study is its relatively small sample size, only 18 and 17 participants in the supplementation and placebo group, respectively. Moreover, the description of loss to follow-up was unclear. Similarly, another RCT also randomly supplemented with 4,000 IU vitamin D_3 daily or a placebo for 6 months; this time subjects were 42 and 39 women with increased IR, respectively (Pamela R. von Hurst et al., 2010). Significant improvements in IR or insulin sensitivity were reported in the vitamin D_3 group. Both of the above RCTs drew positive conclusions.

By contrast, the RCT conducted by Grimnes *et al.* (2011) found different results. They measured participants' vitamin D concentrations after randomly supplemented with 20,000 IU vitamin D_3 twice weekly or a placebo for 6 months. Serum vitamin D concentrations of the study group were increased to 142.7±25.7 nmol/l, and those of the control group were only 42.9±17.3 nmol/l. However, no significant difference in IR was found. In the above three RCTs, the study subjects were not diabetic, and the serum vitamin D levels were significantly increased after using vitamin D supplementation. However, results were inconsistent. As reported by some studies, IR might be affected by lots of factors, such as diets and exercise, which could influence IR by interfering with lipid metabolism and insulin sensitivity (Corcoran et al., 2007; McNaughton et al., 2008). So, other biomarkers, such as insulin secretion and cytokines involved in hyperglycemia should be investigated in future, so as to sufficiently learn more effects of vitamin D supplementation on IR.

Furthermore, studies on patients with diabetes also report conflicting results. A double-blind clinical trial of diabetes patients verified that using vitamin D supplements can improve the patients' IR, even though the daily supplement dose was only 1,500 IU, which was much lower than the doses in the above studies (Shirinzadeh M et al., 2007). However, another RCT of diabetics from Korea (Ryu et al., 2014) also did not show statistically significant differences in IR. In this study, 158 patients with type 2 diabetes were randomized into a vitamin D group and a placebo group. After a 24-week intervention of daily 2,000 IU vitamin D and 200 mg calcium, the two groups had significantly different serum vitamin D concentrations ($p<0.001$) and parathyroid hormone levels ($p=0.003$), but their IR assessments were not significantly different. Moreover, a systematic review, implemented by George *et al.* (2012), investigated fifteen RCTs reporting effects of vitamin D supplement action on IR. The review assessed these trials' quality and found the trials were uneven in study quality and most sample sizes were not big enough. They thought there was insufficient evidence that vitamin D supplements can significantly improve IR. It must be said, though, there were many confounding factors, including different study designs, study populations, sampling, season of the year, loss to follow-up and analysis methods. More trials with bigger sample sizes and better designs are needed before a conclusion can be drawn.

2.3.2.2 Vitamin D and adipocytokines

It is known that adipose tissue is not only an energy store in the human body, but is also an endocrine organ that secretes cytokines. Adipocytokines play important roles in glucolipid metabolism and are closely associated with IR (Yadav et al., 2013). The following section focuses on adiponectin and leptin, which are the most studied adipocytokines.

(a) Adiponectin

Adiponectin is one of the best characterized adipocyte-derived cytokines and one of the most important adipocytokines that protects the body from increased IR. Early studies found that mRNA expression of adiponectin in obese and diabetes patients decreased, resulting in lower adiponectin levels (Kern et al., 2003; Ebinç et al., 2008). In addition, after losing weight, the expression of adiponectin and its serum concentration were normalised (Statnick et al., 2000). Subsequently, animal studies reported that adiponectin levels inversely changed with weight loss and gain in mice with IR (Kubota et al., 2002; Bauche et al., 2006). So far, decreased adiponectin has been demonstrated to be associated with IR, as well as visceral obesity, although their causality was uncertain (Leal & Mafra, 2013; Yadav et al., 2013). The relationship between vitamin D and adiponectin has been of interest in recent years. However, the findings were conflicting. Some cross-sectional data were analysed, and most of the studies (Nimitphong et al., 2009; Vaidya et al., 2011; Teles et al., 2012; Vaidya & Williams, 2012), except for one study (Wright et al., 2013), reported a positive relationship between serum vitamin D and adiponectin. Vaidya *et al.*'s study (2011) also suggested that high BMI could strengthen this association. By contrast, Vaidya and Williams' study (2012) did not show that BMI affects the positive relationship, which was based on two cohorts with larger sample sizes than that of the previous study.

Furthermore, a few prospective studies did not support the positive relationship between vitamin D levels and serum adiponectin. One is the study conducted by Ozkan *et al.* (2009), in which 21 children with vitamin D deficiency rickets were recruited and supplemented with vitamin D to increase their vitamin D status; no

significant increase of adiponectin was found. Similarly, in a small study, 19 peritoneal dialysis (PD) patients, who were vitamin D deficient at baseline and supplemented with cholecalciferol was found to increase their serum vitamin D concentrations from 10.2 ± 4.9 ng/ml to 82.9 ± 56.5 ng/ml (p<0.05). Comparison of the data at baseline and at the end of the treatment did not find significant differences in adiponectin levels despite a significant improvement in patients' IR (Ulutas et al., 2013). The common limitations of both of these studies are small sample size and potential confounders of subjects' background diseases.

Although there was accumulating evidence from observational studies, RCTs investigating the relationship between vitamin D status and adiponectin were limited and only 3 RCTs were found (Table 2.4) (Belenchia et al., 2012; Neyestani et al., 2012; Breslavsky et al., 2013). All three studies showed marginal improvement in IR when using vitamin D supplementation to increase the level of adiponectin. Specifically, in Belenchia's (2012) study, the ratio of leptin to adiponectin decreased although adiponectin did not increase significantly. Neyestani *et al.* (2012) reported significant increase in adiponectin only in the group which was supplemented with both vitamin D and calcium. Breslavsky's (2013) study found a marginal increase in adiponectin. These studies had various study populations, different sample sizes, study durations and vitamin D supplement dose, which might lead to different results, although the implementation of the studies using randomization and double-blinding in these studies indicate good quality.

Table 2.4 A summary of RCTs on the effect of vitamin D supplementation on

adiponectin

Reference	n	Location	Study Population	Study Duration	Dose	Outcomes
Belenchia *et al.*, 2012	35 M/F, 9-19 yr	Missouri, USA	Obese adolescents	6 months	4,000 IU D_3/day	No difference on adiponectin between the groups at 6 month; Serum leptin to adiponectin ratio was significantly lower in V-D_3 group at 6 month.
Neyestani *et al.*, 2012	90 M/F, 30-60 yr	Iran	Patients with type 2 diabetes mellitus	12 weeks	DD group: 500 IU VD_3+150 mg calcium/ day CDD group: 500 IU D_3+250 mg calcium/ day	Vitamin D levels were significantly increased in the two treatment groups; Mean adiponectin was significantly increased only in CDD group compared with the control group (p=0.021).
Breslavsky *et al.*, 2013	47 M/F, Middle aged and elderly people	Israel	Patients with type 2 diabetes mellitus	12 months	VD_3 1,000 IU/day	Vitamin D levels were significantly increased in VD group; HOMA-IR did not change during the study in both groups; Adiponectin in VD group was marginally increased in VD group (p=0.065).

(b) Leptin

Leptin is a polypeptide hormone coded by the obese gene and mainly produced by adipocytes. Recent comprehensive studies show that leptin has extensive biological effects, including suppressing appetite, promoting energy consumption, and adjusting multiple critical enzymes involved in glucolipid metabolism (Yadav et al., 2013). As one of most important adipocytokines, leptin is high in obese individuals and is thought to be related to the occurrence and development of IR (Yadav et al., 2013). Due to the close relationship between obesity and leptin, the effects of vitamin D on

leptin should be investigated so as to explore the potential pathway in the aetiology of IR.

However, there have not been very many studies on this issue; nonetheless, none of these reported finding a relationship between vitamin D and leptin, partially because leptin levels are very different in males and females. For example, a cross-sectional study (Jiménez-Pavón et al., 2013) of 1,053 European adolescents reported both leptin and vitamin D status were independent of IR in females. However, in males, only leptin was reported to be independent of IR. No relationship between leptin and vitamin D was stated (Jiménez-Pavón et al., 2013). Another study of 105 farmers from South Korea (Yeon et al., 2014) also found both vitamin D and leptin were positively correlated with BMI and body fat mass. However, no apparent relationship between vitamin D and leptin was found. A study from Spain explored the relationship between vitamin D and leptin (de Luis et al., 2012). The cross-sectional data were from 62 patients with primary hyperparathyroidism, of whom 32.3% had MetS. However, it did not show a relationship between serum leptin and vitamin D levels. The limitations of the Spanish study are small sample size and potential confounders due to participants' background disease. So, observational studies concurrently with better design, single gender and bigger sample size are needed.

Evidence of an effect of vitamin D supplementation on leptin from RCTs was very limited, too. Few studies were retrieved. Breslavsky *et al*. (2013) supplemented 47 type 2 diabetes patients with vitamin D 1,000 IU or placebo per day for 12 months and found no differences in leptin levels between vitamin D group and control group. Similarly, Belenchia *et al.* (2013) supplemented 35 obese adolescents with 4,000 IU vitamin D or placebo per day for 6 months but found no significant difference between the two groups, in spite of the significant increase in the mean vitamin D concentration of the study group. Furthermore, a prospective study of 14-week 2,000 IU vitamin D per day supplementation on HIV-1-infected patients observed no effects on leptin (van Den Bout-van den Beukel et al., 2008). The results did not show differences in leptin concentration between the baseline and the end date. The common limitation of the above trials was the small sample size, which might be due to various reasons and might have influenced the accuracy of results. To sum up,

evidence of vitamin D benefiting IR by affecting adipokines is small and insufficient.

2.3.2.3 Potential mechanism for vitamin D improving insulin resistance

The exact mechanism by which vitamin D improves IR is uncertain. Below is a summary of recent studies, that discuss potential mechanisms for vitamin D improving IR. Firstly, 1,25-dihydroxyvitamin D, the bioactive molecule of vitamin D in the body, increases insulin sensitivity of target organs in two ways. On the one hand, it directly promotes the mRNA expression and transcriptional activation of insulin receptor in target organs, including skeletal muscle, adipose tissue and liver (Maestro et al., 2002). On the other hand, it protects β-cells from inflammatory response, immune response, and apoptosis and helps β-cells to maintain insulin secretion, thus playing a role in improving IR and diabetes mellitus (Giulietti et al., 2007; Wolden-Kirk et al., 2011; Takiishi et al., 2013). So, vitamin D deficiency would weaken those protective effects and be a risk factor in IR progress. Secondly, the occurrence of IR might be affected by genetic inheritance and living surroundings. Some vitamin D-associated gene polymorphisms were suggested to affect IR, including the best known DBP and VDR (McDermott et al., 1997; Baier et al., 1998; Chang et al., 2000; Hirai et al., 2000; Pani et al., 2000). However, there were also some contrary findings. For instance, Ye *et al.*'s (2001) did not find evidence to support the genetic variants of VDR being associated with the susceptibility of type 2 diabetes.

2.3.3 Vitamin D and hypertension

High blood pressure is also prevalent among middle-aged and old adults worldwide and quite a number of observational and RCT studies suggest that vitamin D insufficiency could be involved in the pathogenesis of hypertension.

2.3.3.1 Evidence for the effect of vitamin D on hypertension

(a) Observational study

Several cross-sectional studies have explored the relationship of serum vitamin D level and hypertension, with mixed results (Wuerzner et al., 2012; Tamez et al., 2013). Perhaps because blood pressure measurement is easy, the bulk of studies were of cross-sectional design and with large sample sizes, and only a few of studies were prospective studies. Among them, most of the cross-sectional studies reported that vitamin D deficiency or insufficiency was associated with increased blood pressure, including some population-based studies (Hintzpeter et al., 2007; Scragg et al., 2007; Hyppönen et al., 2008). Although several cross-sectional studies failed to find significant results. One was on a Chinese population of 1,420 Chinese adults aged 20-83 years old (Li et al., 2012). The null result could be caused by its limitations, such as non-random sampling and potential interference from confounders (no physical exercise information collected). Snijder *et al.* (2007) also found no evidence of an inverse relationship between vitamin D and hypertension. However, among the 1,205 participants of the study, 32.6% were reported using anti-hypertension medication, which may have influenced the result. In addition, Reis *et al.* (2007) explored the relationship between MetS and vitamin D level and blood pressure was one of assessment items. Data analysis showed no significant correlation between serum vitamin D concentration and hypertension, as the stratified OR values from low to high vitamin D level were 0.92, 0.90, 0.88, 1.28 (p=0.6) in males and 0.77, 0.80, 0.81, 1.01 (p=0.8) in females. However in the study, the mean serum vitamin D concentrations in men and women were 108.9 ± 0.3 and 101.6 ± 0.2 nmol/L, respectively, both of which are much higher than the cutoff value of vitamin D sufficiency. That meant almost all the participants were vitamin D sufficient, so the null relationship was not a surprise.

The relationship between low vitamin D level and risk of hypertension was also explored in some prospective studies. In 1994, Jorde *et al.* (2010) measured serum vitamin D and blood pressure in 4,125 participants who were not using anti-hypertension treatment. The data of 2,385 participants of the baseline group were collected 14 years later. The data analysis showed an inverse correlation between serum vitamin D and systolic blood pressure in 1994, but did not show any significant association with blood pressure data in 2008. So the study supported the inverse relationship but did not verify a causal relationship. By contrast, Forman *et al.* (2007) conducted a prospective study and found that the participants with serum deficient vitamin D levels had OR values of 2.67 (95% CI: 1.05-6.79) for incident hypertension after a 4-year follow-up compared to those with sufficient vitamin D levels. The significant result led the team to conduct another study, which was a nested case-control study (Forman et al., 2008). They reported the OR value of lowest quartile of vitamin D compared to the highest quartile was 1.66 (95% CI: 1.11-2.48) for hypertension. The participants with vitamin D deficiency had a multivariable odd ratio of 1.47 (95% CI: 1.10-1.97) for hypertension compared to those with sufficient vitamin D levels. So the study suggested that serum vitamin D was inversely related to blood pressure and was an independent risk factor for hypertension. In addition, results in both the studies were adjusted for some potential confounders.

In addition, there were several prospective studies exploring the effect of vitamin D intake from foods on blood pressure (Forman et al., 2005; Wang et al., 2008). In Forman's study, three prospective cohorts were included and almost 200,000 participants returned questionnaires. After more than 10 years follow-up, the results did not show a significant association between vitamin D intake and risk of hypertension. Conversely, in another prospective study investigating the effect of vitamin D in dairy foods on blood pressure, 28,886 US women were surveyed by semi-quantitative questionnaire. After 10 years follow-up, the OR values of low-fat dairy products intake across increasing quintiles for risk of hypertension were 1.00, 0.98, 0.97, 0.95, 0.89 (p=0.001). Also, compared with the lowest quintile, the highest quintile of vitamin D intake had 0.95 OR value for hypertension. The data analysis suggested vitamin D might be protective against hypertension. Although the two

studies reported conflicting results, the data collecting method of semi-quantitative questionnaire might have produced recall bias and intake estimation bias, which would affect the results..

(b) Intervention study

There are a total of eight RCTs reporting the effects of vitamin D supplementation on blood pressure in humans in the past five years (Table 2.5). Among them, only two studies were specially designed to explore the effects on blood pressure (Forman et al., 2013; Nasri et al., 2014). One is Nasri *et al.*'s study (2014), in which 60 diabetic patients were given 50,000 IU vitamin D_3 supplements or a placebo weekly for 12 weeks. The results showed that the treatment group had significant increase in vitamin D level (p=0.001) and significant decrease in both systolic (from 120 to 110 mmHg, p=0.001) and diastolic BP (from 80.5 to 76.3 mmHg, p=0.046) compared to before the treatment. There were also significant decreases in both systolic and diastolic BP between the groups after the treatment. So Nasri *et al.* concluded that vitamin D supplementation was beneficial to blood pressure in type 2 diabetes patients. Another study specially designed to explore the effects on blood pressure was on African American participants (Forman et al., 2013). A total of 283 healthy participants were recruited and randomly stratified into four groups in order to receive different doses of vitamin D supplements (1,000, 2,000, 4,000 IU cholecalciferol) or placebo. After 3-month treatment, only systolic BP showed a significant, but modest decrease for the three supplement groups (p = 0.04) in spite of obvious increases in serum vitamin D levels in all three treatment groups. No other significant changes were found within or between groups. In both RCTs, the serum vitamin D levels were significantly increased after treatment but had different results in effects on BP. Some potential confounders, such as lifestyle and daily exercise that were not analyzed, might have influenced the results. Study group was another factor possibly leading to different results. For the above two studies, one was for diabetes patients; another was for healthy participants. So the two populations had different physiology and pathology, which could have influenced the study results.

Witham *et al.*'s (2014) RCT recruited 68 patients with blood pressure above 140/90

mmHg and used vitamin D supplements to increase their serum vitamin D concentration. After 6-month intervention which significantly increased serum vitamin D in the vitamin D group, no significant changes in BP between and within the groups were reported. There also were two RCTs using calcium and vitamin D to explore the effect on blood pressure (Margolis et al., 2008; Daly & Nowson, 2009). However, no significant changes were observed in either study. In both the trials, doses of vitamin D supplements were small, 800 IU and 400 IU per day, respectively, while study durations were much longer than those of other studies. Margolis *et al.*'s study even lasted 7 years, together with huge sample size (36282), for which participants' compliance with treatments was not easy to control and low compliance rate might be an influential factor. The remaining RCTs (Toxqui et al., 2013; Wamberg et al., 2013) also did not show evidence of vitamin D being beneficial to hypertension.

Table 2.5 A summary of RCTs on the effect of vitamin D_3 supplementation on blood pressure.

Reference	n	Study Population	Duration	Dose for Intervention	Baseline 25(OH)D In Intervention group	Final 25(OH)D In Intervention group	Outcomes
Witham, *et al.*, (2014)	68	Patients with supine office blood pressure>140/90 mmHg	6 months	100,000 IU, oral, every two months	42 (±16) nmol/L	Not given	Vitamin D treatment did not reduce 24-hour ambulatory blood pressure. (Systolic:+3mmHg, 95%CI:-4 to +11, p=0.33; Diastolic: -2mmHg, 95%CI:-6 to +2, p=0.29).
Nasri, *et al.*, (2014)	60	Patients with type 2 diabetes	12 weeks	50,000 IU, oral, weekly	83.9(±52) nmol/L 8.3% deficiency; 45% insufficiency; 45% normal	164 (± 57) nmol/L	Mean systolic BP decreased from 121 to 110 mmHg (p<0.01). Mean diastolic BP decreased from 80.5 to 76.3 mmHg (p<0.01). Both mean systolic and diastolic BP were less than those of control group (p<0.01).
Witham *et al.*, (2013)	50	Healthy South Asian women with vitamin D level less than 75nmol/L.	8 weeks	Single dose of 100,000 IU was received at baseline.	27±13 nmol/L	Not given and only noted a significant increase 8 weeks later.	Marginal decrease without significance was showed in diastolic BP intra-group. No significant changes were found in systolic BP at 4 weeks and 8 weeks later.
Toxqui, *et al.*, (2013)	109	Young women with low iron stores	16 weeks	200 IU, Vit D and iron fortified milk per day	62.3 ±20.8 nmol/L	71.2±21.1 nmol/L	Both systolic and diastolic BP of D-fortified group were significantly decreased compared before and after treatment (p_s=0.017; p_d=0.01); No significant differences in Both systolic and diastolic BP of the two groups.
Forman *et al.*, (2013)	283	Blacks aged 30 to 80 years	3 months	1,000, 2,000, 4,000 IU VD for three matched groups,	16.3, 14.5, and 15.6 ng/ml for 1,000, 2,000, 4,000 IU groups' median values, respectively, p=0.86	29.7, 34.8, 45.9 ng/ml for 1,000, 2,000, and 4,000 IU groups' median values (p<0.01 in three groups)	Three treatment groups had changes of systolic mean BP from 124.7, 122.8, and 130.4 to 122.5, 120.0, 126.6 for 1,000, 2,000 and 4,000 IU groups, respectively (p=0.04) after 3-month treatment;

Reference	n	Study Population	Duration	Dose for Intervention	Baseline 25(OH)D In Intervention group	Final 25(OH)D In Intervention group	Outcomes
				respectively.			No significant decreases in diastolic BP compared baseline with 3-month treatment in the three treatment groups and placebo groups as well (P=0.37).
Wamberg *et al.*, (2013)	52	Subjects aged 18-50 years with BMI >30kg/m^2 and plasma 25(OH)D <50nmol/L	26 weeks	7,000 IU, oral tablets per day	33.0 ±10.8 nmol/L	110.2±21.2 nmol/L	No significant changes in both systolic and diastolic BP in VD group (p_s=0.11; p_d=0.61). No significant difference in both systolic and diastolic BP between two groups after treatment (p_s =0.61; p_d=0.68).
Daly *et al.*, (2009)	140	Caucasian men aged > 50 years.	2 years	Daily 400 ml fat-reduced milk fortified with 1,000mg calcium and 800 IU VD.	78 ±23 nmol/L,	78±23 nmol/L + 4.8 (mean change)	No significant changes in both systolic BP and diastolic BP in treatment group before and after treatment. No significant difference between the two groups in both systolic BP and diastolic BP.
Margolis *et al.*, (2008)	36282	Postmenopausal women aged 50-79 years old.	7 years (mean follow-up)	Daily 1,000mg calcium plus 400 IU VD.	Normal distribution crossing from vitamin D deficiency to sufficiency	Not given	No significant difference between the two groups in both systolic and diastolic BP (0.22 mmHg and 0.11 mmHg, respectively). In non-hypertensive subjects at baseline, the odd ratio value of Ca/VD treatment for incident hypertension was 1.01 (95%CI: 0.96-1.06).

There were two meta-analyses summarizing some of these trials (Kunutsor et al., 2013; Kunutsor et al., 2014). One meta-analysis by Kunutsor *et al*. (2013) included 11 RCTs, in which 7 studies measured serum vitamin D concentration and 4 studies measured dietary vitamin D intake so as to see the effects on blood pressure after a period of treatment. Another meta-analysis by Kunutsor *et al*. (2014) included 16 RCTs, in which most used cholecalciferol and only one used ergocalciferol and all the studies measured serum vitamin D. However, both the meta-analyses were unable to conclude that vitamin D intake or supplementation is beneficial in treatment of hypertension, and suggested more RCTs were needed. In summary, the evidence that vitamin D is associated with hypertension was mainly from observational studies. More interventional studies are needed.

2.3.3.2 Possible mechanisms linking vitamin D with blood pressure

Summarizing previous studies regarding the possible mechanisms of roles of vitamin D in adjusting blood pressure, the following aspects were found. Firstly, vitamin D has been thought a negative regulator for renin-angiotensin system (RAS) so as to participate in controlling blood pressure (Yan Chun Li et al., 2004). RAS is an important humoral regulation system, which play a crucial role in adjusting blood pressure and water, salt and electrolyte balance (Kobori et al., 2007). So, it is clear that activating RAS is one of mechanisms by which hypertension develops. An animal experiment showed that vitamin D inhibited genetic transcription of renin therefore negatively modulating RAS (Li et al., 2002). In the experiment, giving an inhibitor of vitamin D synthesis to wild mice led to increased renin levels, while giving them a supplement of 1,25-dihydroxyvitamin D decreased renin levels. Meanwhile, VDR-null mice showed much higher renin and plasma angiotensin II than wild mice. Another study demonstrated that mice with 1α-hydroxylase gene knockout had increased blood pressure and up-regulated RAS (Zhou et al., 2008). Giving these mice 1,25-dihydroxyvitamin D, significantly improved the symptoms. A further study demonstrated that 1,25-dihydroxyvitamin D-receptor complex might interfere with the formation of renin gene promoters, and thus suppress the activity of RAS (Yuan et al., 2007). So, overall, animal studies linking vitamin D with RAS

and blood pressure regulation were convincing.

In human studies, as early as 1986 Resnick *et al.* (1986) reported that plasma renin activity was inversely associated with $1,25(OH)_2D$ level in patients with hypertension. Forman *et al.* (2010) recruited 285 healthy participants and gave them high sodium balance. They found that the participants with vitamin D insufficiency (<29.9 ng/mL) had higher serum angiotensin II levels (p=0.03) and more insensitive renal plasma flow responses to angiotensin II (p=0.009) compared to those with sufficient vitamin D status. The results suggested that low vitamin D status might cause up-regulation of RAS in healthy individuals. In a RCT conducted by Sugden *et al.* (2008), the vitamin D group had a significant decrease in systolic BP after treatment compared with the control group. Meanwhile, the angiotensin levels in vitamin D supplemented groups exhibited a declining trend with the treatment, although the change did not reach significance. So, vitamin D acting as a negative regulator of RAS has been supported by some human studies, although they were still very limited.

Secondly, $1,25(OH)_2D$ regulates blood pressure by suppressing parathyroid hormone (PTH). It was known that vitamin D deficiency is accompanied by increased PTH, and PTH is positively associated with hypertension. The relationship has been verified by some epidemiological and clinical studies (Fliser et al., 1997; Jorde et al., 2000; Snijder et al., 2007; Taylor et al., 2008), although the evidence could not be generalized. The exact mechanism by which PTH elevates blood pressure is ambiguous. However, some studies suggested PTH could increase vascular tone and stiffness, renin level and IR as well, therefore increasing blood pressure (Fitzpatrick et al., 2008).

Thirdly, it was suggested that vitamin D protects vascular endothelium from various deleterious effects and therefore benefits blood pressure. The influence of vitamin D on blood vessels might be indirect and complex, as VDR and 1α-hydroxylase are found in vascular endothelial cells and vascular smooth muscle cells (Zehnder et al., 2002). Animal studies showed that vitamin D could improve vascular tone by combining with VDR and then reducing calcium influx into vascular endothelial

cells in spontaneously hypertensive rats (Wong et al., 2008). Another animal intervention study also reported that young rats with vitamin D deficiency had double mesenteric artery myogenic tone compared to the control rats (Tare et al., 2011). Experiments in vitro also showed that $1,25(OH)_2D$ could protect vascular endothelium from advanced glycation end products in a high glucose environment (Talmor et al., 2008). In human studies, Aoshima *et al.* (2012*)* examined the effects of $1,25(OH)_2D$ on human vascular smooth muscle cells and reported that $1,25(OH)_2D$, and its analogue, could protect vascular smooth muscle cell mineralization.

To sum up, the potential mechanism by which vitamin D benefits blood pressure by protecting vascular has been explored by some animal studies and laboratory studies, but clinical studies are still needed. In addition, the effects of vitamin D improving insulin sensitivity and inflammation were supported by some studies, via which vitamin D indirectly benefits blood pressure (Rammos et al., 2008). So, the pathways linking vitamin D with blood pressure might be extensive and broad.

2.3.4 Vitamin D and dyslipidemia

Dyslipidemia is one of main components of MetS and one of the most common causes of IR. It is known that IR is common to many risk factors of CVD. Relative to other components of MetS, studies on the relationship between dyslipidemia and vitamin D were more inconclusive.

2.3.4.1 Observational studies

As early as 1994, Lin *et al.* (1994) treated uremic patients on haemodialysis by intravenous 25(OH)D three times weekly for eight weeks. They reported a significant decrease in plasma triglyceride compared with before treatment (from 2.24±0.34 to 1.80±0,29, $P<0.05$). Since then, many observational studies have demonstrated that increased vitamin D level was associated with normal blood TG, TC, LDL and HDL (Gausch et al., 2012; Huang et al., 2013b; Lee et al., 2009; Martins et al., 2007; Ponda et al., 2012b; Rodríguez-Rodríguez et al., 2011).

However, these studies were difficult to compare, as they had different study protocols, age groups, ethnicities and sample size. Also, they often had partial, not all, lipid profiles with significant results. For instance, a study of 2,708 patients with IR and type 2 diabetes were conducted in China (Y. Huang et al., 2013). The data showed that TC, TG and LDL had a weak, decreasing trend with the increase of vitamin D level, but no relationship between vitamin D and HDL was reported. A study in America also identified vitamin D level was inversely associated with plasma TG concentration in normal adults surveyed in NHANES Ⅲ, whereas not associated with TC, HDL and LDL (Martins et al., 2007). Another population-based cross-sectional study of males from Europe also reported that age-adjusted vitamin D concentration was negatively related with TG (p<0.01) and mean TG values were 33% higher in the lowest quintile of vitamin D level compared to the highest quintile, although the relationship was attenuated after adjustment for IR assessment (Lee et al., 2009). No other correlations were found.

In addition, different study design led to different findings. Ponda *et al.* (2012b) undertook a cross-sectional analysis on 107,811 patients' plasma testing records across the US between September 2009 and February 2011. They found that patients with optimal vitamin D status had significantly lower TC, LDL, and TG and higher HDL compared with those with deficient vitamin D levels. Based on these positive results, they also did a retrospective analysis (Ponda et al., 2012a). The results showed that in patients with vitamin D increase during study duration, their TC also went up significantly. So the serial studies with the same study group had conflicting results. It is noticed that the study group were patients with various diseases, so more potential confounders, such as other medications, some pathological changes, their BMI and their physical exercise, could interfere with the results.

From the above observational studies, it seems that usually some, but not all, lipid markers were related to vitamin D status. More observational studies specially designed for exploring this relationship between vitamin D and lipid markers are needed in the future.

2.3.4.2 Intervention study

There have been several of intervention studies investigating the effects of vitamin D supplementation on metabolic biomarkers and CVD risk factors, often including serum lipid profiles, although the studies specially designed for lipid markers were limited. The summary of RCTs on this issue in recent years is in Table 2.6. From the table, it is evident that only three studies (Kane et al., 2013; Rahimi-Ardabili et al., 2013; Zhou et al., 2013) were specifically designed to examine the effect of vitamin D on lipid profiles. Also, only three studies reported any significant improvement on plasma lipid markers after vitamin D supplementation (Asemi et al., 2013; Rahimi-Ardabili et al., 2013; Zhou et al., 2013). In all the studies in Table 2.6, vitamin D supplementation effectively improved the vitamin D levels. However, most of the RCTs did not get significant improvements in plasma lipid profiles. This may be because the study durations were not long enough to get evident improvement on blood lipid by vitamin D, although the vitamin D levels were significantly increased. For example, some of them had relatively short durations, with only two or three months except for the 12-month duration of Wood *et al.*'s study. Also, some studies (Ponda et al., 2012a; Kane et al., 2013) recruited subjects with vitamin D deficiency, which seemed easier to get the evident effects of vitamin D supplementation on the lipid profiles.

In addition, there were two meta-analyses which had summarized data from multiple studies. Both the meta-analyses indicated that RCTs of vitamin D supplementation did not have significant lipid modulating effects (Elamin et al., 2011; Wang et al., 2012). The authors of these two studies also suggested that more large-scale RCTs with adequate supplement dose and study duration are needed.

Table 2.6 A summary of RCTs on the effect of vitamin D_3 supplementation on serum lipid levels.

Reference	N*	Subjects	Duration	Dose for intervention	Baseline 25(OH)D	Final 25(OH)D in intervention group	Outcomes
Asemi *et al.*, (2013)	n_1=27 n_2=27	Women with gestational diabetes mellitus	6 weeks	Total 100,000 IU vitamin D-3 provided two times during the study	22.44±14.31 ng/ml	38.95±24.72 ng/ml	TC significantly decreased from 211.35±43.22 to 200.31±41.63 mg/dl in vitamin D group (p=0.01). LDL significantly decreased from 121.84±36.37 to 111±38.54 mg/dl (p=0.003). TG decreased without significance (p=0.67). LDL had a little increase of 1.55±10.11 mg/dl (p=0.45).
Witham *et al.*, (2013)	n_1=25 n_2=25	Healthy South Asian women with vitamin D level less than 75nmol/L.	8 weeks	Single dose of 100,000 IU was received at baseline.	27±13 nmol/L	Not given and only noted a significant increase 8 weeks later.	TC, TG and LDL had a little decreases at 4 weeks and 8 weeks later and were no significances compared with placebo group (all p values were greater than 0.05). There were no change and difference in HDL intra-group and inter-groups after treatment.
Kane *et al.*, (2013)	n_1=26 n_2=23	The elderly with vitamin D deficiency	12 weeks	Different doses (from 1,000 to 2,000 IU) were taken in different study durations so as to make all subjects in the treatment group get a significant increase.	16.2±4.5 ng/ml	32.7±6.2 ng/ml	No significant changes and differences were reported on any lipid markers within the treatment group and between groups.

Reference	N*	Subjects	Duration	Dose for intervention	Baseline 25(OH)D	Final 25(OH)D in intervention group	Outcomes
Heravifard *et al.*, (2013)	The total number of three groups was 90.	The subjects aged 30-60 years old with type 2 diabetes	12 weeks	PD group: 150mg calcium + no VD per day; DD group: 150mg calcium + 500 IU VD per day; CDD group: 250mg calcium + 500 IU VD per day	Not given, but did not differ significantly among the groups (p=0.945).	PD: 37.2±44 nmol/L (P=0.136); DD: 77.7±28.6 nmol/L (p<0.001); CDD: 74.6±39.5 nmol/L (p<0.001).	TC, LDL and HDL did not get any significant changes within and between groups. Apo A1 was significantly increased in all three groups (mean changes 0.22 ± 0.38, 0.20 ± 0.27 and 0.01 ± 0.35 g/L, respectively, p = 0.047).
Rahimi-Ardabili *et al.*, (2013)	n_1=24 n_2=26	Women with polycystic ovary syndrome and vitamin D deficiency.	2 months	150,000 IU vitamin D every 20 days.	7±2.8ng/ml	22.9±6.14ng/ml	TC, TG, and VLDL were significantly decreased from 196.6 +/- 32.8 to 179.1 +/- 34.1 mg/dl, 156.8 +/- 73.0 to 130.5 +/- 56.5 mg/dl, and 31.4 +/- 14.6 to 26.1 +/- 11.3 mg/dl, respectively (p<0.05). HDL, LDL and APO-AI did not have differences before and after treatment.
Zhou *et al.*, (2013)	n_1=21 n_2=22	Obese men with BMI>28km/m^2 for study group; Healthy normal weight men with BMI<24km/m^2 for control group.	8 weeks	50,000 IU weekly for both groups.	Study group: 46.1±9.1 nmol/L; Control group: 52.8±17.8 nmol/L.	Study group: 116.7±20.3 nmol/L Control group: 181.3±30.2 nmol/L.	The effect of increasing vitamin D level was weaker in obese group than that in normal weight group. Mean HDL was improved to normal level in study group. There were not significant decreases in TC,TG and LDL in both groups.

Reference	N*	Subjects	Duration	Dose for intervention	Baseline 25(OH)D	Final 25(OH)D in intervention group	Outcomes
Salehpour *et al.*, (2012)	n_1=39 n_2=38	Healthy premenopausal women with overweight and obesity	12 weeks	25ug per day	75±22 nmol/L	113.2±32 nmol/L	A significant increase of 0.07±0.2 in vitamin D group was found and the *p* value was 0.037 compared with placebo group (-0.03±0.2). ApoA-I was significantly increased too The mean LDL level in the vitamin D group, however, had gone up instead of decrease, while the placebo group had a significant decrease in LDL.
Ponda, *et al.*, (2012b)	n_1=76 n_2=75	Normal adults with vitamin D <20ng/ml and one or more CVD risk factors.	8 weeks	50,000 IU weekly	13.4±5.3 ng/ml	43.0±12.3 ng/ml	No significant changes were reported in vitamin D group before and after treatment (the changes of TC, HDL, LDL and TG were 1.2±21.1, 0.3±6.4, -0.3±18.6, 6.1±50 mg/dl, respectively).
Wood *et al.*, (2012)	n_1=102 n_2=101 n_3=102	Postmenopausal women aged 60-70 years old.	12 months	n_1: 400 IU per day n_2: 1,000 IU per day n_3: placebo	n_1: 32.74±12.9 nmol/L n_2: 32.41±13.8 nmol/L. n_3: 36.18±17.1 nmol/L. (*p*=0.13)	Not given. But the mean changes were known: n_1: +33.04 nmol/L n_2: 42.90nmol/L n_3: -2.72nmol/L	TC, TG, LDL and HDL did not have significant changes within any group before and after treatment and among groups.

Note: n_1 is sample size of study group; n_2 is sample size of placebo group.

2.3.4.3 Potential mechanism of effects of vitamin D on lipids

The mechanisms by which vitamin D affects serum lipids are unclear. However, some potential pathways have been suggested. One could be by way of a complex of vitamin D and VDR, which was found to block adipogenesis in vitro (Blumberg et al., 2006). After VDR combining with calcitriol, the complex would down-regulate the expression of C/EBPβ mRNA and C/EBPβ nuclear protein, which are the essential proteins of adipogenesis. On the contrary, in the absence of calcitriol, the unliganded VDR would promote the lipid accumulation. However, the results of the in vitro studies are inconclusive; more studies in vivo are required to further validate these results. Unexpectedly, VDR-null mice did not have raised body lipid profiles as reported in the animal study (Wong et al., 2009). In this study, the VDR-null mice showed less fat mass accumulation and lower plasma TC and TG levels compared with wild mice (C57BL/6). Even with a high-fat diet, the VDR-null mice maintained a lower fat mass, a slower growth rate and had lower blood lipids (Wong et al., 2009). The discrepancy of these in-vitro and in-vivo studies indicates that the potential mechanisms of vitamin D acting on adipogenesis might be extensive and complicated. Another potential mechanism might involve PTH. It is known that increased PTH, which may occur secondary to hypovitaminosis D, is associated with the components of MetS and cardiovascular diseases (Kamycheva et al., 2004; C. Huang et al., 2013). So PTH was suggested to be one of the mediators, by which vitamin D benefits blood lipid in humans (Jorde & Grimnes, 2011), although there is currently little evidence to support this.

2.4 Vitamin D measurement

With the great advances in our understanding of vitamin D roles, the demands of measuring vitamin D levels in clinical medicine are getting more urgent. However, inter-laboratory variability and poor between-assay comparability on 25(OH)D assessments have always existed.

2.4.1 Challenges and needs on vitamin D measurement

Although many kinds of testing technology and commercial kits were developed, 25(OH)D is considered a difficult analyte to measure, and is affected by its own or other metabolites' physical and chemical properties (Carter, 2012).

2.4.1.1 Matrix effects

Serum vitamin D derivatives are highly lipophilic and tightly bind to VDBP. However, the saturation level of VDBP binding with vitamin D derivatives in the circulation is very low, only around 2% to 5% (White & Cooke, 2000). This feature leads to some problems in extracting vitamin D metabolites from serum during the process of vitamin D measurement. The most troubling thing is matrix effects, which always interferes with the vitamin D assessment (Hollis, 2008).

2.4.1.2 Cross-reactivity

In serum, other hydroxylated vitamin D metabolites are present besides 25(OH)D, such as $1,25(OH)_2D$ and $24,25(OH)_2D$. During the immunoextraction procedure, those metabolites would be extracted together with 25(OH)D and would also react with the antibodies against 25(OH)D, therefore interfering with the assay. So the results of the immunoassay often overestimated the actual concentrations (Zerwekh, 2004; Singh et al., 2006).

2.4.1.3 Separate measurement for 25(OH)D_3 and 25(OH)D_2

Originally, most researchers did not think there were significant differences between vitamin D_3 and vitamin D_2 except for small differences in their molecular structures. So it was acceptable to measure the total vitamin D. However, recently, researchers have found that vitamin D_2 has much less bioactivity than vitamin D_3 (Finch et al., 1999; Armas et al., 2004). In addition, it is necessary to monitor the changes of vitamin D_2 concentrations when vitamin D_2 is used for supplementation. So, it is essential to be able to discriminate between vitamin D_3 and vitamin D_2.

2.4.2 Different types of assay

Basically, 25(OH)D assays are categorized into two major types: immunoassay methods and chromatographic methods. Both types of methods have their own advantages and disadvantages. The following section will discuss these methods in detail based on their principles and gradual improvements in the techniques.

2.4.2.1 Immunoassay

(a) Radioimmunoassay (RIA)

The RIA for vitamin D was developed on the basis of antigen-antibody reaction. It utilizes the competitive relationship between 25(OH)D in sample and ^{125}I-labeled 25(OH)D (tracer) on the reactions with a specific antibody, of which the amount is known, then calculating the concentration of sample 25(OH)D (Hollis et al., 1993). On that basis, DiaSorin (Stillwater, MN, US) developed the first RIA test kit in 1996. The assay is non-automated and involves two basic steps; rapid extraction and equilibrium RIA. The aim of the first step is extracting 25(OH)D from the sample, in the process of which, inevitably, some other hydroxylated metabolites are also extracted. Consequently, these non-25(OH)D metabolites would cross-react with the antibody in the second detecting step. Herein, the cross-reactivity between 25(OH)D_2 and anti-25(OH)D antibody is an inherent requirement of the assay to ensure the result reflects the total 25(OH)D. Cavalier *et al.* (2011a) showed that the cross-reactivity for 25(OH)D_2 of the assays based RIA was approximately 100%.

That means the kit is suitable for measuring the total 25(OH)D. However, the cross-reactivity between other metabolites and the antibody would interfere with the detection, which often causes overestimation.

From the above description, we can see the main disadvantages of DiaSorin RIA are: potential overestimation of results; cannot discriminate between $25(OH)D_3$ and $25(OH)D_2$; safety issues related to the use of ^{125}I irradiation; errors induced by individuals' manual operation. Its advantages are relatively higher accuracy and more throughputs (Carter et al., 2004; J. M. W. van den Ouweland et al., 2010). So the assay is often chosen as the reference method for other methods, and it is currently commonly used in clinical laboratories.

There is another RIA kit produced by Immunodiagnostic Systems Limited (IDS, Fountain Hills, AZ). However, the IDS RIA uses sheep derived anti-25(OH)D polyclonal antibody during the detecting procedure, which suppresses the activity of $25(OH)D_2$, thereby leading to underestimation of $25(OH)D_2$, and the total 25(OH)D as well, especially in studies where the participants are given vitamin D_2 treatment (Hollis, 2000).

(b) Automated immunoassay

Recent progress on vitamin D measurement has resulted in the development of several automated immunoassays, which combine the immunological technique and competitive chemiluminesence technology. These assays do not require the extraction procedure and can use untreated serum or plasma samples because the vitamin D binding proteins are inactivated during the incubation. Results are derived from scanning the chemiluminescent signals, which are generated by 25(OH)D binding to fluorescence-labeled anti-25(OH)D antibodies (Binkley et al., 2010; Heijboer et al., 2012). The commonly used assays among these automated methods include DiaSorin Liaison Total, Roche Elecsys, and Siemens Centaur.

DiaSorin Liaison is initially introduced a fully automated immunoassay system which is often used as a method for total 25(OH)D. So, compared to the RIA, the Liaison method is less labor-intensive and has higher throughput as well as not requiring the use of radioactive ^{125}I. As for the precision and accuracy, the conclusions differ in different studies. In the research study (Wagner et al., 2009) using DiaSorin RIA as the reference method, the Liaison showed a strong correlation and good agreement with DiaSorin RIA. The total precision was also similar to the reference assay. However, in Farrell *et al.*'s (2012) study, which compared the Liaison to liquid chromatography-tandem mass spectrometry (LC-MS/MS), Liaison showed more variable performance and less precision than the LC-MS/MS. In another study, which compared Liaison with LC-MS/MS, the results reflected more random error in Liaison despite its reasonable agreement on data with LC-MS/MS (Binkley et al., 2010). However, compared to other automated immunoassays, Liaison showed better agreement with LC-MS/MS results (Farrell et al., 2012). So Liaison could be a good choice for those being limited by research funding without access to LC-MS/MS.

Roche Elecsys is a direct electrochemiluminescence immunoassay specific for $25(OH)D_3$, because the cross-reactivity of $25(OH)D_2$ with this Roche assay is very low (Cavalier et al., 2011b). Therefore the assay is prone to underestimate serum vitamin D in participants who use vitamin D_2 supplements and is not suitable for interventions using vitamin D_2 supplementation. However, its accuracy has been thought to be poorer than Liaison. In the study reported by Wagner *et al.* (Wagner et al., 2009), the data coming from the Roche assay tended to overestimate lower 25(OH)D concentrations and underestimate higher 25(OH)D concentrations compared to Liaison. Another study also found that Roche assay apparently overestimated $25(OH)D_3$ levels compared to the LC-MS/MS (J. M. W. van den Ouweland et al., 2010). It was suggested that matrix effects and cross-reactivity between other vitamin D metabolites and polyclonal sheep antibody against 25(OH)D employed by the Roche assay interfered with the measuring procedures and led to inaccuracy (J. M. W. van den Ouweland et al., 2010). So, standardizing the assay and controlling matrix effects are important to increase its accuracy.

Siemens Centaur is another automated immunoassay that was developed recently and the literature about the method is limited. A study by Heijboer *et al.* (2012) compared the Siemens Centaur and other automated assays with LC-MS/MS. The Siemens Centaur showed a concentration-dependent variance. Also in a study undertaken by Farrell *et al.* (2012), the results from the assay displayed excessive bias compared to Liaison. So, the Siemens Centaur could be improved in precision and accuracy.

In addition, there is automated competitive chemiluminescence instrumentation called Nichols Advantage-automated Protein Binding Assay (NA-CLPBA; San Clemente, CA). In this assay, the employed competitive binder is human vitamin D binding protein (DBP) rather than anti-vitamin D antibody, so it is not an immunoassay but a competitive protein binding assay (CPBA) (Hollis, 2008). However, it has been found that the assay failed to detect the increase in vitamin D concentration in people who had been supplemented with vitamin D_2 (Terry et al., 2005; Lensmeyer et al., 2006). Furthermore, studies showed that this method always markedly overestimated vitamin D concentration in the people who did not use D_2 supplement (Lensmeyer et al., 2006; Carter et al., 2007). In a comparative study for DiaSorin RIA, IDS RIA, IDS EIA, Nichols and HPLC, the accuracy of the Nichols assay was least and its bias was up to more than 30% (Carter et al., 2004). So, the validity of the CPBA has been questioned and should be improved.

(c) Enzyme immunoassay (EIA)

Comparatively, the method of EIA has been less used. The principle of EIA was that labeled, specific enzymes catalyze the reactivity of vitamin D and its antibody, and then the signal of the tracer that reflects the detected vitamin D concentration can be measured. The commonly used EIA commercial kit is introduced by IDS (Fountain Hills, AZ, US). However, its tendency for overestimation for 25(OH)D is one of its disadvantages and the situation would be more obvious in automated EIA than manual EIA (Cavalier et al., 2011a). In Carter *et al.*'s comparative study (2004), the accuracy of the results by IDS EIA was the sixth place and is only a little better than Nichols.

To sum up, more or less inaccuracy in results and inability to discriminate between $25(OH)D_3$ and $25(OH)D_2$ are the main limitations of the immunoassays. The standardization of the calibrations of these assays and manipulation rules needed to be identified so as to improve their accuracy and precision. Comparatively, DiaSorin Liaison and DiaSorin RIA are more reliable than other immunoassays. Besides, the automated instrumentation is more operator-dependent although they are less time-consuming and have a bigger throughput than manual methods. In addition, a study found that the vitamin D binding protein (DBP) concentration could affect the measurement performed by most immunoassays (Heijboer et al., 2012). How big the influences are and how the influences should be avoided or suppressed needs to be further investigated.

2.4.2.2 Chromatographic methods

The chromatography method is a modern technology that precisely separates mixtures of chemical substances. The basic principle of the technology is that passing the stationary phase of chromatographic apparatus, compounds in the sample that have dissolved in the mobile phase can interact with stationary phase and undergo absorption, attraction, affinity, resistance and so on with different strength due to different molecular structures, thereby having different retention time and then flowing out from the stationary phase successively in different periods (Sadek, 2000). Furthermore, chromatography can be connected with a pump that can produce higher pressure to push the liquid mobile phase moving more quickly and separate the compounds more effectively. This is high performance liquid chromatography (HPLC). Then, separated compounds can be further analyzed qualitatively and quantitatively by coupled analysis systems, such as ultra-violet detection (UV) and spectrometry.

(a) High performance liquid chromatography (HPLC)

HPLC has been considered a reliable quantitative method for $25(OH)D_3$ as well as $25(OH)D_2$, and quite a few studies employed it as a reference method against other

vitamin D assays (Neyestani et al., 2007; Binkley et al., 2010). On the technical level, HPLC can get a very high level of accuracy and precision. However, there has been significant variability among the different laboratories with the methods for vitamin D assessment (Lensmeyer et al., 2006). The main limitation of this method is the absence of common standards for technological reference and appropriate calibration material (Binkley et al., 2010). Other defects include (Lai et al., 2010); the equipment is expensive; the operation and maintenance are highly expertise-dependent; large sample volumes are needed; less sample throughput compared to other assays. On that condition, the HPLC-UV is considered unsuitable for extensive use.

(b) Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

LC-MS/MS method is a further developed technology for separating and analyzing compounds with higher selection and sensitivity than HPLC, so it is more suitable to analyze 25(OH)D_3, 25(OH)D_2, and other vitamin D metabolites simultaneously and has been the gold standard for measurements of vitamin D and its metabolites (Johannes M. W. van den Ouweland et al., 2010). However, the LC-MS/MS has similar limitations to HPLC due to similar operating procedures between them, despite more expensive costs and higher expertise-dependence. A further complication is ion suppression during the testing procedures, which could interfere with the technology of mass spectrometry (Jessome & Volmer, 2006). Using deuterated 25(OH)D_3 and 25(OH)D_2 as internal standards can minimize the interference, while the difficulty in separating these 25(OH)-epi-D is another concern that would lead to overestimation, for the compounds have a very similar molecular mass and chemical configuration. Aiming at the issue, some studies explored newer reference measurement procedures (RMPs), in which sufficient chromatographic resolution was suggested so as to improve its analytical performance (Tai et al., 2010; Stepman et al., 2011).

In summary, the LC-MS/MS is an accurate and sensitive method and it has been regarded as the gold standard for vitamin D assessment, although modest

inter-laboratory variability has existed. The common standards of measurement procedures and calibrations will improve accuracy and inter-laboratory agreement. Meanwhile, its essential high expenses, requirement for specific technical expertise and relatively lower sample throughput are the main barriers to wider clinical use.

2.5 Summary

The present review comprehensively presented the physiology of vitamin D, its potential functions, overview of MetS and the associations between 25(OH)D and the components of MetS, which mainly include obesity, increased insulin resistance, hypertension and dyslipidemia. Although the mechanisms of the effects of vitamin D on extra-skeletal system are still being elucidated, and the causality between vitamin D deficiency and obesity is still uncertain, studies with observational designs have provided much evidence for the relationships between vitamin D deficiency and the ingredients of MetS. However, the number of intervention studies is far from sufficient and the majority of RCTs report conflicting results or were inconclusive. The reasons are multiple, and could be caused by different study groups, small sample sizes, insufficient doses, undesirable effects of vitamin D supplementation, short study duration and poor control for potential confounders. So, more intervention studies with better designs and implementation are needed in the future. Even so, vitamin D supplementation has been thought to be a helpful and hopeful treatment means for MetS and cardiovascular diseases. In addition, different vitamin D measurement assays were reviewed. The DiaSorin RIA and LC-MS/MS are the two most frequently-used methods. The former is cheaper and the latter is more accurate and precise.

Chapter 3. Materials and methods

3.1 Study design

Based on the aims of the study, which were to see the vitamin D status of the certain group and explore the relationship between the vitamin D status and MetS, a cross-sectional design was adopted. Some quantitative and qualitative data were collected so as to test the hypotheses of the study from multiple perspectives. On the one hand, in some previous studies (Hill et al., 2008; Janssen et al., 2013; Kimlin et al., 2014; Rockell et al., 2005), drinking, smoking and outdoor exercise were the most common influencing factors of vitamin D status in daily living behaviors. On the other hand, waist circumference, body mass index, blood lipid series, blood glucose and blood pressure are the key indicators of the MetS. So, the study collected data of the above-mentioned indicators to solve the study aims.

3.1.1 Collection of qualitative data

The study collected qualitative data including alcohol drinking status, smoking status, daily physical exercise and past medical history using a specially designed questionnaire (Appendix 1). Specifically, drinking status and smoking status were classified into “none”, “light”, “medium” and “heavy” classifications to describe the degree of smoking and drinking. Based on the pilot survey, it was ruled that “light” was using a pack of cigarettes (20 cigarettes) for more than a week or drinking sporadically, “medium” was using one pack of cigarettes for more than one day and less than a week or often drinking, “heavy” was using one pack of cigarettes or more per day and daily drinking. “Light exercise” was no special time for physical exercise. “Medium exercise” was doing exercise up to an intensity that would produce sweat for less than one hour per day and “heavy exercise” was doing physical exercise up to an intensity that would produce sweat for more than one hour per day. Past medical history, treatments and current situation regarding chronic diseases, like hypertension, diabetic mellitus and dyslipidemia were noted.

3.1.2 Collection of quantitative data

Blood pressure, height, weight, waist circumference and hip circumference were collected. A specially assigned nurse practiced in using the apparatus made the anthropometry measurements of the participants.

Measurement of height and weight: In order to measure height and weight as accurately as possible, the participants were told to have defecated before the test was done. Also, they were asked to only wear a single layer of clothing and take off shoes and caps for height and weight measurement. The participants stood on the platform of the measuring apparatus in an upright posture with both feet touching. The top of heads of the individuals was scanned by infrared scanner which connects with the measuring apparatus. The height and weight values were shown on the display screen after 5 seconds. The body mass indexes (BMI) were calculated using Adolphe Quetelet's formula: BMI= weight (kg)/height $(m)^2$.

Measurement of waist circumference (WC): The measuring tape was made of leather, which provided soft tactile sensation for the participants and was not stretched. The participants were asked to stand upright, with both feet touching and arms hanging freely. The tape was placed at the midpoint between the lowest rib and iliac crest, and measurement made with sufficient tension after individuals purposefully exhaled (Klein et al., 2007). The WC measurements were taken three times and recorded to the nearest 0.1cm. The mean values were used for statistical analysis.

Measurement of hip circumference (HC): The participants were also asked to stand upright with feet touching. The measurement was made at the widest point between the iliac crest and buttock (Bengtsson et al., 1993). The values were recorded to the nearest 0.1cm and the mean values were adopted from three measurements.

Measurement of blood pressure (BP): The BP of each participant was measured by a trained operator using an automatic sphygmomanometer. The participants were in

seated position and their measured arms were easily put on the table which was about level with their hearts when they sat. Before the measurement, the participants were asked not to perform any strenuous exercise for at least half an hour, and to rest for ten minutes prior to measurement. The study uniformly measured the left arm for all the participants so as to control bias (Pickering et al., 2005).

3.1.3 Blood biochemical markers

Routine biochemical markers: Markers included serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and fasting glucose. The participants were asked to have fasted for twelve hours before having venous blood samples taken from median cubital vein. All blood sampling for those routine biochemical markers was handled by two nurses who had professional training and national nurse certificates. The biochemical markers were tested by the automatic biochemistry analyzer (Hitachi, 7600-020ISE) at the Health 100 Check-up Center in Taiyuan City.

Serum 25-hydroxy vitamin D: Blood samples were collected on specialized filter paper. The dried blood spots (DBS) method was previously verified to be a useful blood repository, by which the vitamin D measurement had sufficient precision and accuracy (Eyles et al., 2009; Eyles et al., 2010; Higashi et al., 2011). The blood collection was taken from a finger and the procedure strictly complied with manufacturer's instructions of the kit. The filter paper was provided by the NHS vitamin D laboratory in Sandwell and West Birmingham Hospital in UK, currently registered with Vitamin D External Quality Assessment Scheme (DEQAS). This laboratory was also responsible for the vitamin D measurement. The testing method was tandem mass spectrometry (LC-MS/MS), using Waters XEVO TQ-MS after performing a chemical derivatization (4-phenyl-1,2,4-triazoline-3,5-dione, PTAD) on the extracts of 3 mm punches taken from the dried blood spots.

Diagnosis of MetS: The diagnosis of MetS in the current study was according to the diagnosis criteria of the International Diabetes Federation (IDF) (Alberti et al., 2005), which were the most commonly accepted for Chinese participants. The criteria were;

central obesity specific for Chinese people (waist circumference; greater than or equal to 80 cm in females and 90 cm in males). Plus, any two items of the following; raised triglycerides (>1.7mmol/L) or specific treatment for this lipid abnormality; reduced HDL-cholesterol (<1.03mmol/L in men and <1.29 mmol/L in women) or specific treatment for this lipid abnormality; raised blood pressure (systolic ≥ 130 mmHg or diastolic ≥85 mmHg) or treatment for previously diagnosed hypertension; raised fasting plasma glucose (≥5.6 mmol/L) or previously diagnosed type 2 diabetes.

Diagnosis of obesity and central obesity: For Asian, overweight is defined as BMI of 23.0 to 27.49 kg/m^2 and obesity is defined as BMI ≥ 27.5 kg/m^2 (Barba et al., 2004). Abdominal obesity was also defined as waist circumference ≥ 85 cm in men and ≥ 80 cm in women in the current study, based on the suggestion made by the China Obesity Task Force in 2002 (Zhou & Coorperative Meta-Analysis Group Of China Obesity Task, 2002) and ATP □ (Alberti et al., 2009).

Vitamin D sufficiency criteria: Serum vitamin D concentrations were described using the definitions provided by the NHS vitamin D laboratory, which were also commonly accepted in the area. Sufficient vitamin D status was considered to be greater than 50nmol/L; mild vitamin D insufficiency was between 25-50nmol/L; moderate vitamin D insufficiency was between 12.5-25nmol/L; and severe vitamin D deficiency was lower than 12.5nmol/L (Munns et al., 2006).

3.2 Study sample

Two hundred adult volunteers aged 20 to 80 years old, living in Taiyuan city, China were recruited. The inclusion criteria were as follows; non-manual workers; volunteered for the study; and answered the questionnaire survey. Participants were excluded if they were diagnosed as having chronic liver disease, kidney diseases, severe cardio-cerebral vascular diseases and cancer, and participants who were pregnant.

3.3 Recruitment of the participants

The participants were individuals who went to Health 100 Check-up Center and took normal health checks during December 2013 and January 2014, when it was the winter season in Taiyuan City. Health 100 Check-up Center is a private, eligible medical institution with national practice license in China. The center specifically provides physical check-up services for public.

Two staff members in the Health 100 Check-up Center introduced the study, with Information Sheet (Appendix 2), to people who went to the centre reception to make appointments. Being informed the study, people who wanted to participate in the study were then interviewed based on the contents of the Interview Questions (Appendix 3). If the interviewer was unwilling to answer any question due to privacy or had been diagnosed some chronic disease, he or she was needless to continue. The preliminarily screen like that for participants could reduce the exclusion rate after data collection. The individuals who were thought to continue to take the study would receive the questionnaire (Appendix 1) and asked to fill it out so as to collect qualitative data. These data included smoking, drinking and daily exercise. Other data collection was conducted on the day when they came to the Check Center again for physical examination. The sample collection did not finish until the final number of participants reached 200. The sample size was statistically significant according to the sample size calculation (Appendix 4). Because some participants coming to the Health Check Center would be staff of a corporation, which ordered the routine physical check-up, around 10 to 15 volunteers were recruited per day so as to avoid too concentrated sources of participants and control for selection bias better.

3.4 Ethics

The project (application 12/46) was approved by Massey University Human Ethics Committee (MUHEC), 2012. All the participants were informed of the study details. They were also told how to manage the data and how to use them in the study. As stated in the Information Sheet (Appendix 2), all the data would be stored in a locked

cabinet and only the research teams could access them. After finishing the study, all data on paper would be stored in a secure archive for ten years before being destroyed. Electronic data also would be kept on the researcher's personal computer, with safe password, for ten years before being deleted. After the prescreening interviews, all the participants signed the Consent Forms (Appendix 5). Following participation in the study, their vitamin D results were sent to them, together with a personalized suggestion on how to get sufficient vitamin D.

3.5 Statistical analysis

All data were analyzed using SPSS version 21 (IBM Corporation, USA). The variables were tested for normality by the Kolmogorov-Smirnov Test. The data were expressed as mean ± SD or median (inter-quartile range). The t-test was performed to compare two groups of the data with normal distribution. Manny-Whitney U test was performed to compare two or more groups of the data with non-normal distribution. Multiple linear regression analysis was used for evaluating the effects of the variables on the vitamin D status. Multivariate logistic regression analysis was used for evaluating the effects of vitamin D and other factors on MetS. A p value of less than 0.05 was considered to be statistically significant.

Chapter 4. Results

A total of 200 participants who went to Health 100 Check-up Center for routine physical examination were recruited for this study according to the inclusion criteria. All 200 people provided fingertip blood samples for vitamin D measurement. However, 11 participants did not have waist and hip circumference measurements and venous blood collection taken. Another 25 participants ordered a different packaged service, which was cheaper and only provided TC and TG assessments in lipid profiles. So, there were a total of 167 participants who could be diagnosed whether they had MetS or not. Of these participants, 50 were diagnosed MetS.

4.1 General characteristics

The general characteristics of the quantitative variables and their mean or median value, comparisons by gender are presented in Table 4.1.

Table 4.1 Descriptive and biochemical characteristics of the participants

	n	Male	n	Female	*p*-value
Age (y) (median, IQR)	73	49.0 (31.0, 60.5)	127	48.0 (35.0, 55.0)	0.51
WC (cm) (median, IQR)	69	89.75 (82.84, 96.83)	120	77.13 (73.63, 82.84)	<0.01
SBP (mmHg) (median, IQR)	69	132.0 (118.0, 142.5)	120	118.5 (110.0, 131.0)	<0.01
DBP (mmHg) (median, IQR)	69	82.0 (75.0, 92.0)	120	73.0 (67.0, 79.8)	<0.01
Glu (mmol/L) (median, IQR)	69	6.01 (5.78, 6.24)	120	5.67 (5.31, 6.07)	<0.01
TG (mmol/L) (median, IQR)	69	1.78 (1.15, 2.55)	120	1.15 (0.77, 1.76)	<0.01
BMI (kg/m^2) (mean±SD)	69	25.9±2.8	120	23.6±3.1	<0.01
WHR (mean±SD)	69	0.91±0.65	120	0.82±0.62	<0.01
TC (mmol/L) (mean±SD)	69	5.08±0.87	120	4.95±0.10	0.36
LDL (mmol/L) (mean±SD)	58	3.29±0.76	106	3.12±0.81	0.17
HDL (mmol/L) (median, IQR)	58	1.23 (1.11, 1.33)	106	1.46 (1.29, 1.65)	<0.01

IQR: inter-quartile range; SD: standard deviation; WC: waist circumference; HC: hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Glu: fasting glucose; TG: triglyceride; BMI: body mass index; WHR: waist circumference/hip circumference; TC: total cholesterol; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol.

Of the 200 participants, there were 73 males (36.5%) and 127 females (63.5%). The ages of the male group and the female group were not statistically different. Excluding 11 participants who did not provide venous blood analysis and body measurement, 189 participants (69 men (36.51%) and 120 women (63.49%)) were analyzed for BMI, WC, HC, WHR, BP, fasting glucose and lipid profiles. There were no significant differences in age, TC and LDL by genders. However, there were significantly different BMI, WC, HC, WHR, BP, fasting glucose, TG and HDL between the gender groups, with females' status being better compared with males.

4.2 Vitamin D status

There were a total of 200 participants measured for 25(OH)D concentrations.

Table 4.2 Mean values of 25(OH)D in males and females

	Male (73)	Female(127)	
	Median (IQR)	Median (IQR)	*p*-value
T25(OH)D(nmol/L)	44.0 (32.3, 55.4)	32.7 (25.8, 43.8)	<0.01
25(OH)D_3 (nmol/L)	37.9 (28.2, 48.9)	27.2 (21.8, 37.7)	<0.01
25(OH)D_2 (nmol/L)	3.6 (2.8, 5.00)	3.3 (2.8, 5.7)	0.89

IQR: inter-quartile; 25(OH)D: 25-hydroxyvitamin D; 25(OH)D_3: 25-hydroxyvitamin D_3; 25(OH)D_2: 25-hydroxyvitamin D_2.

In Table 4.2, the median values of total 25(OH)D in males and females were presented. They were 44.0 and 32.7 nmol/L, respectively. There were significantly higher total 25(OH)D and 25(OH)D_3 concentrations in males than those in females (p<0.01; p<0.01). However, the difference of 25OH)D_2 status in males and females was not statistically significant (p=0.89). Overall, males' vitamin D status was better than that of females in the present study.

The numbers and the constituent ratios of different 25(OH)D levels by gender are presented in Table 4.3.

Table 4.3 Numbers and constituent ratios of 25(OH)D in males and females

25(OH)D (nmol/L)	< 12.5	12.5-25	25-50	≥50
number in males (% in total)	2 (2.74%)	6 (8.22%)	40 (54.79%)	25 (34.25%)
number in females (% in total)	0 (0%)	29 (22.83%)	79 (62.20%)	19 (14.96%)
number in total (% in total)	2 (1%)	35 (17.5%)	119 (59.5%)	44 (22%)
Total			156 (78%)	44 (22%)

25(OH)D: 25-hydroxyvitamin D.

On the whole, 78% of the participants were vitamin D insufficient and only 22% were vitamin D sufficient. Over half (59.5%) of the participants had 25(OH)D values in the range 25-50nmol/L. Males (34.25%) were more likely to have sufficient vitamin D than females (14.96%).

Table 4.4 shows the comparisons of total 25(OH)D by age groups between genders. The vitamin D levels in different age groups in males were not statistically different. By contrast, the median values of 25(OH)D showed an increasing tread with increase age in females. Also, the vitamin D status in the group of younger than 40 is significantly lower than that of the group older than 65 years in females.

Table 4.4 Comparisons of total 25(OH)D (nmol/L) by age rang in males and females

	Male (73)		Female (127)		
Age	n	Median (IQR)	n	Median (IQR)	*p*-value
<40	27	43.7[a] (35.2, 57.1)	46	29.3[a] (24.1, 40.9)	<0.01
40--65	34	44.1[a] (29.2, 57.9)	73	33.7[ab] (27.4, 43.9)	0.04
≥65	12	45.4[a] (28.2, 52.6)	8	46.8[b] (25.8, 61.2)	0.59

Different superscripts (a, b) represent a significant difference between groups in the same column at $p<0.05$; IQR: inter-quartile range; 25(OH)D: 25-hydroxyvitamin D.

4.3 Multiple linear regression analyses of 25(OH)D concentration

A large multivariate model (Campolongo et al., 2007) was used to screen out the factors with more influence on the 25(OH)D status. Analyzed variables included age, gender, BMI, WC, HC, SBP, DBP, fasting glucose, TC, HDL, LDL, TG, and three lifestyle factors. The results of the large model analysis were presented in Table 4.5. Variable selection was conducted by in-and-out stepwise method. After that, multiple linear regression analysis was conducted for the chosen factors. In Table 4.6, the results of multiple linear regression analysis were presented, in which gender, fasting glucose and smoking were the independent factors for 25(OH)D.

Table 4.5 The large multivariate model analysis for 25(OH)D concentration

	B	SE	*p*-value	F-value
Intercept	37.98	26.20	0.15	2.10
Age	0.10	0.11	0.34	0.90
gender 1=male 2=female	-12.39	3.83	<0.01	10.44
BMI	-0.30	0.63	0.63	0.23
WC	0.43	0.25	0.09	2.93
HC	0.13	0.25	0.61	0.26
SBP	-0.02	0.10	0.83	0.05
DBP	-0.12	0.15	0.43	0.61
Fasting Glu	-2.06	0.10	0.04	4.27
TC	-2.51	2.31	0.28	1.18
HDL	8.78	5.95	0.14	2.18
LDL	2.98	2.32	0.20	1.66
TG	-0.58	1.88	0.76	0.09
Smoking:1=none 2=light 3=medium 4=heavy	-4.38	2.09	0.04	4.40
Drinking:1=none 2=light 3=medium 4=heavy	1.26	2.11	0.55	0.36
Exercise: 1=none 2=light 3=medium 4=heavy	-1.90	2.75	0.50	0.48

25(OH)D: 25-hydroxyvitamin D; MetS: metabolic syndrome; BMI: body mass index; WC: waist circumference; HC: hip circumference; TC: total cholesterol; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; TG: triglyceride;

Table 4.6 Multiple linear regression analysis for 25(OH)D concentration

	B	SE	*p*-value	F-value
Intercept	58.23	16.19	<0.01	12.94
gender 1=male 2=female	-12.12	3.30	<0.01	13.48
WC	0.24	0.14	0.09	2.91
Glu	-2.32	0.91	0.01	6.48
Smoking: 1=none 2=light 3=medium 4=heavy	-4.04	1.88	0.03	4.60

Using step regression method to filter variables and the variable including point was 0.1 and excluding point was 0.05.

WC: waist circumference; Glu: fasting glucose.

4.4 The relationships between vitamin D and the components of metabolic syndrome

4.4.1 Comparison of 25(OH)D concentrations by body mass index

The participants were divided into three groups by BMI; normal (BMI<23), overweight (23≤BMI<27.5), and obese (BMI≥27.5). As shown in Table 4.7, the three groups did not have significant differences in vitamin D status by gender.

Table 4.7 Comparisons of 25(OH)D (nmol/L) by BMI in males and females (n)

BMI	<23		23-27.5		≥27.5		
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	*p*-value
Male	11	42.0 (32.2, 49.9)	39	44.1 (28.3, 53.7)	19	40.7 (32.5, 62.6)	0.92
Female	61	32.6 (24.8, 41.2)	45	35.0 (28.8, 44.0)	14	31.2 (26.6, 55.8)	0.45
Total	72	32.9 (26.8, 43.4)	84	36.5 (28.7, 49.8)	33	40.0 (29.4, 58.2)	0.07

IQR: inter-quartile range; 25(OH)D: 25-hydroxyvitamin D; BMI: body mass index.

4.4.2 Comparison of serum 25(OH)D concentrations by waist circumference

All the participants were divided into two groups; non-abdominal obesity and abdominal obesity, based on their WC values for both genders. In Table 4.8, the 25(OH)D status of the groups with and without abdominal obesity were not significantly different in either males or females (p=0.21; p=0.49). However, the vitamin D status in all the participants with abdominal obesity was significantly higher than those without abdominal obesity (p=0.02).

Table 4.8 Comparison of 25(OH)D (nmol/L) status by WC in males and females

	Non-abdominal obesity		Abdominal obesity		
	n	Median (IQR)	n	Median (IQR)	*p*-value
Males	21	42.00 (29.75, 47.45)	48	46.35 (32.68, 59.03)	0.21
Females	81	32.70 (24.80, 42.70)	39	34.00 (27.50, 45.20)	0.49
Total	102	33.55 (27.08, 44.08)	87	38.00 (29.60, 52.80)	0.02

IQR: inter-quartile range; 25(OH)D: 25-hydroxyvitamin D; WC: waist circumference.

4.4.3 Comparison of 25(OH)D concentrations in people with or without metabolic syndrome.

As shown in Table 4.9, the 25(OH)D status of the participants with MetS was significantly higher than that of the group without MetS (p=0.05). For both men and women individually, the 25(OH)D concentrations of the participants with MetS did not have significant differences compared those without MetS.

Table 4.9 Comparison of 25(OH)D (nmol/L) status by MetS in males and females

	MetS		Non-MetS		
	n	Median (IQR)	n	Median (IQR)	*p*-value
Males	28	47.45 (32.68, 61.58)	32	43.95 (33.1, 53.03)	0.40
Females	22	34.05 (27.50, 42.73)	85	31.80 (24.80, 42.70)	0.41
Total	50	37.15 (28.70, 53.88)	117	34.30 (27.2, 44.50)	0.05

25(OH)D: 25-hydroxyvitamin D; MetS: metabolic syndrome; Min.: minimum; Max: maximum.

4.4.4 Prevalence of MetS, obesity and central obesity in different 25(OH)D levels

The prevalence of MetS, overweight+obesity, and abdominal obesity was presented in Table 4.10. There were 167 participants who provided enough data to diagnose whether they had MetS or not. Among them, 50 subjects were diagnosed MetS, meaning the prevalence was 29.94%. Meanwhile, there were 189 participants who could be diagnosed whether they had overweight+obesity or abdominal obesity or not, based the BMI and WC values. The prevalence of overweight+obesity and abdominal obesity was 61.90% and 46.03%, respectively. No significant difference

in the prevalence of MetS and abdominal obesity were found between the groups with vitamin D sufficiency and insufficiency (p=0.08, p=0.07). However, the prevalence of overweight+obesity in the group of vitamin D sufficient adults was significantly higher than that in the group of vitamin D insufficient (p=0.02).

Table 4.10 Prevalence of MetS, obesity and central obesity in different vitamin D levels

	Vitamin D sufficient	Vitamin D insufficient	*p*-value	Total incidence
MetS (n)	42.42% (14/33)	26.87% (36/134)	0.08	29.94% (50/167)
Overweight+obesity (n)	78.05% (32/41)	57.43% (85/148)	0.02	61.90% (117/189)
Abdominal obesity (n)	58.54% (24/41)	42.57% (63/148)	0.07	46.03% (87/189)

MetS：metabolic syndrome.

4.4.5 Comparison of various parameters in different vitamin D levels

Based on the total 25(OH)D concentrations, all participants were divided into vitamin D sufficient group and vitamin D insufficient group. The comparisons of all indicators in different vitamin D levels were presented in Table 4.11. Compared with the vitamin D sufficient group, BMI, WHR, LDL, WC, Glu were significantly lower in the vitamin D insufficient group (p<0.05). There were no significant differences between the two groups in age, blood pressure, TC, TG and HDL.

Table 4.11 Comparison of variables in different 25(OH)D levels

	n	Vitamin D sufficient	n	Vitamin D insufficient	*p*-value
Age (y) (median, IQR)	44	50.5 (32.0, 60.5)	156	45.0 (35.0, 55.0)	0.19
WC (cm) (median, IQR)	41	86.10 (77.78, 94.58)	148	79.20 (74.73, 87.55)	0.02
SBP (mmHg) (median, IQR)	41	125.0 (112.5, 140.0)	148	123.0 (111.0, 137.0)	0.71
DBP (mmHg) (median, IQR)	41	78.0 (71.5, 84.5)	148	75.0(69.0, 84.0)	0.38
Glu (mmol/L) (median, IQR)	41	5.94 (5.69, 6.23)	148	5.78 (5.44, 6.11)	0.03
BMI(kg/m^2) (mean±SD)	41	25.52±2.94	148	24.15±3.22	0.02
WHR (mean±SD)	41	0.88±0.07	148	0.85±0.07	0.01
TC(mmol/L) (mean±SD)	41	5.05±0.99	148	4.98±0.95	0.71
TG (mmol/L) (median, IQR)	41	1.30 (0.88, 2.17)	148	1.30 (0.84, 2.12)	0.87
HDL (mmol/L)(median, IQR)	33	1.31 (1.15, 1.49)	131	1.36 (1.23, 1.57)	0.11
LDL(mmol/L) (median, IQR)	33	3.44±0.83	131	3.11±0.77	0.03

IQR: inter-quartile range; SD: standard deviation; 25(OH)D: 25-hydroxyvitamin D; Min.: minimum; Max: maximum; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Glu: fasting blood glucose; BMI: body mass index; WHR: waist circumference/hip circumference; TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol.

4.4.6 Multiple logistic regression analyses of metabolic syndrome

A large multivariate model was also used for screening factors and then multiple logistic regression analysis was performed for multiple correlation analysis. The results of the large model analysis for all variables are presented in Table 4.12. Gender, WC, HC and TG were filtered out by in-and-out stepwise method. Then they were analyzed by multiple logistic regressions for MetS (Table 4.13). Table 4.13 shows that gender, WC, HC, and TG are the independent risk factors of MetS with statistical significance.

Table 4.12 The large multivariate model analysis for MetS

	β	SE	*p*-value	OR
Intercept	-113.50	107.20	0.29	.
Age	-0.04	0.04	0.31	0.96
Gender (Male=1, Female=2)	6.48	1.85	<0.01	650.56
25(OH)D	-1.83	1.06	0.08	0.16
BMI 1(<23), 2(27.5>BMI≥23), 3(≥27.5)	-1.03	0.90	0.30	0.36
Abdominal obesity (no=1; yes=2)	2.31	1.59	0.15	10.06
WC	-0.15	1.21	0.90	0.86
HC	0.61	1.07	0.57	1.84
WHR	56.48	120.90	0.64	>999
SBP	0.03	0.04	0.52	1.03
DBP	0.00	0.06	0.95	1.00
TC	0.37	0.80	0.65	1.44
HDL	-1.89	2.89	0.51	0.15
LDL	-0.77	0.78	0.33	0.47
TG	2.48	0.75	<0.01	11.97
Glu	-0.09	0.32	0.78	0.91
Smoking (none=1, light=2, medium=3, heavy=4)	0.25	0.53	0.64	1.28
Drinking (none=1, light=2, medium=3, heavy=4)	0.50	0.61	0.42	1.64
Exercise (light=2, medium=3, heavy=4)	0.94	0.92	0.31	2.57

MetS: metabolic syndrome; r: correlation coefficient; SE: standard error; OR: odds ratio; 25(OH)D: 25-hydroxyvitamin D; BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist circumference/hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; TG: triglyceride; Glu: fasting glucose.

Table 4.13 Multiple logistic regression analysis for MetS

					95%CI	
	β	SE	*p*-value	OR	Lower	Upper
Intercept	-53.91	10.55	<0.01	.	.	.
gender (1=male 2=female)	3.96	1.03	<0.01	52.45	6.94	396.41
WC	0.37	0.08	<0.01	1.45	1.24	1.69
HC	0.11	0.06	0.04	1.12	1.01	1.25
TG	1.97	0.45	<0.01	7.14	2.93	17.39

Using step regression method to filter variables and the variable including point was 0.1 and excluding point was 0.05.

MetS: metabolic syndrome; β: partial regression coefficient; SE: standard error; OR: odds ratio; CI: confidence interval; WC: waist circumference; HC: hip circumference; TG: triglyceride.

Chapter 5. Discussion

5.1 Introduction

The purpose of the present study was to learn the vitamin D status in residents in Taiyuan City in China, and to explore the relationships between vitamin D levels and MetS-related parameters. The rationale was based on some studies that presented a high prevalence of vitamin D deficiency in different Chinese groups, and the research, which indicated a certain relationship between vitamin D levels and the biomarkers related with MetS worldwide. The present study recruited 200 non-manual urban dwellers with Chinese food culture and same seasonal period. In the current study, participants' fingertip blood sample was analyzed for 25(OH)D measurement. Physiological and biochemical indices of cardiovascular health were collected during visits to the Health 100 Check-up Center. In addition, their WC and HC were measured, and then BMI and WHR values were calculated. Some qualitative indicators of lifestyle, including smoking, drinking and daily exercise were investigated.

5.2 Interpretation of the results

5.2.1 The 25(OH)D status of the participants

The present study was conducted in winter (December and January) in the northern city Taiyuan in China. The results of 25(OH)D measurement showed that the prevalence of 25(OH)D insufficiency was relative high and 78% out of the 200 participants had total 25(OH)D values less than 50nmol/L. Only 22% had sufficient 25(OH)D concentrations. However, the number of the participants with severely deficient 25(OH)D levels was few. Only 2 participants had 25(OH)D levels lower than 12.5nmol/L (1%). Most of the participants (67%) had mild to moderate vitamin D insufficiency.

The results of the present study of residents of Taiyuan city (38°N) were similar to those of the previous studies being conducted in northern China, showing the high prevalence of vitamin D insufficiency in Chinese (Zhou et al., 2003; Woo et al., 2008;

Wang et al., 2009; Zhou et al., 2010). For instance, Woo *et al.*'s study (2008) reported over 90% of women aged 18 to 40 years had vitamin D status less than 50 nmol/L in Beijing (40°N); Zhou *et al.*'s study (2010) also reported 86% prevalence of vitamin D insufficiency (<50 nmol/L) in the middle-aged and old participants in Shenyang (42°N). However, comparisons with other research are difficult, since different studies adopted different cutoff values of 25(OH)D or had used different vitamin D assessment assay.

The prevalence of vitamin D insufficiency in Taiyuan City could be mainly caused by the following factors. The most important one likely is environmental factors. In the winter season, the zenith angle of the sun is increased and more ultraviolet radiation is absorbed by the earth's ozone layer, which leads to less ultraviolet radiation reaching the earth's surface, thereby affecting the synthesis of $25(OH)D_3$. Second of all, Taiyuan City is a traditional industrial city and air pollution has been high for many years. It was reported that in December and January during the period from 2008 to 2012, dust hazy weather occurred on average up to 14 days/month (Li et al., 2014). The dust haze mostly occurred during the daytime from 8 am to 1 pm, which significantly decreased the surface solar radiation intensity and duration compared with no-haze days (Li et al., 2014). Hazy weather also hindered residents from doing outdoor exercise to avoid respiratory infection, thereby reducing their time of sun exposure. Most of the office workers in the present study largely had a sedentary lifestyle, which led them to short sunshine exposure time. The interviews in the present study showed that even those people who were conscious of the importance of healthy lifestyle to their health, seemed to seldom have special time to do outdoor exercise.

In addition, the dietary intake of $25(OH)D_2$ of the participants might be limited. Taiyuan City is located inland and having vitamin D-containing foods, such as sea foods which contain vitamin D (Holick, 2008b), is not common. Also, foods being fortified with vitamin D in China are limited. Interestingly, there are thirteen participants of the current study who had very high $25(OH)D_2$ concentrations with no extra vitamin D_2 supplementation. Their $25(OH)D_2$ concentrations were higher than 10nmol/L, even up to 34.9nmol/L, much higher than the median values (3.60 nmol/L in males and 3.30 nmol/L females, respectively). Possibly, they had some foods

containing high vitamin D_2, such as mushroom. However, the phenomenon needs further investigation, possibly by dietary intake assessment, which was not conducted in the present study.

5.2.2 Women had lower vitamin D status than men

The present study showed that the status of total 25(OH)D and 25(OH)D_3 in females was lower than in males. The vitamin D status being lower in women than men was reported in some (Bolland et al., 2006; Lu et al., 2012; Borissova et al., 2013; Yoshimura et al., 2013), but not all (Yan et al., 2000; Kimlin et al., 2014) of previous studies. One of the reasons for this gender difference could be the effect of adipose tissue on vitamin D level. It well known that, the fat mass percentage is different in males and females. On average, well-nourished healthy women have a higher percentage of body fat than that of men (Frisch, 1994). Adipose tissue could be a storage depot of vitamin D, because vitamin D is fat soluble and adipose tissue easily trap the vitamin D molecules thereby leading to a decreased vitamin D concentration in the blood stream (Rosenstreich et al., 1971; Wortsman et al., 2000). Alternatively, the effect of volumetric dilution could explain the lower concentration of vitamin D in people with more adipose tissue (Drincic et al., 2012). This means in an individual with larger fat mass, the fat mass in effect has diluted vitamin D, so less vitamin D is present in circulation.

Other possible explanations could be hormonal changes and use of estrogen contraceptives. Hedlund *et al.* (2013) found that use of estrogen contraceptives could explain part of variation in 25(OH)D concentrations. Janssen *et al.* (2013) reported that estradiol level was negatively related with vitamin D level in postmenopausal women. However, Yoshimura *et al.*'s study (2013) did not find that vitamin D deficiency was associated with the menstrual status in female participants. So, recent studies regarding the relationship between vitamin D status and the estrogen or menstrual cycle are limited and inconclusive.

5.2.3 Women under 40 years had the poorest vitamin D status

The present study divided all the participants into three groups according to different age ranges; less than 40 years, between 40 and 65 years, and older than 65 years. The results showed no significant differences in the 25(OH)D serum concentrations among the three age groups in males. However, in females, there was an interesting finding, which was that the younger participants aged less than 40 years had a significantly lower 25(OH)Ds level compared with those older than 65 years. The result differs to some of the previous studies (Yan et al., 2000; Lu et al., 2012). This also goes against the accepted acknowledge, which is the ability of vitamin D photosynthesis in the elderly is markedly diminished compared with the young (Maclaughlin & Holick, 1985).

However, the modifiable determinants of serum 25(OH)D are various and extensive (Kimlin et al., 2014). In the female participants of the present study, the younger group and the older group had some common determinants of vitamin D status, such as season, living location, and similar habitual clothing cover, and skin color. However, there could have been confounding variables not investigated in the current study that differed between the younger and older women. Furthermore, the sampling method may have introduced selection bias in the present study. There were very few women older than 65 years (n=8), while the women under 40 years were more (n=46).

5.2.4 Potential factors associated with the 25(OH)D status

In the present study, gender, fasting glucose and smoking had significant independent influence on the 25(OH)D level in this population. The relationship between gender and 25(OH)D level has been widely debated. Some studies support the current study (Rockell et al., 2005; Hill et al., 2008; Janssen et al., 2013). While other studies disagree that gender was a significant determinant on vitamin D status (Palacios et al., 2012; Vierucci et al., 2013). Rockell *et al.* (2005) thought gender might be a surrogate marker for sun exposure based on their association between gender and physical activity. However, in the present study, daily exercise was also analyzed and was not associated with 25(OH)D status whereas gender was. The point of difference

might be the different study populations. Rockell and colleagues' study (Rockell et al., 2005) was on children. They reported that sex differences in children were much weaker compared with adults. For adult females, estradiol was suggested as a negative factor for vitamin D status (Janssen et al., 2013) and might be the basis of gender being one of the influencing factors for vitamin D status.

Smoking was also a factor with independent negative relationship with vitamin D status in the present study. Studies (Brot et al., 1999; Hermann et al., 2000; Supervia et al., 2006) designed especially to investigate the effects of smoking on bone metabolism, found that smoking decreased vitamin D level. Possible mechanisms suggested by authors were liver damage and depression of vitamin D-PTH system (Supervia et al., 2006; Breitling et al., 2011). However, in some previous studies (Bjork et al., 2013; Janssen et al., 2013), smoking was analyzed by multiple regressions model as one of the potential factors, and the result did not show that smoking had independent influence on the vitamin D status. More studies with large scale and better designs are needed.

Unexpectedly, in the present study, fasting glucose was also negatively associated with vitamin D status, although the relationship was weak. It is well known that IR and increased fasting glucose are the principle pathophysiology of many metabolic diseases (Fujita, 2007) and many studies were conducted on the effects of vitamin D on IR. However, few previous studies found evidence of the effects of fasting glucose or IR-related parameters on vitamin D levels. So, no reasonable mechanism has been proposed for the results for the moment. Possibly, changed liver micro-environment due to hyperglycemia and increased IR might be the basic pathological mechanism (Vanitallie & Bentley, 1955).

Interestingly, exercise and drinking, of which there was some evidence for influence on vitamin D status in previous studies (Lamberg-Allardt et al., 2001; Janssen et al., 2013), were not associated with vitamin D in the present study. For exercise, the possible explanation was that outdoor walking was the main exercise type in participants of the present study. Previously, Heuvel *et al.* (Heuvel et al., 2013) reported no association of walking with vitamin D status. Exercise types performed at higher intensity, such as gardening and cycling were reported as being associated

with vitamin D levels (Heuvel et al., 2013). None of the participants in the present reported performing gardening, cycling, and other higher intensity exercise.

Excessive alcohol consumption leads to impaired liver function, malnutrition, and disorder in bone metabolism, thereby decreasing the 25(OH)D levels (Malik et al., 2009; Naude et al., 2012). However, most participants in the current study did not usually drink any alcohol. Few participants reported heavy drinking (daily intake). Furthermore, it was suggested that moderate wine or beer can reduce risk of vitamin D deficiency, as wine or beer contains polyphenols and B vitamins (Bountziouka et al., 2012), which are antioxidants and anti-inflammatory and beneficial to health (Bognar et al., 2013). However, investigations on the relationship of wine and beer consumption with vitamin D are limited currently. So, the dual nature of alcohol on the body makes the effects of alcohol consumption on vitamin D status obscure.

5.2.5 The relationship between serum vitamin D and the components of metabolic syndrome

5.2.5.1 High prevalence of metabolic syndrome and obesity

High prevalence of MetS, overweight+obesity, and abdominal obesity was found in the present study. The prevalence of MetS was 29.94%, this is high compared with some previous investigations for the same age groups (Janus, 2003; Gu et al., 2005; Xi et al., 2013). The prevalence of overweight+obesity, and abdominal obesity were 61.90% and 46.03%, respectively, which were similar to the results of one study (Wang et al., 2014), and higher than some other studies in China (Hou et al., 2013; Jin et al., 2013). One of the reasons for the discrepancies could be different diagnostic criteria of MetS, overweight, and obesity adopted by different studies. Currently, the definitions of MetS and obesity are controversial even for same ethnicity. Some expert organizations, such as WHO, IDF, ATP and CDS suggested various diagnostic criteria. The study conducted by Xi *et al*. (2013) reported that for the same group, the prevalence of MetS using NCEP criteria and CDS criteria reached 21.5% and 10.5%, respectively. So, using different diagnostic criteria could induce a large discrepancy on the prevalence of MetS. The present study adopted the

IDF criteria, in which there were different cutoff values in WC especially for different races.

Other reasons for the discrepancy in the prevalence of MetS and obesity could be due to when the study was done, location, and study group. Liu *et al*. (2013) suggested that from 2001 to 2010, the prevalence of MetS experienced an enormous increase (from 50.4% to 58.1%) in old people in Beijing. Hou *et al*. (2013) also reported a significant increase in the prevalence of central obesity over a ten-year study period. The trend was in keeping with the changes in lifestyle following the dramatic socioeconomic development in China over the last decade or so. In addition, prevalence of MetS could be impacted by life habits, including diets, exercise and living surroundings (Buscemi et al., 2014). So there are many confounders impacting study results. So, it is foreseeable that the prevalence of MetS and obesity were higher than those from some years ago and could be different to studies over the same time period in different locations and population groups.

5.2.5.2 Comparisons of metabolic syndrome variables in the group with and without vitamin D sufficiency

Currently, there was some evidence of vitamin D deficiency being associated with MetS and obesity, although the evidence was mainly from observational studies whereas limited from interventional studies (Jorde & Grimnes, 2011; Renzaho et al., 2011; Dolinsky et al., 2013; Saneei et al., 2013). Although, the potential mechanisms of vitamin D benefit to obesity and MetS were plausible (Luong & Nguyen, 2013; Vanlint, 2013).

However, there was no significant relationship between MetS and vitamin D insufficiency in the present study. In other words, whether vitamin D was sufficient or not, did not impact on the prevalence of the MetS. Further analysis of multiple logistic regressions for MetS also verified that vitamin D insufficiency did not predict MetS after removing interference effects of other confounders. Furthermore, there was no difference in the prevalence of abdominal obesity between the groups with and without vitamin D insufficiency. Interestingly, the present study found a

higher prevalence of overweight+obesity in those who were vitamin D sufficient. These findings are opposite to previous studies (Brock et al., 2010; González-Molero et al., 2013), in which the subjects with vitamin D deficiency had higher risk of MetS than the subjects with vitamin D sufficiency. For this discrepancy, one of the reasons could be sample selection bias. The present study adopted non-random sampling (volunteer response) which could lead to an impaired representativeness of the whole population. That meant the sample could have discrepancies in various characteristics compared with the whole population (residents in Taiyuan City), which could cause difference between the results of the present study and other studies which do show a relationship between vitamin D insufficiency and MetS. Another possible explanation was the errors. The sample size of the present study was relative small for a prevalence survey. The small size could lead to bigger sampling error. Furthermore, measurement error also could not be ignored. For example, the WC measurement was the mainstay of the diagnosis for obesity and MetS. However, WC measurement was likely to be more affected by measurement error during the manipulations (Verweij et al., 2013).

Additionally, BMI, WHR, Glu, and LDL were significantly higher in the group with vitamin D sufficiency than the group with vitamin D insufficiency. This is also opposite to some of the previous studies (Rodriguez-Rodriguez et al., 2010; Alfawaz & Megeid, 2013; Garcia-Bailo et al., 2013; Saneei et al., 2013), but agreed with the study conducted by Abiaka *et al.* (Abiaka et al., 2013; Garcia-Bailo et al., 2013) in BMI and WHR. These discrepancies might be caused by the effects of other factors, which included the measured variables, such as exercising, drinking, and smoking, and also included some factors not measured and unknown, such as dietary intake, unknown medication use, education level, use of estrogen contraceptives, and genetic inheritance (Cai et al., 2012; Belfki et al., 2013; Zhao et al., 2014).

So, in the present study, there were no significant differences in the prevalence of MetS and abdominal obesity, and CVD-related variables between the vitamin D sufficient group and insufficient group. MetS contains several components and this multiple pathophysiologic conditions make the studies of MetS be too general. For example, the present study investigated a wide range of biomarkers, which involved all the components of MetS. However, only fasting glucose represented glucose

metabolic status. Actually, fasting glucose was not enough to roundly reflect the glucose metabolism and insulin sensitivity. Being investigated more biomarkers might get significant results. So, study on the relationship of individual components of MetS and vitamin D status might be more useful.

5.3 Limitations and self-reflection

The present study has several limitations. Firstly, the study is a cross-sectional design, of which the quality control during the stage of design is critical. However, the consideration for the potential confounders during the preparation phase was insufficient, which directly led to a flaw in the questionnaire. Therefore, the following information collection could have nonresponse bias or information bias. For instance, the answers for the question “do you use some medication or supplementation and if you do, what are they” in the questionnaire were general. Only a few participants answered “have” and listed them. If the question had been replaced with multiple choices, the information collection would be better. The similar situation occurred in the collection of “exercise” information, which would be collected in more detail. Later, following data analysis, this was realized and the influence of some confounders on the results was noted but nothing could be done to remedy the situation. Secondly, the sampling method of the present study was not completely random, which led to the big sampling error and low representation of Taiyuan City population. Thirdly, the cross-sectional design did not have the power to make certain the causality found in the analysis. Also, the sample size is relatively small as a cross-sectional study, which might lead to bigger sampling error. In addition, in communication with a few participants, they revealed they were not satisfied with the questionnaire survey and body measurement components of the current study, which led to some missing data.

5.4 Conclusions

Vitamin D insufficiency was highly prevalent in tested non-manual workers in Taiyuan City in China during winter season. Vitamin D status of the women was lower than that of the men. Among the females, the young women had worse vitamin D status than the older women. So, in the present study, female gender, increased fasting glucose and smoking were significant determinants for vitamin D insufficiency. Nonetheless, the vitamin D insufficiency was not associated with the risk factors for MetS in the present study. However, female gender, increased WC, and raised TG were associated with higher risk of MetS.

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Appendices

Appendix 1

Vitamin D status and relationship between vitamin D and risk factors of metabolic syndrome: A study in Taiyuan City in China

Personal Information Survey Code Number___

PARTICIPANT INFORMATION	Name_____ Gender_____ Age_____ Address_____ Day Phone_____
EXERCISE	Types of activity_____ Frequency_____ Typical duration_____ Location_____
LIFE STYLE	Occupation_____ Working Time_____ Working Load_____ Average daily sitting time_____ Average time outdoors_____ Smoking History and Daily Amount _____ Drinking History and Daily Amount _____
MEDICATION USE	Do you use some medication or supplementation and if you do, what are they?
MEDICAL HISTORY	
FAMILY MEDICAL HISTORY	

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Vitamin D status and relationship between vitamin D and risk factors of metabolic syndrome: A study in Taiyuan City in China

中国居民血清维生素 D 水平与代谢综合征关系的研究

个人信息调查　　编号_____

基本信息	姓名_____　性别_____　年龄_____ 住址_____　联系电话_____
身体锻炼情况	频率_____　持续时长_____ 运动方式_____ 地点（室内还是室外）_____
生活方式	职业_____ 工作时长_____ 每日工作量_____ 平均每日坐卧时长_____ 平均每日户外时长_____ 吸烟史_____每日吸烟量_____ 饮酒史 _____饮酒量_____
目前服用药物及保健品	如服用任何药物或保健品，请列出：
个人病史	
家族病史	

Appendix 2

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Vitamin D status and relationship between vitamin D and risk factors of metabolic syndrome: A study in Taiyuan City in China

INFORMATION SHEET

Researcher's introduction.
My name is Xiaoning Yan and I am undertaking this research as part of the requirements for a Master Degree in Human nutrition. My supervisor is Assoc. Professor Jane Coad in the Institute of Food, Nutrition and Human Health (IFNHH), Massey University, Palmerston North, New Zealand. Two qualified doctors in the Health 100 Check-up Center in Taiyuan City, under the supervision of Mrs Rengli Yue, will also be involved in the project.

Project description.
Residents in Taiyuan City could easily have vitamin D insufficiency due to short sunshine time and heavy contamination. Meanwhile, there is emerging evidence suggesting that vitamin D status may be associated with prevalence of metabolic syndrome, which usually progress to diabetes and increase the risk of cardiovascular disease. Metabolic syndrome (MetS) is diagnosed in the presence of any three of markers, central obesity, high triglycerides, low HDL cholesterol, high fasting glucose, and high blood pressure. The disease has been getting much more common in China, and more noticeably, affecting younger people. This study will investigate the vitamin D status in 200 participants attending the Health 100 Check-up Center in Taiyuan City for their usual health check, will also explore the relationship between vitamin D status and markers of MetS. The Health 100 Check-up Center will provide the data relating to MetS of eligible individuals who agree to take part in the study and also agree with the check-up center providing their data to the researchers. This data will be about body composition (height, weight and body circumferences) and biochemical data (lipid profile and fasting glucose from blood samples). In addition to the normal health checks carried out by the Health 100 Check-up Center, participants will have their vitamin D levels measured by a simple finger prick procedure and will complete a personal information survey about their lifestyle. We hypothesize that general population living in Taiyuan City will have sufficient serum vitamin D concentrations and vitamin D serum concentration is not associated with

the risk of metabolic syndrome.

Participant identification and recruitment

We would like to invite 200 participants who go to Health 100 Check-up Center in Taiyuan City in China for their routine physical examination and are non-manual workers aged 20 to 80 years to join our study.

To fit in to our study you should:

- Not have been diagnosed with cardiovascular or cerebrovascular diseases.
- Not have serious dermotosis and hepatorenal dysfunction.
- Not have cancer.
- Not be pregnant.

The included subjects should pay the expenses of their routine physical examination as normal but the cost of the vitamin D measurement will be free.

Project Procedures:

Individuals who attend the Health 100 Check-up Center will be invited to participate in the study. Those who intend to take part in the study will be asked some questions about their lifestyle and medical history so as to judge whether they meet the inclusion criteria. They also will complete a personal information survey and have a blood sample taken by a finger prick procedure for measurement of vitamin D status in addition to the routine medical check-up by Health 100 Check-up Center. Please notice you should fast 8 hours before you come to take the physical check-up.

The extra time involved (in addition to the time of the routine medical check-up) will be about 45 minutes.

Your check-up results and conclusion will be sent to you by the check-up center in two weeks after check-up except for vitamin D concentration which will be got later and sent by us. Noticeably, if you are found some serious diseases, including liver and kidney diseases and cancer, you will be eliminated from the study, but I still very appreciate you for your participation and wish you recovering quickly.

The Measurement items involved in the study

1. Measurements for body height, weight, waist and hip circumferences, and blood pressure will be conducted as normal as part of the routine medical check-up by staff at the Health 100 Check-up Center. This information will be made available to the research team.
2. Blood samples:

The normal trained staff at the Health 100 Check-up Center will take the normal blood samples as part of the routine medical check-up at Health 100 Check-up Center. The information about fasting blood glucose and lipid profile will be made available to the research team. In addition, a finger-prick blood sample will be made for vitamin D measurement.

Data Management
When you join the study, you will be given a study code number and thereafter all information about you will be filed with the code number, and stored in a locked filling cabinet accessed by research team only. When the information from all the subjects has been pooled and made anonymous, it will be written up in the dissertation. No names will be used, just the designated numbers.

All personal data will be destroyed at the end of the study once the results are analyzed. Scientific data, filed on paper, will be shredded and electronic data will be deleted from our computer records and databases after 10 years. For the first 5 years it will be stored in a secure archive where all data is stored in boxes labeled by barcode only. It is accessible by nominated staff only.

Participant's Rights
Each individual has right to refuse the invitation without any reason. If you decide to participate, you have the right to:

- Decline to answer any particular question;
- Withdraw from the study at any time;
- Ask any questions about the study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give permission to the research team;
- Be given access to a summary of the project findings when it is concluded.

Compensation for Injury
If physical injury results from your participation in this study, you should visit Health 100 Check-up Center again and find treatment. You also can make claim to local higher authority and ask reasonable compensation.

Project Contacts
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If you are interested in the study or have any questions about the project please contact Xiaoning Yan who will be happy to discuss the project and answer your questions.

Statements

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application (12/46). If you have any concerns about the conduct of this research, please contact Dr Brian Finch, Chair, Massey University Human Ethics Committee: Southern A, Telephone 06 350 5799 × 8717, Email: humanethicsoutha@massey.ac.nz

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中国居民血清维生素 D 水平及其与代谢综合征关系的研究
课题情况说明

INFORMATION SHEET

研究人员

燕小宁，新西兰梅西大学食品营养健康学院硕士研究生；
Jane Coad 女士，新西兰梅西大学食品营养健康学院副教授，硕士生导师；
另有太原市美年大健康体检中心相关工作人员协助数据收集。

研究课题简介

太原市位于中国北方，由于其冬天的日照时间短，严重的空气污染等原因， 太原居民很可能有着较高的维生素 D 缺乏可能性。越来越多的研究表明，维生素 D 水平与代谢综合征的发生可能有一定关联，而后者通常会进展为糖尿病且为心脑血管病变的危险因素之一。代谢综合征包含一系列的代谢异常，可表现为中心性肥胖，甘油三酯增高，高密度胆固醇减低，血糖增高及血压升高。以上症状出现三项即可考虑诊断本病。在我国，代谢综合征的发生越来越普遍，并且有低龄化的趋势。本研究将要对符合我们研究纳入标准并同意我们使用您的相关体检数据的 200 名到太原市美年大健康体检中心进行常规体检的客户进行相关数据采集及分析，以了解他们的维生素 D 水平及其与代谢综合征相关指标之间的关系。美年大健康体检中心将会提供给我们所有研究对象的与脂类代谢相关的体检数据，包括身高，体重，腰围，臀围等人体测量数据，及血脂系列和空腹血糖检测结果。对研究对象而言，除了进行该体检中心提供的常规检查项目，我们还要对其进行血维生素 D 检测和进行一项与生活方式有关的问卷调查。本课题假定维生素 D 水平与脂类代谢相关指标之间有一定联系。

研究对象的确定及招募

我们将邀请 200 名到美年大健康体检中心进行常规体检的客户参与我们的研究。这些研究对象要求是年龄在 20 到 80 岁之间的非体力劳动者。此外，您还要符合以下条件：

- 无严重心脑血管病患者；
- 无严重皮肤病及肝肾功能异常；
- 非癌症患者
- 非孕妇；

所有研究对象的维生素 D 检测将是免费进行，但其余的体检费用仍需自理。

数据收集过程

所有愿意参加本研究项目的体检客户将会通过一个简单面谈以判断其是否符合我们的纳入标准。正式成为我们研究对象的客户还将完成一份个人信息调查表，内容包括个人的生活方式及每日运动量等方面。生化检测方面除正常体检所需

的项目外，我们将额外针刺取少量指尖血样以进行血维生素 D 水平的检测。请注意在参加体检之前您应当禁食 8 小时以确保生化检测的准确性。整个数据收集过程约 45 分钟（不含其余体检项目所需时间）。各项体检结果及结论将在 2 周之内得出并由体检中心发送给您，维生素 D 水平的检测将会晚些时候进行，结果也将由我们给您寄出。在此需要说明的是，如果体检发现您患有肝肾疾病和癌肿我们将会终止您在本课题的参与，但我们仍然非常感谢您的参与并祝愿您早日康复！

本研究所涉及的检测项目：

1. 人体测量：身高，体重，腰围及臀围的测量；
2. 生化检测：血维生素 D 检测；
3. 此外，血压，血脂系列及空腹血糖的测量值将由美年大健康体检中心提供与研究小组。

数据管理：

每位研究对象都将拥有自己的编号，并且其所有的相关信息都将用此编号标注。所有的研究数据都将存放于研究人员掌管的安全文件柜中。当研究所需的信息收集完毕后，这些数据将应用到研究论文中，并且只用编码标注，研究对象的真实姓名不会出现在论文当中。

研究完毕后，所有的个人信息都将被销毁，而研究数据将存放于设置有条形码的密封文件盒中，只有专人可以接触，为期五年。同时这些数据将以电子文档方式存放于专人保管的电子设备中，为期十年。

研究对象的权利：

任何被邀请者有权不提供任何理由拒绝参与本研究。而如果你决定参与本项研究，你仍然有权：

- 拒绝回答你认为难以接受的问题；
- 随时可以选择退出本研究；
- 在研究进行当中的任何时候提出任何关于本项研究的疑问；
- 在明了本人姓名在论文当中不会出现的前提下，选择提供其他个人信息；
- 查看课题结束后的研究结论。

伤害赔偿：

如果由于参与此次研究而发生任何身体伤害，您可以再次到美年大健康体检中心检查伤情并予以治疗。您也可以寻求上级主管部分投诉并要求补偿。

项目联系人：

燕小宁 项目承担人 新西兰梅西大学食品营养健康学院
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联系地址：太原市长风东街建设路口 （030006）
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如果您对我们的研究课题感兴趣或有任何疑问，请随时与燕小宁联系，她将非常高兴回答您的问题并进行讨论！

声明

该项目已通过梅西大学人类研究伦理委员会审阅并同意 (Southern A, 申请编号: (12/46). 如果你对本研究还有任何疑问，请联系梅西大学人类研究伦理委员会主席，Brian Finch 博士，联系方式：Massey University Human Ethics Committee: Southern A, 电话：06 350 5799 × 8717, 电子邮箱: humanethicsoutha@massey.ac.nz

Appendix 3

Vitamin D status and relationship between vitamin D and risk factors of metabolic syndrome: A study in Taiyuan City in China

Interview Questions

1. Could you describe your daily job please?

2 Would you like to do activities out door? For example, physical exercise or enjoy the sunshine.

3. How often do you do medical check-up?

4. How do you think your health status recently?

5. Were you diagnosed some disease before? What is it? What treatment did you take? How are things going recently?

5. Would you like to take part in the study? Are you sure it?

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中国居民血清维生素D水平及其与代谢综合征关系的研究

研究对象初筛提问

1. 请问您从事什么性质的工作呢？能简单描述么？
2. 平日喜欢外出活动么，比如身体锻炼，晒太阳等？
3. 你通常多久来做一次体检呢？
4. 最近感觉身体状况怎么样啊？
5. 以前得过什么病么？当时是怎样治疗的？现在情况怎么样？
6. 你愿意参加我们这项研究么？你确定么？

Appendix 4

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Vitamin D status and relationship between vitamin D and risk factors of metabolic syndrome: A study in Taiyuan City in China

Researcher: Xiaoning Yan

Supervisor: Jane Code

Complementary explanation calculation for Sample Size:

The research project is a cross sectional study. So the calculation for sample size is based on the equation (see the attached reference):

$$n = \frac{\left(\frac{P[1-P]}{\frac{A^2}{Z^2} + \frac{P[1-P]}{N}} \right)}{R}$$

Where: n = sample size required

N=number of people in the population

P = estimated variance in population, as a decimal.

A=precision desired, expressed as a decimal.

Z=based on confidence level: 1.96 for 95%confidence, 1.6449 for 90% and 2.5758 for 99%.

R=estimated response rate, as a decimal.

In term of the project:

- N is the number of non-manual people with ages of 20-80 years old in the Xiaodian District in Taiyuan City where is the location of Health 100 Check-up Center. We do not know the exact number of the group. However, according to the Sixth National Population Census Statistics made in 2010 (http://wenku.baidu.com/view/df394d4d767f5acfa1c7cd36.html), the number of population in Xiaodian District is about 750,000 and the people with age of 15-64 years old occupy about 78% of the whole population. Also, our target population is non-manual workers, so we temporarily estimate the number of the target people is at least 80000-100,000. In fact, it is hard to estimate, while the figure is too insignificant to affect the result of n when it is put into the equator.
- P is estimated degree of variability in the population. According to some studies (Liu, 2007; Chen, 2010), the average incidence of Metabolic Syndrome (MetS) in similar population in some places in China is about 10%. So, we use 0.1 to be the estimated degree of variability.
- A is adopted usual value with statistical significance: 0.05.
- Z is adopted the corresponded value of 95% confidence: 1.96.
- R would be 100%. Because the subjects are the clients of Health 100 Check-up Center and the collecting method is based on an interview by which we make sure the people agree to participant the study and all of them will sign the Consent Form.

In summary, we put the above values into the equator and get the result:

$$n = \frac{P(1-P)}{\frac{A^2}{Z^2} + \frac{P(1-P)}{N}} = \frac{0.1(1-0.1)}{\frac{0.05^2}{1.96^2} + \frac{0.1(1-0.1)}{100000}} = 138$$

References:

Chen, F.M., Guo, Z.R., Hu, X.S. & Wu, M. (2010). Relationship between the basic risk factors and the RR of MS in cohort. *Anhui Medical and Pharmaceutical Journal* 14(7): 786-789.

Liu, J., Zhao, D., Wang, W., Liu, J., Sun, J.Y. & Wu, Z.F. (2007). Incidence of the metabolic syndrome and its risk factors. *Journal of Cardiovascular & Pulmonary Diseases* 26(2): 65-68.

Appendix 5

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Vitamin D status and relationship between vitamin D and risk factors of metabolic syndrome: A study in Taiyuan City in China

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. I also agree with the Health 100 Check-up Center to provide my check-up data to the researchers. The vitamin D concentration determined by the study can be advised to a general practitioner. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: _____ Date: _____

Full Name (printed): _____

Statements

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application (12/46). If you have any concerns about the conduct of this research, please contact Dr Brian Finch, Chair, Massey University Human Ethics Committee: Southern A, Telephone 06 350 5799 × 8717, Email: humanethicsoutha@massey.ac.nz

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PARTICIPANT CONSENT FORM

知情同意书

我已经阅毕课题研究情况说明书(IS)，对课题具体内容有清楚得了解并且同意该体检中心向该课题研究人员提供我的体检数据。此次研究中获知的维生素 D 血液浓度可以用于医疗参考。 所有与课题相关的疑问已经明了，并且我清楚在课题进行当中的任何时候我仍然有权提出疑问。

我同意在课题研究情况说明书(IS)所陈述的条件下参与该项目。

签名：_____ 日期：_____

签名(工整体)：_____

声明

该项目已通过梅西大学人类研究伦理委员会审阅并同意 (Southern A, 申请编号: (12/46). 如果你对本研究还有任何疑问，请联系梅西大学人类研究伦理委员会主席，Brian Finch 博士，联系方式：Massey University Human Ethics Committee: Southern A, 电话：06 350 5799 × 8717, 电子邮箱：humanethicsoutha@massey.ac.nz