Prevalence of metabolic syndrome and its components and their association with vitamin D status among Iranian adults

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Abstract

Metabolic syndrome, which is highly prevalent worldwide, is a clustering of several cardio-metabolic risk factors and is associated with an increased risk of cardiovascular and other chronic diseases. The exact causes of metabolic syndrome are not yet fully acknowledged; however, the most significant risk factors are: being overweight/obese, genetics, aging and inactivity. Moreover, there is evidence suggesting that vitamin D deficiency might be associated with the incidence of chronic diseases such as metabolic syndrome; nevertheless, available data are conflicting regarding this association. Vitamin D deficiency is becoming a re-emerging public health problem globally which could be a consequence of modern lifestyles. In Iran, both metabolic syndrome and vitamin D deficiency are growing public health problems. Therefore, this research aimed to investigate the prevalence of metabolic syndrome and vitamin D deficiency and their trends based on a cohort study among Iranian adults. This study was also designed to examine the association of metabolic syndrome and its components with vitamin D status.

For this thesis, a longitudinal study and a nested case-control study were designed. The longitudinal study was designed based on the Isfahan Cohort Study, which is an ongoing longitudinal population-based study of adults 35 years and older at baseline (2001). In 2001, information was gathered by trained health professionals regarding demographic and socio-economic characteristics, height, weight, family and medical history, as well as behaviours related to cardiovascular risk factors such as dietary intake, smoking behaviour, and physical activity. Fasting plasma glucose, lipid profiles and other biochemical parameters were measured using fasting blood samples. All measurements were repeated in 2007 and 2013 for all participants using the same methods as the 2001 survey. The nested case-control study was designed based on a

sub-sample of cohort participants. Serum vitamin D concentration was assessed from frozen serum samples of case-control study participants.

Metabolic syndrome was found to be highly prevalent among Iranian adults. Moreover, the risk of developing cardiovascular diseases was significantly higher in individuals with metabolic syndrome compared to those free of metabolic syndrome. Among all tested definitions, the AHA-NHLBI was found to be the best predictor of cardiovascular diseases among the studied population.

The prevalence of metabolic syndrome, hypertension, low high density lipoprotein (HDL), central obesity and diabetes/glucose intolerance increased from 2001 to 2013, while the prevalence of hypertriglyceridemia decreased during this period. On the other hand, socio-demographic factors, such as older age, gender and education levels were found to be associated with changes in metabolic syndrome and its components. On the other hand, vitamin D deficiency was also highly prevalent among the studied population, however, its prevalence decreased during the study period from 2001 to 2013. Finally, this study failed to find any association between vitamin D status and the incidence of metabolic syndrome and its components.

The findings of the present study highlight the need to use a population specific definition of MetS to predict cardiovascular diseases. In addition, there is an urgent need to control the development of metabolic syndrome and its components. Individuals with metabolic syndrome, or any component of metabolic syndrome, may benefit from interventions which improve their lifestyle factors, including physical activity and a healthy diet. Although this study did not find any association between vitamin D deficiency and the incidence of metabolic syndrome, there is a need to improve vitamin D status, since vitamin D is required for bone health and calcium homeostasis.

Statement of Originality

This thesis represents the original research conducted by Hossein Khosravi Boroujeni in the School of Medicine, Griffith University, and has not been submitted for a degree or diploma at any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Hossein Khosravi Boroujeni

Dedication

I would like to dedicate my thesis to my dearest wife, Gilda, who has been a constant source of energy and encouragement during the challenges of my study and life.

I also dedicate this thesis to my beloved parents, for their endless love, support and encouragement.

Acknowledgment

There are a number of great people without whom this thesis might not have been completed. First and foremost, I have to thank my Principal supervisor, Associate Professor Faruk Ahmed, whose patience and knowledge fostered my personal and professional development throughout my research. Second, I thank Dr Hai Phung and Associate Professor Shu-Kay Ng for their brilliant ideas, guidance and support throughout this study.

I would also like to express a special word of thanks to Prof Nizal Sarrafzadegan, my external supervisor and director of Isfahan cardiovascular Research Institute (ICRI). She has been supportive since the days I began working on my proposal and gave me the freedom and encouragement I needed to proceed and develop.

Also, I would like to thanks ICRI personals, especially Dr Hamidreza Roohafza, Mrs Minoo Dianatkhah and the ICRI Lab team, for their effort, motivation and support during this study. Without their help, this research may not have been achievable. I am also very grateful to my friend Mr Terry Mitchell for his assistance and support throughout the writing of my thesis.

My very special thanks go to my respectful and lovely family and friends whom made my candidature expedition a brilliant experience. Thank you for your understanding, encouragement and support in my many moments of crises.

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Glossary of Terms

%	Percentage
<	Less than
>	Greater than
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
2hpp	2 hour plasma glucose
25(OH)D	25-hydroxy vitamin D
AHA	American Heart Association
ANOVA	Analysis Of Variance
ASCVD	Atherosclerotic Cardiovascular Disease
ATP III	Adult Treatment Panel III
BMI	Body Mass Index
cAMP	cyclic Adenosine Monophosphate
CDS	Chinese Diabetes Society
CHD	Coronary Heart Disease
CINDI	Countrywide Integrated Non-Communicable Disease Intervention
COPD	Chronic Obstructive Pulmonary Disease
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DRIs	Dietary Reference Intakes
EGIR	European Group for the Study of Insulin Resistance
ELISA	Enzyme Linked Immune Sorbent Assay
FDA	Food and Drug Administration
FBS	Fasting Blood Sugar
FPG	Fasting Plasma Glucose
FFQ	Food Frequency Questionnaire
g	Gram
Hb	Haemoglobin
HDL	High Density Lipoprotein
ICRI	Isfahan cardiovascular Research Institute
IDF	International Diabetes Federation
IGT	impaired glucose tolerance

IM	Institute of Medicine
IU	International Unit
JIS	Joint Interim Statement
LDL	Low Density Lipoprotein
MAP kinase	Mitogen-Activated Protein Kinase
MED	Minimal Erythemal Dose
MEDLINE	Medical Literature Analysis and Retrieval System Online
ml	Millilitre
ng	Nanogram
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung, and Blood Institute
nm	Nanomole
PCOS	Polycystic Ovary Syndrome
PTH	Parathyroid Hormone
RCT	Randomised Control Trial
SBP	Systolic Blood Pressure
SD	Standard Deviations
SE	Standard Error
TG	Triglyceride
TNF-α	Tumour Necrosis Factor-α
T2DM	Type 2 Diabetes Mellitus
T1DM	Type 1 Diabetes Mellitus
UVB	Ultraviolet B
VDR	Vitamin D Receptor
VLDL	Very Low Density Lipoprotein
WBCs	White Blood Cells
WC	Waist Circumference
WHO	World Health Organization

Publications in Support of this Thesis

The PhD candidate has prepared six manuscripts throughout this research. The first manuscript is a review article, which is part of the literature review, and is published in International Journal for Vitamin and Nutrition Research. The Results Chapter of this thesis presents other manuscripts as a series of papers. Among them, one manuscript is published in "BMC public health", another one is published in "Metabolic Syndrome and Related Disorders" and others are currently under review for publication.

The mentioned manuscripts are as follows:

- **Khosravi-Boroujeni, H**., Ahmed, F., & Sarrafzadegan, N. (2016). Is the Association between Vitamin D and Metabolic Syndrome Independent of Other Micronutrients?. International Journal for Vitamin and Nutrition Research, 20, 1-16.

- **Khosravi-Boroujeni, H.**, Ahmed, F., Sadeghi, M., Roohafza, H., Talaei, M., Dianatkhah, M., & Sarrafzadegan, N. (2015). Does the impact of metabolic syndrome on cardiovascular events vary by using different definitions?. BMC public health, 15(1), 1.

- **Khosravi-Boroujeni, H.**, Sarrafzadegan, N., Sadeghi, M., Roohafza, H., Talaei, M., Ng, SK., Phung, H., Pourmogaddas, A., & Ahmed, F. (2017). Secular trend of metabolic syndrome and its components in a cohort of Iranian adults from 2001 to 2013. Metabolic Syndrome and Related Disorders.

- **Khosravi-Boroujeni, H.**, Sarrafzadegan, N., Sadeghi, M., Roohafza, H., Ng, SK., Pourmogaddas, A., & Ahmed, F. Factors influencing the changes in metabolic syndrome components in a twelve years cohort of Iranian adults. Submitted to *Diabetology & Metabolic Syndrome.*

- **Khosravi-Boroujeni, H.**, Sarrafzadegan, N., Sadeghi, M., Roohafza, H., Ng, SK., Pourmogaddas, A., & Ahmed, F. Prevalence and trends of vitamin D deficiency in a longitudinal study of Iranian adults. Submitted to "Journal of Nutritional Science and Vitaminology"..

- **Khosravi-Boroujeni, H.**, Sarrafzadegan, N., Sadeghi, M., Roohafza, H., Ng, SK., Pourmogaddas, A., & Ahmed, F. Association between metabolic syndrome and serum vitamin D levels in a longitudinal study of Iranian adults. Submitted to Metabolic Syndrome and Related Disorders.

Structure of thesis

This thesis is presented as a series of published and unpublished papers in agreement with the Griffith University Regulation. This "thesis as a series of papers" format has an introduction (chapter 1), literature review (chapter 2), methodology (chapter 3), results (chapter 4) and discussion (chapter 5). The papers are presented as part of the literature review and result chapters (Figure 1). Papers formatted to meet the requirements of the journals which they have been submitted/published.

This format results in unavoidable repetitions, particularly in the literature review, method and material sections, however, I tried to minimize such repetitions. The references were combined into the end of the thesis to decrease duplications.

Each manuscript in the result chapter has its own abstract, introduction, material and method, results, tables and discussion. Each manuscript also has a short preface, which serves to link the results to address the aims of the thesis and explains the contribution of the candidate as the author of these papers.

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Figure 1: Structure of thesis based on Griffith University Regulation

Acknowledgment of Papers Included in this Thesis

Included in this thesis are articles and manuscripts which are co-authored with other researchers. The contribution of the PhD candidate to each manuscript is summarized at the front of the related sections. Individuals who contributed in each study, but who do not qualify as authors, are included in the acknowledgment parts.

Candidate: Hossein Khosravi-Boroujeni (06/12/2016)

Principal supervisor: Faruk Ahmed (06/12/2016)

CHAPTER ONE: INTRODUCTION

1.1 Background

Metabolic syndrome (MetS) is a collection of metabolic abnormalities, including central obesity, hypertension, dyslipidaemia and glucose intolerance, which increase the risk of cardiovascular disease (CVD) (McNeill *et al.*, 2005b), diabetes mellitus (Resnick *et al.*, 2003), and other chronic diseases (Lakka *et al.*, 2002a). MetS has serious consequences, with an increased risk of cardiovascular morbidity and mortality. Previous studies have also shown that CVD death and all-cause mortality were higher in individuals with MetS (Liu *et al.*, 2014), even in individuals free of CVD or diabetes at baseline (Gami *et al.*, 2007a).

As a result of the increasing prevalence of obesity and sedentary lifestyles, the prevalence of MetS has been increasing globally (Aballay *et al.*, 2013; Eckel *et al.*, 2005). Recent studies have shown that nearly one-third of Americans (Ramphal *et al.*, 2014) and 21.7% - 30.7% of Australians (Cameron *et al.*, 2007) are living with MetS. Similarly, MetS is highly prevalent among Europeans (24%) (Athyros *et al.*, 2005) and Middle Eastern populations (28-42%) (Sorkhou *et al.*, 2003). A population-based study in Iran also reported a very similar prevalence (27%) of MetS in adults (Ostovaneh *et al.*, 2014).

Differences in genetic predisposition, age, gender, level of physical activity, and dietary pattern are known to influence the prevalence and development of MetS (Cameron *et al.*, 2004). In particular, various dietary factors, such as excess energy intake, high consumption of salt, saturated and trans fat, and low intake of fibre have been found to be positively associated with the prevalence of MetS (Djousse *et al.*, 2010). In addition, a range of micronutrient deficiencies, such as vitamin D (Hyppönen *et al.*, 2008; Lu *et*

al., 2009; Strange *et al.*, 2015), calcium (Moore-Schiltz *et al.*, 2015), and magnesium (Moore-Schiltz *et al.*, 2015) deficiencies, have also been associated with the prevalence of MetS. In recent years, it has been suggested that vitamin D deficiency is one of the most important risk factors of MetS and its components (Hyppönen *et al.*, 2008; Lu *et al.*, 2009; Strange *et al.*, 2015).

Current literature has also indicated that vitamin D deficiency is highly prevalent globally and has been considered as a re-emerging public health problem (Moniz *et al.*, 2005; Souberbielle *et al.*, 2016; Souberbielle, 2016). For example, the prevalence of vitamin D deficiency was 36% in young adults in the United States (Ramphal *et al.*, 2014), while studies in Australia have also reported a high prevalence of vitamin D deficiency (33% and 40% in men and women respectively), despite the high levels of sunshine (Quaggiotto *et al.*, 2014). The prevalence was even higher in specific population groups (80% of women and 70% of men living in hostels or nursing homes) in Australia (Diamond *et al.*, 2005; Flicker *et al.*, 2003). A high prevalence of vitamin D deficiency/insufficiency (61.4%-87.8%) was also reported in Middle-Eastern countries (Ardawi *et al.*, 2012; Gaafar & Badr, 2013). In Iran, vitamin D deficiency is highly prevalent, and its prevalence varies from 30.6% to 72.3% across different population groups (Habibesadat *et al.*, 2014; Hossein-Nezhad *et al.*, 2009).

The National Health and Nutrition Examination Survey and several other studies have found that lower levels of 25-hydroxy vitamin D (25(OH)D) were related to diabetes, hypertension, atherosclerosis, stroke, congestive heart failure, myocardial infarction, microalbuminuria and decreased kidney function (Bhatt *et al.*, 2013; Çankaya *et al.*, 2015; Chiu *et al.*, 2004; Chonchol & Scragg, 2006; Cigolini *et al.*, 2006; de Boer *et al.*, 2007; Gao *et al.*, 2015; Heidari *et al.*, 2015; Kendrick *et al.*, 2009; Krause *et al.*, 1998; Martins *et al.*, 2007; Scragg *et al.*, 2004, 2007; Targher *et al.*, 2006; Tomaino *et al.*,

2015; Turetsky *et al.*, 2015; Wang *et al.*, 2008). It has been suggested that non-calcemic roles of vitamin D are responsible for these diseases because most cells and tissues in the body have vitamin D receptors (Nagpal *et al.*, 2005). However, the findings of the studies on the relationship between vitamin D and MetS are equivocal. For example, several studies have reported a significant inverse association between serum vitamin D levels and the prevalence of MetS (Botella-Carretero *et al.*, 2007; Ford *et al.*, 2005; Gagnon *et al.*, 2012; Hyppönen *et al.*, 2008; Liu *et al.*, 2005; Maki *et al.*, 2009), while others did not find any association (Amirbaigloo *et al.*, 2013; Reis *et al.*, 2007; Rueda *et al.*, 2008). The inconsistencies in findings from different studies might be due to the differences in study populations, ethnicity, criteria used to define MetS and vitamin D deficiency, and/or other potential confounders. For example, some micronutrients such as vitamin A (Zulet *et al.*, 2008), zinc (Kechrid *et al.*, 2012), calcium (Moore-Schiltz *et al.*, 2015), and magnesium (Moore-Schiltz *et al.*, 2015) are associated with MetS and also related to the activation and function of vitamin D (Bettoun *et al.*, 2003; Craig *et al.*, 1999; Rude *et al.*, 2009). Therefore, they could affect the association between vitamin D and MetS. In a recent review article, we highlighted the potential interactions of vitamin A, zinc, magnesium and vitamin D with MetS as well as its components (Khosravi-Boroujeni *et al.*, 2016a), which might justify some of the inconsistencies in the studies.

In addition, most of the previous studies employed a cross-sectional study design to explore the association between vitamin D deficiency and MetS and its components, with only a few conducted longitudinal study designs. Therefore, in this research, a longitudinal study design was employed to examine the secular trends of MetS prevalence and to explore the effect of changes in MetS risk factors on the prevalence of MetS. In addition, we examined whether vitamin D deficiency was independently

associated with MetS and its components. The conceptual framework of this study is presented in Figure 1.1.

1.2 Significance and innovative nature of the research

Globally, the prevalence of MetS is increasing rapidly. It has been linked with cardiovascular morbidity and mortality, and all causes of mortality (Lakka *et al.*, 2002a). MetS and its components are influenced by a number of risk factors such as age, physical activity, diet, smoking and family history of diseases (Grundy *et al.*, 2005). In Iran, nearly one-third of the population are currently suffering from MetS and thus it is recognized as a significant public health problem in the country (Azizi *et al.*, 2003; Ostovaneh *et al.*, 2014). Therefore, it is of benefit to determine the risk factors that are associated with MetS in the Iranian population so that effective intervention strategies can be developed to prevent this syndrome.

Vitamin D deficiency is also highly prevalent among the Iranian population (Hovsepian *et al.*, 2011). As mentioned earlier, several studies in recent years have shown an association between vitamin D status and MetS (Botella-Carretero *et al.*, 2007; Cheng *et al.*, 2010; Chiu *et al.*, 2000; Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Lu *et al.*, 2009; Pereira *et al.*, 2002), while a good number of studies failed to demonstrate such an association (Bonakdaran & Varasteh, 2009; Liu *et al.*, 2009; Reis *et al.*, 2007; Rueda *et al.*, 2008; Tai *et al.*, 2008). Although some factors such as diversity in sample size, age group, ethnicity and polymorphism were identified as the major reasons for this inconsistency, the effect of other potential confounding factors is undeniable. Moreover, different studies have used different definitions to define MetS, which could misclassify the subjects as either being normal or suffering from MetS, and thus the definition can influence the result of a study (Cameron *et al.*, 2007). Therefore, the present study, first,

compared different definitions of MetS to identify the best predictor for CVD events in a cohort of an Iranian population (Khosravi-Boroujeni *et al.*, 2015).

To date, very few studies have investigated the association between vitamin D deficiency and MetS among the Iranian population. Moreover, these studies were cross-sectional in nature, except one cohort study which demonstrated no association between vitamin D deficiency and MetS (Amirbaigloo *et al.*, 2013). In addition, these studies were limited by not considering all potential confounders. Clearly, there is a need for a well-designed study to determine whether vitamin D deficiency is associated with MetS among the Iranian population while taking into account potential confounding factors. Further, both in Iran and other countries, most of the previous studies, even those which used cohort study design, reported this association based on a one-time assessment of vitamin D and MetS components, which meant that they could not adjust the effect of changes in the risk factors over time while assessing this association (Amirbaigloo *et al.*, 2013). It is important to recognize that MetS develops gradually due to long-term exposure to various risk factors. Furthermore, the prevalence of MetS components may change over time as a result of changes in their risk factors. For example, changes in lifestyle might change the MetS risk factors, such as physical activity level and dietary intake of an individual. Thus, single point assessment is considered inadequate, as this indicates only the current situation. To establish the causal relationship, it is important to look at secular trends of MetS and its components in addition to the potential risk factors. Therefore, the current study examined the secular trends of vitamin D deficiency, MetS and its components over a period of 12-years, using data from three points in time (2001, 2007 and 2013) among Iranian adults. The longitudinal effect of MetS risk factors on the prevalence of MetS, or its components, was also explored. Additionally, this study investigated the association between vitamin D and MetS after

controlling for potential confounders. Moreover, the severity of vitamin D deficiency and MetS definition might also influence the association between vitamin D and MetS. Consequently, this study investigated the association between vitamin D deficiency and MetS using different definitions of MetS and different stages of vitamin D deficiency.

To the best of our knowledge, this study is the first study that has examined the influence of serum vitamin D concentrations on MetS, based on three time assessments (2001, 2007 and 2013) in a cohort study. Thus, this study could explain the association between serum vitamin D levels and MetS or its components more accurately. Further, the findings of this study contribute to, and enrich, the current knowledge about the association between vitamin D deficiency and MetS and its components, and may explain part of the inconsistencies. The present study also demonstrated a high prevalence of MetS as well as increasing trends of MetS and its components from 2001 to 2013. Given the serious health consequence of MetS, the present findings have created an opportunity to convince government and decision-makers to take appropriate measures and to finance the public sector to engage in the prevention and control of MetS. The results of this study, in relation to the high prevalence of vitamin D deficiency among the studied population, might also be a basis for encouraging medical professionals and individuals to treat the vitamin D deficiency. Finally, the findings of this study could be a basis for future investigations into controlling MetS, as well as vitamin D deficiency, in the country.
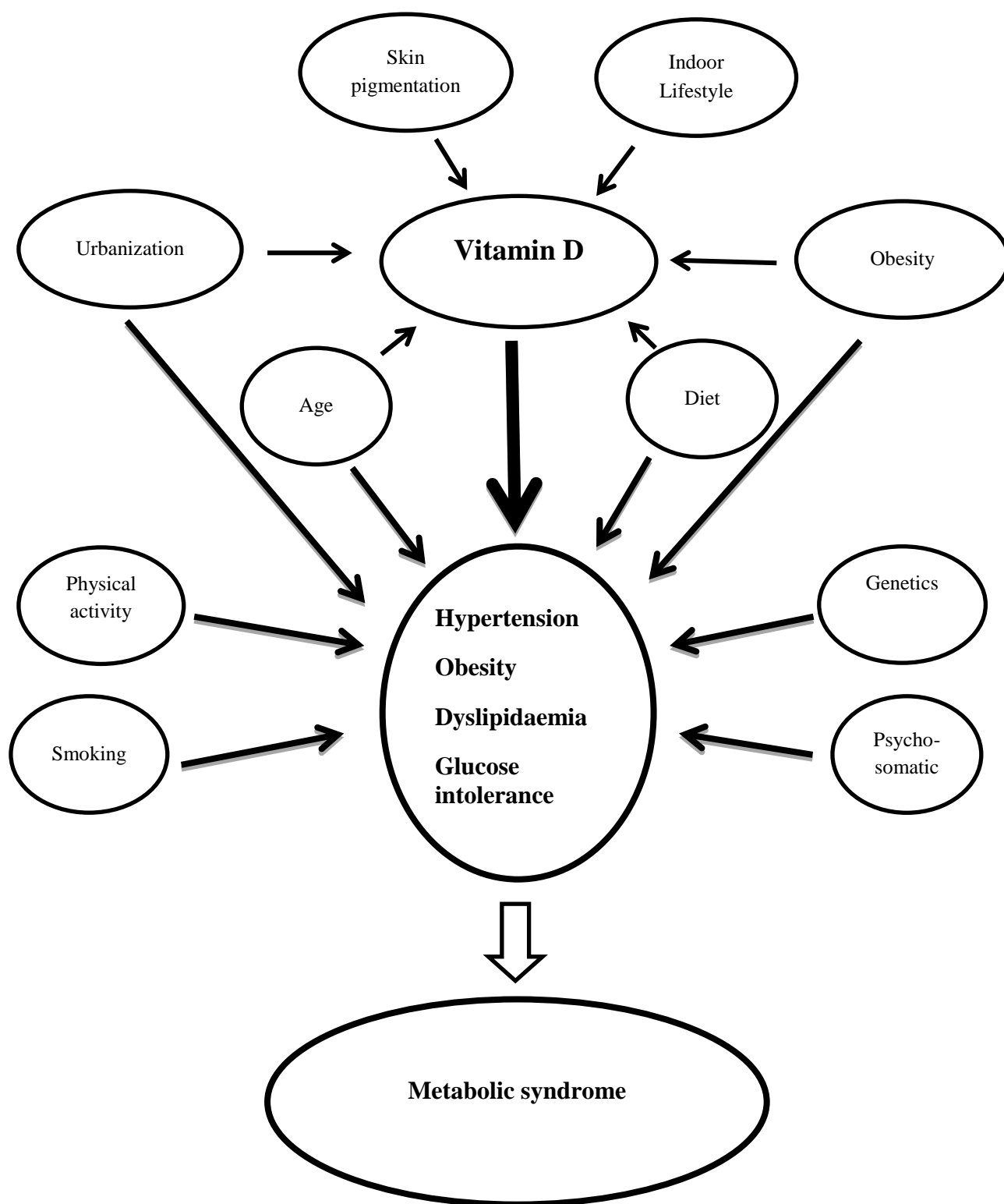
Figure 1. 1: The conceptual framework of the research

1.3.1 Main aim

The overall aim of this research was to examine the prevalence of MetS and its components as well as vitamin D deficiency, and to investigate the association between vitamin D status and MetS and its components in a longitudinal study among Iranian adults.

1.3.2 Specific aims/objective

1. To identify the best possible definition of MetS according to its prevalence and its impact on CVD events.

2. To determine the secular trends of MetS and its components among Iranian adults during 12 years of follow-up.

3. To examine the influence of changes in MetS risk factors, including BMI, dietary factors, physical activity or smoking, on MetS and its components.

4. To assess the prevalence of and trends in vitamin D deficiency in a sample of Iranian adults during 12-years of follow-up.

5. To determine the association between vitamin D levels and MetS and/or its components by considering the possible confounders, and to compare these associations using different definitions of MetS and severity of vitamin D deficiency.

1.3.3 Study questions

1. Are the prevalence of MetS and its effect on CVD events influenced by different definitions?

2. What is the secular trend of MetS and its components over a 12-year time period?

3. Do changes in BMI, dietary factors, physical activity or smoking influence the prevalence of MetS and its components?

4. What were the prevalence of and trends in vitamin D deficiency in Iranian adults from 2001 to 2013?

5. Is vitamin D deficiency associated with MetS and/or its components, and are these associations influenced by potential confounders, different MetS definitions, and severity of vitamin D deficiency?

CHAPTER TWO: LITERATURE REVIEW

2.1 Preface

The focus of this literature review is on MetS, its components, and related risk factors especially vitamin D deficiency. First part of this review focuses on a brief history of MetS, definitions, current prevalence, its consequences, and risk factors. The second part of this review describes vitamin D features, functions, prevalence and risk factors of vitamin D deficiency. Furthermore, it explains the association between vitamin D deficiency and MetS and summarizes the findings of reviewed studies. It also reviews the available evidence regarding the mechanism and findings of the association of vitamin D deficiency with abnormal glucose metabolism, lipid profiles, blood pressure and obesity. Finally, the last section presents our review article regarding the association between vitamin D and MetS which highlights the possible roles of other micronutrients which may affect the association between MetS and vitamin D.

2.2 Metabolic syndrome

2.2.1 Brief historical review

The term 'Metabolic Syndrome' is now used globally; however, it has a history of over 80-years of development. In 1923, a Swedish physician described a cluster of health conditions which include hyperglycaemia, hypertension, and gout (Kylin, 1923). In 1947, a prominent study reported that android, or male-type obesity, was related to metabolic abnormalities, regularly seen with diabetes mellitus and cardiovascular diseases (Vague *et al.*, 1979). Four decades later, Reaven underlined the clinical importance of this syndrome and described the presence of metabolic abnormalities with insulin resistance as a principal pathophysiological feature, although obesity was not included (Reaven, 1988). He used the term 'Syndrome X' for this abnormality, however, MetS is also known by other names such as the 'Deadly Quarter' and 'Insulin Resistance Syndrome' (Zimmet *et al.*, 2005).

MetS is defined as a clustering of risk factors, including central obesity, hypertension, glucose intolerance and dyslipidaemia which increases the risk of developing CVD (McNeill *et al.*, 2005b), diabetes mellitus (Resnick *et al.*, 2003), and other chronic diseases (Lakka *et al.*, 2002a).

2.2.2 Definition of MetS

The following provides a detailed discussion of the most widely accepted definitions of MetS. Several expert groups have attempted to define MetS as a simple method for predicting CVD (Table 2.1). The World Health Organization (WHO) consultation group first attempted to provide a global definition. Its definition is based on the statement that insulin resistance is one of the major primary contributors to MetS. Therefore, the

presence of insulin resistance [or its alternate, such as impaired glucose tolerance (IGT) or diabetes] plus at least two other components is required for the diagnosis of MetS (World Health Organization, 1999). The European Group for the Study of Insulin Resistance (EGIR) suggested an adjusted version of the definition for non-diabetic subjects only and used fasting plasma glucose as it is easier to use in epidemiological studies and does not require the measurement of insulin sensitivity (Balkau & Charles, 1999). The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) for the prevention of coronary heart disease (CHD) presented the ATP III definition to facilitate diagnosis in clinical practice. In this definition, insulin resistance was not a component of MetS, and glucose abnormalities had only the same weight as other components (Expert Panel on Detection, 2001). The American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) agreed to the ATP III definition, but decreased the threshold for impaired glucose tolerance (IFG) from 110 to 100 mg/dl (Grundy *et al.*, 2005).

Additionally, the International Diabetes Federation (IDF) suggested a simple definition which could be used easily in any country (International Diabetes Federation, 2006). Based on this new definition, MetS is defined as having central obesity plus any two of the four other factors. The rationale for this definition is that central obesity is highly correlated with insulin resistance and is more strongly associated with the other features of MetS (Carr *et al.*, 2004). One key point of this definition is that the same criteria should not be applied to central obesity for different ethnic groups and countries, thus, waist circumference (WC) thresholds for abdominal obesity have been recommended by the IDF and other organizations (Table 2.2) (Alberti *et al.*, 2009b).

Finally, a harmonized definition of MetS [a Joint Interim Statement (JIS)] prepared by a number of organizations, including IDF, NHLBI, AHA, the International Atherosclerosis Society, the World Heart Federation, and the International Association for the Study of Obesity, developed joint criteria to define MetS. They argued that a single cut-off point for WC in defining abdominal obesity is not suitable across all population groups and that the cut-off point for WC should be based on ethnicity. Moreover, they stated that there should not be any mandatory component for diagnosis of MetS and any three out of five components are sufficient for such a diagnosis (Alberti *et al.*, 2009a).

Table 2.1: MetS definitions

	WHO	EGIR	NCEP ATP III	IDF	AHA	JIS
Definitions	Glucose intolerance, IGT or diabetes and/or insulin resistance together with two or more of the following:	Insulin resistance (defined as hyperinsulinaemia—top 25% of fasting insulin values among the non-diabetic population). Plus two of the following:	Three or more of the following five risk factors:	Central obesity plus two other features	Three or more of the following five risk factors:	Three or more of the following five risk factors:
Fasting plasma glucose	—	≥ 6.1 mmol/l (110 mg/ dl) but non-diabetic	≥ 5.6 mmol/l (100 mg/ dl)	≥5.6 mmol/l (100 mg/ dl) or diagnosed type 2 diabetes	≥ 5.6 mmol/l (100 mg/ dl)	≥ 5.6 mmol/l (100 mg/ dl)
Blood pressure	≥140/90 mmHg	≥ 140/90 mmHg or treatment	≥ 130/ 85 mmHg	≥130/85 mmHg or treatment	≥ 130/ 85 mmHg	≥ 130/ 85 mmHg
Triglycerides	≥ 1.7 mmol/l (150 mg/ dl)	> 2.0 mmol/l (178 mg/ dl)	≥ 1.7 mmol/l (150 mg/ dl) or treatment	≥1.7 mmol/l (150 mg/ dl) or treatment	≥ 1.7 mmol/l (150 mg/ dl) or treatment	≥ 1.7 mmol/l (150 mg/ dl) or treatment
HDL-cholesterol	Men: < 0.9 mmol/l (35 mg/ dl) Women: < 1.0 mmol/l (39 mg/ dl)	< 1.0 mmol/l (39 mg/ dl) or treatment	Men: < 1.03 mmol/l (40 mg/ dl) Women: < 1.29 mmol/l (50 mg/ dl)	Men: <1.0 mmol/l (39 mg/ dl) Women: <1.3 mmol/l (40 mg/ dl) or treatment	Men: < 1.03 mmol/l (40 mg/ dl) Women: < 1.29 mmol/l (50 mg/ dl)	Men: < 1.03 mmol/l (40 mg/ dl) Women: < 1.29 mmol/l (50 mg/ dl)
Obesity	Men: waist–hip ratio > 0.90 Women: waist–hip ratio > 0.85 and/or BMI > 30 kg/m2	Men: WC ≥ 94 cm Women: WC ≥ 80 cm	Men: WC > 102 cm Women: WC > 88 cm	Men: WC ≥94cm , Women: WC ≥80cm* or BMI >30kg/m²	Men: WC > 102 cm Women: WC > 88 cm	Ethnic cut-offpoint for WC **
Microalbuminuria	Urinary albumin excretion rate ≥ 20 µg/min or albumin:creatinine ratio ≥ 30 mg/g	—		—	—	

*Population- and country-specific definitions (Alberti *et al.*, 2009b)

** based on Iranian cut-off point, WC ≥ 95 for both sexes

Table 2.2: Recommended WC thresholds for abdominal obesity (Alberti *et al.*, 2009b)

Population	Organization (Reference)	Recommended WC Threshold for Abdominal Obesity	
		Men	Women
Europid	IDF	≥94 cm	≥80 cm
Caucasian	WHO	≥94 cm (increased risk)	≥80 cm (increased risk)
		≥102 cm (still higher risk)	≥88 cm (still higher risk)
United States	AHA/NHLBI (ATP III)	≥102 cm	≥88 cm
		≥94cm (recent guideline)	≥80 cm (recent guideline)
Canada	Health Canada	≥102 cm	≥88 cm
European	European Cardiovascular Societies	≥102 cm	≥88 cm
Asian (including Japanese)	IDF	≥90 cm	≥80 cm
Asian	WHO	≥90 cm	≥80 cm
Japanese	Japanese Obesity Society	≥85 cm	≥90 cm
China	Cooperative Task Force	≥85 cm	≥80 cm
Middle East, Mediterranean	IDF	≥94 cm	≥80 cm
Sub-Saharan African	IDF	≥94 cm	≥80 cm
Ethnic Central and South American	IDF	≥90 cm	≥80 cm

2.2.3 Prevalence

As a consequence of the ageing of the population, the increasing prevalence of obesity and modern lifestyles, an outstanding increase in the prevalence of MetS has been taking place worldwide (van Vliet-Ostaptchouk *et al.*, 2014). For example, one in every three Americans is suffering from MetS (Mitchell *et al.*, 2013), while in Scandinavian populations it has been reported that 25% of the population suffers from this disorder (Qiao *et al.*, 2009). Moreover, the FINRISK cohort found that MetS was present in 22.2% of women and 28.8% of men in the general Finnish population (Ilanne-Parikka *et al.*, 2004). A meta-analysis of cross-sectional studies on Brazilian adults also showed a high prevalence of MetS (29.6%) (de Carvalho Vidigal *et al.*, 2013).

On the other hand, the prevalence of MetS is dependent on the definition used, sex, race, and ethnicity of the population. In Australia, the prevalence of MetS is defined using different definitions, including WHO, EGIR, ATP III and IDF, which indicated a prevalence of 21.7%, 13.4%, 22.1% and 30.7%, respectively (Cameron *et al.*, 2007). A study in China also reported that the prevalence of MetS, based on the definitions of ATPIII, IDF and Chinese Diabetes Society (CDS) criteria, was 21.3%, 18.2% and 10.5%, respectively (Xi *et al.*, 2013).

Iran, like other developing countries, has encountered some socioeconomic transitions and, simultaneously with lifestyle changes and the development of obesity, the prevalence of MetS has increased. Based on the Nationwide Study of the Prevalence of MetS, the age-standardized prevalence was 34.7 and 37.4 based on the ATP III and IDF criteria respectively (Delavari *et al.*, 2009). Previous studies have also shown that MetS is highly prevalent among Iranians, with more than 30% of adults (Azizi *et al.*, 2003) and almost 10% of adolescents (Esmaillzadeh *et al.*, 2006) being affected.

Figure 2.1 presents MetS prevalence studies from ten different countries that used ATP III criteria.
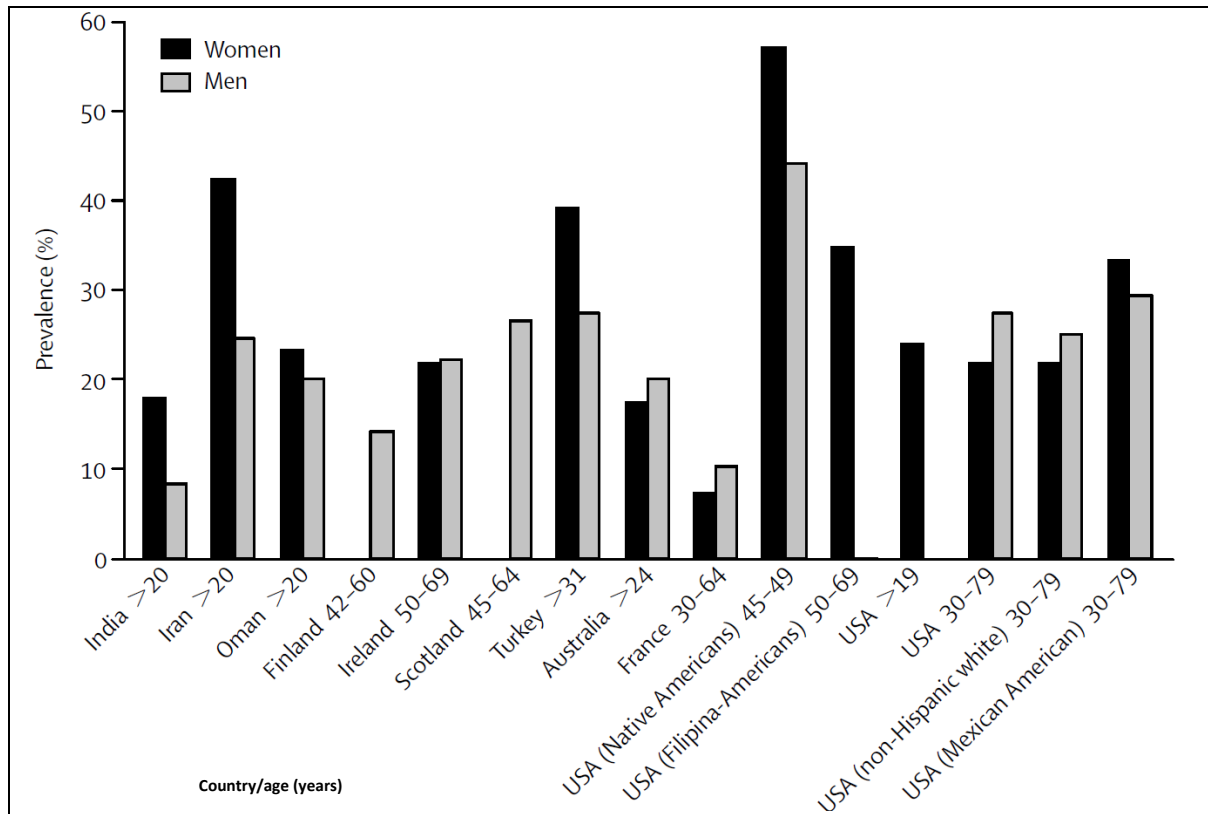
Figure 2.1: Worldwide prevalence of the MetS based on ATPIII definition (Cameron *et al.*, 2004; Eckel *et al.*, 2005).

2.2.4 Consequences of MetS

MetS is associated with stroke (Lin *et al.*, 2015), coronary heart disease (Wild *et al.*, 2013) and myocardial infarction (Islam *et al.*, 2013). These complications of MetS, as well as non-alcoholic hepatitis and the development of some cancers (Reaven, 2004a) are the leading causes of death in developed countries (Lakka *et al.*, 2002a). Five years of follow-up study in non-diabetic individuals found a 9 to 34 times increased risk of developing diabetes in individuals with MetS (Klein *et al.*, 2002). In another study on non-diabetic peritoneal dialysis patients, the risk of mortality was two times greater in patients with MetS compared to those without MetS (Prasad *et al.*, 2013). Another study revealed that MetS increased the risk of dyspnea and comorbidity in chronic obstructive

pulmonary disease (COPD) patients (Ozyilmaz *et al.*, 2015). An analysis of the Scandinavian Simvastatin Survival Study also showed a 1.5 times increased risk for major coronary events (myocardial infarction, angina, or sudden cardiac death) among subjects with MetS (Girman *et al.*, 2004a). Results of a prospective study among Finnish men showed that CVD deaths and all-cause mortality were higher in individuals with MetS, even in individuals free of CVD or diabetes at baseline (Lakka *et al.*, 2002a). Additionally, a systematic review and meta-analysis of longitudinal studies over 170,000 subjects found that the risk of CVD death and all-cause mortality in MetS patients, compared with individuals without MetS, were 2 and 1.6-fold higher, respectively (Gami *et al.*, 2007a).

2.2.5 MetS risk factors

The pathophysiology of MetS is complex and has been only partially explained. Being overweight, genetics, increase in age, smoking, sedentary lifestyle, physical inactivity, and excess caloric intake are known to be the most important risk factors of MetS (Bradshaw *et al.*, 2013; Djousse *et al.*, 2010; Kelliny *et al.*, 2008; Pattyn *et al.*, 2013; Sun *et al.*, 2012; Terán-García & Bouchard, 2007). Moreover, dietary factors, such as consumption of fat, saturated fat, trans fat, whole grains, carbohydrate quality and quantity, simple sugars, fibre, salt, and alcohol, are also related to MetS (Djousse *et al.*, 2010; Heinonen *et al.*, 2014; Khayef & Sabaté, 2016).

Increased physical activity levels were found to be associated with decreased body weight, visceral obesity, high blood pressure, modified insulin sensitivity, increased HDL cholesterol and a decreased plasma triglyceride level (Lakka *et al.*, 2003). Further,

a sedentary lifestyle has been linked to increased risk of MetS and its components (Eskelinen *et al.*, 2015).

A study conducted by Chen *et al.* (2008) found that smoking stimulates insulin resistance, increases the circulating white blood cells (a marker of inflammation), and also increases the plasma levels of adiponectin. The authors have also reported a dose-dependent relationship of smoking with MetS, high triglyceride levels and low levels of HDL. High intake of saturated fatty acids also increased the risk of MetS and type 2 diabetes (Mukwevho & Joseph, 2014).

Moreover, it was reported that intake of carbohydrates from refined grains was positively associated with MetS (Song *et al.*, 2014). In addition, diets rich in whole-grain foods and fibre were related to lower insulin concentrations, indicating that the source and quality of dietary carbohydrates might affect insulin action and insulin resistance (McKeown *et al.*, 2004). A low-carbohydrate diet is also associated with improved insulin sensitivity among obese individuals (McKeown *et al.*, 2004). A positive association was observed between a high glycemic index diet or high glycemic load diet and the risk of MetS and its components, including central obesity, hypertriglyceridemia, high blood pressure, high fasting glucose and low HDL cholesterol (Juanola-Falgarona *et al.*, 2015).

Central obesity, as a component of MetS, consists of both the visceral fat and subcutaneous adipose tissue. Visceral obesity could lead to insulin resistance and an altered metabolic profile and is therefore a marker of metabolic abnormalities (Bergman *et al.*, 2006). It is also proposed that subcutaneous fat has a strong association with insulin resistance as visceral fat (Goodpaster *et al.*, 1997). Adipose tissue is not only limited to the storage of lipids but it also acts as an endocrine structure which releases several cytokines, including tumour necrosis factor-α (TNF-α) and interleukin (IL)-6

(Weisberg *et al.*, 2003). Thus, it has been suggested that central obesity is causally implicated in the pathophysiology of the MetS (Lemieux, 2004).

Reducing the MetS risk factors is highly recommended for the prevention and treatment of MetS. In this regard, the first step is a cardiovascular risk assessment and then lifestyle modification, including: increased physical activity; improved dietary intake, including calorie restriction; reduced intake of salt, total saturated fat and trans fat; and increased intake of fibre (Alberti *et al.*, 2006).

In addition, there are some vitamins and minerals that are related to the incidence of MetS. Previous studies have found a potential association of vitamin D (Hyppönen *et al.*, 2008; Lu *et al.*, 2009; Strange *et al.*, 2015), calcium (Moore-Schiltz *et al.*, 2015), vitamin A (Zulet *et al.*, 2008), zinc (Kechrid *et al.*, 2012) and magnesium (Moore-Schiltz *et al.*, 2015) with MetS and its components (biochemical or anthropometrical measurements). Among these factors, vitamin D is one of the most obvious elements considered to be associated with MetS and its components (Diaz *et al.*, 2016; Hyppönen *et al.*, 2008; Kramkowska *et al.*, 2015; Lu *et al.*, 2009; Strange *et al.*, 2015). However, the association between vitamin D and MetS remains controversial (Chiu *et al.*, 2004; Diaz *et al.*, 2016; Hyppönen *et al.*, 2008; Kramkowska *et al.*, 2015; Lu *et al.*, 2009; Prasad & Kochhar, 2015; Strange *et al.*, 2015).

2.3 Vitamin D

2.3.1 Brief historical review

The role of vitamin D in bone health has been studied and demonstrated extensively and the use of vitamin D as a treatment for bone diseases is a longstanding topic. Schuette, in 1824, was the first scientist who prescribed cod-liver oil for the treatment of rickets, a bone disease with a long history (McCollum, 1957). McCollum and his co-workers, in 1914, isolated a fat-soluble factor from butter fat that was considered essential for the prevention of eye and bone diseases, and for normal growth (McCollum & Davis, 1914) and called the fat soluble factor 'vitamin A'. Afterwards, they discovered that heated fats could not prevent eye diseases, but still could treat rickets. They concluded that there should be another fat-soluble factor and called it 'vitamin D' (McCollum *et al.*, 1922). In the meantime, it was discovered that sunlight or ultraviolet (UV) light irradiation could also cure rickets (Chick *et al.*, 1922). Finally, the chemical structure of this factor was discovered in other studies and it was found that it could be produced in animals and plants by UV irradiation (Wolf, 2004). Although vitamin D is traditionally classified as a vitamin, it performs a role similar to that of a hormone (DeLuca, 2004).

2.3.2 Structure

The structure of vitamin D and all its metabolites are similar to steroid hormones, which are related to cholesterol. In comparison to other steroid hormones, the structure of vitamin D is flexible and rotates around each of the five carbon–carbon single bonds that result in rapid conformational changes (Norman *et al.*, 2004). There are two physiologically related forms of vitamin D that are slightly different in chemical

structure and are known as Calciferol. These two forms include Cholecalciferol (vitamin D_3), which is derived from animal sources and is produced photochemically in the vertebrates' skin, and Ergocalciferol (vitamin D_2), which is formed by invertebrates, plant and fungal sterols in the presence of UV irradiation (Hollis, 1996). The structural difference between these two forms of the vitamin is in their side chains (Figure 2.2).
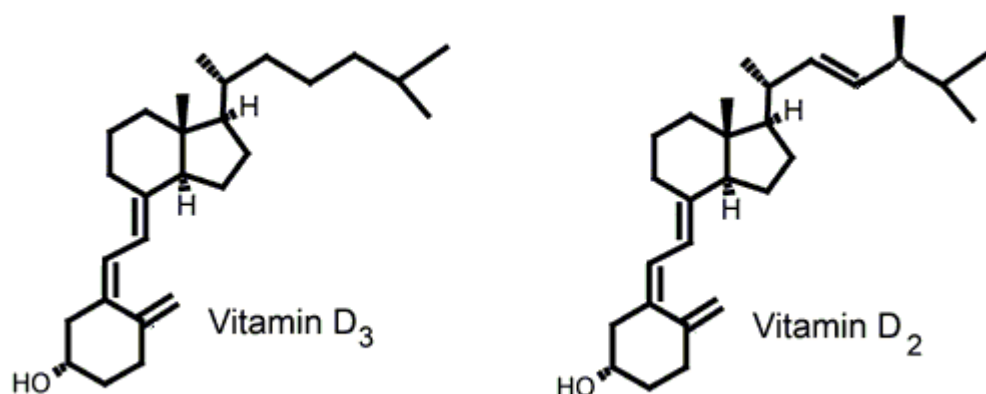
Figure 2.2: The structural difference between vitamin D2 and D3

2.3.3 Synthesis

Ergosterol was produced 750 million years ago by the initial forms of life that used calcium for their structure, such as phytoplankton and diatom (Holick, 2004b). Synthesis of vitamin D involves ultraviolet B (UVB) radiation in the skin of humans, animals, and plants. 7-dehydrocholesterol is the precursor for the cholesterol biosynthetic pathway that is formed in fairly large quantities in the skin of vertebrate animals, although there are few exceptions, such as male rats, dogs, cats and bats (How *et al.*, 1994). During exposure to sunlight, UVB is absorbed by the 7-dehydrocholesterol

in the epidermal and dermal cells and results in a change, and a break, in the B-ring construction and forms pre-vitamin D_3 that is unstable and isomerizes to vitamin D_3 (Figure 2.3) (Holick, 2004b).
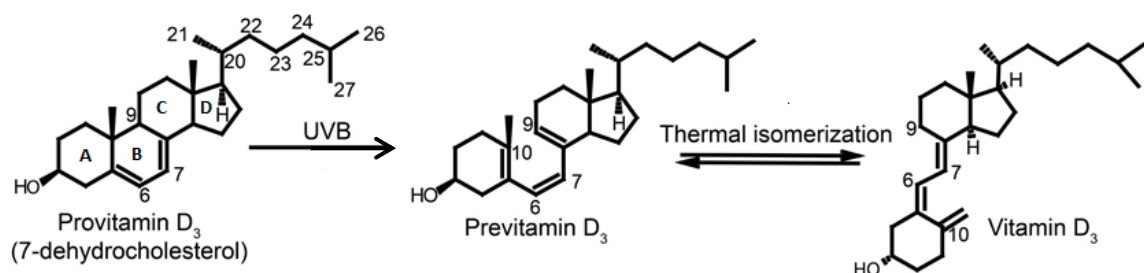
Figure 2.3: Synthesis of vitamin D in epidermis

These forms of vitamin D are not biologically active and have to change to the active form in the liver and kidney. Vitamin D binding protein binds to vitamin D_3 and transports it into the bloodstream to target cells for metabolism. In the liver, 25-hydroxylase convert the vitamin D_3 to 25-hydroxyvitamin D_3 (25(OH)D_3). In the second part, 1,25 hydroxylase in the kidney converts the vitamin D_3 to calcitriol (1,25-dihydroxyvitamin D_3 or 1,25(OH)$_2$$D_3$) which is the biologically active form of vitamin D (figure 2.4) (Holick *et al.*, 1971).
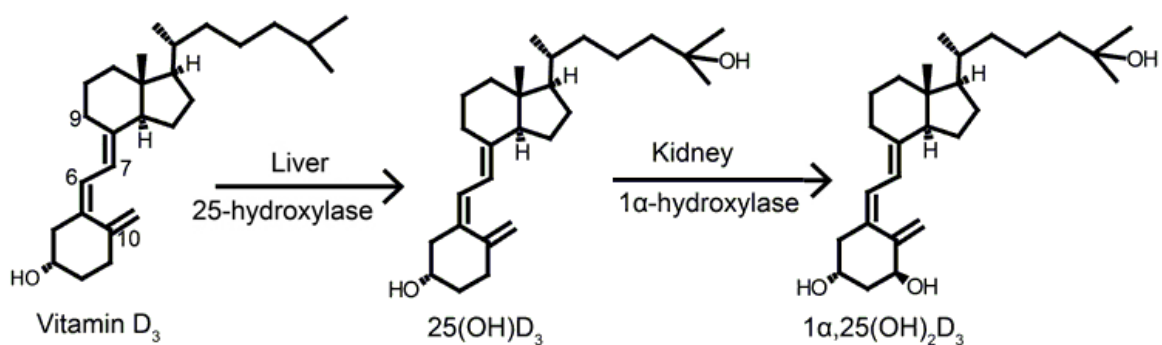
Figure 2.4: Activation of vitamin D in liver and kidney

These vitamin D activation processes have been found in other tissues including the intestine, adrenal gland, kidney, lung, bone, colon, prostate, breast, parathyroid cells and epidermal cells (Holick, 2004b; Lehmann *et al.*, 2001).

2.3.4 Sources of vitamin D

There are two sources of vitamin D: endogenous, that is created in the skin; and exogenous, that is found in foods and supplements. The exogenous source cannot adequately meet the body's requirements. The dietary requirement, based on Dietary Reference Intakes (DRIs), is between 600 to 800 IU for adults; however, some studies recommended a much higher amount of vitamin D to sustain adequate levels (Heaney *et al.*, 2003; Vieth *et al.*, 2007). There are limited foods that naturally contain vitamin D and almost all foods have less than 400 IU of vitamin D per 100 g edible portion. The best sources are oily fish, such as mackerel (325 IU/100g), salmon (345 IU/100g), and sardines (500IU/100g), as well as cod liver oil (1360 IU/15ml). Beef liver (15 IU/100g) and whole egg (20 IU) are other natural sources of vitamin D (Meerza *et al.*, 2010). Moreover, in some countries, certain foods such as milk, orange juice, margarine,

vegetable oil, cereals, and bread are fortified with vitamin D (Calvo *et al.*, 2004; Tangpricha *et al.*, 2003; Tylavsky *et al.*, 2006).

Most individuals obtain vitamin D from casual exposure to sunlight to fulfil the body's requirement. The body skin has a great capacity to produce vitamin D. Being exposed to enough sunlight on a sunny beach to cause a slight skin pinkness is equal to an oral dose of 20,000 IU vitamin D (Holick, 2002). The vitamin D that is synthesised during the sunny seasons (spring, summer, and autumn), stored in body fat, and can be mobilized during winter to satisfy body requirements. Even in older people who have a lower amount of skin 7-dehydrocholesterol, the skin exposed to sunlight could make adequate vitamin D (Chuck *et al.*, 2001; Holick *et al.*, 1989). To calculate the amount of solar UVB irradiation required to synthesise an adequate amount of vitamin D, the skin colour, percentage of the body exposed, and minimal erythemal dose (MED) (which can make equal to 10,000-25,000 IU of oral vitamin D) should be known. In the summer at noon, the exposure time for one MED for pale skin and dark skin is 4-10 and 60-80 minutes respectively (Holick, 2004a). Vitamin D can be produced naturally during sunny seasons (spring, summer, and fall) from 10 a.m. to 3 p.m., with the best time being near noon when the UVB to UVA ratio is highest (Holick, 2004a). When the sunlight streams from a lower angle in the sky, (in the early morning or late afternoon), UVB photons have to pass through more of the ozone layer. Therefore, fewer UVB photons reach the earth and vitamin D production in the skin is minor (Holick, 2004a).

According to U.S. Food and Drug Administration's (FDA) nutritional guidelines, vitamin D supplementation up to 2000 IU/day is effective and safe (Hathcock *et al.*, 2007), but higher amounts of vitamin D supplementation might be associated with health problems. However, a review study concluded that vitamin D consumption of up to 10,000 IU/day is safe (Hathcock *et al.*, 2007). It has been shown that symptoms

related to hypercalcemia, including diminished appetite, polyuria, itchiness, thirst, diarrhoea and calcification (especially in the aorta, kidney, lung, heart and also subcutaneous tissue), are the most common symptoms of vitamin D intoxication (DeLuca, 2004) and occur if intake is more than 40,000 IU/day and when serum 25(OH)D level is higher than 150-200 ng/dl (Vieth, 1999).

2.3.5 Function

The best-known role of vitamin D is stimulating calcium absorption and therefore is causative to optimal mineralization of bone and reduced risk of bone fracture. Consequently, it is not surprising that vitamin D deficiency is associated with a variety of bone diseases, including an increased risk of fractures, osteoporosis, rickets, and various tooth disorders (Holick, 2007). To control bone and calcium metabolism, vitamin D interrelates with its nuclear receptor in the bone, intestine and kidney (Haussler *et al.*, 2013; Holick, 2009; Wang *et al.*, 2012b). Moreover, vitamin D plays an essential role in muscle function, and more recently, it has been shown that a low vitamin D status is associated with a number of chronic diseases, infections, and cancers (Peterlik & Cross, 2005).

A large number of different cells and organs in the body, such as osteoblasts, brain, gonads, immune cells, myocytes, stomach, vascular endothelial cells, skin, pancreas, and lymphocytes, have $1,25(OH)_2D$ nuclear receptors, called the vitamin D receptor (VDR) (Haussler *et al.*, 2013; Holick, 2009; Mathieu & Adorini, 2002; Wang *et al.*, 2012b). So, it is quite obvious that $1,25(OH)_2D$ has different biologic functions (Holick, 2010b).

It has been understood that vitamin D, by acting as a ligand for the receptors, stimulates biological reactions in more than 30 target tissues and can produce genomic biological responses and regulate more than 200 genes, such as those related to insulin secretion and production, renin production and cytokine release (Wilfinger *et al.*, 2014).

The rapid pathway for $1,25(OH)_2D$ action causes a diversity of biological responses within seconds to minutes. The receptor involved in this pathway is the membrane binding protein that is linked to the fast calcium absorption from the duodenum (Chang *et al.*, 2015; Nemere *et al.*, 1984). Interconnecting the $1,25(OH)_2D$ and its rapid response receptor may activate membrane receptors for peptide hormones and growth factors (Kahn *et al.*, 2006) and activate several signalling pathways, such as intracellular calcium, protein kinase C, cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAP kinase) (Norman *et al.*, 2001). Others have reported another pathway in the ligand binding area of VDR that is linked to the rapid response receptors (Mizwicki *et al.*, 2004). The genomic effect is influenced by the interaction between vitamin D and VDR and subsequent activation of steroid receptor complex in the nucleus. The interaction between vitamin D and VDR induces heterodimerization of VDR and finally leads to the enhanced gene transcription (McPhee *et al.*, 2010). The biological responses that happen through the genomic pathway involve long processes, such as gene transcription, and are not rapid (Holick, 2004b).

2.3.6 Vitamin D assessment

Vitamin D deficiency is diagnosed by assessing the circulating vitamin D level. The serum 25(OH)D level is considered the best measure of body vitamin D status and reveals the total vitamin D production from exposure to UVB irradiation and dietary

intake (Wang *et al.*, 2008). Although $1,25(OH)_2D$ concentrations show the active form of vitamin D, it is not a good measurement of vitamin D status, because it has a short half-life in serum (almost 4 hours) and its serum level is controlled and affected by the parathyroid hormone, calcium and phosphate concentrations. In contrast, serum 25(OH)D has a half-life (three weeks) and its concentration is primarily dependent on substrate concentration (Zerwekh, 2008).

2.3.7 Vitamin D deficiency criteria

It is important to note that, in the context of examining the association between vitamin D and chronic diseases, different studies have used different criteria for defining vitamin D deficiency. The reason for this is that the investigators have used different symptoms to determine the threshold for vitamin D deficiency and sufficiency, thus there is an ongoing disagreement over the optimal ranges for serum 25 (OH)D levels. The institute of medicine (IM) defined vitamin D deficiency when serum 25(OH)D levels are lower than 20 ng/ml, and vitamin D adequacy when serum 25(OH)D levels are between 20 and 50 ng/ml (Food and Nutrition Board. Institute of Medicine, 2011). Other researchers have defined insufficiency when serum 25(OH)D levels are between 20 and 30 ng/ml (Hollis & Feldman, 1997).

The optimum serum 25(OH)D level was defined based on homeostatic mechanisms, including the effects of vitamin D on bone mineral density, the increase of serum PTH level, or the synthesis of $1,25(OH)_2D$ in case of vitamin D deficiency (Lips, 2004). The most common principle used to define the optimal serum 25(OH)D level has been based on the negative relationship between vitamin D and PTH level. Under the threshold

point for serum 25(OH)D level, vitamin D supplementation decreases the serum PTH level (Lips, 2004).

In populations with vitamin D deficiency, the synthesis of $1,25(OH)_2D$ is related to the availability of the serum 25(OH)D, and therefore vitamin D supplementation resulted in an increase in serum $1,25(OH)_2D$ levels (Lips *et al.*, 1988). Further, when the baseline serum level of 25(OH)D was lower than 12 ng/ml, an increase in serum $1,25(OH)_2D$ level occurred and it was concluded that the threshold for the serum 25(OH)D level was 12 ng/ml (Lips *et al.*, 1988). Zittermann reported a different serum 25(OH)D cut-off for defining different stages of vitamin D deficiency based on different symptoms (Zittermann, 2006) (Table 2.3).

Some health experts have set the cut-off value of 20 ng/ml to define vitamin D deficiency based on the fact that a serum 25(OH)D level below this cut-off caused impaired bone health in 97.5% of the population, thereby proposing that the serum level above this cut-off value represents normality (Hollis, 2005). Others proposed that the normal range starts somewhere between 20 and 60 ng/ml and some suggested the normal serum 25(OH)D threshold level is the point (at ~32 ng/ml) when elevated serum PTH levels are seen (Lips *et al.*, 1988; Moradzadeh *et al.*, 2008). Furthermore, another bone expert group, when studying the bone density or muscle strength factors in extensive populations of normal American women or whilst studying the correlations between serum 25(OH)D levels and breast, colon, and prostate cancer, suggested that the sufficiency level should be at 40 ng/ml or even higher (Bischoff-Ferrari *et al.*, 2004; Bischoff-Ferrari *et al.*, 2006).

Based on more than 1000 studies regarding vitamin D, the IOM concluded that, since documents from non-bone related disease outcomes were inconclusive, or contradictory, vitamin D status criteria should be based on bone health findings and serum vitamin D

levels lower than 20 ng/ml, which has been suggested for deficiency (Food and Nutrition Board. Institute of Medicine, 2011).

Table 2.3: Classification of different stages of vitamin D status **(Zittermann, 2006)**

Stage of vitamin D status	25(OH)D concentrations (ng/ml)*	25(OH)D concentrations (nmol/L)*	Biochemical/clinical symptoms
Deficiency	4-10	10-25	Severe hyperparathyroidism, calcium malabsorption, rickets, osteomalacia, myopathy
Insufficiency	10-20	25–50.0	Elevated PTH levels, low intestinal calcium absorption rates, reduced bone mineral density, subclinical myopathy
Hypovitaminosis D	20-28 to 40	50–70 to100	Low body stores of vitamin D, slightly elevated PTH levels
Adequacy	28-40 to 100	70–100 to 250 **	No disturbances of vitamin D-dependent functions
Toxicity	>100	>250	Intestinal calcium hyperabsorption, hypercalcemia

*To convert nmol/L values to ng/ml, divide by 2.50

2.3.8 Risk factors of vitamin D deficiency

As there is not an adequate amount of vitamin D in the usual diet of most people (Vieth *et al.*, 2004), vitamin D production in body skin is an important way to cover the body requirement. Since the number of ultraviolet B photons absorbed by 7-dehydrocholesterol is associated with the production of vitamin D_3 in the body skin, any factor which decreases this absorption, or reduces the 7-dehydrocholesterol amount, will cause a reduction in vitamin D_3 production in the body skin.

Present human lifestyles, including indoor lifestyles, use of sunscreens and other sun prevention strategies, disturb vitamin D production in the body skin. The proper use of a sunscreen reduces the total number of UVB photons and the capacity of the skin to synthesise vitamin D_3 from 7-dehydrocholesterol by up to 99% (Holick, 1994; Matsuoka *et al.*, 1987). However, the fact that some people tan indicates that a sufficient amount of sunlight penetrates the epidermis and makes sufficient amounts of vitamin D_3 in the skin. Urbanisation and westernized lifestyle have become one of the important contributing factors for vitamin D inadequacy. It usually leads to indoor activities and is also linked to air pollution in some cities that may reduce the UVB absorption (Hosseinpanah *et al.*, 2010). Moreover, clothing style and inadequate vitamin D intake are known to affect vitamin D status (Gannagé-Yared *et al.*, 2000). For example, Turkish premenopausal women wearing traditional Islamic style clothing are reported to suffer from vitamin D deficiency (Alagöl *et al.*, 2000). Further, UVB radiation might differ by latitude, residential status (rural or urban), the length of exposure, time of exposure (season and hour) and skin pigmentation grade (Hagenau *et al.*, 2009; Webb *et al.*, 1988).

Skin pigmentation is a natural and efficient sunscreen that has evolved to protect against solar radiation. It prevents penetration of UV photons to the subcutaneous layer of the

skin. Consequently, people with darker skin produce less amount of vitamin D_3 in their epidermis (Clemens *et al.*, 1982; Hagenau *et al.*, 2009) and are, thus, required to spend 10-50 more time in sunlight in comparison with white people to produce equal vitamin D_3 (Clemens *et al.*, 1982).

Moreover, older people are particularly more susceptible to vitamin D deficiency, because of a reduction in vitamin D synthesis in the skin and also due to lower 7-dehydrocholesterol levels (MacLaughlin & Holick, 1985). As a result, 40–100% of elderly people in Europe and the United State are vitamin D deficient (Nadir *et al.*, 2010). Obesity is another important risk factor for vitamin D deficiency and it is assumed that the high quantity of subcutaneous fat sequesters vitamin D (Arunabh *et al.*, 2003). The high prevalence of vitamin D deficiency among obese and older people might also be due to lower sunlight exposure and outdoor activity (Michos *et al.*, 2010; Scragg & Camargo, 2008a).

2.3.9 Prevalence of vitamin D deficiency

Vitamin D deficiency is highly prevalent in different population groups worldwide (Moniz *et al.*, 2005) and it has now been recognised as a pandemic in the 21st century (Holick, 2010a). A study of higher socioeconomic populations in India has found that more than 85% of the individuals were moderate to severely vitamin D deficient (Gunjaliya *et al.*, 2015). In addition, the prevalence of vitamin D deficiency (serum 25(OH)D < 20 ng/mL) was 75.2% in north-western China (Zhen *et al.*, 2015). Vitamin D deficiency (<50 nmol/L) was also common in Australia (31%), a sunny country (Daly *et al.*, 2012). The prevalence was also higher in women and men living in hostels or nursing homes (80% and 70%, respectively) (Flicker *et al.*, 2003). In Kuwait, a Middle-

Eastern country, a high prevalence of vitamin D deficiency/insufficiency (61.4%) was also reported (Gaafar & Badr, 2013). In Saudi Arabia, the situation was even worse and the vitamin D deficiency prevalence was 87.8% in adults (Ardawi *et al.*, 2012). Although there are no national data in Iran regarding the prevalence of vitamin D deficiency, different small studies reported vitamin D deficiency from 26.1% (serum 25(OH)D <16 ng/ml in female and < 14.5 in male) (Bonakdaran & Varasteh, 2009) to 72.3% (serum 25(OH)D ≤ 14 ng/ml) in different population groups (Hossein-Nezhad *et al.*, 2009).

2.3.10 Vitamin D deficiency in Iran

Although Iran is a sunny country, vitamin D deficiency is highly prevalent in this region (Table 2.4). Low dietary vitamin D, indoor lifestyles, cultural clothing and veils, use of sunscreens and other sun prevention strategies, as well as the natural skin pigmentation, disturb skin vitamin D production (Hossein-Nezhad *et al.*, 2009).

A study of diabetic patients reported that the prevalence of vitamin D deficiency [serum 25(OH)D less than 16.6 ng/ml (41.5 nmol/L) in females and 14.5 ng/ml (36.25 nmol/L) in males] was 26.1% (Bonakdaran & Varasteh, 2009). Moreover, a study among pregnant women reported vitamin D deficiency (serum 25(OH)D <10 ng/ml) in 70.6% of these women (Maghbooli *et al.*, 2008). Another study of pregnant women and their newborn found that 86% of the mothers and 75% of the newborns were vitamin D deficient (25(OH)D <10 ng/ml) during winter whereas, during summer, 46% of women and 35% of newborns were vitamin D deficient (Kazemi *et al.*, 2009). The authors pointed out that a higher percentage of body fat during pregnancy and lower outdoor activity might be the cause of the high prevalence of vitamin D deficiency. Similarly, a

high prevalence (86%) of vitamin D deficiency and insufficiency was observed in school-aged children (<14.8 ng/ml) (Neyestani *et al.*, 2012).

Furthermore, in a study among the healthy adult population, the prevalence of severe and moderate vitamin D deficiency was 9.3% and 56.4%, respectively (Hashemipour *et al.*, 2006). In another survey among adults, the prevalence of mild, moderate and severe vitamin D deficiencies were 19.6%, 23.9%, and 26.9%, respectively (Hovsepian *et al.*, 2011). A recent study reported that 83.4% of adult participants had vitamin D deficiency or insufficiency (serum 25(OH)D<30ng/ml) (Shamsian *et al.*, 2016). Similarly, the prevalence of vitamin D deficiency and insufficiency (serum 25(OH)D ≤14 ng/ml) was 72.3% among healthy adults (Hossein-Nezhad *et al.*, 2009). A multi-centre study conducted in five cities in metropolitan areas showed that vitamin D deficiency (serum 25(OH)D <10 ng/ml) was 44.2%-47.2% for different age groups among men and 37.5%-54.2% among women (Heshmat *et al.*, 2008). Other studies also reported a high prevalence of vitamin D deficiency or insufficiency among the Iranian population (Alipour *et al.*, 2014; Bonakdaran *et al.*, 2016; Ebrahimi *et al.*, 2014). It is evident from the above studies that there is a wide variation (range 26%-86%) in the reported prevalence of vitamin D deficiency in Iran. These variations in prevalence were due to the differences in population groups, sample size and cut-offs used to define vitamin D deficiency, which is evident in Table 2.4.

Table 2.4: prevalence of vitamin D deficiency in Iran

study	Type of study and subjects	Method	Output
Shamsian *et al.* 2016	cross-sectional study 1110 patents referred to city laboratories	< 20 ng/ml as vitamin D deficiency 20–30 ng/ml as vitamin D insufficiency	Deficiency 68.8% and insufficiency 14.6%
Bonakdaran *et al.* 2016	Cross-sectional, 846 subjects	< 20 ng/ml as vitamin D deficiency	vitamin D deficiency was 80.7% and 79.0% in subjects with or without MetS
Alipour et al. 2014	cross-sectional study, 538 women	Vitamin D deficiency less than 35 nmol/L	prevalence of vitamin D deficiency was 69%
Ebrahimi *et al.* 2014	cross-sectional study, 1047 students	Vitamin D deficiency: <15 ng/mL Insufficiency: 15-20 ng/mL	Prevalence of vitamin D deficiency and insufficiency were 61.2% and 18.9%, respectively (sex and age-adjusted)
Ziaee *et al.* 2013	cross-sectional study	Dietary vitamin D intake	90-95% of participants received less than the minimum daily recommended
Hovsepian *et al.,* 2011	cross-sectional 1,111 healthy adult	vitamin D deficiencies Mild (20-30 ng/ml,) moderate (10-20 ng/ml) severe (<10 ng/ml)	The prevalence of mild, moderate and severe vitamin D deficiencies was 19.6%, 23.9%, and 26.9%, respectively, among the adult population
Neyestani *et al.,* 2012	cross-sectional 1111 children aged 9–12 years	Severe deficiency (vit D $\leq$ 5) Moderate deficiency (5<vit D $\leq$ 10) Mild deficiency (10<vitamin D $\leq$ 14.8) Normal (vit D $\geq$14.8) ng/ml	Approximately 86% of the children had vitamin D deficiency, with 38.3% being severely deficient and 25% moderate deficient. Prevalence of vitamin D deficiency was higher in girls than in boys
Bonakdaran and Varasteh, 2009	cross-sectional 119 type 2 diabetic patients	deficiency vit D <16.6 ng/ml in female vit D <14.5 ng/ml in male	The prevalence of vitamin D deficiency was 26.1% among the diabetic patients.

Table 2.4 Continued …

Hossein-Nezhad et al., 2009	cross-sectional 646 healthy population aged from 20-79 years	deficiency (vit D ≤14 ng/ml)	Prevalence of vitamin D deficiency was 72.3%
Kazemi et al., 2009	Cross-sectional 67 full-term pregnant mothers	Hypovitaminosis (vit D <10 ng/ml)	Hypovitaminosis D prevalence was 86% in the women and 75% in the newborns during winter and 46% in the mothers and 35% in the newborns during summer
Heshmat et al., 2008	random cluster sample of healthy adults (ranged 20 to 69 years old) 5232 subjects from five urban metropolitan cities	Severe deficiency (vit D ≤ 5) Moderate deficiency (5<vit D ≤ 10) Mild deficiency (10<vitamin D ≤ 14) Normal (14.2<vit D ≤ 60) ng/ml	The prevalence of moderate to severe vitamin D deficiency was 47.2%, 45.7% and 44.2% in age groups of <50, 50-60 and 60≤ years, respectively, among men, and among women in the same age groups it was 54.2%, 41.2% and 37.5%, respectively.
Maghbooli et al., 2008	cross-sectional 741 pregnant women	deficiency (vit D <10 ng/ml) severe deficiency (vit D <5 ng/ml)	Vitamin D deficiency was found in 70.6% of pregnant women
Hashemipour et al., 2006	cross-sectional 1210 adult	deficiency (vit D ≤5 ng/ml) moderate deficiency (vit D > 5 ng/ml but vit D < 10 ng/ml)	The prevalence of severe and moderate vitamin D deficiency was 9.3% and 56.4%, respectively.

2.4 Vitamin D and diseases

Previous studies observed that people living at higher latitudes, who have the lowest exposure to sunlight, had a higher risk of many chronic diseases such as hypertension (Rostand, 1997), multiple sclerosis (Hernán *et al.*, 1999) and common cancers (Ahonen *et al.*, 2000; Grant, 2002) and of dying of common cancer (Apperly, 1941). Recent studies have found that the risk of all-cause and cardiovascular-related mortality was higher in people with the minimum amount of serum 25(OH)D (Dobnig *et al.*, 2008; Melamed *et al.*, 2008; Zittermann *et al.*, 2012). Its deficiency has also been found to be associated with diabetes, hypertension, atherosclerosis, congestive heart failure, myocardial infarction, cardiovascular risk, stroke, and kidney dysfunction (Chiu *et al.*, 2004; Chonchol & Scragg, 2006; Cigolini *et al.*, 2006; de Boer *et al.*, 2007; Holick, 2007; Kendrick *et al.*, 2009; Martins *et al.*, 2007; Scragg *et al.*, 2004, 2007; Targher *et al.*, 2006; Wang *et al.*, 2008; Zittermann *et al.*, 2003).

2.4.1 Vitamin D and MetS

The association between vitamin D deficiency and MetS has been studied previously using different study designs, sample sizes, and covariates. Several studies in different populations have documented an inverse association between serum 25(OH)D concentration and the prevalence of MetS, and indicated that inadequate vitamin D intake and decreased serum vitamin D level was implicated as a MetS risk factor (Botella-Carretero *et al.*, 2007; Cheng *et al.*, 2010; Chiu *et al.*, 2000; Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Lu *et al.*, 2009; Pereira *et al.*, 2002). However, there are other studies which did not find any significant association between vitamin D levels and MetS (Bonakdaran & Varasteh, 2009; Liu *et al.*, 2009; Reis *et al.*, 2007; Rueda *et al.*, 2008; Tai *et al.*, 2008).

The majority of the available literature regarding vitamin D and MetS came from cross-sectional studies which examined the association on one specific occasion. For instance, a population-based cross-sectional study among Chinese individuals (aged 50-70 years) found that lower levels of serum 25(OH)D were associated with a higher risk of MetS after controlling the confounders (Lu *et al.*, 2009). They also reported that this association was stronger in obese and overweight people. Hyppönen *et al.* (2008), basing their study on British Birth Cohort data (age 45 years) reported that a lower serum vitamin D level was related to MetS, as well as some of its components, including abdominal obesity, glucose, blood pressure, and triglycerides, but not HDL cholesterol. Other studies also reported that serum 25(OH)D levels might be an independent risk factor of MetS (Ford *et al.*, 2005; Kim, 2015; Reis *et al.*, 2008). On the contrary, an investigation on obese patients reported a significant association between vitamin D deficiency and MetS [0.42 (95% CI: 0.19, 0.96)], however, after adjustment for age, sex, fat mass and season of sampling, this association disappeared [0.63 (95% CI: 0.26, 1.52)] (Rueda *et al.*, 2008). Reis *et al.* (2007) in a cross-sectional study of 1070 participants, 44–96 years of age, also failed to find any association between serum 25(OH)D concentration and MetS in both sexes [2.02 (95% CI: 0.96, 4.24)]. Similarly, an analysis of Korean National Health and Nutrition Examination Surveys in post-menopausal women did not find any significant associations between serum 25(OH)D levels and the prevalence of MetS [0.96 (95% CI: 0.69–1.34)] (Kim *et al.*, 2012). This study adjusted for age, sex, education level, income, alcohol consumption, smoking, physical activity, season, vitamin supplementation, and residential district. A similar result was also evident in women's health study data [1.05 (95% CI: 0.84, 1.32), derived from 10,066 middle-aged US women, aged ≥45 years, who were free of diabetes, cardiovascular disease or cancer, and had not used post-menopausal hormones (Liu *et al.*, 2005). This study adjusted

the association for smoking, exercise, total calories, alcohol, multivitamin, and parental history of myocardial infarction.

The inferences drawn from cross-sectional studies are generally weak and are less reliable compared to results of longitudinal studies. However, results from available longitudinal studies are also inconsistent. A study in Australia observed 4164 adults (25 years and older and free of MetS at baseline) from 42 randomly selected districts for 5 years (Gagnon *et al.*, 2012) and showed that lower levels of serum 25(OH)D [the first (<18 ng/ml) and second (18–23 ng/ml) quintiles] were related to an increased risk of MetS after adjusting for a large number of confounding variables, except for dietary intake and PTH. As unhealthy diet and higher levels of PTH increase the risk of MetS, adding them to the model may attenuate the risk of MetS. Vitezova *et al.* (2015) in a longitudinal study of adults in the Netherlands, aged 55 years or older at baseline, revealed that higher 25(OH)D levels were associated with a lower prevalence of MetS and some of its components, including HDL-C, TG, WC, and serum glucose. Similarly, Kayaniyil *et al.* (2014) undertook a three-year follow-up of adults without MetS and diabetes at baseline in Canada which indicated an independent and inverse association between baseline 25(OH)D levels and the incidence of MetS. However, this study only reported on the association between serum vitamin D levels and fasting serum glucose, but not with other MetS components. In contrast, another study of Australian adults found that serum vitamin D levels were not associated with the risk of MetS at the five-year follow-up, after adjustment for baseline insulin resistance (Gagnon *et al.*, 2012). Forouhi *et al.* (2008) in another longitudinal study, after a 10-year follow-up, demonstrated that baseline 25(OH)D levels are not associated with MetS incidence, after multivariate adjustment. A nested case-control study on 324 pairs of Iranians aged 20-years and older with no MetS at baseline (matched for sex, age, duration of follow-up, and month of entry), and followed for a

mean duration of 6.8 years, was also unable to provide adequate evidence for the association between vitamin D and the incidence of MetS (Amirbaigloo *et al.*, 2013).

An intervention study with dietary vitamin D or sun exposure reported a decrease in the prevalence of MetS and an improvement in HDL cholesterol after 6 and 12 months (Al-Daghri *et al.*, 2012). However, the intervention could not resolve vitamin D insufficiency and the study did not have a control group. Finally, a recent meta-analysis of vitamin D deficiency and MetS, which analysed the results of 18 studies, concluded that MetS is inversely associated with serum vitamin D levels only in cross-sectional studies [0.87 (95% CI: 0.83, 0.92)] but not in cohort studies [1.00 (95% CI: 0.98, 1.02)] (Ju *et al.*, 2013).

In conclusion, while several studies have found an association between vitamin D status and MetS or its components (Al-Daghri *et al.*, 2012; Botella-Carretero *et al.*, 2007; Boucher *et al.*, 1995; Chiu *et al.*, 2004; Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Lind *et al.*, 1995; Liu *et al.*, 2009; Maki *et al.*, 2009; Martini & Wood, 2006; Parker *et al.*, 2010; Reis *et al.*, 2008; Scragg *et al.*, 2004; Smotkin-Tangorra *et al.*, 2007) and found that vitamin D deficiency could be an independent risk factor for MetS (Boucher, 1998; Chiu *et al.*, 2004; Lind *et al.*, 1995), not all of these studies have taken into account all potential confounders. On the other hand, there are a number of studies that could not demonstrate any relationship between vitamin D status and MetS (Bonakdaran & Varasteh, 2009; Chon *et al.*, 2014; Melamed *et al.*, 2008; Reis *et al.*, 2007; Rueda *et al.*, 2008; Tai *et al.*, 2008) or, if using multivariate models, the association was not statistically significant (Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Liu *et al.*, 2009). The discrepancy in findings might also be the result of using different categories of vitamin D deficiency, different definitions of MetS and the effect of other potential confounders. Clearly, based on the current literature, to properly understand the association between vitamin D concentration and MetS, more needs to be learned about the association between this vitamin and the components of MetS.

2.4.2 Vitamin D and abnormal glucose metabolism (Diabetes, Insulin deficiency, and Insulin resistance)

It has been hypothesised that inadequate glycemic control in the winter months might be due to lower vitamin D production in the skin and lower circulating vitamin D concentrations (Campbell *et al.*, 1975; Ishii *et al.*, 2001). It is suggested that 25-hyroxyvitamin D concentration is associated with systemic inflammation, insulin secretion, beta-cell function and insulin sensitivity (Kayaniyil *et al.*, 2010) and that it might play a role in diabetes improvement through the effect on intracellular calcium (Pittas *et al.*, 2007b). Inflammatory factors are related to β-cell dysfunction and insulin resistance (Pittas *et al.*, 2007b), and it has been reported that vitamin D down-regulates the production of some cytokines (Ozfirat & Chowdhury, 2010; Pearce & Cheetham, 2010). Vitamin D may also play a role in β-cell function, which seems to be applied via binding of its active form to VDR in pancreatic β-cells (Johnson *et al.*, 1994). Moreover, insulin secretion is related to calcium levels (Milner & Hales, 1967), therefore vitamin D may affect pancreatic β-cells and insulin secretion through the regulation of intracellular calcium (Fujita *et al.*, 1978). Calcium is also necessary for insulin function in insulin-responsive tissues (Wright *et al.*, 2004), thus, vitamin D may also affect insulin sensitivity. Moreover, vitamin D deficiency may be associated with insulin resistance through increasing body fat, PTH levels and also through the renin angiotensin system (Reis *et al.*, 2007). However, most of these mechanisms have been proven in animal models but not in humans.

Several cross-sectional studies have demonstrated an association between vitamin D status and glucose metabolism. For example, in a study on Americans (n=6288), a negative association was found between serum 25(OH)D concentration and glucose level and diabetes risk, even after controlling for some confounders (Scragg *et al.*, 2004). Other studies also reported the association between low serum concentration of vitamin D and insulin

resistance, diabetes and impaired glucose level (Liu *et al.*, 2009; Mattila *et al.*, 2007; Scragg *et al.*, 1995a). Moreover, different analyses of the National Health and Nutrition Examination Survey (NHANES) data on 18825 adult participants, aged ≥20 years, showed an inverse association between serum 25-hydroxyvitamin D concentration and the presence of diabetes (Martins *et al.*, 2007; Scragg *et al.*, 2004), but this was observed only in non-Hispanic whites but not in blacks (Scragg *et al.*, 2004). On the other hand, Reis *et al.* (2008) study on non-diabetic individuals, using the same NHANES dataset, could not find any association between vitamin D and glucose level after adjustment for potential confounders. Other cross-sectional studies also did not find any associations between serum 25(OH)D levels and blood glucose, insulin levels and insulin functions (Gannagé-Yared *et al.*, 2009; Gulseth *et al.*, 2010).

Longitudinal and intervention studies also reported diverse results. In a cohort study on subjects aged ≥30 years and free of diabetes (PROMISE), insulin sensitivity and β-cell function were associated with serum 25(OH)D concentration, even after controlling for potential confounders (Kayaniyil *et al.*, 2010). In other longitudinal studies in Australia, the US and UK, serum 25(OH)D levels were inversely associated with serum glucose, insulin resistance, and incidence of diabetes mellitus (Forouhi *et al.*, 2008; Need *et al.*, 2005; Vacek *et al.*, 2012). The Australian Diabetes, Obesity, and Lifestyle Study, after a 5 year follow-up, also found an independent and inverse association between serum 25(OH)D and fasting glucose, but not with 2h plasma glucose (Gagnon *et al.*, 2012). On the contrary, the Women's Health Study of 39,876 women working as health professionals, aged ≥45 years, (controlled for potential confounders) could not find any association between the incidence of type 2 diabetes and dietary vitamin D intake (Liu *et al.*, 2005). Wareham *et al.* (1997), in a cohort of 40-65 year old participants in England, also reported no association between IGT and serum vitamin D status. Further, the Nurse's Health study in 83,000 women with no history of

diabetes, cancer and CVD, found no association between the intake of vitamin D and diabetes (Pittas *et al.*, 2006). Similarly, a recent meta-analysis of prospective cohort studies showed no association between vitamin D intake and type 2 diabetes (Zhao *et al.*, 2014).

On the other hand, a double-blind, randomized, controlled trial, which studied 314 Caucasian adults without diabetes, revealed that a daily intake of 700 IU vitamin D plus 500 mg calcium for three years may lessen the increases in glycaemia and insulin resistance (Pittas *et al.*, 2007a). Similarly, using three doses of 120,000 IU vitamin D improved postprandial insulin sensitivity (Nagpal *et al.*, 2009). von Hurst *et al.* (2010), in a study in New Zealand using daily 4,000 IU vitamin D for 6 months, reported improvement in insulin resistance and sensitivity, but no change in insulin secretion. On the contrary, a double-blind placebo-controlled study used 40,000 IU vitamin D supplementation per week for one year on overweight or obese subjects, 21–70 years old, did not support a positive effect of vitamin D supplementation on glucose tolerance after adjustment for confounders (Jorde *et al.*, 2010a). Moreover, the Women Health Initiative study on postmenopausal women aged 50–79 years after a median follow-up time of 7 years showed that daily intake of 400 IU vitamin D did not reduce the risk of developing diabetes (de Boer *et al.*, 2008). In the same way, weekly 40,000 IU cholecalciferol supplementation for 6 months in 36 European type 2 diabetics aged 21-75 years did not significantly change fasting glucose, insulin, and Hb_{A1C} (Jorde & Figenschau, 2009). Other interventional studies also found no effect of vitamin D supplementation on blood glucose or insulin concentrations or insulin sensitivity (Borissova *et al.*, 2003; Tai *et al.*, 2008). It has been deduced that vitamin D supplementation could be effective mostly in patients with low vitamin D levels and impaired glucose metabolism (Borissova *et al.*, 2003; Isaia *et al.*, 2001).

The inconsistencies of the results from the different studies on the association between vitamin D and glucose homeostasis could be due to different participant characteristics,

including age group, having glucose intolerance or diabetes, using serum vitamin D and dietary intake or different doses of supplementation, VDR polymorphisms or ethnic differences (Malecki *et al.*, 2003). Moreover, previous studies did not consider the potential confounders such as dietary factors. They also assessed this association based on different categories for vitamin D deficiency, which might also be a possible reason for the inconsistencies.

2.4.3 Vitamin D and Lipid profiles

Some studies have shown that individuals with high levels of serum vitamin D have more favourable lipid profiles in comparison with individuals with low serum vitamin D levels (Chaudhuri *et al.*, 2013; Ponda *et al.*, 2012). On the other hand, low levels of vitamin D have been found in some individuals with increased risk of cardiovascular disease and dyslipidaemia (John *et al.*, 2005). The actual mechanisms by which vitamin D may affect lipid profiles is not clear, however, it has been suggested that the inverse association between serum vitamin D concentrations and lipid profiles is via the reduction in the intestinal absorption of dietary lipids as well as a decrease in lipid synthesis with increasing levels of serum vitamin D. Moreover, vitamin D may control cholesterol levels by modifying the synthesis of bile acid from cholesterol through VDR at a genetic level (Gonzalez & Moschetta, 2014). Vitamin D is also suggested to have a role in the reduction of LDL-C as a result of binding LDL-C to 25(OH)D (Teramoto *et al.*, 1995).

Results of studies considering the association between vitamin D and lipid profiles are inconsistent; moreover, these associations were not the same for all of the evaluated lipid profiles. For instance, vitamin D insufficiency was associated with decreased HDL-C in a cross-sectional study on 217 obese individuals (Smotkin-Tangorra *et al.*, 2007). *Hyppönen et*

al. (2008), in a study on Europeans, found a negative relationship between circulating 25(OH)D levels and serum triglyceride after controlling for the potential confounders. However, they could not find any association between vitamin D levels and HDL cholesterol. Similarly, NHANES III study on 15,088 individuals, aged ≥20 years, found that lower serum 25(OH)D levels were associated with a greater prevalence of hypertriglyceridemia (Martins *et al.*, 2007). However, another study using the same database, after adjusting for more confounders including calcium, energy intake, and PTH, did not find any association (Reis *et al.*, 2008). Chiu *et al.* (2004) investigated 126 healthy, glucose-tolerant subjects living in California and found an inverse association of vitamin D levels with LDL and total cholesterol but not with triglyceride and HDL cholesterol. They also reported that none of the participants was vitamin D deficient. Moreover, a study on healthy British Bangladeshi adults aged 35−65 years, found an independent relationship between higher levels of serum vitamin D and Apo A-I concentrations, but not for cholesterol, triglyceride, LDL, HDL and Apo B (John *et al.*, 2005). An additional study on 108,711 Americans, using the baseline variables serum vitamin D level, was negatively associated with total cholesterol, LDL, and triglyceride, while positively associated with HDL; however, a longitudinal analysis of this study found that vitamin D deficiency was associated with a mean increase in both total and HDL cholesterol but not with LDL cholesterol and triglyceride (Ponda *et al.*, 2012). Serum 25(OH)D levels were also found not to be independently associated with serum lipids in a population-based study among 1,203 Chinese, older than 52 years (Chen *et al.*, 2014). Likewise, other cross-sectional studies could not find an independent association between serum vitamin D level and lipid profiles (Gannagé-Yared *et al.*, 2009; Rueda *et al.*, 2008).

In addition, a retrospective study on 10,800 US participants found an association between vitamin D deficiency and hyperlipidaemia (Vacek *et al.*, 2012). Results from a five-year follow up based on the Australian Diabetes, Obesity, and Lifestyle Study also showed that

serum 25(OH)D levels were independently and inversely associated with triglycerides, but not with HDL cholesterol (Gagnon *et al.*, 2012). Similarly, in a prospective study on 142 Dutchmen aged 70-88 years, HDL-cholesterol was not related to serum 25(OH)D concentration (Baynes *et al.*, 1997). Al-Daghri *et al.* (2012) in a 12-month prospective study on 59 non-diabetic, overweight and obese adults showed that HDL cholesterol was significantly lower among patients with vitamin D deficiency; moreover, vitamin D supplementation or sun exposure increased HDL and decreased high triglyceride prevalence. Furthermore, a double-blind study on healthy, overweight individuals revealed that vitamin D supplementation (3320 IU/d for 12 months) decreased triglyceride levels but increased LDL cholesterol levels (Zittermann *et al.*, 2009). On the contrary, daily vitamin D plus calcium supplementation (300 IU vitamin D_3 and 500 mg calcium) for 12 months in post-menopausal women could not change HDL, LDL and total cholesterol levels (Heikkinen *et al.*, 1997). Similarly, vitamin D and calcium supplementation (1000 mg of calcium and 800 IU of vitamin D_3 for two years) (Daly & Nowson, 2009) or UV radiation (twice weekly for 12 weeks) (Carbone *et al.*, 2008) had no effect on plasma lipids or lipoprotein concentration.

Overall, although a large majority of the observational studies reported an association between a high vitamin D level and lipid profiles, a good number of studies failed to demonstrate such an association. In addition, this association has not been proven in interventional studies. Further, a systematic review and meta-analysis of randomized-controlled trials regarding the effect of vitamin D supplementation on plasma lipid profiles revealed that vitamin D supplementation increases LDL cholesterol levels, while it does not have any significant effect on triglycerides, HDL and total cholesterol (Wang *et al.*, 2012a). Corresponding changes for LDL, triglycerides, HDL-C, and total cholesterol, were 3.23 mg/dl (95% CI: 0.55, 5.90), -1.92 mg/dl (95% CI: -7.72, 3.88), -0.14 mg/dl (95% CI: -0.99, 0.71) and 1.52 mg/dl (95% CI: -1.42, 4.46), respectively. A recent review article, which also

reviewed the studies regarding the association between vitamin D and lipid profiles, indicated that there is a lack of sufficient evidence to reach a firm conclusion on the association between vitamin D status and lipid profiles (Challoumas, 2014). Based on the reported studies, it appears that the associations between vitamin D status and lipid profiles varied based on different population groups, levels of various lipids and lipoproteins, study designs and serum vitamin D levels. In addition, consideration of potential confounders, while assessing the association between vitamin D status and lipid profiles, appears to be important.

2.4.4 Vitamin D and Blood pressure

Epidemiological studies have exhibited a higher prevalence of hypertension in the higher latitudes and higher blood pressure in the winter months (Nadir *et al.*, 2010; Woodhouse *et al.*, 1993). Moreover, primary hypertension is related to low blood levels of ionized calcium (Mccarron *et al.*, 1987) and higher PTH levels (Jorde *et al.*, 2005). Thus, researchers suggested that the vitamin D level might be associated with blood pressure (Michos *et al.*, 2010). There are some hypotheses which linked vitamin D deficiency with hypertension. First, vitamin D may affect blood pressure through its negative association with the renin-angiotensin system (Vaidya & Williams, 2012). However, there is other evidence suggesting that the effect of vitamin D on the reduction of blood pressure in hypertensive animals might be independent of renin levels. It has been proposed that vitamin D deficiency, via an increase in PTH, may contribute to the development of hypertension (Chen *et al.*, 2015). Moreover, vitamin D may affect blood pressure through vascular smooth muscle and vascular endothelial function (Chen *et al.*, 2010). It has been shown that vitamin D controls the proliferation of endothelial cells and vascular smooth muscles (Mitsuhashi *et al.*, 1991). Moreover, VDR and $1,25(OH)_2D$ in vascular smooth muscle and endothelial cells control the

effects of inflammatory cytokines on the vascular system (Molinari *et al.*, 2011). Although the above-mentioned mechanisms have been verified in animal models, questions still remain about their validity for humans and there is discrepancy among human studies which have investigated the association between vitamin D and blood pressure.

Cross-sectional data from NHANES III indicated that higher levels of serum 25(OH)D were associated with lower systolic and diastolic blood pressure and lower prevalence of blood pressure (Martins *et al.*, 2007; Scragg *et al.*, 2007). Similarly, in an investigation on 6,810 British aged from 44-46 years, low serum vitamin D levels were associated with high blood pressure (Hyppönen *et al.*, 2008). Rueda *et al.'s* (2008) investigation on severely obese patients (mean BMI = 46.7±5.3 kg/m2) reported that individuals in the highest quartile of serum 25(OH)D level were less likely to have high blood pressure, but this association disappeared after adjustment for potential confounders. On the other hand, in another observational study on healthy subjects, serum vitamin D level was not associated with blood pressure, although the subjects were not vitamin D deficient (Chiu *et al.*, 2004). Other studies on non-obese university students (Gannagé-Yared *et al.*, 2009), New Zealand residents (Scragg *et al.*, 1995b) and different analysis of NHANES (Ford *et al.*, 2005), also found no correlation between vitamin D status and blood pressure.

In addition, a retrospective study on 10,800 US adults also showed an association between vitamin D deficiency (25(OH)D <30 ng/ml) and hypertension (Vacek *et al.*, 2012). Moreover, a nested case-control from the Nurses' Health Study on non-obese, non-hypotensive women at baseline reported an inverse and independent association between serum vitamin D level and the risk of developing hypertension (Forman *et al.*, 2008). An inverse association between baseline serum 25(OH)D level and hypertension was also reported in other prospective studies (Al-Daghri *et al.*, 2012; Forman *et al.*, 2007; Forouhi *et*

al., 2008). On the contrary, the Australian Diabetes, Obesity, and Lifestyle Study could not find any association after a 5-year follow-up (Gagnon *et al.*, 2012).

UV irradiation in patients with untreated mild hypertension for three months increased serum vitamin D concentrations and significantly decreased systolic and diastolic blood pressure (6mm Hg reduction) (Krause *et al.*, 1998). Moreover, eight weeks supplementation with vitamin D (800 IU/d) and calcium (1200 mg/d) in elderly hypovitaminosis women caused a decrease in PTH and decrease in systolic blood pressure (7 mmHg) and heart rate in comparison with calcium supplementation alone (Pfeifer *et al.*, 2001). However, supplementation with 2,800 IU of vitamin D per day in hypertensive patients with low 25-hydroxyvitamin D for eight weeks had no significant effect on blood pressure (Pilz *et al.*, 2015). Rolf Jorde *et al.* (2010), also in a double-blind placebo-controlled study in northern Norway on overweight or obese subjects, aged from 21–70 years, found that vitamin D supplementation (40,000 IU per week) for one year had no effect on blood pressure. In this study, all of the participants were given calcium supplementation. Daily supplementation of 1000 mg of calcium and 400 IU of vitamin D_3 for seven years in post-menopausal women also did not decrease either blood pressure or the risk of developing hypertension (Margolis *et al.*, 2008). Daly *et al.* (2009) also failed to find any association between two years of vitamin D supplementation (1000 mg Ca and 800 IU vitamin D per day) and blood pressure in obese men older than 50 years.

A meta-analysis on vitamin D supplementation found weak evidence for the beneficial effect of vitamin D on blood pressure (significant decrease in diastolic blood pressure in hypertensive individuals but not a significant fall in systolic blood pressure). They also reported a small reduction in patients with hypertension, but not in individuals without hypertension (Witham *et al.*, 2009). Finally a recent meta-analysis of randomized placebo-

controlled clinical trials indicated that vitamin D supplementation, for a minimum of four weeks, did not have any effect on systolic or diastolic blood pressure (Beveridge *et al.*, 2015). In conclusion, inconsistent data do not allow us to confirm, or refute, the association between vitamin D and blood pressure. Study designs, serum vitamin D levels, doses of vitamin D supplementation, the presence of hypertension and its diagnosis, the studied population and controlled covariate are some of the possible reasons for these results. It is also important to note that blood pressure is affected by several factors, such as obesity, dietary intake, salt intake, physical activity, body homeostasis and genetics; therefore, more carefully designed studies are needed to determine the independent effect of vitamin D status on blood pressure.

2.4.5 Vitamin D and Obesity

Vitamin D deficiency or insufficiency is common in obese individuals (Bell *et al.*, 1985; Compston *et al.*, 1981) and several studies have confirmed the association between adiposity and serum 25(OH)D levels (Earthman *et al.*, 2011). Other studies have confirmed the link between serum vitamin D concentrations and lipid storage and adipose tissue and found the VDR in adipocytes (Kamei *et al.*, 1993). These findings suggest the possible beneficial effect of vitamin D supplementation on these metabolic abnormalities. Several mechanisms have been suggested to explain the association between vitamin D deficiency and obesity. First, it is likely that obese individuals have low social and outdoor activities; moreover, they might wear more clothes to hide the obesity which may reduce their sun exposure and vitamin D synthesis in their body. However, the Framingham study adjusted for outdoor activity but still found an association between vitamin D deficiency and adiposity, which may reject this hypothesis (Cheng *et al.*, 2010). Additionally, the association between lower levels of serum vitamin D and obesity might be due to deposition of vitamin D in body fat tissues, which

decreases its bioavailability and transportation in the body (Wortsman *et al.*, 2000). Similarly, it has been reported that after UV irradiation, the increase in the circulating concentration of vitamin D is lower in obese individuals compared to normal-weight individuals (Wortsman *et al.*, 2000). Alternatively, it has been proposed that obesity could be a consequence of vitamin D deficiency through increasing PTH and intracellular calcium, thus increasing lipogenesis (Wood, 2008). It has also been proposed that vitamin D via VDR functions could inhibit lipogenesis (Martini & Wood, 2006).

Cross-sectional studies found that BMI and body fat mass were negatively related to vitamin D status, and vitamin D levels were significantly lower in obese individuals (Maki *et al.*, 2009; Parikh *et al.*, 2004). Moreover, in the Framingham study, BMI and WC were negatively related to vitamin D levels (Liu *et al.*, 2009). A recently published cross-sectional study also revealed the association between serum vitamin D concentration and abdominal obesity and showed that vitamin D levels were strongly associated with adiposity phenotypes (Zhang *et al.*, 2013). It has also been reported that higher body weights were independently associated with lower vitamin D status (Weishaar *et al.*, 2016). Other studies also confirmed this association (Hyppönen *et al.*, 2008; Kremer *et al.*, 2009; Liel *et al.*, 1988; Liu *et al.*, 2005).

Moreover, in the NHANES III study the prevalence of obesity was higher in the lower quartiles of vitamin D level (Martins *et al.*, 2007). In addition, this study showed a significant inverse association between serum 25(OH)D concentration and abdominal adiposity. However, another analysis on this data reported that the relationship between body weight and vitamin D levels is not consistent across different ethnic groups and reported that a low level of vitamin D was associated with higher BMI in white women, but not in African American women (Nesby-O'Dell *et al.*, 2002). Scragg *et al.* (1995), in their study in New Zealand, also found that serum level of 25(OH)D was unrelated to BMI. A large cross-

sectional and cohort study of serum vitamin D and obesity based on 25,616 adults aged 19–55 years indicated that vitamin D deficiency (less than 500 nmol/L) independently increased the odds ratio for the incidence and prevalence of obesity (Mai *et al.*, 2012). The same study also reported that vitamin D adequacy was related to less weight gain. Another longitudinal study in Australia also found an inverse association between serum 25(OH)D levels and WC (Gagnon *et al.*, 2012). Moreover, 12 weeks of vitamin D supplementation decreased abdominal fat in vitamin D deficient elderly women (Kim *et al.*, 2014). However, vitamin D supplementation (3,332 IU/day) for 12 months had no significant effect on weight loss (Zittermann *et al.*, 2009). In a recently published randomized double-blind clinical trial, vitamin D supplementation also had no significant effect on body weight, fat mass or WC (Sadiya *et al.*, 2016).

Results of systematic reviews were also equivocal. For example, a recently published systematic review and meta-analysis of observational studies revealed that vitamin D deficiency was independently associated with obesity (Pereira-Santos *et al.*, 2015). On the contrary, Pathak *et al.* (2014), in their meta-analysis of randomized control trials, indicated that vitamin D supplementation, without calorie restriction, has a small effect on BMI [−0.139 (95% CI: −0.322, 0.043)] and could not reduce body fat and adiposity [−0.262 (95% CI: −0.940, 0.417)]. The later review also concluded that studies on older populations were less likely to find any association; moreover, a higher percentage of women reflected the positive effect of vitamin D on weight loss. Different ethnicity, age, sex, physical activity, dietary intake, genetics, sample size and other confounder factors may be responsible for these inconsistencies.

In conclusion, although a possible association between vitamin D deficiency and obesity has been reported by previous studies, they could not demonstrate causality. Moreover, intervention studies failed to demonstrate any positive effects from vitamin D

supplementation. However, the effect of confounders such as age, sex, ethnicity, physical activity and dietary intake in this association is undeniable.

2.4.6 Studies regarding vitamin D deficiency and MetS or its components in Iran

Different studies in Iran also found different results while examining the association between serum vitamin D and MetS or its components (Table 2.5). Some studies reported a positive association between vitamin D and MetS (Bonakdaran & Varasteh, 2009; Ghanei *et al.*, 2014; Goshayeshi *et al.*, 2012; Hossein-Nezhad *et al.*, 2009); however, others did not find any association (Amirbaigloo *et al.*, 2013; Bonakdaran *et al.*, 2016). An association between the components of MetS and vitamin D was reported in some (Ahmadi *et al.*, 2015; Asemi *et al.*, 2013a; Asemi *et al.*, 2013b; Hosseinpanah *et al.*, 2011; Maghbooli *et al.*, 2008; Nikooyeh *et al.*, 2011; Salehpour *et al.*, 2012; Shab-Bidar *et al.*, 2011a; Shab-Bidar *et al.*, 2012), but not in all of the studies (Ardabili *et al.*, 2012; Bonakdaran *et al.*, 2016; Bonakdaran *et al.*, 2010; Kaviani *et al.*, 2012; Mozaffari-Khosravi *et al.*, 2012; Rafraf *et al.*, 2014).

A cross-sectional study on 120 Rheumatoid arthritis patients reported a protective role for vitamin D against MetS (defined by ATP III criteria). However, they acknowledged that uncontrolled confounders may affect the association between serum 25(OH)D and MetS (Goshayeshi *et al.*, 2012). Similarly, in another cross-sectional study on 119 type 2 diabetic patients, a higher percentage of MetS (defined according to the ATP III criteria) was reported in vitamin D deficient subjects (Bonakdaran & Varasteh, 2009). In this study, vitamin D deficiency was defined as serum 25(OH)D <16.6 ng/ml in females and serum 25(OH)D <14.5 ng/ml in males. Ghanei *et al.* (2014), in their cross-sectional study on 250 subjects (aged >20 years), reported a high prevalence of vitamin D deficiency among participants (98.4% of subjects with MetS and 88.3% of those without MetS). The same study also

reported that serum vitamin D concentrations were significantly lower in those individuals with MetS (defined according to the ATP III criteria). These results were also confirmed by another study on 646 healthy individuals which found a significant association between vitamin D deficiency (serum 25(OH) D < 14 ng/ml) and MetS (defined according to WHO criteria). Moreover, vitamin D deficiency had a significant relationship with obesity and was also associated with hypertension in women only (Hossein-Nezhad *et al.*, 2009).

On the contrary, Bonakdaran *et al.* (2016), in a cross-sectional study on 846 healthy adults, found no significant difference in serum vitamin D level between individuals with, or without, MetS (MetS was defined using IDF criteria). In this study, vitamin D deficiency (25(OH)D <20 ng/ml) was very common in participants (80.7% and 79.0% of individuals with or without MetS, respectively). Salekzamani *et al.* (2010) also could not find any significant difference in serum 25(OH)D levels (serum 25(OH)D ≤11 ng/ml, 11-20 ng/ml and ≥20 ng/ml) between individuals with, and without, MetS (defined according to the ATP III criteria) after controlling for some covariates. An additional study on 216 girls (aged 14–17 years) reported vitamin D deficiency (25(OH)D <20 ng/ml) in 96% of participants (Rafraf *et al.*, 2014), with a higher mean serum vitamin D level in individuals with MetS (defined by ATP III criteria). However, after controlling for some confounders, an inverse association was found only between serum vitamin D concentrations and fasting glucose.

Additionally, in a nested case-control study on 251 matched pairs (aged >30 years), an inverse association was found between serum 25(OH)D levels (<10 ng/ml, 10-14.9 ng/ml, and ≥15 ng/ml) and fasting plasma glucose (FPG) as well as cardiovascular outcomes (Hosseinpanah *et al.*, 2011). However, Amirbaigloo *et al.* (2013), in another nested case-control study of 324 adults matched pairs, who were free of MetS (defined by IDF criteria) at baseline, could not find any association between vitamin D levels (serum 25(OH)D <20, 20-

30 and >30 ng/ml) and MetS. This study adjusted the results for WC, BMI, FBS, SBP, DBP, TG, HDL-C, and smoking status but not dietary intake and physical activity.

The results from interventional studies were also inconsistent. A randomised control trial (RCT) was conducted on 90 type 2 diabetic subjects (aged 30–60 years) who were provided with either: plain yogurt drink; or vitamin D-fortified yogurt drink (500 IU vitamin D and 150 mg calcium /250 ml); or vitamin D plus calcium-fortified yogurt drink (500 IU D3 and 250 mg calcium /250 ml) for 12 weeks. This study found that vitamin D-fortified yogurt drink improved glycaemic status in type 2 diabetic subjects and reduced fat mass, WC and BMI (Nikooyeh *et al.*, 2011). Shab-Bidar *et al.* (2011) also provided a fortified yogurt drink (containing 170 mg calcium /250 ml or 170 mg calcium plus 500 IU vitamin D per 250 ml yogurt drink) twice-a-day for 12 weeks for type 2 diabetic subjects and reported an improvement in glycaemic status and lipid profiles (TG, LDL, TC, HDL) in the vitamin D-fortified group. The same researchers, in a clinical trial on 350 type 2 diabetic patients and 350 non-diabetic subjects, reported that 1000 IU vitamin D per day may not effectively improve insulin resistance and related disorders (Shab-Bidar *et al.*, 2011a). Furthermore, an RCT on 48 pregnant women (aged 18–40 years) found that vitamin D supplementation (400 IU/d for 9 weeks) significantly decreased FPG, SBP, DBP, serum CRP, and insulin concentrations while it significantly increased insulin sensitivity; however, it has no effect on lipid profiles (Asemi *et al.*, 2013a). On the contrary, another RCT on 45 gestational diabetes subjects reported that 300,000 IU of vitamin D supplementation (single injection) did not change HOMA-IR index, BMI, WC or blood pressure status; however, it improved the serum vitamin D level (Mozaffari-Khosravi *et al.*, 2012). Kaviani *et al.* (2012) also reported that vitamin D supplementation (50,000 IU vitamin D per week for 8 weeks) on healthy adults (aged ≥ 65) did not improve fasting plasma glucose and insulin resistance. Moreover, one more double-blinded trial on 50 women with PCOS (Polycystic ovary syndrome) with

vitamin D deficiency (aged 20-40 years), found that insulin sensitivity and insulin resistance did not change significantly after receiving three oral supplementations of 50,000 IU of vitamin D_3 (every 20 days) for 2 months (Ardabili *et al.*, 2012).

In summary, the reported studies have shown a high prevalence of vitamin D deficiency among the Iranian population. However, the findings of these studies regarding the association between vitamin D deficiency and MetS or its components are equivocal. The differences in these findings were likely due to the use of different definitions of vitamin D deficiency and/or MetS, as well as the consideration of different covariates while not taking into account all potential confounders.

2.4.7 Confounding factors of the association between MetS and vitamin D

Despite the many studies that have been undertaken on the association between vitamin D and MetS or its components, there are inconsistencies in the results. Some studies reported that different populations, VDR polymorphisms, small sample sizes, and other confounders were responsible for these differences (Reis *et al.*, 2007). In addition to the usual confounders including age, sex, BMI, physical exercise, smoking, ethnicity, and season, other confounders might play an important role in this association.

As MetS is a multi-component disorder, factors affecting its components can all affect MetS, and should be considered as potential confounders. Among these factors, physical activity (Strasser, 2013) and dietary factors (Esmaillzadeh *et al.*, 2012; Khosravi-Boroujeni *et al.*, 2012) are associated with body weight, hypertension, lipid profiles and, thus, with MetS.

Physical activity has been considered as the cornerstone of weight management (Mozaffarian *et al.*, 2011). Because physical activity is related to skeletal muscle movements and consumes energy, it improves the efficiency of the cardiorespiratory system and is related to many

health-related benefits, and reduced risk of several chronic diseases including MetS (Hahn *et al.*, 2009), obesity (Ekelund *et al.*, 2011), cardiovascular disease (Manson *et al.*, 2002), and type 2 diabetes mellitus (Tuomilehto *et al.*, 2001). On the other hand, vitamin D supplementation was associated with muscle strength improvement. Furthermore, muscle strengthening exercise was associated with an increase in the serum vitamin D level (Scott *et al.*, 2010). It was proposed that both physical activity and skeletal muscle may affect the synthesis or absorption of vitamin D (Scott *et al.*, 2010). Sunlight exposure during outdoor physical activities might be the reason for the association between physical activity and high vitamin D concentration (Lips *et al.*, 1987).

Dietary intakes related to MetS, including a unhealthy diet, high calorie intake (Mendoza *et al.*, 2007), dietary fat (van Dijk *et al.*, 2009), sugar sweetening (Khosravi-Boroujeni *et al.*, 2012) and some vitamin and mineral deficiencies, such as magnesium, zinc, calcium and vitamin A, should also be considered as potential confounders.

Table 2.5: Studies on vitamin D and metabolic disorders in Iran

Author	Type of study and subjects	Method	Output
Bonakdaran et al., 2016	Cross-sectional, 846 subjects	vitamin D deficiency: < 20 ng/ml vitamin D insufficiency: 20–30 ng/ml	There was no significant difference in serum 25OHD levels between participants with or without MetS
Rafraf et al., 2014	Cross-sectional, 216 adolescent girls	Vitamin D deficiency: <20 ng/ml	96% of the participants had vitamin D deficiency. Mean serum vitamin D was higher in participants with MetS. Adjusted association only found an inverse association between serum vitamin D levels and fasting glucose
Ghanei et al., 2014	Cross-sectional, 250 subjects older than 20years old	Vitamin D deficiency: <20 ng/ml	98.4% of subjects with MetS and 88.3% of those without MetS had vitamin D deficiency. Serum vitamin D levels were negatively associated with MetS
Salehpour et al., 2012	double-blind, randomized, placebo-controlled 42 women	25 μg/d vitamin supplementation for 12 weeks	increasing 25(OH)D concentrations by vitamin D supplementation led to reduction in body fat mass
Amirbaigloo et al., 2013	nested case–control (MetS/ No) 324 matched-pairs, aged 20 or older	Vitamin D category: <20, 20-30, >30 ng/ml matched by sex, age, duration of follow-up, and month of entry to the study. adjusted for WC, BMI, FBS, SBP, DBP, Tg, HDL-C, and smoking status	No association was found between different serum vitamin D levels and incidence of MetS
Hosseinpanah et al., 2011	nested case control 251 matched-pairs, aged > 30 years	Vitamin D category: <10, 10-14.9, and ≥15 ng/ml matched by age, sex and the month of entry adjusted for BMI, FPS, SBP, DBP, cholesterol, triglyceride, HDL-C, smoking status, degree of physical activities, and premature CVD familial history	serum 25OHD concentration has an independent association with cardiovascular outcomes and FPG

Table 2.5 Continued…

Nikooyeh et al., 2011	randomized controlled trial 12 weeks 90 diabetic subjects Aged 30–60 years	Supplement 1) plain yogurt drink (150 mg Ca/250 mL), 2) vitamin D–fortified yogurt drink (500 IU vitamin D3 and 150 mg Ca/250 mL), or 3) vitamin D + calcium–fortified yogurt drink (500 IU D3 and 250 mg Ca/250 mL) twice per day for 12 wk.	Daily intake of a vitamin D fortified yogurt drink, either with or without added calcium, improved glycaemic status in T2D patients and improved FM%, WHR, WC, BMI
Mozaffari-Khosravi et al., 2011	randomized controlled trial 45 gestational diabetes mellitus	300 000 IU of vitamin D3 supplementation	80% of mothers had a degree of vitamin D deficiency The median baseline HOMA-IR index did not differ in the two groups not BMI, WC, BP resistance improved after delivery insulin
Kaviani et al., 2012	interventional healthy adults aged ≥ 65	50,000 IU vitamin D supplementation per week for 8 weeks	Vitamin D supplementation had no significant effect on FPG level but significantly increased the prevalence of insulin resistance
Asemi et al., 2013	double-blind randomized controlled clinical trial 54 women with GDM	50,000 IU vitamin D supplementation, 2 times during the study (at baseline and at day 21 of the intervention) adjusted for baseline values	Supplementation had beneficial effects on glycaemia and total cholesterol and LDL-C but did not affect inflammation and oxidative stress
Kelishadi et al., 2014	triple-masked controlled trial 50 participants, aged 10 to16 years	300,000 IU vitamin D supplementation for 12-weeks	serum insulin and triglyceride concentrations, as well as HOM -IR and C-MetS decreased significantly, but total cholesterol, LDL-C, HDL-C, fasting blood glucose, and blood pressure did not change
Asemi et al., 2013	randomized, double-blind, placebo-controlled clinical trial 48 pregnant women aged 18–40 y old	400 IU/d vitamin D supplementation for 9 weeks	significant decrease in serum hs-CRP, and insulin concentrations and a significant increase in the Quantitative Insulin Sensitivity and significant decrease in fasting plasma glucose, systolic blood pressure and diastolic blood pressure but not in lipid profiles was reported

Table 2.5 Continued…

Ardabili et al., 2012	randomized, placebo-controlled, double-blinded trial 50 women with PCOS 20 to 40 years old	50000 IU of vitamin D or a placebo (1 every 20 days) for 2 months, receive 3 oral treatments	Reported a significant increase in insulin secretion in the vitamin D group, but this was not significant compared with the placebo group. The fasting serum insulin and glucose levels and the insulin sensitivity and homeostasis model assessment of insulin resistance did not change significantly
Shab-Bidar et al., 2011b	randomized double-blind clinical trial 100 subjects with T2D	Yogurt drink (containing 170 mg calcium and no vitamin D/250 mL, n1=50) or vitamin D3-fortified yogurt drink (containing 170 mg calcium and 500 IU/250 mL, n2=50) twice a day for 12 weeks	Perfected vitamin D status was accompanied by improved glycaemic status, lipid profile (Tg, LDL, TC, HDL) and endothelial biomarkers in T2D subjects.
Shab-Bidar et al., 2012	12-week randomized controlled trial, 100 subjects with T2D	plain yogurt drink (170 mg calcium, n1=50) or vitamin D3-fortified doogh (170 mg calcium and 500 IU/250 mL, n2=50) twice a day	improvement of the systemic inflammatory markers
Shab-Bidar et al., 2011a	case-control study 350 T2DM patients and 350 non-diabetic	D3-fortified yogurt drink 1000 IU/d	supplementation was not effective enough to improve insulin resistance and related morbidities
Goshayeshi et al., 2012	cross-sectional 120 Rheumatoid arthritis patients	Vitamin D category: 5<Vit D≤10 and 10<Vit D≤14	Vitamin D had a positive association with MetS in RA patients. Vitamin D has also a negative relation with BMI in these patients
Baradaran et al., 2012	cross-sectional 259 ambulant medical staff adults aged 20–64 years	Vitamin D deficiency: <10 ng/ml	no significant association was found between vitamin D level and BMI
Salekzamani et al., 2010	case-control study 200 women (100 with MetS, 100 age- and residence area-matched control)	vitamin D deficiency: < 27.5 nmol/L vitamin D insufficiency: 27.5–50 nmol/L adjusted for BMI, WC and FM	There was no significant difference in vitamin D status between the two groups. C-reactive protein (hsCRP) concentration was significantly higher in the MetS group, higher plasma glucose concentrations were observed in subjects with vitamin D deficiency

Table 2.5 Continued…

Garakyaraghi et al., 2010	cross-sectional 95 heart failure patients	vitamin D deficiency: <10 ng/ml vitamin D insufficiency: 10-20 ng/ml hypovitaminosis D: 20-40 ng/ml excess: >100 ng/ml	prevalence of low vitamin D status was 84.2% Hypovitaminosis D is very prevalent in heart failure patients
Bonakdaran and Varasteh, 2009	cross-sectional 119 type 2 diabetic patients	vitamin D deficiency: <16.6ng/ml in females and <14.5 in males	hypovitaminous D and CVD was not significantly correlated. Patients with vitamin D deficiency had significant differences in body mass index, MetS, and high sensitive C-reactive protein compared to patients with sufficient vitamin D. The fasting blood sugar, glycosylated hemoglobin, lipid profiles, homocysteine, uric acid, and insulin resistance were not related to vitamin D deficiency
Maghbooli et al., 2008	cross-sectional 741 pregnant women	vitamin D deficiency: <10 ng/ml severe vitamin D deficiency: <5 ng/ml	a positive correlation was found between vitamin D concentrations and insulin sensitivity
Hossein-Nezhad et al., 2009	cross-sectional 646 healthy population aged between 20-79 years	vitamin D deficiency: ≤14 ng/ml adjusted for age and sex	In vitamin D deficient men, the prevalence of the MetS was significantly higher than normal vitamin D group Obesity prevalence was significantly higher in men of 40 years or older with vitamin D deficiency The prevalence of obesity, hyperglycaemia, and HTN were significantly higher in vitamin D deficient women (aged ≥40 years)
Neyestani, 2008	Cross-sectional 90 subjects with either type 1 diabetes mellitus, T2DM, or apparently healthy subjects	Vitamin D category: Severe deficiency: ≤ 5 ng/ml Moderate deficiency 5- 10 ng/ml Mild deficiency: 10-14.8 ng/ml	Mean serum level of 25(OH)D in patients with T2DM was significantly higher than in T1DM, at least in the cold seasons, vitamin D status of the healthy subjects may not be higher than that of T1DM patients
Bonakdaran et al., 2010	cross-sectional 119 type 2 diabetic patients	vitamin D deficiency: <16.6ng/ml in females and <14.5 in males	Reported an association between hypovitaminos D and inflammatory markers but not for glycaemic status and lipid profile

2.5 Is the Association between Vitamin D and Metabolic Syndrome Independent of Other Micronutrients?

2.5.1 Preface

As mentioned before, the association between vitamin D and MetS or its components has been studied by previous researchers, however the results are inconclusive. Different covariates have been mentioned for the discrepancy between the results of different studies. In this section, we briefly reviewed the literature regarding the association between vitamin D and MetS and proposed new covariates, including vitamin A, zinc and magnesium, which are potentially associated with vitamin D function as well as the development of MetS.

2.5.2 Statement of contribution to co-authored published paper:

This section includes a co-authored manuscript published in International Journal for Vitamin and Nutrition Research. The status of the co-authored paper, including all authors, is: Hossein Khosravi-Boroujeni, Faruk Ahmed, and Nizal Sarrafzadegan. (2016). Is the Association between Vitamin D and Metabolic Syndrome Independent of Other Micronutrients? Int J Vitam Nutr Res. 2016 Jul 20:1-16.

Because of the copyright status of the paper, a post-print of our manuscript has been inserted in this section.

2.5.3 The research candidate has made the following contributions to this study:

- ✓ Developed the study idea

- ✓ Systematic review of literature

- ✓ Extract the information from related articles

- ✓ Prepared the manuscript and submitted to the journal revise and resubmission.

Hossein Khosravi-Boroujeni (06/12/2016)

Principal Supervisor: Faruk Ahmed (06/12/2016)

2.5.4 Abstract:

The incidence of metabolic syndrome (MetS) has been increasing globally and it is recognized as a major public health problem because MetS is associated with increased risk of diabetes, stroke, cancer, and other chronic diseases. Recently, MetS has been linked to vitamin D deficiency. However, the evidence on the association between vitamin D deficiency and the risk of MetS remains inconclusive. This review therefore aims to depict the existing evidence related to MetS and vitamin D deficiency, and examined some of the possible confounders which may affect the association between vitamin D status and risk of MetS

Earlier studies on the association between vitamin D deficiency and MetS have adjusted for the effect of some confounders including, age, sex, body mass index, race, physical activity, smoking, alcohol consumption and energy intake. However, these studies failed to consider other potential confounders. There is evidence that vitamin A, zinc (Zn), and magnesium (Mg) play important roles in the activation and function of vitamin D and interact with gene expression. Furthermore, these micronutrients are also related to several components of the MetS including glucose intolerance, dyslipidemia, and obesity. Thus, there could be an interaction between these micronutrients, vitamin D and MetS.

In conclusion, this review highlights the possible interactions of vitamin A, Zn, Mg, and vitamin D with MetS and its components. The findings reinforce the need for further well designed studies that take into account all potential confounders, including other micronutrients such as vitamin A, Zn, and Mg status to investigate the independent association of vitamin D status with MetS and its components, and also to scrutinize for possible interactions among other nutrients which may have similar confounding effects.

2.5.5 Introduction

Metabolic syndrome (MetS) is defined as a cluster of biochemical and physiological abnormalities and it is associated with increased risk of developing non-communicable diseases (Meigs *et al.*, 2006). Despite the significant global effort to control the MetS risk factors, its prevalence has been increasing persistently (Mozumdar & Liguori, 2011) and it has now been recognized as one of the major public health problems globally (Sarrafzadegan *et al.*, 2008; Zimmet *et al.*, 2005).

Although the exact causes of MetS have not yet clearly elucidated, several risk factors and unhealthy behaviours, including diet and lifestyle changes have been attributed to this condition. In recent years, the occurrence of MetS has been linked to vitamin D deficiency (Maki *et al.*, 2009; Parker *et al.*, 2010). However, the present literature on the association between vitamin D deficiency and the incidence of MetS or its components remains inconclusive (L Gulseth *et al.*, 2013). For example, some studies have reported an inverse association between serum vitamin D level and risk of MetS (Hyppönen *et al.*, 2008; Maki *et al.*, 2009; Parker *et al.*, 2010; Scragg *et al.*, 2004); while others have failed to demonstrate such association (Bonakdaran & Varasteh, 2009; Melamed *et al.*, 2008; Rueda *et al.*, 2008). Similarly, the available literature on the findings of the association between serum vitamin D concentration and MetS components is also mixed. Some, but not all, studies reported a significant association between vitamin D deficiency and glucose intolerance (Gagnon *et al.*, 2012), lipid profiles including total cholesterol, LDL, HDL and triglyceride (Ponda *et al.*, 2012), blood pressure (Vimaleswaran *et al.*, 2014) and obesity (González *et al.*, 2015).

Further, a systematic review and meta-analysis has shown a negative association between blood 25(OH) vitamin D concentrations and the risk of MetS only in cross-sectional studies but not in longitudinal studies (Ju *et al.*, 2013). Although some of the studies examining the association between vitamin D status and MetS have taken into account some potential

confounders, the role of other components that were not assessed is undeniably likely. Among the nutrients, some vitamins or minerals such as vitamin A, Zn and Mg have a role in vitamin D activation and/or function. They also take part in controlling some of the MetS components including, central obesity, glucose intolerance, dyslipidemia and hypertension and altogether the development of MetS. Thus it is reasonable to expect a possible interaction of these micronutrients in the association between vitamin D and MetS. Although other micronutrients may also have similar interaction effects on the association between vitamin D and MetS, unfortunately there is no data in the current literature.

In order to fill this gap, the present review aims to briefly describe the current knowledge of the MetS and vitamin D including the extent of the problem, and subsequently examines some of the possible confounders which may influence the association between vitamin D status and risk of MetS.

2.5.6 Literature search

This review used a number online search engine including, PubMed (MEDLINE), Cochrane Library (Central), and Web of Science to identify the relevant human studies published up to June 2015. First, the literature search was conducted to identify any studies that reported the association between MetS and vitamin D. Second, in order to identify possible confounders among nutrients, the search focused on the literature that investigated the association or interaction between MetS and vitamin D and possible confounders including vitamin A, Zn, Mg, iodine, vitamin K, vitamin C, vitamin E, and calcium. A possible interaction was only reported for vitamin A, Zn, Mg and calcium in previous studies. However, calcium has been already assessed as a confounder in the association between MetS and vitamin D (Ford *et al.*, 2005; Liu *et al.*, 2005). Thus the combinations of vitamin D and vitamin A, Zinc or

Magnesium were used for the database search. Titles and abstracts were individually reviewed to include relevant articles. Reference lists of selected articles were also searched for other possible related articles. Altogether, only 8 human studies published in peer reviewed journals were eligible for the analysis (Table 2.5.2).

2.5.7 Metabolic syndrome

MetS has received much attention in the past decades as it helps to identify the risk of both type-2 diabetes and cardiovascular diseases (CVD) (Cameron *et al.*, 2004; Talaei *et al.*, 2012a). MetS increases the risk of diabetes mellitus, stroke, coronary heart disease, myocardial infarction and other chronic diseases (Lakka *et al.*, 2002b; McNeill *et al.*, 2005a; Pyorala *et al.*, 2000; Resnick *et al.*, 2003). These diseases are the leading causes of all deaths in both the developed and developing countries (Lakka *et al.*, 2002a). It has also been reported that CVD death and all-cause mortality were higher in MetS participants, even if they were free of diabetes and CVD at baseline (Lakka *et al.*, 2002a). As a consequence of sedentary lifestyles, the population ageing and the increasing prevalence of obesity, the prevalence of MetS has been increasing worldwide (Eckel *et al.*, 2005). Globally, the reported prevalence of MetS varies from 10 % to more than 80% in different populations based on different definitions (Kaur, 2013).

Because of the importance of identifying the individuals with MetS, various expert groups/panels have attempted to define diagnostic criteria for MetS. Table 2.5.1 shows different definitions of MetS proposed by different expert groups. The first definition of MetS proposed by the World Health Organization (WHO) consultation group was based on the importance of insulin resistance in diagnosing the MetS (World Health Organization, 1999). Then the European Group for the Study of Insulin Resistance (EGIR) suggested a

modification of WHO definition which incorporated fasting plasma glucose as an easier method for glucose intolerance (Balkau & Charles, 1999). The National Cholesterol Education Program, Third Adult Treatment Panel (NCEP ATP III) definition did not obligatorily require to measure glucose intolerance, rather suggested the presence of any 3 of the 5 components: central obesity, raised blood pressure, high triglycerides, low HDL-cholesterol and fasting hyperglycaemia (Expert Panel on Detection, 2001). Finally the International Diabetes Federation (IDF) proposed central obesity as the most important component of MetS, and thus recommended that the presence of central obesity along with any 2 of the 4 additional factors (raised blood pressure, high triglycerides, low HDL-cholesterol, and fasting hyperglycaemia) were required to define MetS (Alberti *et al.*, 2006).

2.5.8 Risk factors of metabolic syndrome

A wide variety of risk factors has been associated with the increased incidence of MetS. Overweight and obesity (Kelliny *et al.*, 2008), ageing (Grundy *et al.*, 2004b), smoking (Reaven, 2002), physical inactivity (Grundy *et al.*, 2005), excess caloric intake (Djousse *et al.*, 2010) and genetics (Terán-García & Bouchard, 2007) were reported to be the most important risk factors. Other studies reported an association between MetS or its components and dietary factors, including consumption of fat (Riccardi *et al.*, 2004), dairy products (Sadeghi *et al.*, 2014a), grains (Khosravi-Boroujeni *et al.*, 2013), simple sugars (Khosravi-Boroujeni *et al.*, 2012), salt and alcohol consumption (Djousse *et al.*, 2010). Furthermore, some minerals and vitamins were also found to be associated with the incidence of MetS. For example, studies have shown an association of MetS or it components with vitamin D (Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Lu *et al.*, 2009), calcium (Liu *et al.*, 2005), vitamin A (Zulet *et al.*, 2008), Zn (Kechrid *et al.*, 2012) and Mg (Barbagallo *et al.*, 2003). Among these

micronutrients, vitamin D is one of the most noticeable nutrients that has recently received much attention worldwide.

2.5.9 Vitamin D

Vitamin D deficiency or insufficiency is a widespread problem in different populations (Ben-Shoshan, 2012; Koenig & Elmadfa, 2000; Moniz *et al.*, 2005) and it has been estimated that up to a billion people may be affected globally (Holick, 2007), emphasising this as a probable global pandemic in the 21st century (Plehwe, 2003). Vitamin D can be synthesised in the skin through exposure to ultraviolet (UV) light from sun (which is a major source of vitamin D) or can be obtained through dietary intake which exerts minor influence to vitamin D status. This form of vitamin D is not biologically active and needs to be converted to the active form in the body. First, vitamin D is converted to 25-hydroxyvitamin D [25(OH)D] in the liver by , and then in the kidney it is converted to 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] which is the active form of vitamin D (Holick *et al.*, 1971). Other studies also confirmed that other tissues such as skin, lymph nodes, pancreas, and colon also have 1-alphahydroxylase enzyme and can produce [1,25(OH)$_2$D] (Hewison *et al.*, 2000). Moreover, 1,25(OH)$_2$D has a short half-life and its serum concentrations are affected by parathyroid hormone (PTH), calcium and phosphate concentrations. Thus serum 1,25(OH)$_2$ vitamin D level is not a good indicator of vitamin D status (Zerwekh, 2008). To date, serum 25(OH)D is considered to be the best measure of body vitamin D status (Wang *et al.*, 2008). Although there is no consensus on vitamin D sufficiency levels, vitamin D deficiency has been defined by most experts as a serum 25(OH) vitamin D level lower than 50 nmol/L (20 ng/mL) (20 ng/mL) (Bischoff-Ferrari *et al.*, 2006; Holick, 2006; Zittermann, 2006). However, some other studies proposed

a cut-off point of below 80 nmol/L (32 ng/mL) to define vitamin D insufficiency (Gómez *et al.*, 2003).

As vitamin D synthesis in the skin is the most important source of this vitamin, any factor that reduces the exposure to sunlight or decreases the concentrations of 7-dehydrocholesterol will cause vitamin D deficiency. Older individuals, who have lower 7-dehydrocholesterol levels (MacLaughlin & Holick, 1985) and lower ability to synthesize vitamin D in the skin (Holick *et al.*, 1989), are more susceptible to vitamin D deficiency. Consequently, in Western countries, 40 −100 % of elderly people are vitamin D deficient (Michos *et al.*, 2010; Nadir *et al.*, 2010). High prevalence of vitamin D deficiency is also observed among obese individuals, may be because of trapped vitamin D in subcutaneous fat (Arunabh *et al.*, 2003; Wortsman *et al.*, 2000) or lower sunlight exposure and outdoor activity (Michos *et al.*, 2010; Scragg & Camargo, 2008b). Urbanisation and westernized lifestyles, including indoor lifestyles, sun prevention strategies and clothing style, as well as skin pigmentation inhibit vitamin D production in the skin (Gannagé-Yared *et al.*, 2000).

2.5.10 Vitamin D functions

The best known classical role of vitamin D is related to calcium metabolism and maintaining bone health (Holick, 2007). For this function, vitamin D interrelates with its nuclear receptor in the related tissues including bone, intestine and kidney (Deluca & Cantorna, 2001; Holick, 2002, 2009). The non-genomic function of vitamin D, which has been described in many cell types, induces increases in the concentrations of intracellular calcium (Boland *et al.*, 2002). It has been shown that even in individuals with genetic defects of vitamin D metabolism, vitamin D is essential for active calcium absorption. Alternatively bone mineralization can be increased by high doses of calcium (Lips, 2006). In addition, vitamin D produces biological

reactions in more than 30 target tissues and can generate genomic biological responses and regulates more than 200 genes, including those related to insulin, renin, and cytokines (Norman *et al.*, 2001). In addition, the 1-alpha-hydroxylase enzyme (Stöcklin & Eggersdorfer, 2013) and 1,25(OH)$_2$D nuclear receptors, known as vitamin D receptors (VDR), have been found in a large number of different cells and organs including skin, osteoblasts, brain, gonads, immune cells, vascular endothelial cells, pancreas, and lymphocytes (Manolagas *et al.*, 1985; Mathieu & Adorini, 2002; Stumpf *et al.*, 1979).

Vitamin D performs its hormone-like functions to adjust its target genes via binding to VDR (Haussler *et al.*, 1997). The vitamin D function acts through a single receptor that recognizes vitamin D-responsive elements (VDREs) which are repeated sequences of nucleotides. The 3' arm of these sequences binds to VDR and the 5' arm binds to retinoic acid X receptor (RXR) (DeLuca, 2004). VDR, in cooperation with RXR, forms a heterodimer that binds to VDREs (Jones *et al.*, 1998). Simultaneously, VDR binds other proteins and an activator required for the gene transcription (McPhee *et al.*, 2010) that is related to several functions. Moreover, different genes are selective for the coregulator (inhibitory or stimulatory) that with the VDR heterodimer regulates their transcription (Oda *et al.*, 2003).

It has previously been reported that individuals living at higher latitudes, and having lowest exposure to sunlight, are at a higher risk of various chronic diseases (Rostand, 1997). Recent studies have indicated that the risk of all-cause and cardiovascular related mortality are higher in individuals with low serum vitamin D levels (Dobnig *et al.*, 2008; Melamed *et al.*, 2008). Low vitamin D status is also associated with diabetes, hypertension, atherosclerosis, congestive heart failure, myocardial infarction, cardiovascular risk, stroke, kidney dysfunction, infections, and cancers (Chiu *et al.*, 2004; Chonchol & Scragg, 2006; Cigolini *et al.*, 2006; de Boer *et al.*, 2007; Holick, 2007; Kendrick *et al.*, 2009; Krause *et al.*, 1998; Martins *et al.*, 2007; Peterlik & Cross, 2005; Rodríguez-Rodríguez *et al.*, 2014; Scragg *et al.*,

2004, 2007; Targher *et al.*, 2006; Wang *et al.*, 2008; Zittermann *et al.*, 2003). However, most recently, Theodoratou et al (Theodoratou *et al.*, 2014) have carried out an umbrella review of the evidence across systematic reviews and meta-analyses of observational studies and randomised controlled trials that were related to vitamin D and various diseases and failed to draw a firm conclusion about the beneficial effects of vitamin D and health outcomes (Theodoratou *et al.*, 2014).

2.5.11 Vitamin D and metabolic syndrome

Several epidemiologic studies have reported an inverse association between MetS and serum 25 hydroxyvitamin D concentrations (Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Lu *et al.*, 2009). In addition, other studies have shown that individuals with vitamin D deficiency were up to three times more at risk of developing MetS than individuals with normal vitamin D status (Botella-Carretero *et al.*, 2007; Chiu *et al.*, 2004). As MetS is defined by its components, any association between vitamin D deficiency and MetS components can influence the association between vitamin D and MetS. As indicated earlier, studies have found an inverse association between vitamin D status and diabetes (Holick, 2004b; Scragg *et al.*, 2004). It has been suggested that vitamin D, via its effect on intracellular calcium (Pittas *et al.*, 2007b), is associated with inflammation, pancreatic β-cell function and insulin resistance (Kayaniyil *et al.*, 2010). Moreover, adipose tissues and skeletal muscles, which are related to peripheral insulin sensitivity, have VDR (Holt *et al.*, 2005; Wild *et al.*, 2004). Pancreatic β-cells and the insulin gene promoter also have a VDR section (Ramachandran *et al.*, 2003), which could explain the role of vitamin D on glucose homeostasis. Adequate vitamin D status has also been found to be associated with blood pressure, probably by its role on the down-regulation of renin and angiotensin and therefore, reducing blood pressure

(Li *et al.*, 2002). 1,25(OH)$_2$D also supresses the production of some components of renin, angiotensin in pancreatic islets (Leung & Cheng, 2010). Furthermore, the presence of VDR in vascular smooth muscles and endothelial cells may justify the vascular effect of vitamin D (Florentin *et al.*, 2010). Vitamin D deficiency was also found to be associated with obesity (Martins *et al.*, 2007). It has been proposed that vitamin D may work with calcium to increase postprandial fat oxidation (Chan She Ping-Delfos & Soares, 2011) or may inhibit adipogenesis because of its role on gene expression (Kong & Li, 2006). It has also been proposed that obese individuals need more vitamin D for stronger bones to support their greater weight, or may have lower vitamin D production as a consequence of clothing habits or restricted outdoor activity and less exposure to UVB (Holick, 2004a).

Although several investigations accepted vitamin D deficiency as a risk factor for MetS (Al-Daghri *et al.*, 2012; Botella-Carretero *et al.*, 2007; Boucher, 1998; Boucher *et al.*, 1995; Chiu *et al.*, 2004; Ford *et al.*, 2005; Lind *et al.*, 1995; Liu *et al.*, 2009; Martini & Wood, 2006; Reis *et al.*, 2008; Smotkin-Tangorra *et al.*, 2007), several studies could not demonstrate any significant association (Bonakdaran & Varasteh, 2009; Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Liu *et al.*, 2009; Melamed *et al.*, 2008; Reis *et al.*, 2007; Rueda *et al.*, 2008; Tai *et al.*, 2008).The discrepancy between studies on the association between vitamin D and MetS or its components has been justified by population differences in exposure to UVB irradiation, residential place, skin exposure to sunlight, different dietary intake, and genetic differences (Reis *et al.*, 2007). Although some of these studies controlled possible confounders including, age, sex, BMI, race, physical activity, smoking, alcohol consumption, and energy intake, the roles of other confounders are irrefutable. For example, vitamin A, Zn, Mg, iodine, vitamin K, vitamin C, vitamin E, and calcium are related to vitamin D, MetS or its components. Based on the current literature, vitamin A, Zn, and Mg are related to the activation and function of vitamin D as well as MetS and/or its components but these micronutrients were

not considered as possible confounders in previous studies. Therefore, it is likely that these micronutrients could act as potential confounders while examining the association between vitamin D and MetS and/or its components. Table 2.5.2 summarizes the human studies investigating the interaction between vitamin D and other nutrients. The following section will describe the findings of available literature which examined the possible links between these micronutrients and vitamin D and MetS.

2.5.12 Possible role of vitamin A

Vitamin A deficiency is a major public health problem in low income countries (West Jr *et al.*, 2007). The main cause of vitamin A deficiency is inadequate dietary intake of vitamin A over a long period, which leads to inefficient body stores and the inability to meet the physiological needs (World Health Organization, 2009). Retinol, retinoic acid (RA), or pro-vitamin A (β-carotene and other carotenoids) are the sources of vitamin A in the diet (Shils & Shike, 2006).

It has been reported that the plasma levels of vitamin A were inversely associated with MetS prevalence (Godala *et al.*, 2014). It has also been shown that plasma carotenoids concentrations were significantly lower in MetS participants when compared with healthy individuals and the serum carotenoids levels were significantly reduced with an increased number of MetS components (Coyne *et al.*, 2009; Ford *et al.*, 2003; Suzuki *et al.*, 2011). Antioxidant activity of carotenoids that protect against oxidative stress has been suggested as a possible mechanism to reduce MetS (Suzuki *et al.*, 2011). In addition, vitamin A intake was found to be inversely associated with several biochemical and anthropometric measurements (body weight, BMI, waist circumference and waist hip ratio) that were linked to MetS manifestations (Zulet *et al.*, 2008).

Vitamin A along with vitamin D stimulates production of proteins through modulation of gene expression (Germain *et al.*, 2006). Binding of RA to nuclear RA receptors (RARs) and retinoid X receptors (RXRs) forms heterodimers that regulate the expression of specific target genes (Germain *et al.*, 2006). This complex also supports VDR signalling and prevents the degradation of vitamin D (Sánchez Martínez *et al.*, 2006). To regulate gene expression and transcription, RXR requires to form a heterodimer complex with VDR (VDR:RXR heterodimer) (Bettoun *et al.*, 2003) (Figure 2.5.1). Synergistic or antagonistic interactions between vitamin A and vitamin D have been reported in in-vitro studies (Haussler *et al.*, 1998). It has also been reported that higher vitamin A intakes cause more prevalent and severe osteoporosis (Schrijver & van den Berg, 2003). A high dose of retinol as an antagonist of vitamin D could cause osteoporosis, rickets and non-bony vitamin D related diseases (J Boucher, 2011). In human studies, it has been demonstrated that retinol antagonizes the serum calcium response to vitamin D (Johansson & Melhus, 2001). The interaction between vitamin A and vitamin D has been confirmed by other studies (Cheng *et al.*, 2014; Schmutz *et al.*, 2015).

Thus vitamin A may act as a potential confounder while examining the association between vitamin D and MetS and hence it is important to take its effect into account in order to investigate an independent association of vitamin D and MetS.

2.5.13 Possible role of zinc

Zn deficiency is one of the major mineral deficiencies throughout the world (Shils & Shike, 2006). The Food and Agriculture Organization (FAO) reported that around 50 % of the world's population are at a risk of inadequate intake of Zn (Brown & Wuehler, 2000). The

catalytic role of Zn has been discovered in many enzymes and around 300 Zn metalloenzymes have been found (Blasie & Berg, 2002).

Zn plays an essential role in antioxidant systems including glutathione peroxidase, superoxide dismutase, and catalase (McCall *et al.*, 2000) and decrease production of inflammatory cytokine through Zn-finger protein regulation (Prasad, 2009). Thus Zn could be related in the pathophysiology of the MetS (Arnaud *et al.*, 2012). The association between Zn and MetS components is contradictory. There are studies which reported that surplus Zn, via the renin-angiotensin system, might increase blood pressure (Kasai *et al.*, 2012), and Zn supplementation reduces plasma HDL-C concentrations (Hughes & Samman, 2006). However, others recommended Zn supplementation as a safe intervention to decrease the risk of MetS (Hashemipour *et al.*, 2009). Moreover, Zn affects the body fat deposition and the insulin activity (Lee *et al.*, 2005a). It has also been connected with pancreatic β-cells insulin secretion, and so is associated with diabetes and obesity (Naik *et al.*, 2009). Zn deficiency and its metabolic disorders are also associated with the pathogenesis of several chronic diseases (Maret, 2005).

 Zn is a vital component of steroid hormone receptors and is bound to the DNA binding domains of zinc-finger proteins. Removal of Zn from zinc-finger proteins changes the structure, and causes dysfunction and probably degradation of the proteins (Shils & Shike, 2006). Intracellular Zn binds to VDR and influences the activity of vitamin D dependent genes (Craig *et al.*, 2001). Zn also improves the effect of vitamin D on the activity of alkaline phosphatase and DNA synthesis (Yamaguchi & Kitajima, 1991). Zn deficiency causes bone calcification disorders which is comparable with vitamin D deficiency (Xu *et al.*, 1992). It has also been described that Zn enhances the activity of vitamin D dependent promoters (Lutz *et al.*, 2000). In human studies, Zn supplementation particularly in the presence of vitamin D increases bone mass (Ekbote *et al.*, 2015).

Overall, Zn appears to help in various functions of vitamin D in the body, and may also be related to some components of MetS. Thus, Zn status in individuals could be a very important factor when examining the association between vitamin D status and the risk of MetS.

2.5.14 Possible role of magnesium

Mg deficiency has been frequently reported in patients with hypertension (Barbagallo *et al.*, 2003), dyslipidemia (Guerrero-Romero & Rodriguez-Moran, 2000), diabetes (de Lourdes Lima *et al.*, 1998) and cardiovascular diseases (Abbott *et al.*, 2003). Mg plays important roles in a wide range of biological reactions and is responsible for more than 300 essential metabolic responses (Maguire & Cowan, 2002; Wolf & Cittadini, 2003). Mg is connected with calcium homeostasis (van den Bergh *et al.*, 2008) and is also associated with the metabolism of vitamin D (Rude *et al.*, 2009). It is essential for the binding of vitamin D to its carrier protein and thus helps in the transportation of vitamin D in the body, and is necessary for the conversion of inactive vitamin D into active forms 25(OH) D and 1,25(OH)$_2$D in the liver and kidney, respectively (Reddy & Sivakumar, 1974; Rude *et al.*, 1985). Earlier investigations found a higher prevalence of Mg insufficiency or deficiency in individuals with lower concentrations of serum vitamin D (Sahota *et al.*, 2006). Other studies revealed that Mg deficiency is associated with reduction in the active form of vitamin D [1,25(OH)$_2$D] and causes resistance to pharmacological doses of vitamin D (Rude *et al.*, 2009) and is related to vitamin D resistant rickets (Rude *et al.*, 1985). Conversely, Mg supplementation reduces the resistance to vitamin D treatment for rickets (Reddy & Sivakumar, 1974). Additionally, a positive association between vitamin D levels and serum Mg has been reported in human studies (Al-Daghri *et al.*, 2014; Deng *et al.*, 2013; Gandhe *et al.*, 2013).

Vitamin D supplementation was also associated with increased serum Mg concentrations in obese individuals (Farhanghi *et al.*, 2009).

On the other hand, Mg deficiency and/or low Mg intakes are associated with MetS (Huang *et al.*, 2012) and its components, including insulin resistance (McCarty, 2005), diabetes mellitus (Song *et al.*, 2004), hypertension (Barbagallo *et al.*, 2003), dyslipidemia (Guerrero-Romero & Rodriguez-Moran, 2000) and cardiovascular diseases (Abbott *et al.*, 2003). It has been suggested that Mg intake and intracellular Mg influence insulin secretion (Guerrero-Romero *et al.*, 2004; Kandeel *et al.*, 1996) and insulin function (Takaya *et al.*, 2004) through their effect on calcium homeostasis, stimulation and transcription of some enzymes and nuclear proteins, and oxidative stress (Barbagallo *et al.*, 2003). Mg as a calcium antagonist can inhibit the intracellular calcium mobilization (Touyz, 2003), increase sodium excretion in urine and control blood pressure (Touyz, 2003). Mg also acts as a co-factor for several enzymes affecting lipid metabolism (Kazue *et al.*, 1997) and can decrease the absorption of fatty acids and cholesterol in intestine by forming an un-absorbable soap and decrease energy intake which may prevent obesity (Drenick, 1961).

A recent study also showed a possible interaction between serum vitamin D, Mg intake and mortality (Deng *et al.*, 2013). Because of the association between Mg deficiency and MetS, and the effect of Mg on the serum level of vitamin D and its activation, a potential interaction between vitamin D, Mg and MetS is conceivable.

2.5.15 Strengths and limitations

While this review revealed a complex interaction between vitamin D, MetS and other micronutrients, the major limitation is that there is limited literature that has reported on this interaction. However, the strength of this study is that it is the first review that collated the

available documents to address the interaction of other micronutrients with vitamin D and MetS.

2.5.16 Conclusion

Despite the increasing evidence on the importance of vitamin D in the prevention of metabolic diseases, presently there are significant inconsistencies in available literature regarding the association between vitamin D status and MetS, which made it difficult to draw a firm conclusion. This review also highlights possible interactions of vitamin A, Zn, Mg and vitamin D with MetS and its components. Finally, this review reinforces the need for further studies by controlling the confounding effects of other nutrients to confirm the association between vitamin D status and MetS and its components and also to look for possible interactions among other nutrients which may have similar confounding effects.

2.5.17 Acknowledgements

Table 2.5.1: Metabolic syndrome definitions

	WHO (1999)	EGIR (1999)	NCEP ATP III	IDF
Definitions	insulin resistance together with two or more of the following:	Insulin resistance or impaired fasting glucose (IFG) plus two of the following:	Three or more of the following five risk factors:	Central obesity plus 2 other features
Fasting plasma glucose	__	≥ 6.1 mmol/l (110 mg/ dl) but non-diabetic	≥ 5.6 mmol/l (100 mg/ dl)	≥5.6 mmol/l (100 mg/ dl) or diagnosed type 2 diabetes
Central obesity	Men: waist–hip ratio > 0.90 Women: waist–hip ratio > 0.85 and/or BMI > 30 kg/m2	Men: waist circumference ≥ 94 cm Women: waist circumference ≥ 80 cm	Men: waist circumference > 102 cm Women: waist circumference > 88 cm	Men: waist circumference ≥94cm , Women: waist circumference ≥80cm[*] or BMI >30kg/m²
Blood pressure	≥140/90 mmHg	≥ 140/90 mmHg or treatment	≥ 130/ 85 mmHg	≥130/85 mmHg or treatment
Triglycerides	≥ 1.7 mmol/l (150 mg/ dl)	> 2.0 mmol/l (178 mg/ dl)	≥ 1.7 mmol/l (150 mg/ dl) or treatment	≥1.7 mmol/l (150 mg/ dl) or treatment
HDL-cholesterol	Men: < 0.9 mmol/l (35 mg/ dl) Women: < 1.0 mmol/l (39 mg/ dl)	< 1.0 mmol/l (39 mg/ dl) or treatment	Men: < 1.03 mmol/l (40 mg/ dl) Women: < 1.29 mmol/l (50 mg/ dl)	Men: <1.0 mmol/l (39 mg/ dl) Women: <1.3 mmol/l (40 mg/ dl) or treatment

Table 2.5.2: Human Studies mentioned the interaction between vitamin D and other nutrients

Authors (year), Country	Participants	Study design	Results and comments
Vitamin A and vitamin D			
Schmutz et al. 2015, USA	15,998 adults	Cohort	Negative associations between serum vitamin D and risk of dead was reported only among individuals with serum retinylesters <7.0 µg/dL. A possible interaction between serum vitamin D and vitamin A levels is probable.
Cheng et al. 2014, USA	14,254 50-69 years	Case-Control	Vitamin D supplementation was associated with a lower risk of total lung cancer among individuals who had vitamin A intake ≥1,500 µg/day. Vitamin A may support vitamin D in cancer prevention.
Johansson et al. 2001, Sweden	9 healthy subjects	Intervention	Intake of vitamin D plus retinal weakened the calcium response to vitamin D. Vitamin A intake antagonizes the quick intestinal calcium response to vitamin D in man.
Zinc and vitamin D			
Ekbote et al. 2015, India	31 children	Intervention	Adding zinc to calcium and vitamin D supplementation increased the bone mineral content. Zinc supplementation particularly in the presence of vitamin D increase bone mass.
Magnesium and vitamin D			
Gandhe et al. 2013, India	30 healthy and 30 type 2 diabetics	Case-Control	A positive association between vitamin D and magnesium levels was detected. Vitamin D has the potential to decrease insulin resistance and also can affect magnesium status.
Al-Daghri et al. 2014, Saudi Arabia	126 adult	Intervention	Magnesium levels significantly increased after the vitamin D supplementation. Vitamin D supplementation increases the serum level of magnesium
Deng et al. 2013, USA	12157 adults NHNES study	Cross-sectional and Cohort	High Mg intake was diminished the risk of vitamin D. A possible metabolic interaction between vitamin D and deficiency magnesium is stated.
Farhanghi et al. 2009, Iran	82 women (17-50 years)	Intervention	Vitamin D supplementation increased serum Magnesium level in obese individuals. In obese people vitamin D can modify low serum Magnesium levels

Figure 2.5.1: function of vitamin D and vitamin D in gene expression
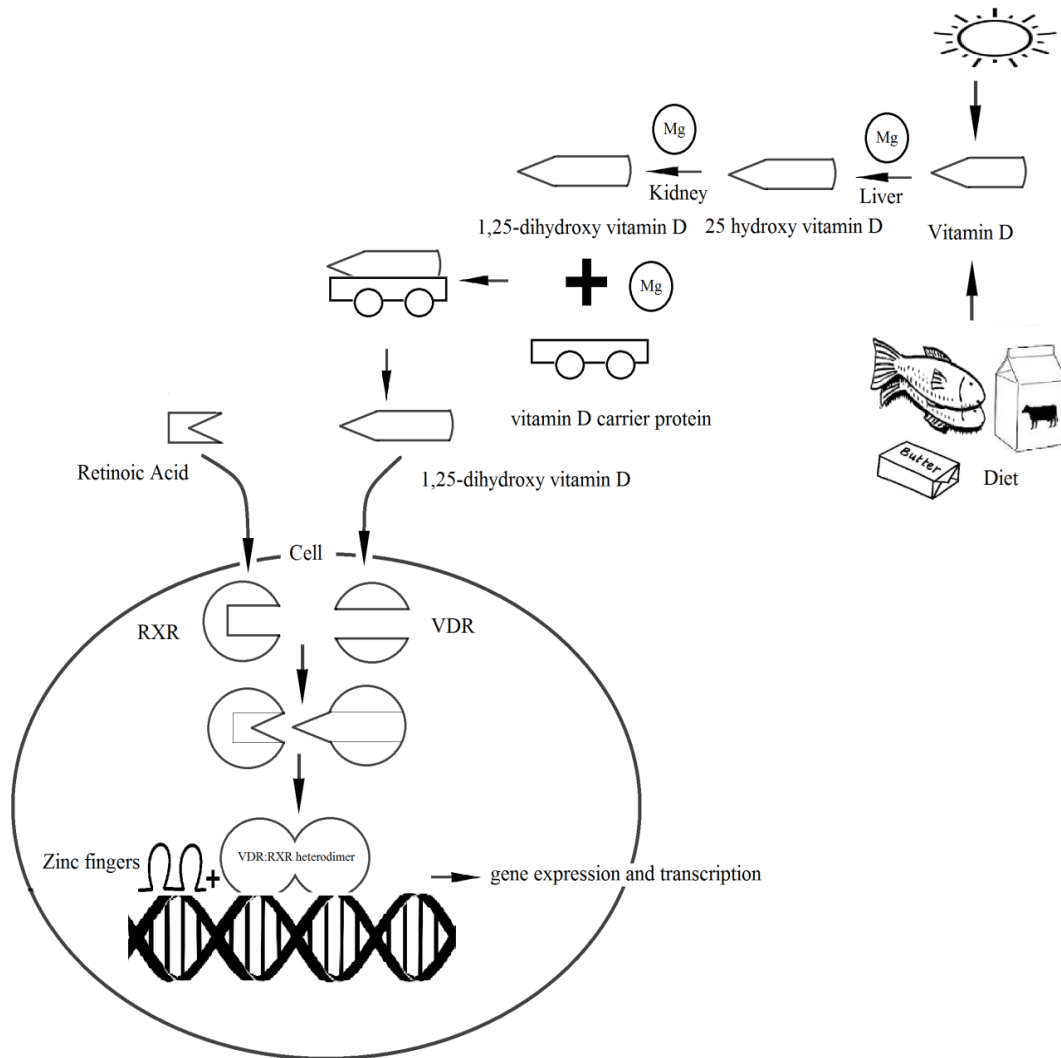
2.6 Summary

MetS, which is highly prevalent globally and its prevalence continues to rise (Alberti *et al.*, 2009b), has been known for decades as a risk factor for a number of chronic diseases, such as diabetes mellitus and cardiovascular disease. To control the prevalence of MetS and its complications, it is important to appreciate that not only recognising its risk factors (including lifestyle and dietary factors) but also reducing them, are a public health and clinical responsibility. As well as many traditional risk factors, including obesity, aging, sedentary lifestyle, smoking and high calorie intake, vitamin D deficiency has now been suggested as a possible risk factor for MetS in several studies (Botella-Carretero *et al.*, 2007; Cheng *et al.*, 2010; Chiu *et al.*, 2000; Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Lu *et al.*, 2009; Pereira *et al.*, 2002). However, a number of studies did not demonstrate any association between MetS and vitamin D deficiency (Bonakdaran & Varasteh, 2009; Liu *et al.*, 2009; Reis *et al.*, 2007; Rueda *et al.*, 2008; Tai *et al.*, 2008).

Vitamin D deficiency, which has long been known for its effect on calcium, phosphorus and bone diseases (Holick, 2002), has also been emphasised as a potential pandemic in the 21st century (Mithal *et al.*, 2009). Despite the high worldwide prevalence of vitamin D deficiency, surprisingly, there are limited health policies for preventing vitamin D deficiency due to a lack of strong evidence for the health benefits of adequate vitamin D status other than bone health.

In spite of an overwhelming body of studies related to vitamin D deficiency and MetS or its components, the results are inconclusive. Although several factors, including sample size, age group, ethnicity and polymorphism are addressed regarding the contradictions between results, there are other factors which also need to be considered. Furthermore, different stages of vitamin D deficiency and different definitions of MetS used in different studies might also

explain the differences in findings regarding the association between vitamin D deficiency and MetS.

In Iran, MetS and vitamin D deficiency are significant public health problems (Bonakdaran *et al.*, 2010; Hossein-nezhad & Holick, 2013; Hossein-Nezhad *et al.*, 2015; Ostovaneh *et al.*, 2014). Moreover, the available studies in Iran are mostly cross-sectional or case-controlled and, therefore, it is pivotal to explore the association between vitamin D deficiency and MetS based on a longitudinal study in Iran.

CHAPTER THREE: METHODOLOGY

3.1 Preface

This chapter outlines the research methodology which was employed to answer the research questions and to test the hypotheses of this research. The research reported in this thesis is comprised of two parts. The first part is the secondary analyses of ICS data on socio-economic and biochemical variables related to MetS and its components from 2001 to 2013. The second part is the assessment of vitamin D and examination of its association with MetS in a sub-sample of ICS. This chapter describes the research setting, process of sampling, measuring tools, data collection and follow-up methods. Moreover, it explains the statistical methods used to test the hypotheses.

3.2 Research design and setting

3.2.1 Geographical and Demographic information of Iran

Iran is a Middle Eastern country in Southwest Asia and features vast deserts, great mountains, and large cities. The eastern part of Iran is dominated by a high plateau, with great sand deserts which are surrounded by high mountains, including the Elburz to the north and the Zagros to the west. The central parts of the country are arid and have hot summers, with less than 200 mm of rain per annum. The southern part of Iran, which comprises the coastal plains of the Persian Gulf and the Gulf of Oman, has mild winters and very humid and hot summers. To the North, Iran is bordered by Turkmenistan, Azerbaijan, and Armenia and on the east by Afghanistan and Pakistan. Turkey and Iraq are on the western border. Iran's geological

attributes have attributed to its huge reserves of petroleum and natural gas and, therefore, Iran has an important role in international energy security and the world economy.

Iran has a population of about 80 million (sex ratio: 1.03 male/female) of which 69.1% are urban dwellers. The population growth rate is approximately 1.22%, the birth rate is 18.23 births/1,000 population, and the death rate is 5.94 deaths/1,000 population. Life expectancy at birth for the total population is 70.89 years (male: 69.32 years and female: 72.53 years). The age distributions for 0-14, 15-24, 25-54, 55-64, and 65 years and over are 23.7%, 18.7%, 46.1%, 6.3% and 5.2%, respectively. The major cities and population of Iran are Tehran (capital) with 7.304 million; Mashhad 2.713 million; Esfahan 1.781 million; Karaj 1.635 million; Tabriz 1.509 million; and Shiraz 1.321 million. Tehran, the largest city, is the capital of Iran and is also the cultural, political, industrial and commercial centre of the nation.

Iranian culture has long been a predominant culture of the region, and much of what later became known as Islamic knowledge, such as philosophy, literature, sciences, architecture, and medicine were based on some of the practices transferred from the Persians to the Muslim world (Barrientos & Soria, 2015).

3.2.2 Study site

During the previous decades, Middle Eastern countries have faced great increases in chronic and non-communicable diseases like diabetes and cardiovascular diseases (Wild *et al.*, 2004), and Iran has not been exempted from this (Naghavi *et al.*, 2009). MetS has been reported in one-third of the Iranian population (Azizi *et al.*, 2003; Noshad *et al.*, 2016). The primary data collection for the current study was undertaken in three counties (Isfahan, Arak, and Najaf-Abad) in the central part of Iran in 2001. This study was based on the Isfahan Cohort Study (ICS) and examines the prevalence of MetS and its components and their relationships with

vitamin D status. The ICS is an ongoing population-based longitudinal study of adults, aged 35 years or older, living in urban and rural areas of the three central districts in Iran in 2001 (Sarrafzadegan *et al.*, 2011).

3.2.3 Sampling of the first phase of ICS

Baseline participants of ICS were those who had participated in the baseline survey, entitled Isfahan Healthy Heart Program (IHHP) (Sarraf-Zadegan *et al.*, 2003b). The baseline sampling was a quota sampling to stratify the study population by their living area, either urban or rural, according to the distribution of the population. Quota sampling is a method of selecting individuals for surveys. It essentially selects a certain number of individuals from specific groups. In quota sampling, people are first divided into equal sub-groups, similar to stratified sampling; participants are then selected from each segment based on a specified proportion. This method guarantees the selection of adequate numbers of individuals with appropriate characteristics (Moser, 1952). In this study, census blocks were randomly selected from each county with the possibility of selection proportional to the expected number of households. Within families, one single adult (age-eligible) was randomly selected.

Based on the 1999 national population survey, the population of Isfahan city was 1,777,185 (1,607,000 in urban and 170,185 in rural areas) and the population in Najaf-Abad city was 261,215 (177,392 in urban and 83,823 in rural areas). Isfahan city was split into 93 clusters, Arak into 60 clusters and Najaf-Abad into 47 clusters; of these, 25 clusters from Isfahan, 15 clusters from Najaf-Abad and 23 clusters from Arak were randomly selected. Roughly 5%-10% of the families within these clusters were randomly selected for sampling. Next, one individual aged 19 years or older was randomly selected from each household if he/she was

Iranian, mentally capable and not pregnant. The sample size was calculated for each sex and then divided into age categories (19-24, 25-34, 35-44, 45-54, 55-64, and >65 years) according to the community distribution. Because of the cluster method, and considering the missing rate for cohort surveys, the total sample size was doubled and a total of 12,514 individuals were included in the baseline survey (Sarraf-Zadegan *et al.*, 2003b). Among 12,514 individuals from IHHP, 6,640 adults aged 35 years and over were enrolled into the ICS. Ethical approval was achieved from the Ethics Committee of Isfahan Cardiovascular Research Centre.

3.2.4 Data collection

After obtaining informed written consent, a 30-minute interview was completed for eligible participants by trained interviewers to determine demographic and socioeconomic characteristics. Moreover, health knowledge, family and medical history, as well as attitudes and behaviour related to cardiovascular risk factors, such as dietary intake, smoking behaviour, and physical activity, were also determined. At the beginning, a questionnaire collected information on participant demographics such as age, gender, marital status, education, occupation, and residential area. Participants were also asked to answer questions about smoking, as well as knowledge, attitude and practice of community and health related diseases. They were also asked about their family history of diseases and their recent medical history (physician visits, hospitalization, stroke, coronary symptoms, diseases, and treatments).

3.2.5 Physical activity assessment

Physical activity was assessed using a Baecke questionnaire regarding habitual physical activity. Baecke's questionnaire includes 16 questions following three habitual physical activity scores from the previous year, including occupational and leisure physical activity scores (Baecke *et al.*, 1982). The questionnaire was prepared to collect information on frequency, duration and intensity of physical activity.

Self-reported individual activities were identified and recorded regarding the number of times per day and the number of days per week spent on each activity. The groups of physical activities were consigned based on the rate of energy expenditure as Metabolic Equivalent of Task (MET). Light physical activities were those with a score of <3 METs, and include activities such as household activities, normal pace walking, and recreational swimming. Moderate physical activities were those with a score of 3-6 METs, such as volleyball, badminton, and table tennis. Vigorous physical activities were those with a score of >6 METs including activities such as jogging, running, stair-climbing, cycling, weight training, and vigorous sports such as basketball, soccer, handball and singles tennis. To calculate METs minutes/week, the MET value of each activity is multiplied by the duration of that activity and the number of days per week. For instance, walking MET- minutes/week is 3.3 × walking minutes per day × walking days per week. Total physical activity MET-minutes/week is the sum of all the individual activities' MET-minutes/week scores.

3.2.6 Clinical assessments

Individuals were requested to attend the survey centres for clinical examination and risk factor assessment. They were requested to fast for at least 12 hours prior to the biochemical testing and bring all prescription and non-prescription drugs which are used regularly as well

as their medical records. Once a participant arrived at the survey centre, informed written consent was obtained.

3.2.7 Anthropometry assessment

3.2.7.1 Height and weight measurements

Height was measured in bare feet using a secured metal ruler and recorded to the nearest 0.5 centimetres. Weight was measured without shoes and in light clothing, using the calibrated scale to the nearest 0.5 kilograms. Every morning before starting the work, instruments were calibrated with a standard weight. BMI for each respondent, based on World Health Organization recommendations (World Health Organization, 2000), was computed as weight (kg) divided by the square of the height (m^2).

3. 2.7.2 Waist and hip circumference measurements

The WC was measured at the level of the uppermost edge of the hip bone using a non-stretchable tape measure, as the minimum circumference at or below the costal margin, and the hip circumference was measured at the maximum circumference and recorded to the nearest 0.5 centimetres.

Obesity was defined as waist circumference ≥94cm for men and ≥80cm for women or BMI >30kg/m² (Expert Panel on Detection, 2001). Moreover, it has been assessed based on the Iranian National Committee of Obesity cut-off point for WC (≥90 cm for both genders) (Fereidoun Azizi *et al.*, 2010; Talaei *et al.*, 2012b).

3.2.8 Blood pressure assessment

Blood pressure was taken from the arm (brachial artery) from all participants. Blood pressure was measured by a random-zero sphygmomanometer with an appropriate-sized cuff in a seated position after a 5-minute rest, and repeated after 5 minutes. The average of two blood pressure measurements was reported. Hypertension was defined as SBP $\geq$130 and DBP $\geq$85 mmHg or under treatment (Expert Panel on Detection, 2001).

3.2.9 Laboratory tests

A blood sample was taken from the left antecubital vein into citrate or fluoride monovettes. Haematological parameters, including red blood cell count, haemoglobin (Hb), platelet count, and white blood cell (WBCs) count were measured on fresh blood samples using a cell counter AL820. All collected serum samples from each centre were immediately frozen at -20°C until analysed within 72 hours in the central laboratory of ICRI. The additional serum sample was frozen at -80°C for future potential studies. The central laboratory of ICRI meets the standards of the national standard laboratory of the Ministry of Health in Iran for quality control. External quality control was also applied in the St Rafael University of Brussels in Belgium.

3.2.9.1 Blood glucose assessment

Fasting plasma glucose was measured using fasting blood samples. The 2-hour plasma glucose (2hpp) was assessed two hours after having a glucose solution (containing 50gr glucose). Plasma glucose concentrations were analysed enzymatically by a clinical chemistry

analyser (Eppendorf, Hamburg, Germany). Glucose intolerance was defined as fasting plasma glucose ≥ 100 mg/dl (Expert Panel on Detection, 2001).

3. 2.9.2 Blood lipid assessment

The enzymatic colorimetric method was used to measure serum total cholesterol and triglycerides (Eppendorf, Hamburg, Germany). After dextran sulphate-magnesium chloride precipitation of non-HDL cholesterol, HDL cholesterol was measured by the enzymatic colorimetric method. The LDL cholesterol level was calculated using the Friedewald equation (Friedewald *et al.*, 1972a). Hyperlipidaemia was defined as fasting triglyceride ≥150 mg/dl or fasting HDL cholesterol < 40 mg/dl for men and <50 mg/dl for women or for those under treatment (Expert Panel on Detection, 2001).

3. 2.9.3 Vitamin D assessment

The serum 25-hydroxyvitamin D level, as the best measure of body vitamin D status (Wang *et al.*, 2008), has been assessed from frozen serum samples using Enzyme Linked Immune Sorbent Assay (ELISA; Euroimmun AG, Luebeck, Germany). It has been confirmed that 25-hydroxyvitamin D is very stable in serum when kept frozen for more than 10 years (Hollis, 2008).

3.2.10 Dietary assessment

Dietary habits were evaluated with a validated, 48 item food frequency questionnaire (FFQ). As ICS was a research study, similar to the countrywide integrated non-communicable

disease intervention (CINDI) programme, the FFQ was modified from the CINDI programme questionnaire (Leparki & Nussel, 1987) and translated into Persian by a nutritionist. Some typical Iranian foods that provide total and saturated fat intake were included and items that were not consumed in Iran were excluded from the list. Face-content validity and translation of the questionnaire were evaluated by an expert panel. The FFQ was pre-tested for internal validity among 200 adults who were not among the main study subjects. Participants completed the FFQ twice at a 2-week interval to evaluate test–retest reliability (r=0.8). To assess criterion-related validity, the global dietary index (GDI) score was compared with a single 24 h diet recall in 2400 subjects of the baseline sample.

Global dietary index (GDI) of diet was assessed via 29 frequency questions in seven categories. Table 3.1 shows the questions and related scores. The frequency of answers was scored as 0, 1 or 2, measuring the nutritional value. These indices are calculated from the mean of scores given to dietary substances from specific food (Table 3.1). A lower GDI (>4) shows better dietary behaviour.

3.2.10.1 Dietary vitamin D intake

The main dietary sources of vitamin D are oily fish (salmon, tuna, sardines, anchovies and mackerel), butter, cream, high-fat dairies, margarine, eggs, and liver. The dietary vitamin D intake was assessed by the frequency of consumption of these sources.

Table 3.1: Calculation of the global dietary index, fat consumption index and meat consumption index scores (Mohammadifard *et al.*, 2009b)

	Score		
	0	1	2
Global dietary index			
1 How many times per week do you eat fast foods? (4 questions)	0–1	2–3	4 or more
2 How many serving of fruits or vegetables do you eat in a week? (7 questions)	28 or more	14–28	Less than 14
3 How many times per week do you eat beans, chicken, soya protein or fish? (4 questions)	3 or more	1–2	Less than 1
4 How many times per week do you eat sweets? (6 questions)	0–1	2–3	4 or more
5 How many times per week do you eat hydrogenated oil, ghee, animal fats or butter? (4 questions)	0–1	2–3	4 or more
6 How many times per week do you eat meat, egg or whole dairy products? (4 questions)	0–1	2–3	4 or more
7 How many times per week do you eat non-hydrogenated oil, olive oil? (2 questions)	7 or more	5–6	Less than 5
Fat consumption index			
1 How many times per week do you eat hydrogenated oil? (1 question)	0	1–2	3 or more
2 How many times per week do you eat ghee? (1 question)	0	1–2	3 or more
3 How many times per week do you eat butter? (1 question)	0	1–2	3 or more
4 How many times per week do you eat animal fats? (1 question)	0	1–2	3 or more
5 How many times per week do you eat non-hydrogenated oil? (1 question)	4 or more	1–2	Less than 1
6 How many times per week do you eat olive oil? (1 question)	4 or more	1–2	Less than 1
Meat consumption index			
1 How many times per week do you eat meat? (1 question)	0–1	2–3	4 or more
2 How many times per week do you eat sausages? (2 questions)	0–1	2–3	4 or more
3 How many times per week do you eat liver, lung or kidney? (3 questions)	0	1	2 or more
4 How many times per week do you eat chicken or fish? (2 questions)	3 or more	1–2	Less than 1

3.2.11 Follow-up

After the baseline survey in 2001, telephone interviews have been conducted every 2 years to follow-up participants. In 2007 and 2013, fully designed interviews, as well as biochemical and physical measurements, were repeated in the same way as for the baseline survey.

For the telephone follow-ups, at least five attempts were made to contact the participants or their first-degree relatives if they were dead. If telephone interviews were ineffective, the participants were visited at their residential address for follow-up. After proving the participant's identity, interviews were carried out based on a questionnaire with three main questions; 1) Is he/she alive?, 2) Has he/she been hospitalized for any reason? and 3) Has the he/she experienced any of the following five neurological symptoms (hemiparesis, dysarthria, facial asymmetry, imbalance and transient monocular blindness)?

If the participant was dead or hospitalized, or had neurological symptoms, the date of the events, physician diagnosis, as well as the hospital's name were collected during the interview. If any event had happened, the related questionnaire and the relevant health records were checked. If out-of-hospital deaths occurred, death certificates were requested from the provincial mortality database; moreover, an oral autopsy was conducted by a trained expert nurse with participant's family members using a pre-defined questionnaire including medical history, as well as signs and symptoms.

3.2.12 Confirmation of end points

The reported events were reassessed using the MI and stroke registry database of the Surveillance Department, Isfahan Cardiovascular Research Center. In the case of any discrepancy in diagnoses, or unavailable records, original medical records were examined. Two separate panels of specialists, consisting of four neurologists and cardiologists,

considered all related documents for every patient and made the final decision on all of the five main events (fatal and non-fatal MI, fatal and non-fatal stroke and sudden cardiac death). The related criteria have been described in previous publications (Sarrafzadegan *et al.*, 2011).

3.2.13 MetS criteria

In the proposed study, MetS was diagnosed using different definitions, including WHO, EGIR, ATP III, AHA, IDF and JIS definitions. More details about these definitions have been described in section 2.2.3.

3.2.14 Vitamin D deficiency criteria

Different criteria are used for defining vitamin D deficiency in different studies. However, most of the studies approved that the cut-off point for vitamin D deficiency and inadequacy should be below 25, 50 or 75 nmol/L (Food and Nutrition Board. Institute of Medicine, 2011; Holick, 2007; Need *et al.*, 2008; Organization, 2003). In this study, we used the most accepted criteria for defining vitamin D deficiency and insufficiency which is below 25 nmol/L and 50 nmol/L respectively. However, we also compared other categories (<25, 25-50, 50-75, 75-100, >100 nmol/L) to explore the effect of different categories of vitamin D deficiency on MetS and its components.

3.3 Methodology and sampling of the current research

To study the prevalence and secular trend of MetS and its components, and to examine the influence of changes in the risk factors of MetS and its components, a longitudinal analysis was conducted based on the ICS data set. We explored the changes in the risk factors of MetS and its components between the 2001, 2007 and 2013 samples. The subjects who had the required variables, including MetS components and MetS risk factors, including dietary intake, physical activity, and smoking in 2001, 2007 and 2013 were included in the analyses.

To assess the association between serum vitamin D status and MetS, a nested case-control study design was applied. This study was based on the ongoing cohort of ICS from whom data were collected in 2001, 2007 and 2013. The subjects who had their socio-demographic and dietary information, as well as the biochemical variables and follow-up information, were included in this study. Other inclusion criteria were being free of MetS in 2001, and having frozen serum samples in 2001, 2007 and 2013. In this cohort study, there was a significant loss to follow-up participants because the frozen serum samples were unavailable for assessing the serum vitamin D levels. Therefore, using these criteria, only 370 subjects were eligible for this study. Among these samples, those who were free of MetS in the 2001 survey but had developed MetS in the 2013 survey were chosen as cases. Thus, only 170 cases met the inclusion criteria. Additionally, 200 subjects who were free of MetS in the 2001 survey, and remained free of MetS up to the 2013 survey, were chosen as controls.

3.4 Statistical analysis

Statistical analyses were achieved by using Statistical Package for the Social Sciences (SPSS) version 22.0. SPSS Statistics is a software package which is widely used for statistical analysis.

The two-sided test was used and a probability value of <0.05 was considered statistically significant. Continuous variables were analysed to assess normal distribution. The normal distribution of the variable was determined by descriptive statistics including Skewness and kurtosis tests, histograms and normal probability plots. For continuous variables, data were presented as mean ± standard deviations (SD) or standard error (SE) and for categorical variables they were presented as percentages.

3.4.1 Prevalence of MetS and vitamin D deficiency

The prevalence represents existing cases of a disease and can be seen as a measure of disease status; it is the proportion of people in a population having a disease:

$$Prevalence = \frac{number\ of\ cases\ at\ a\ time\ point}{total\ number\ of\ subjects\ in\ the\ population} \qquad (3.1)$$

We assessed the MetS and its components at three points in time (2001, 2007 and 2013) to identify their prevalence and trends during the 12-year period.

The Generalized Estimating Equation (GEE) (Hanley *et al.*, 2003) was used to determine trends in the prevalence of MetS and its components from 2001 to 2013. To control the effect of aging on the trends of MetS and its components, age was adjusted in logistic regression and prevalence was calculated using the following equation (Bastos *et al.*, 2015):

$$P = \frac{1}{1 + \exp[-(\beta + \beta AGE)]} \qquad (3.2)$$

Where P is the probability of MetS or its components, β and βAGE are regression coefficients.

To control the effect of potential confounders on the trends of vitamin D deficiency and insufficiency, age, BMI, region, season of blood collection and dietary intake of vitamin D were adjusted in the GEE model.

3.4.2 Differences between variables

Differences in continuous and categorical variables in individuals between 2001, 2007 and 2013 were tested using analysis of variance (ANOVA) and Chi-square analysis, respectively. ANOVA is responsible for a statistical test of whether the means of several groups are equal or not, and so this test generalizes the t-test for more than two groups. This is appropriate because using multiple two-sample t-tests results in a higher chance of the statistical type I error. Characteristics between available participants and loss-to-follow-up individuals were examined by the t-test or chi-square test where appropriate. Characteristics of the study subjects (with or without MetS) were compared using the t-test for continuous variables and chi-square test for categorical variables.

3.4.3 The association between vitamin D levels and MetS

The GEE approach was used to test the association between vitamin D levels and dependent variables, including MetS and its components.

The GEE approach is a widely used estimation method for longitudinal models in a variety of statistical methods, including logistic and linear regression, which investigates the association between repeated measurements and correlated responses (Liang & Zeger, 1986). The GEE approach does not require most specification of the multivariate distribution in the repeated measures and is appropriate for both continuous and dichotomous variables (Zeger *et al.*, 1988). The associations among the repeated variables were considered by working correlation structure and combining this structure into the estimation. Moreover, either time-varying or time-stationary variables were considered as covariates.

For the analysis, the GEE approach with an auto-regressive working correlation structure was used. The binary logistic model was used to assess the association between serum 25(OH)D level and categorical dependent variables, such as incidence of MetS. A linear model was used to assess the association between serum 25(OH)D level and continuous dependent variables, including SBP, DBP, FBS, HDL, and BMI. Other predictors, including continuous and categorical variables, were also added in the model to control their effect on the association between serum vitamin D and MetS and its components.

Development of the model was based on univariate analysis and clinical significance. First, univariate analysis was applied to identify important covariates. In univariate analyses, significant differences were found in gender, BMI, WC, FBS, total cholesterol, TG, BP, physical activity and family history of dyslipidemia between cases and controls. WC and total cholesterol were removed from the model because they have a significant association with BMI and triglyceride, respectively. From the perspective of clinical significant dietary score,

dietary sources of vitamin D, family history of diseases, baseline metabolic risk factors, smoking, and season of blood draw were added to the model.

3.5 Ethical consideration and approval

Prior to the study commencement, ethical approval was obtained from Griffith University (GU Ref No: MED/53/14/HREC). Moreover, ethical approval was also obtained from the Ethics Committee of Isfahan Cardiovascular Research Institute. (Appendix A)

CHAPTER FOUR: RESULTS

4.1 Preface

This chapter presents the research findings and answers the research questions. This chapter consists of five sections. The first section explores the effect of different definitions of MetS in predicting CVD events. The second section reports the trend of MetS and its components from 2001 to 2013. Section three describes factors influencing the changes in MetS components from 2001 to 2013. Section four presents the prevalence and trends of vitamin D deficiency over a 12-year period from 2001. Finally, section five reports the association between MetS and serum vitamin D levels.

These five sections are presented as a series of published and unpublished (under review) manuscripts. Thus, these sections are presented based on the journals' accepted format.

4.2 Does the impact of metabolic syndrome on cardiovascular events vary by using different definitions?

4.2.1 Preface

MetS is highly prevalent in the Iranian population and has been considered as a public health problem in the country (Delavari *et al.*, 2009). Although different expert groups use different definitions to identify MetS individuals who are at risk of CVD, there is no exclusive definition. Moreover, the applied definitions in different studies have affected the prevalence of MetS as well as the association between MetS and cardiovascular events (Brown *et al.*, 2008). It could also be a reason for varied results regarding the association between MetS and its risk factors such as vitamin D deficiency. Therefore, in this research, we decided to identify the best possible definition of MetS for the study population. In this section, six definitions of MetS were compared to explore the influence of using different definitions of MetS on predicted risk of CVD and also to identify the best definition for this study population. Figure 4.2.1 presents the flow chart of this study.
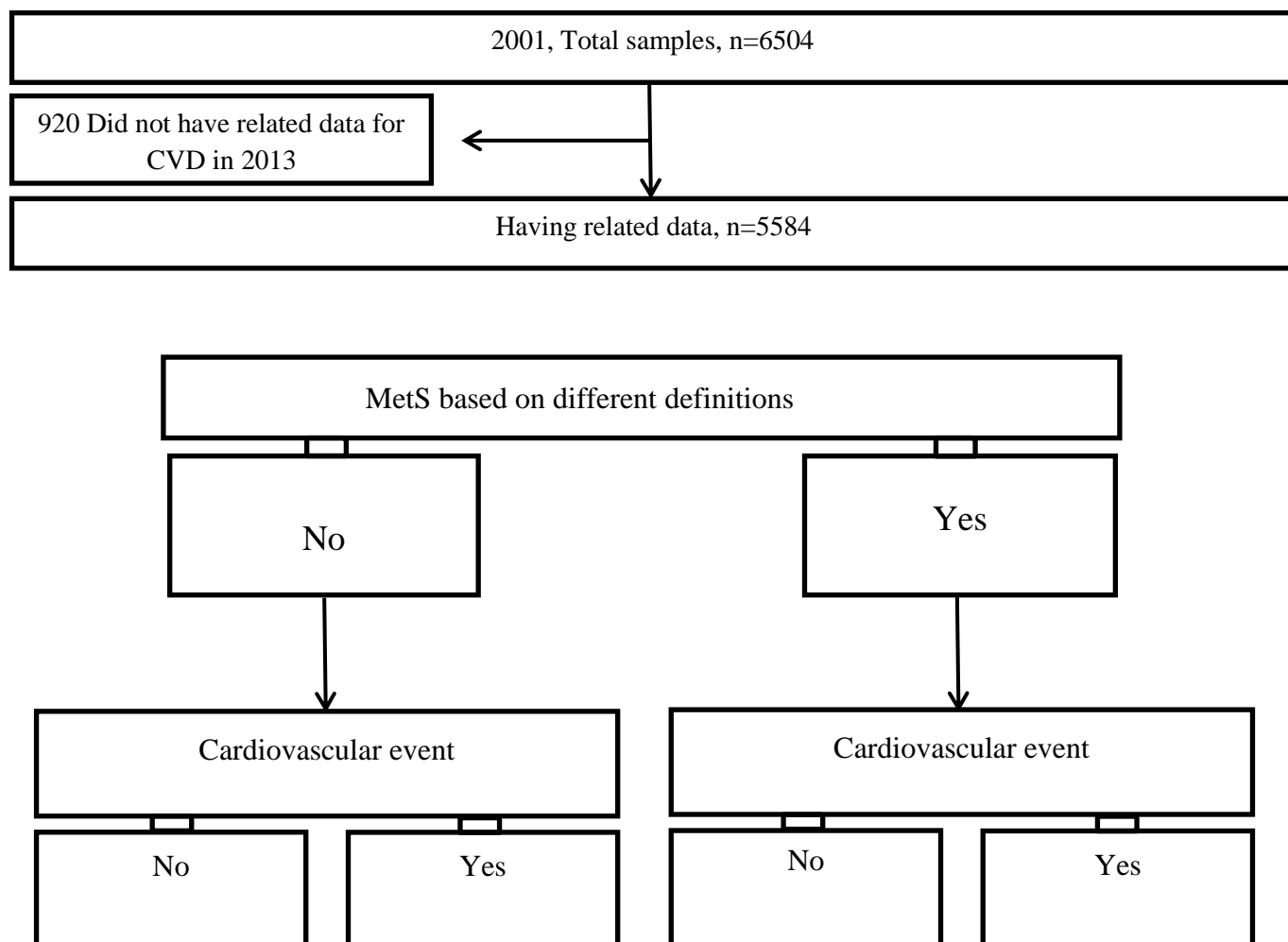
Figure 4.2.1: Flow chart of this study. The same process repeated for each definition of MetS.

4.2.2 Statement of contribution to co-authored published paper:

This section includes a co-authored manuscript published in BMC Public Health. The status of the co-authored paper, including all authors, is: Hossein Khosravi-Boroujeni, Faruk Ahmed, Masoumeh Sadeghi, Hamidreza Roohafza, Mohammad Talaei, Minoo Dianatkhah, Ali Pourmogaddas and Nizal Sarrafzadegan. (2015). Does the impact of metabolic syndrome on cardiovascular events vary by using different definitions?. BMC public health. 2015 Dec 29;15(1):1. DOI 10.1186/s12889-015-2623-3.

4.2.3 The research candidate has made the following contributions to this study:

- ✓ Developed the study design.
- ✓ Clean the data and matching based on different definitions of MetS.
- ✓ Analysed the data and interpreted the findings.
- ✓ Prepared the manuscript and submitted to the journal.

███████

Hossein Khosravi-Boroujeni (06/12/2016)

█████████████

Principal Supervisor: Faruk Ahmed (06/12/2016)

4.2.4 Abstract

Background: Metabolic Syndrome (MetS) is a complex disorder which increases the risk of chronic diseases, including cardiovascular diseases and diabetes mellitus. As a result of modern lifestyles, the prevalence of MetS has been rising globally. This study aims to investigate whether overall prevalence of MetS varies when using different definitions of MetS and to identify the best and most predictive definition of the MetS for cardiovascular disease (CVD) events over 10 years in a cohort of an Iranian population.

Method: Adults aged ≥ 35 years from urban and rural regions in central Iran were selected at baseline and followed up for more than 10 years. Data on socio-demographic characteristics, anthropometry, blood pressure and smoking status were collected at baseline. In addition, various biochemical indices were assessed. MetS was defined based on five available definitions, and cardiovascular events during 10 years follow up were confirmed by an expert group. The hazard ratios were calculated by the Cox proportional hazards model.

Results: The highest prevalence of MetS was observed by using AHA-NHLBI definition (36.9%), followed by JIS definition (31.2%). On the other hand, EGIR (8.8%) provided the lowest prevalence. The risk of developing CVD, irrespective of definitions, was approximately two fold higher in the presence of MetS. After controlling for possible confounders, AHA-NHLBI definition was found to be the best predictor of CVD.

Conclusion: This study demonstrated a great variability in the prevalence of MetS among Iranian adults when using different definitions of MetS. CVD risk was significantly higher in MetS participants, as well as in participants with any risk factors of MetS; however, the AHA-NHLBI definition was found to be the best predictor of CVD. Thus protective measures, including lifestyle modifications, plus control of individual risk factors is necessary to prevent cardiovascular events.

4.2.5 Introduction

Metabolic Syndrome (MetS) is a complex disorder with a collection of related metabolic risk factors which increase the risk of developing chronic diseases, such as atherosclerotic cardiovascular disease (ASCVD) and diabetes mellitus (Panel, 2002). MetS is also associated with other disorders such as fatty liver (Browning *et al.*, 2004), cholesterol gallstones (Chen *et al.*, 2012), polycystic ovary syndrome (Lim *et al.*, 2012), and sleep apnea (Coughlin *et al.*, 2004). In addition, it poses a significant risk of higher morbidity, mortality and financial burden (Malik *et al.*, 2004). The prevalence of MetS has been rising in both developed and developing countries, probably as a consequence of modern lifestyle and the overweight/obesity epidemic (van Vliet-Ostaptchouk *et al.*, 2014). Therefore, MetS is considered a public health, as well as a clinical, problem. During the past decades, due to major lifestyle changes and aging population, the prevalence of MetS, cardiovascular diseases (CVD) and other chronic diseases has been increasing in Iran (Sarrafzadegan *et al.*, 2009a). Based on a national study, MetS has been diagnosed in 34.7 to 37.4 percent of the Iranian population (Delavari *et al.*, 2009). Moreover, high incidence rates for almost all CVD and mortality have been reported in the Iranian population (Nizal Sarrafzadegan *et al.*, 2013). The etiology of MetS has not been clearly defined, thus the definition of MetS is not based on etiology and pathology, but on the predictors of CVD as a primary outcome of MetS. It's diagnostic criteria have been developed on the basis of best clustering of interrelated risk factors of CVD which occur simultaneously and can predict CVD events (Alberti *et al.*, 2009a). In the past, several expert groups have attempted to develop practical diagnostic criteria to characterize individuals who are at high risk of CVD. They included underlying and metabolic risk factors, characterized by insulin resistance or impaired blood glucose, central obesity, dyslipidemia (increase in triglycerides and decrease in high density lipoprotein cholesterol HDL-C levels) and hypertension. However, the suggested criteria

varies to some extent and some individuals might diagnose with one or two definitions but not with others (Expert Panel on Detection, 2001; International Diabetes Federation, 2006; World Health Organization, 1999).

Because there is no exclusive definition for the diagnosis of MetS, its prevalence, incidence and its association with an increased risk of cardiovascular diseases depends on the criteria used (Brown *et al.*, 2008). Thus, this study aims to investigate whether the prevalence of MetS varies when using different definitions of MetS in an Iranian population. In addition, it aims to determine the definition that is the best predictor for CVD events over 10 years in a cohort of an Iranian population.

4.2.6 Material and method

4.2.6.1 Study design

The Isfahan Cohort Study (ICS) is an ongoing, population based, longitudinal study of adults aged $\geq$ 35 years, from urban and rural regions in central Iran. It is designed to display the incidence of CVD and its risk factors, and to determine the Iranian risk assessment values. Participants were selected between January and September 2001 by multistage random sampling and were enrolled to represent the age, gender and urban/rural distribution of their societies. The study details are presented elsewhere (Sarrafzadegan *et al.*, 2011). The study was approved by the Ethics Committee of the Isfahan Cardiovascular Research institute (ICRI) a World Health Organization (WHO) collaborating center and the Griffith University Ethics Committee.

4.2.6.2 Measurements

After obtaining the informed written consent of participants, physical examinations, fasting blood samples, and anthropometric measurements were carried out. Serum triglycerides, fasting blood glucose (FBG), and total cholesterol (TC), were determined using the enzymatic method (McNamara & Schaefer, 1987). Serum HDL-C was measured after precipitation of low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) (Warnick *et al.*, 1985). The LDL-C level was calculated by Friedewald formula (Friedewald *et al.*, 1972b). Weight and height were measured by a calibrated scale and stadiometer with participants wearing light clothes and no shoes. Waist circumference (WC) was measured with non-elastic measuring tape at or below the costal margin (minimal waist) without compressing the tissue. Blood pressure was taken twice at 5 minutes interval in a sitting position with a random-zero sphygmomanometer with an appropriate cuff for adults. The mean value of the two measurements was calculated and applied.

4.2.6.3 Metabolic syndrome definitions

Among the available definitions for MetS, this study selected the most widely practiced definitions which were developed by various international expert groups and organizations. The MetS was defined according to five definitions (table 4.2.1). Based on the WHO definition, insulin resistance is required for diagnosing MetS, along with two other risk factors among central obesity, high triglyceride, low HDL or hypertension (World Health Organization, 1999). The European Group for Study of Insulin Resistance (EGIR) defined MetS only for non-diabetic people (Balkau & Charles, 1999). The National Cholesterol Education Program, Third Adult Treatment Panel (NCEP ATP III) did not emphasise any risk factors, but the presence of any 3 of the 5 risk factors would qualify a person for MetS

(Expert Panel on Detection, 2001). Based on the International Diabetes Foundation (IDF), abdominal obesity is a requirement in MetS definition, and having central obesity plus any other two risk factors are required for the diagnosis of MetS (International Diabetes Federation, 2006). This definition insists on easy-to-use measures in clinical practice, and moreover, emphasises ethnic differences in recognising the cut-off point of abdominal obesity (Alberti *et al.*, 2006). The American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI) accepted the ATP III definition, but reduced the threshold for impaired glucose tolerance (IFG) from 110 to 100 mg/dl (Grundy *et al.*, 2005). A harmonized definition of MetS (a Joint Interim Statement (JIS)) formulated by several organizations including IDF, NHLBI, AHA, the World Heart Federation, the International Atherosclerosis Society and the International Association for the Study of Obesity attempted to develop a unified criteria for defining MetS. They agreed that a single cut-off point for WC is not suitable and should not be a required component. Furthermore, any 3 out of 5 components are adequate for MetS diagnosis (Alberti *et al.*, 2009a).

4.2.6.4 Follow up

With the purpose of verifying CVD events, the follow-up of participants was conducted using telephone call interviews and home visits when required, every two years. The participants were asked about their hospital admissions or any cardiac or neurological symptoms that led to visiting a physician. In 2007 and 2013, interviews, physical examinations and laboratory tests were repeated for all participants. The measurement methods were similar to the 2001 survey. Five main events (fatal and non-fatal MI, fatal and non-fatal stroke and sudden cardiac death) were considered as the indicators of ischemic heart disease. A panel of three cardiologists and one neurologist, unaware of the data related to risk factors, examined all the

documents to confirm the CVD cases. For the purpose of this study, the data on CVD event were recorded up to the 2013 survey.

4.2.6.5 Statistical methods

The prevalence of MetS was calculated by using different definitions in the total samples and also by considering sex, age category, region, education level, and occupation using SPSS crosstab. For univariate analysis, the data were compared between groups by the Student t-test or chi-squared analysis. Based on our variables (the follow up duration and the CVD event), the Cox proportional hazards model was chosen as the best multivariate approach for analysing survival time data to investigate the association of different definitions of MetS and its components with cardiovascular events. In the first model, the analysis was conducted using a crude model, and in the second model, age, sex, smoking status and physical activity were adjusted to remove the effects of covariates. For all analyses, statistical significance was considered at a level of 0.05. All data were analysed by using Statistical Package for the Social Sciences (IBM SPSS Version 22).

4.2.7 Results

Overall, 3,336 females and 3,168 males participated in the first phase of the ICS. When compared between various definitions of MetS, the highest prevalence was observed using the AHA-NHLBI definition (36.9%), followed by JIS (31.2%) and ATP III (30.0%). On the other hand WHO (13.3%) and EGIR (8.8%) provided a much lower prevalence (Table 4.2.2). Considering all definitions, the overall prevalence of MetS was higher in females than in

males. Using WHO and EGIR definitions, the prevalence of MetS rose with increasing age, while it increased only until 65-75y using all other definitions.

Table 4.2.3 shows the presence of CVD events based on the development of MetS using different definitions. Irrespective of the definitions used, MetS was significantly associated with CVD events. Higher values of CVD risk factors (e.g. age, cholesterol, blood glucose and smoking) were also observed in individuals with CVD events. However, HDL-C was an exception in that there was no significant difference in the levels between the CVD events groups.

The risk of developing CVD, considering all definitions, was approximately two fold higher in the presence of MetS (Table 4.2.4). As shown in the crude model, the MetS using the WHO definition predicted the highest risk for CVD followed by the JIS definition (HR: 2.41, 95% CI: 2.05-2.83 and HR: 2.14, 95% CI: 1.86-2.46 respectively). After controlling for possible confounders including age, sex, smoking status and physical activity, the risk of CVD decreased slightly and using the AHA-NHLBI definition was found to be a better predictor than using other definitions (HR: 1.93, 95% CI: 1.66-2.25). When examining the risk of CVD events for each of the abnormal components of MetS, the risk of CVD occurrence was also significantly higher. Among the components, glucose abnormality was found to be a higher predictor of CVD events (HR: 1.83, 95% CI: 1.56-2.15) than the other components.

4.2.8 Discussion

The present study demonstrated that the prevalence of MetS among Iranian adults varies widely when different definitions are used. Using the WHO and EGIR definitions resulted in a much lower prevalence of MetS when compared with other definitions. Regardless of the

definitions, this study also revealed that diagnosing MetS can help identify individuals who are at a higher risk of CVD and can also predict long term CVD events. The AHA-NHLBI definition was found to be the best predictor of CVD followed by the WHO and ATPIII definitions; nevertheless, the hazard risk ratios for all definitions were very close.

Researchers have found that multiple endogenous origin risk factors of CVD may accumulate in one person (He *et al.*, 2006). Thus, MetS has been defined by expert groups as a functional and simple indicator of the risk of CVD, although the predicted risk depends on which definition of MetS is used (He *et al.*, 2006). Some definitions have emphasised insulin resistance as an essential component for the diagnosis of MetS. For instance, according to the WHO definition, without insulin resistance, individuals would not have MetS, even though they have all other criteria (World Health Organization, 1999). Insulin resistance influences hyperglycemia and diabetes mellitus (Reaven, 2004b), and increases lipolysis of stored lipids and free fatty acids (Eckel *et al.*, 1995). Furthermore, it can lead to vasoconstriction and sodium retention which ultimately cause hypertension (Ferrannini *et al.*, 1987). Later, abdominal obesity was detected to be strongly associated with insulin resistance (Carr *et al.*, 2004), impaired glucose tolerance (IGT) (Hayashi *et al.*, 2003), hypertension (Rattarasarn *et al.*, 2003), hyperlipidemia (Nicklas *et al.*, 2003) and increased risk of coronary heart disease (Lamarche *et al.*, 1998). Abdominal obesity is metabolically active and releases bioactive products such as free fatty acids (van Harmelen *et al.*, 2002), inflammatory cytokines and adipokines (Yatagai *et al.*, 2003). Thus, abdominal obesity is implicated as a MetS risk factor, and consequently IDF has considered this kind of obesity to be an essential determinant of the MetS (Carr *et al.*, 2004).

Surprisingly, it was confirmed that individuals having inherent insulin resistance, such as individuals with South Asian ethnicity, can develop insulin resistance and MetS without an excessive degree of obesity, and even with a WC below the cut off points (Abate *et al.*,

2004). In Asian populations the NCEP and ATP III definitions underestimated the population at risk (Tan *et al.*, 2004). Due to the ethnic differences, there has been a proposal for using modified cut-off points for defining central obesity as a risk factor for MetS (International Diabetes Federation, 2006). In the current study, the Iranian National Committee of Obesity cut-off point for WC (≥90 cm for both genders) was used to determine the risk for CVD (Fereidoun Azizi *et al.*, 2010). Further, it was suggested that there should not be any compulsory components (Alberti *et al.*, 2009a).

Previous studies among different population groups in Iran have shown varied prevalence of MetS based on different definitions. However, the patterns of MetS prevalence, using different definitions (Delavari *et al.*, 2009; Zabetian *et al.*, 2007), were very similar to those observed in our study. For instance, in Zabetian et al's study (Zabetian *et al.*, 2007), the prevalence of MetS was 32.1%, 33.2% and 18.4% based on the IDF, ATP III, and WHO definitions respectively. In Delavari et al's study (Delavari *et al.*, 2009) its prevalence was 37.4%, 34.7% and 41.6% based on the IDF, ATP III, and AHA/NHLBI criteria respectively. Other studies have also reported that the prevalence of MetS was approximately 30% in Iranian adults by the ATP III definition (Azizi *et al.*, 2003; Sarrafzadegan *et al.*, 2008). It is likely that using different population criteria, including different age categories, gender ratios, living areas and physical activity levels, might have influenced the reported prevalence of MetS among studies in Iran. Further, the patterns of MetS prevalence in other countries, using different definitions of MetS, have also reported similar variations. An earlier study among Australians reported that one in three was identified with MetS by the IDF definition, while one in five was identified with MstS by using the WHO and ATPIII definitions and it was slightly less when the EGIR definition was used (Cameron *et al.*, 2007).

Previous studies have shown that individuals with MetS were at higher risk of CVD development in the near future (approximately 10 years) even after considering the

confounding effects of other major risk factors such as age, sex, smoking, and hypercholesterolemia (Galassi *et al.*, 2006; Gami *et al.*, 2007b; Girman *et al.*, 2004b; Isomaa *et al.*, 2001; Mottillo *et al.*, 2010; Olijhoek *et al.*, 2004) which is in line with our results. Although the current study found that the WHO and IDF criteria for MetS was related to a high risk for CVD events (HR: 1.92 and 1.65 respectively), their requirements (insulin resistance or abdominal obesity) make it difficult to diagnose high risk individuals without insulin resistance or abdominal obesity. There was also a slight difference in CVD risk between the JIS, AHA-NHLBI and ATP III definitions, which was principally because of the variation in their definition and threshold for impaired fasting glucose and WC. The definition which has higher sensitivity and identified a higher number of individuals who are at risk of CVD is the best. In the current study, the AHA-NHLBI definition was associated with higher prevalence of MetS, as well as higher CVD risk. Thus, AHA-NHLBI definition can be nominated as the best indicator to identify MetS for this study population. On the other hand, as the only difference between the AHA and JIS definitions is the WC threshold, our results showed that the suggested cut-off point for WC in the Iranian population may not be satisfactory and there is a need for redefining the WC cut-off point for the best estimation of CVD risk in this population.

The present study also found a significant association between the risk of CVD and MetS components. The associated risk was higher for glucose intolerance or diabetes (HR: 1.83, 95% CI: 1.56-2.15) than any other MetS components, which is in line with previous research (Bonora *et al.*, 2004). In addition, when the risk of CVD was examined based on the presence of MetS risk factors, more MetS components were associated with a higher risk of cardiovascular events. The risk of CVD for individuals having four and five components of MetS was 2.98 and 6.06 respectively (data not shown). Thus, it may also be necessary to examine the number of components in MetS individuals to identify the individuals at higher

risk of CVD. Nevertheless, other risk factors of MetS including family history of diseases, age, gender, smoking, LDL or total cholesterol levels should be considered for CVD risk factors (Huang, 2009).

This study has some limitations. First, insulin resistance was not assessed directly, but instead, oral glucose tolerance test was used to estimate insulin sensitivity. Nevertheless, it is accepted that this measurement can be linked with insulin resistance (Stumvoll *et al.*, 2000). Further, cohort studies are inherently limited for loss-to-follow up participants. However, the characteristics of individuals did not differ to a great extent as a result of drop-outs. On the other hand, the strength of this study is that to the best of our knowledge, it is the first study that has looked at the HR of CVD events occurrence based on MetS components and different definitions of MetS in a longitudinal study, in urban and rural areas in Eastern Mediterranean countries. Further, this study draws attention to the importance of having a national cut-off point for WC for the Iranian population, which could diagnose the individuals at higher risk of CVD. The present study also emphasises the importance of individual components of MetS for prediction of CVD risk.

In conclusion, this representative sample of Iranian adults revealed a varied prevalence of MetS when using difference definitions of MetS. Further, follow-up of participants for more than 10 years showed that CVD risk was significantly higher in MetS participants, irrespective of the used definitions, as well as in participants with any risk factors of MetS. Overall, the AHA-NHLBI and JIS definitions were better indicators because they were able to capture more individuals with MetS who were not identified by the EGIR and WHO definitions and were also at higher risk of CVD. Finally, the findings of this study emphasise the need for using the best possible population specific indicators for identifying MetS individuals. In addition, there is an urgent need for the development and implementation of appropriate protective measures, including lifestyle modifications, to improve all the MetS

components. Control of individual components to prevent cardiovascular events is also necessary.

4.2.9 Acknowledgment

4.2.10 Author contribution

H.K. designed the concept of the study and prepared the draft manuscript. M.S., H.R. and M.T. effectively worked for the data collection. H.K. and M.D. statistically analyzed the data. F.A. A.P. and N.S. provided guidance on the study design and critically reviewed the manuscript. All authors read and approved the final manuscript.

The authors declare that there is no conflict of interest.

Table 4.2.1: Different definitions of metabolic syndrome

	WHO	EGIR	NCEP ATP III	AHA	IDF	JIS
Definitions	insulin resistance together with two or more of the following:	Insulin resistance or impaired fasting glucose (IFG) plus two of the following:	Three or more of the following five risk factors:	Three or more of the following five risk factors:	Central obesity plus 2 other features	Three or more of the following five risk factors:
Fasting plasma glucose	__	≥ 6.1 mmol/l (110 mg/ dl) but non-diabetic	≥ 6.1 mmol/l (110 mg/ dl)	≥ 5.6 mmol/l (100 mg/ dl)	≥5.6 mmol/l (100 mg/ dl) or diagnosed type 2 diabetes	≥ 5.6 mmol/l (100 mg/ dl)
Central obesity	Men: waist–hip ratio > 0.90 Women: waist–hip ratio > 0.85 and/or BMI > 30 kg/m2	Men: waist circumference ≥ 94 cm Women: waist circumference ≥ 80 cm	Men: waist circumference > 102 cm Women: waist circumference > 88 cm	Men: waist circumference > 102 cm Women: waist circumference > 88 cm	Men: waist circumference ≥94cm , Women: waist circumference ≥80cm* or BMI >30kg/m²	Ethnic cut point for waist circumference *
Blood pressure	≥140/90 mmHg	≥ 140/90 mmHg or treatment	≥ 130/ 85 mmHg	≥ 130/ 85 mmHg	≥130/85 mmHg or treatment	≥ 130/ 85 mmHg
Triglycerides	≥ 1.7 mmol/l (150 mg/ dl)	> 2.0 mmol/l (178 mg/ dl)	≥ 1.7 mmol/l (150 mg/ dl) or treatment	≥ 1.7 mmol/l (150 mg/ dl) or treatment	≥1.7 mmol/l (150 mg/ dl) or treatment	≥ 1.7 mmol/l (150 mg/ dl) or treatment
HDL-cholesterol	Men: < 0.9 mmol/l (35 mg/ dl) Women: < 1.0 mmol/l (39 mg/ dl)	< 1.0 mmol/l (39 mg/ dl) or treatment	Men: < 1.03 mmol/l (40 mg/ dl) Women: < 1.29 mmol/l (50 mg/ dl)	Men: < 1.03 mmol/l (40 mg/ dl) Women: < 1.29 mmol/l (50 mg/ dl)	Men: <1.0 mmol/l (39 mg/ dl) Women: <1.3 mmol/l (40 mg/ dl) or treatment	Men: < 1.03 mmol/l (40 mg/ dl) Women: < 1.29 mmol/l (50 mg/ dl)

* based on Iranian cut off point, Waist Circumference ≥ 90 for both sexes

Table 4.2.2: Prevalence of metabolic syndrome by sex and age groups based on having MetS using different definitions

	MetS definition					
	WHO	EGIR	ATPIII	AHA-NHLBI	IDF*	JIS
Total	13.3%	8.8%	30.0%	36.9%	28.0%	31.2%
Sex						
Male	10.6%	6.9%	20.7%	21.4%	25.5%	29.1%
Female	15.9%	10.7%	38.8%	51.7%	30.2%	33.2%
Age Categories						
35-45	7.6%	5.8%	19.9%	28.4%	19.0%	21.2%
45-55	13.1%	8.5%	32.7%	40.6%	31.4%	34.2%
55-65	19.8%	12.7%	40.1%	47.4%	37.0%	42.2%
65-75	21.3%	13.0%	43.4%	46.9%	37.9%	42.3%
>75	21.9%	14.4%	30.5%	33.3%	27.8%	33.7%

* Waist Circumference $\geq$ 90 for both sexes

Table 4.2.3: Present of cardiovascular events based on different definition of metabolic syndrome

		Cardiovascular event		
		No	Yes	P
Presence of MetS by different definitions	MetS (N)	N (%)	N (%)	
WHO definition	Yes (766)	568 (11.8)	198 (25.5)	<0.001
	No (4818)	4241 (88.2)	577 (74.5)	
EGIR definition	Yes (507)	386 (8.0)	121 (15.6)	<0.001
	No (5077)	4423 (92.0)	654 (84.4)	
ATPIII definition	Yes (1701)	1337 (27.8)	364 (47.0)	<0.001
	No (3883)	3472 (72.2)	411 (53.0)	
AHA-NHLBI definition	Yes (2093)	1681 (35.0)	412 (53.2)	<0.001
	No (3491)	3128 (65.0)	363 (46.8)	
IDF* definition	Yes (1595)	1266 (26.3)	329 (42.5)	<0.001
	No (3989)	3543 (73.7)	446 (57.5)	
JIS definition	Yes (1773)	1397 (29.0)	376 (48.5)	<0.001
	No (3811)	3412 (71.0)	399 (51.5)	
Cardiovascular risk factors				
Age (years)		49.8 ± 11.3	58.0 ± 11.6	<0.001
FBS (mg/dl)		87.0 ± 29.9	101.1 ± 48.5	<0.001
Total Cholesterol (mg/dl)		212.2 ± 51.5	228.8 ± 55.8	<0.001
HDL-C (mg/dl)		46.9 ± 10.4	47.0 ± 10.6	0.87
LDL-C (mg/dl)		127.6 ± 42.9	138.4 ± 46.3	<0.001
Triglyceride (mg/dl)		188.2 ± 101.7	217.4 ± 114.5	<0.001
Waist circumference (cm)		94.4 ± 12.2	97.4 ± 12.4	<0.001
BMI		26.6 ± 4.4	27.2 ± 4.7	<0.001
Systolic BP (mmHg)		120.1 ± 20.0	133.5 ± 24.2	<0.001
Diastolic BP (mmHg)		77.7 ± 11.2	83.1 ± 12.8	<0.001
Daily Physical Activity (Mets/h)		882.4 ± 544.9	755.1 ± 562.9	<0.001
Smoking				<0.05
Current smoker		769 (16.0)	139 (18.0)	
Past smoker		270 (5.6)	64 (8.3)	
Never smoker		3763 (78.4)	570 (73.7)	

* Waist Circumference ≥ 90 for both sexes

Data presented as number (percent) or mean ± Standard deviation

Table 4.2.4: Hazard Ratio of CVD occurrence based on different definitions of metabolic syndrome and its components

Metabolic syndrome definitions	Crude HR (95% CI)	P-Value	Adjusted HR** (95% CI)	P-Value
WHO	2.41 (2.05-2.83)	<0.001	1.92 (1.62-2.26)	<0.001
EGIR	2.03 (1.67-2.47)	<0.001	1.66 (1.37-2.03)	<0.001
ATPIII	2.12 (1.84-2.44)	<0.001	1.87 (1.61-2.16)	<0.001
AHA-NHLBI	1.98 (1.72- 2.28)	<0.001	1.93 (1.66-2.25)	<0.001
IDF*	1.93 (1.67-2.23)	<0.001	1.65 (1.43-1.91)	<0.001
JIS	2.14 (1.86-2.46)	<0.001	1.80 (1.56-2.08)	<0.001
Metabolic syndrome components				
Obesity	1.51 (1.31-1.75)	<0.001	1.44 (1.24-1.67)	<0.001
High triglyceride	1.67 (1.43-1.95)	<0.001	1.60 (1.37-1.87)	<0.001
High LDL-C	1.49 (1.28-1.74)	<0.001	1.30 (1.11-1.52)	<0.01
Low HDL-C	1.07 (0.91-1.25)	0.404	1.19 (1.01-1.39)	<0.05
Hypertension	2.51 (2.18-2.89)	<0.001	1.79 (1.53-2.08)	<0.001
Glucose intolerance/ Diabetes	2.21 (1.88-2.59)	<0.001	1.83 (1.56-2.15)	<0.001

*Waist Circumference ≥90 for both sexes

**Adjusted Model for age, sex, smoking status and physical activity

4.3 Secular trend of metabolic syndrome and its components in a cohort of Iranian adults from 2001 to 2013

4.3.1 Preface

Despite the great improvements in the therapeutic control of MetS and its components, they are still significant global health problems (Grundy, 2008; Khosravi-Boroujeni *et al.*, 2015). Thus, it is of great clinical and public health interest to follow the trends in the prevalence of MetS and its components to adjust the therapeutic and preventive measures so that the consequences of the chronic diseases can be minimized.

Previous studies in Iran have reported an increase in the prevalence of MetS as well as its components, including obesity and diabetes, but they were based on cross-sectional studies. Moreover, these studies have used different definitions of MetS. As reported in section one, we found that the AHA-NHLBI definition is the best possible definition of MetS to predict CVD in the Iranian population. Therefore, we examined the 12-year trends of MetS and its components in our studied population from 2001 using the AHA-NHLBI definition. We also investigated the age adjusted trend for controlling the effect of aging in this cohort study. Figure 4.3.1 presents the flow chart of participant follow-up from 2001 to 2013.
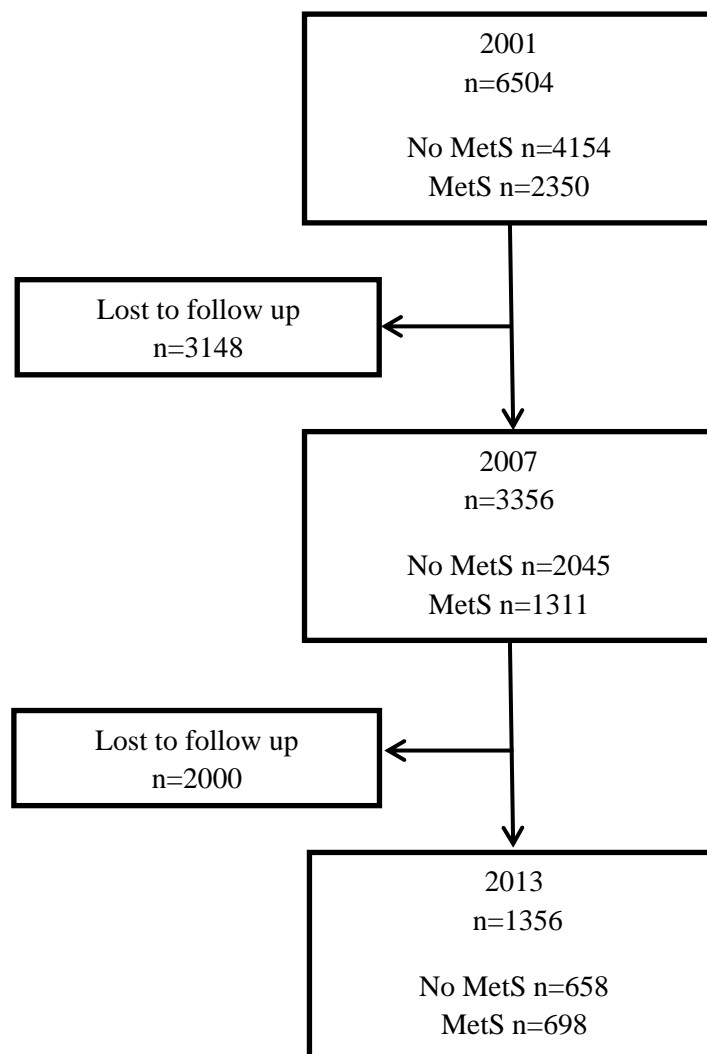
Figure 4.3.1: Consort diagram showing the process of subject selection and loss to follow-up information during 2001 to 2013.

4.3.2 Statement of contribution to co-authored published paper:

This section includes a co-authored manuscript published in "Metabolic Syndrome and Related Disorders" journal. The status of the co-authored paper, including all authors, is: Hossein Khosravi-Boroujeni, Nizal Sarrafzadegan, Masoumeh Sadeghi, Hamidreza Roohafza, Mohammad Talaei, Shu-Kay Ng, Hai Phung, Ali Pourmogaddas, and Faruk Ahmed. (2017). Secular Trend of Metabolic Syndrome and Its Components in a Cohort of Iranian Adults from 2001 to 2013. Metabolic Syndrome and Related Disorders. doi: 10.1089/met.2016.0073

4.3.3 The research candidate has made the following contributions to this study:

- ✓ Developed the study design.
- ✓ Cleaned and prepared the variables for this study.
- ✓ Analysed the data and interpreted the findings.
- ✓ Prepared the manuscript and submitted to the journal.

██████

Hossein Khosravi-Boroujeni (06/12/2016)

██████████

Principal Supervisor: Faruk Ahmed (06/12/2016)

4.3.4 Abstract:

Background: Metabolic syndrome (MetS) and its components increase the risk of developing cardiovascular diseases, type 2 diabetes and all-cause mortality. Reports on the trends of MetS and its components in longitudinal studies are scarce, especially in low- and middle-income countries. This study was designed to investigate the prevalence and trends of MetS and its components in a cohort of Iranian adults from 2001 to 2013.

Methods: Participants were followed up for 12 years in a longitudinal population-based study of 6500 adults aged 35 years and older in 2001. Participants were randomly selected from 3 provinces in central Iran. Socio-demographic characteristics, anthropometry, blood pressure and various biochemical indices were collected in 2001, 2007 and 2013. Secular trend and age-adjusted trend of MetS and its components were calculated from 2001 to 2013.

Results: The standardized prevalence of MetS, hypertension, low HDL-C, abdominal obesity and diabetes/IGT increased over the 12 years (6.9, 5.5, 12.0, 2.3 and 18.7% respectively), while the prevalence of hypertriglyceridemia decreased by 15.5% during this period. The prevalence of MetS, low HDL-C and abdominal obesity were higher in females than males in all three phases. Moreover, the increases in the prevalence of these metabolic abnormalities were higher in the rural population than in the urban population.

Conclusion: The present study underscored the increasing trends in MetS and most of its risk factors, thus, to prevent an increase in the cardiovascular risk factors there is a need to improve lifestyle by education, screening and treatment of abnormalities.

Keywords: metabolic syndrome, hypertension, dyslipidemia, obesity, Insulin Resistance.

4.3.5 Introduction:

Metabolic syndrome (MetS) is a collection of abnormalities which comprise, abdominal obesity , glucose intolerance, hypertriglyceridemia, low level of high-density lipoprotein cholesterol (HDL-C), and hypertension (Alberti *et al.*, 2009a). MetS increases the risk of developing cardiovascular diseases (CVD), type 2 diabetes, other diseases and all causes mortality (Browning *et al.*, 2004; Chen *et al.*, 2012; Grundy, 2007). Consequently, MetS has been considered as a global health problem (Grundy, 2008). During the past decades, the prevalence of MetS has increased worldwide, most likely as a consequence of modern lifestyle (van Vliet-Ostaptchouk *et al.*, 2014). A continuing increase in the prevalence of MetS has been reported among western populations (Mozumdar & Liguori, 2011). Our recent study in Iran also showed that the prevalence of MetS varies from 13 to 37 percent based on different diagnostic criteria (Khosravi-Boroujeni *et al.*, 2015). An increase in the prevalence of MetS has also been reported in Iran, possibly as a result of major lifestyle changes and population aging (Sarrafzadegan *et al.*, 2009a; Sarrafzadegan *et al.*, 2013a). Furthermore, evidence exists for an increased prevalence of some components of MetS such as abdominal obesity and glucose intolerance (Whiting *et al.*, 2011). These are also independently associated with the increased risk of chronic diseases such as CVD and diabetes mellitus (Eckel *et al.*, 2005; Khosravi-Boroujeni *et al.*, 2015).

Investigating the trend of MetS and its components is important in order to determine health policies, allocate community and health resources and to promote measures to reduce trends of increasing prevalence. Most of the previous studies on the prevalence of MetS and its components used cross-sectional data, and limited reports using data extracted from longitudinal studies are available. This study was designed to investigate the trend of MetS prevalence and its components in a cohort of Iranian adults from 2001 to 2013. It also aimed to calculate the prevalence according to gender, age categories, and rural/urban residency.

4.3.6 Method and Material

4.4.6.1 Study design

This study was designed as part of the Isfahan Cohort Study (ICS) that started in 2001 through 2013. ICS is a longitudinal population-based study of 6500 adults 35 years and older at baseline (2001), from 3 provinces in central Iran. Multistage random sampling was used to select samples which represent their society's distribution of age, gender and residential area (urban/rural). More details about the ICS have been published elsewhere (Sarrafzadegan *et al.*, 2011). The current study was approved by Isfahan Cardiovascular Research institute (ICRI) and the Griffith University ethics committees.

4.3.6.2 Measurements

Following fasting blood sampling, blood pressure and anthropometric measurements and physical examinations were carried out. Weight, height and waist circumference (WC) were measured by trained researchers. Blood pressure was taken twice in a sitting position and the mean value was computed. Global dietary index (GDI) of diet was calculated via questions from consumption of specific food groups including sweets, hydrogenated oils, animal fats, red meat, fast foods, fruit and vegetables. Smaller GDI shows better dietary behaviour.
Serum triglycerides, total cholesterol (TC) and fasting blood sugar (FBS), were measured using the enzymatic method (McNamara & Schaefer, 1987). Serum HDL-C was determined after separation of very low-density lipoprotein (VLDL) and low-density lipoprotein cholesterol (LDL-C) (Warnick *et al.*, 1985). The Friedewald formula was used to calculate the LDL-C level (Friedewald *et al.*, 1972b). All subjects provided informed written consent for their participation. The measurements were repeated in 2007 and 2013 for all participants

using the same methods, however, participants were followed every two years by phone calls looking for the occurrence of fatal and non-fatal myocardial infarction or stroke or sudden cardiac death (Sarrafzadegan *et al.*, 2011).

4.3.6.3 Definitions and criteria

MetS was identified based on the revised version of the ATP III criteria, adjusted by the American Heart Association (AHA) and the National Heart, Lung, and Blood Institute (NHLBI). Based on our previous study (Khosravi-Boroujeni *et al.*, 2015), this diagnostic criterion was the best predictor of CVD in the studied population. According to this criteria, having three or more components, as listed below, is required for diagnosing MetS (Grundy *et al.*, 2005). Impaired glucose: FBS $\geq$ 5.6 mmol/l (100 mg/ dl) or being on hypoglycemic medication; Abdominal obesity : men, waist circumference $\geq$ 102 cm and women, waist circumference $\geq$ 88 cm; Elevated blood pressure: SBP/DBP $\geq$ 130/ 85 mmHg or being on blood pressure treatment; Hypertriglyceridemia: triglyceride (TG) $\geq$ 1.7 mmol/l (150 mg/ dl) or treatment; Low HDL-C: men < 1.03 mmol/l (40 mg/ dl) women < 1.29 mmol/l (50 mg/ dl). Combinations of MetS components were considered based on the presence of different components of MetS.

4.3.6.4 Statistical analysis

The difference of the characteristics between available participants and loss-to-follow-up individuals were examined by independent sample t-test. Variables are presented as the mean (Standard deviation) or percentage. The mean value for each of the individual characteristics between the 3 phases was compared using Generalized Estimating Equation (GEE). GEE is used for repeated measurements with missing values such as cohort data (Twisk, 2013).

Analyses were stratified by gender, age and residential area categories to consider their effect on the prevalence of MetS and its components. For age, participants were divided into five groups: 35-45, 45-55, 55-65, 65-75 and >75 years based on the age in 2001, 2007 and 2013 phases.

Generalized Estimating Equation (GEE) was used to determine trends in the prevalence of MetS and its components from 2001 to 2013. To control the effect of aging on the trends of MetS and its components, age was adjusted in logistic regression and through the following equation, prevalence was calculated (Bastos *et al.*, 2015).

$$P = \frac{1}{1 + \exp[-(\beta + \beta AGE)]}$$

Where P is the probability of MetS or its components, β and βAGE are regression coefficients calculated from the data. Statistical analysis was carried out using SPSS statistical software package version 22 (SPSS Inc., Chicago, IL, USA).

4.3.7 Results

Available participants and loss-to-follow-up individuals were comparable in terms of gender (male: 48.6% vs 48.7%; female; 51.4% vs 51.3%), age (49.2 vs 51.3 years) and MetS (37.4% vs 35.8%). Characteristics of individuals in 2001, 2007 and 2013 showed that both systolic and diastolic blood pressure significantly increased from 2001 to 2013. The increasing trends were also observed in FBS levels, WC and BMI. On the contrary, decreasing trends were detected for total cholesterol, LDL-C, HDL-C, triglyceride and physical activity (Table 4.3.1).

When examining the trend, an upward trend was revealed for an overall prevalence of MetS (15.4 % increase) and most of its components, including elevated blood pressure (18.2 % increase), Low HDL-C (14.4 % increase), abdominal obesity (8.1 % increase) and

diabetes/IGT (26.5% increase), while the prevalence of hypertriglyceridemia decreased from 2001 to 2013 (16.9% decrease). Moreover, it has shown that the prevalence of MetS, elevated blood pressure, hypertriglyceridemia and diabetes/IGT has been increased in older age groups (except age group >75years and sometimes age group 65-75 years) in each phase. The prevalence of MetS, low HDL-C and abdominal obesity was higher in females than males in all three phases, however, no specific patterns were observed in other components of MetS.

The age-standardized prevalence of MetS and its components showed an increasing trend in the prevalence of MetS (6.9% increase), elevated blood pressure (5.5% increase), low HDL-C (12.0% increase), abdominal obesity (2.3% increase) and diabetes/IGT (18.7% increase) but not in hypertriglyceridemia (15.6% decrease) (Figure 4.3.2). These trends were observed, to some extent, in each age category (Figure 4.3.3). The prevalence of MetS, low HDL-C and abdominal obesity was higher in females than males in all three phases. When the population was divided based on their residential area, an upward trend was also observed in MetS, elevated blood pressure, low HDL-C, abdominal obesity and diabetes/IGT for both rural and urban populations. Dietary changes to unhealthy diet were also observed in both populations. However, the increases in the prevalence of these metabolic abnormalities were higher in the rural population compared to the urban population. Moreover, the prevalence of MetS and its components was higher in urban areas in 2001 and lower in 2013 compared to rural areas (Table 4.3.2). Overall, of all the components, the prevalence of diabetes/IGT increased the most, followed by low HDL-C.

Age-adjusted odds of MetS and its components between 2001, 2007 and 2013 were presented in table 4.3.3. Results indicated that the risk of MetS, elevated blood pressure, low HDL-C (except for females in 2007) and impair glucose were significantly higher in 2007 and 2013 compared to 2001 independent of age and gender. On the contrary, the risk of hypertriglyceridemia has been significantly decreased in 2007 and 2013. Moreover, the age-

adjusted odds ratio for abdominal obesity decreased in 2007 and increased in 2013 compared to 2001.

4.3.8 Discussion

This study investigated the prevalence and trends of MetS and its components among Iranian adults in a cohort study from 2001 to 2013. The current study revealed that the prevalence of MetS and most of its components were increased over the 12 year period. This study also showed an increase in the prevalence of MetS with an increase in age up to the age of 75 years followed by a decrease in its prevalence. Several factors might be responsible for this reduction, the most possible is the decrease in appetite and abdominal obesity in elderly (Hickson, 2006). Frequent medical check-up and treatment in people older than 75 years might be another cause for this result. Moreover, older people may not be interested in modern lifestyles such as the changes in dietary behaviour were not significant among individuals older than 65 years old (data not shown). Finally, individuals with MetS may develop other diseases that lead to premature death (Ahmed *et al.*, 2012). Furthermore, a small sample size in this age group compared to other age groups might have also affected the proportion of individuals with metabolic abnormalities. In the present study, a similar pattern was also observed in the components of MetS and thus confirmed the findings of previous studies (Ford *et al.*, 2002; Hildrum *et al.*, 2007) which reported that the prevalence of MetS increases strongly with age. The Third National Health and Nutrition Survey (NHNS), on American adults, revealed that the MetS prevalence increased with age from 20–29 to 60–69 years (Ford *et al.*, 2002). Another study showed that the prevalence of MetS and its components increased, and the highest prevalence was reported at the ages of 60–69 years (Kuzuya *et al.*, 2007). Although more risk factors emerge as age increase, this result may

indicate that aging affects the MetS prevalence independent of other related factors such as genetics and environment. It has also been proposed that a lifetime accumulation of unhealthy behaviors, such as low physical activity; an unhealthy dietary intake; obesity; untreated dyslipidemia and hypertension; changes in insulin and other hormone secretion; and other physiological and environmental factors may lead to a higher prevalence of MetS in older ages (Grundy *et al.*, 2005; Kraja *et al.*, 2006).

To eliminate the effect of aging, which is an intrinsic part of cohort studies, we calculated the age-adjusted prevalence of MetS and its components from 2001 to 2013 which showed an increase in the prevalence of MetS by 6.9 percent from 2001 to 2013. The contributors to this increase were elevated blood pressure, low HDL-C, abdominal obesity and diabetes/IGT (5.5, 12.0, 2.3 and 18.7% respectively). Interestingly, the increases in the prevalence of MetS, elevated blood pressure, Low HDL-C, abdominal obesity and diabetes/IGT, over a period of 12 years, were higher in men. Although the reason for this result is not evident, less concern for diet, health and appearance by men compared to women may result in a metabolic worsening in men (Kiefer *et al.*, 2005).

When calculating changes in the prevalence of MetS components from 2001 to 2013, an increase in the prevalence of diabetes/IGT was higher than other components and also indicated that the risk of diabetes increased in the recent years. Glucose intolerance is one of the most important characteristics of MetS (World Health Organization, 1999). Glucose intolerance increases free fatty acids (Eckel *et al.*, 1995) while insulin resistance can result in sodium retention and vasoconstriction which eventually initiate hypertension (Ferrannini *et al.*, 1987). The prevalence of diabetes is increasing as a result of aging, population growth, lifestyle changes, such as urbanization and physical inactivity, and the increasing prevalence of obesity (Wild *et al.*, 2004). The prevalence of diabetes is expected to be double in 2030 compared to 2010 (Unwin *et al.*, 2009). In developing countries, the number of people with

diabetes has been increasing over recent decades, mostly due to rapid socio-economic growth (Ramachandran *et al.*, 2012). In addition, abdominal obesity is significantly associated with glucose intolerance/insulin resistance (Carr *et al.*, 2004), hypertension (Rattarasarn *et al.*, 2003) and hyperlipidemia (Nicklas *et al.*, 2003). As a result, abdominal obesity is a critical factor in the pathogenesis of MetS. Our results also reflect an increasing trend in abdominal obesity and decreasing trend in physical activity after 12 years (Sarrafzadegan *et al.*, 2008). During past decades a dramatic change has occurred regarding the way that people move, eat and drink. These transformations have conflicted with personal biology and consequently, body composition has been affected, as illustrated by rising obesity in both developed and developing countries (Popkin *et al.*, 2012; Sarrafzadegan *et al.*, 2014). It has been estimated that the prevalence of obesity will increase by 33% over the next two decades and 51% of the population will be obese by 2030 (Finkelstein *et al.*, 2012).

On the other hand, the association of sedentary lifestyle with central adiposity, insulin resistance (Thorp *et al.*, 2009) and hypertension (Nualnim *et al.*, 2012) has been reported by previous studies. After all, an inverse association between the amount of physical activity and MetS has been documented (Hahn *et al.*, 2009). Urbanization and rapid economic change have been found to be associated with decreased physical activity generally and occupational, domestic, and transport-related physical activities (Ng *et al.*, 2009). It has also been revealed that information and communication technology and transportation changes are the most important reasons for decreasing physical activity (Pratt *et al.*, 2012).

A decreasing trend, over the 12 years period, in the prevalence of hypertriglyceridemia is another important result of this study. Previous studies also reported a decreasing trend in the level of serum lipids after 6 to 20 years (Beltrán-Sánchez *et al.*, 2013; Carroll *et al.*, 2012; Cohen *et al.*, 2010). Although there is no clear reason for the decreasing prevalence of hypertriglyceridemia in our study population, there could be several possible explanations for

this decreasing trend. The favorable trends in serum lipids observed in the current study may be due, in part, to an increase in clinical screening and recognition of dyslipidemia and also an increase in the proportion of the population who received lipid-lowering treatment (Carroll *et al.*, 2012). Moreover, healthy lifestyle changes, such as a decrease in the consumption of carbohydrates and *trans*-fatty acids has been suggested as a possible reason for this trend (Vesper *et al.*, 2012). There is also some evidence that the decreasing trend in triglyceride levels is attributable to a reduction in cigarette smoking and carbohydrate intake (Carroll *et al.*, 2012). Iranians traditionally used to have a high carbohydrate and saturated fat diet that could increase triglyceride levels. However, their diet has been gradually changed and the portion of carbohydrates and saturated fat consumed has decreased in recent years (Ghassemi *et al.*, 2002). Moreover, it is possible that the existing public educational programs (Sarrafzadegan *et al.*, 2006) may have contributed to this change. In ICS, prevalence of smoking had decreased from 16.7% in 2001 to 6.7% in 2007 and 2.8% in 2013. Moreover, mean consumptions of bread and rice were also decreased during this period (data are not shown).

Another interesting result of our study is the changes in the pattern of MetS and its components between rural and urban areas. Although in 2001, as expected, the metabolic abnormalities were worse in urban areas, the inverse situation was observed in 2013. Rural areas in Iran also embraced lifestyle changes by changing their diet and decreased physical activity as a result of modern technology. It is possible that this transformation could have an important effect on abdominal obesity and, consequently, on other components of MetS. It has been reported that by increasing income, a higher prevalence of obesity was regularly reported in rural areas and among poor families due to increased intakes of refined carbohydrates, added sugars and fats. Moreover, the increase in the prevalence of obesity was higher in rural (3.9%) than urban (2.5%) residence (Popkin *et al.*, 2012).

The strengths of this study include the assessment of MetS and its components, 3 times over a period of 12 years, based on a cohort data to remove the effect of diversity in the studied population. While previous studies dealt with the prevalence of MetS and its components over time using cross-sectional studies, at different points in time. Besides, in the current study the revised version of ATP III criteria, which was the best predictor of CVD in the studied population based on our previous study (Khosravi-Boroujeni *et al.*, 2015), was used to diagnose MetS. The primary limitation of the present study was the loss to follow-up individuals over the 12 year period which is inevitable in cohort studies. Moreover, the use of medications was based on self-reported data which may be inaccurate.

In conclusion, this study reveals a significant increasing trend in the age-standardized prevalence of MetS from 2001 to 2013 among the Iranian population. Interestingly the increased prevalence of MetS observed in both rural and urban areas. Aging, low physical activity, abdominal obesity and glucose intolerance were the main determinants for increasing the prevalence of MetS. Thus, to prevent an increase in the cardiovascular risk factors and the risk of other diseases related to MetS, there is an urgent need for developing an appropriate intervention programmes to control MetS and its components.

4.3.9 Acknowledgment

4.3.10 Author's contribution:

H K-B contributed to the design of the study, analyzed the data and wrote the manuscript; MS, HR, MT and AP collected the data; S-K N and HP provided guidance in performing statistical analysis and critical revision of the manuscript; NS contributed to the design of the study and critical revision of the manuscript; FA provided guidance on the overall design of the study, contributed to the interpretation of results and critical revision of the manuscript. All authors have read and approved the final manuscript.

Table 4.3.1: Characteristics of individuals in 2001, 2007 and 2013 separated by sex

	2001			2007			2013		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
	3336	3168	6504	1711	1645	3356	700	656	1356
Age (years)	50.60 (11.4)	51.4 (12.0)	51.01 (11.7)	54.8 (10.0)	56.1 (10.7)	55.4 (10.4) [*]	58.4 (8.5)	60.1 (9.6)	59.2 (9.1) [**]
Systolic blood pressure(mmhg)	122.5 (21.9)	121.2 (20.4)	121.9 (21.2)	122.2 (19.9)	124.0 (19.7)	123.1 (19.9)	128.8 (17.9)	128.4 (17.4)	128.6 (17.7) [*]
Diastolic blood pressure(mmhg)	78.9 (12.1)	78.2 (10.9)	78.5 (11.6)	76.4 (11.5)	76.9 (11.6)	76.6 (11.6) [*]	81.9 (13.4)	83.9 (11.4)	82.9 (12.5) [**]
Fasting blood sugar (mg/dl)	89.2 (32.5)	88.2 (32.9)	88.7 (32.7)	101.4 (37.0)	98.5 (33.6)	99.9 (35.4) *	109.1 (43.3)	104.0 (30.9)	106.7 (37.8) [**]
Total cholesterol (mg/dl)	220.4 (52.9)	207.8 (51.4)	214.3 (52.5)	222.2 (44.7)	206.4 (41.9)	214.4 (44.1)	207.8 (39.8)	192.3 (40.4)	200.3 (40.8) [*]
HDL cholesterol (mg/dl)	48.4 (10.5)	45.3 (10.1)	46.9 (10.4)	48.9 (11.4)	42.9 (9.6)	45.7 (11.1) *	47.5 (17.8)	41.0 (22.3)	44.4 (20.4) [**]
LDL cholesterol (mg/dl)	134.1 (43.4)	123.6 (42.8)	128.9 (43.4)	127.6 (30.3)	120.3 (28.7)	124.0 (29.8) [*]	115.1 (28.4)	108.0 (26.4)	111.7 (27.7) [**]
Triglyceride (mg/dl)	189.8 (101.5)	194.7 (168.0)	192.2 (104.6)	171.5 (101.9)	175.8 (117.9)	173.6 (110.0) [*]	155.6 (85.8)	155.1 (83.5)	155.3 (84.7) [**]
Waist circumference	96.5 (12.8)	92.3 (11.5)	94.5 (12.4)	95.8 (12.0)	92.9 (10.5)	94.4 (11.4)	98.0 (12.2)	97.4 (10.3)	97.7 (11.3) [*]
BMI	27.8 (4.72)	25.4 (3.9)	26.6 (4.5)	28.7 (4.6)	26.1 (3.8)	27.4 (4.4) [*]	29.6 (4.9)	26.8 (4.0)	29.0 (4.7) [**]
Physical activity (mets/h)	648.9 (422.5)	1089.3 (571.4)	863.4 (546.8)	659.7 (386.2)	877.2 (836.9)	766.3 (656.6) [*]	693.6 (412.3)	861.8 (719.7)	775.1 (587.7) [*]
Global dietary index	4.9 (1.7)	5.1 (1.8)	5.0 (1.7)	4.8 (2.0)	4.8 (2.0)	4.8 (2.0) [*]	5.3 (1.9)	5.3 (1.9)	5.2 (2.0) [**]

Data are presented as Mean (Standard deviation)

*P < 0.05 compared with 2001 values

**P < 0.05 compared with 2007 values

Table 4.3.2: Age-standardized prevalence of MetS and its components from 2001 to 2013 separated by sex and residency area

		2001			2007			2013		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
MetS*										
	Total	20.2	50.6	35.6	26.3	46.7	36.7	33.9	50.8	42.5
	Urban	22.7	52.6	37.9	26.9	45.4	36.1	34.1	48.2	41.1
	Rural	14.0	45.6	29.8	24.2	49.9	38.3	39.8	60.5	48.2
MetS Components										
Elevated Blood Pressure*										
	Total	32.9	37.7	35.5	35.7	31.4	33.6	40.6	37.7	39.0
	Urban	31.9	36.5	34.4	36.6	30.9	33.8	40.5	35.7	37.9
	Rural	35.6	41.2	38.3	33.5	32.6	38.3	42.0	45.4	49.2
Hypertriglyceridemia*										
	Total	59.6	58.8	59.0	49.7	46.6	47.9	45.9	41.5	43.4
	Urban	62.6	60.0	61.1	51.3	47.2	49.0	46.8	40.4	43.3
	Rural	52.0	55.6	53.4	44.6	45.0	44.6	43.6	46.8	45.1
Low HDL-C*										
	Total	31.4	57.8	44.8	42.2	55.9	49.1	50.7	62.4	56.8
	Urban	32.8	58.1	45.8	43.2	54.9	48.9	50.7	61.4	56.2
	Rural	28.2	56.8	42.5	39.2	58.4	49.3	50.2	66.8	59.6
Abdominal obesity *										
	Total	21.4	77.6	50.1	17.6	74.8	47.2	26.4	75.9	52.4
	Urban	24.9	82.9	54.5	15.3	71.9	43.8	25.2	73.9	50.5
	Rural	12.4	63.4	38.5	24.2	82.0	56.3	29.8	82.3	58.2
Diabetes/IGT*										
	Total	12.9	19.6	14.4	24.6	28.5	26.5	32.2	33.9	33.0
	Urban	14.3	16.2	15.2	25.8	27.6	26.5	32.5	31.1	31.7
	Rural	9.2	15.0	12.1	20.9	30.7	26.3	31.8	43.9	38.3

Prevalence is reported in percentage.

* a significant change (p<0.05) between 2001, 2007 and 2013

Impaired glucose: FBS $\geq$ 5.6 mmol/l (100 mg/ dl), **Abdominal obesity :** men: waist circumference > 102 cm and women: waist circumference > 88 cm, **Elevated Blood Pressure:** SBP/DBP $\geq$ 130/ 85 mmHg, **Hypertriglyceridemia:** Tg $\geq$ 1.7 mmol/l (150 mg/ dl), **Low HDL-C**: men: < 1.03 mmol/l (40 mg/ dl) women: < 1.29 mmol/l (50 mg/ dl).

Table 4.3.3: Age-adjusted odds ratios for the prevalence of MetS and its components from 2001 to 2013

		Total		Male		Female	
		Odd ratio	95% CI	Odd ratio	95% CI	Odd ratio	95% CI
MetS							
	2001	1:00	-	-	-	-	-
	2007	1.13	1.05-1.22	1.48	1.31-1.67	0.96	0.87-1.07
	2013	1.88	1.68-2.09	2.81	2.38-3.32	1.46	1.24-1.70
Elevated Blood Pressure							
	2001	1:00	-	-	-	-	-
	2007	1.25	1.16-1.36	1.56	1.38-1.75	1.02	0.91-1.15
	2013	2.78	2.46-3.14	3.12	2.61-3.74	2.51	2.12-2.97
Hypertriglyceridemia							
	2001	1:00	-	-	-	-	-
	2007	0.66	0.61-0.71	0.61	0.57-0.70	0.56	0.48-0.66
	2013	0.50	0.45-0.56	0.45	0.38-0.53	0.69	0.62-0.77
Low HDL-C							
	2001	1:00	-	-	-	-	-
	2007	1.09	1.02-1.18	1.46	1.30-1.63	0.87	0.78-0.96
	2013	1.70	1.52-1.91	2.35	2.01-2.73	1.32	1.12-1.55
Abdominal obesity							
	2001	1:00	-	-	-	-	-
	2007	0.92	0.86-0.98	0.85	0.74-0.96	0.95	0.83-1.08
	2013	1.38	1.25-1.54	2.06	1.75-2.43	1.19	1.01-1.44
Diabetes/IGT							
	2001	1:00	-	-	-	-	-
	2007	2.35	2.07-2.46	2.34	2.05-1.67	2.38	2.11-2.68
	2013	4.53	4.01-5.12	4.88	4.08-5.82	4.29	3.62-5.07
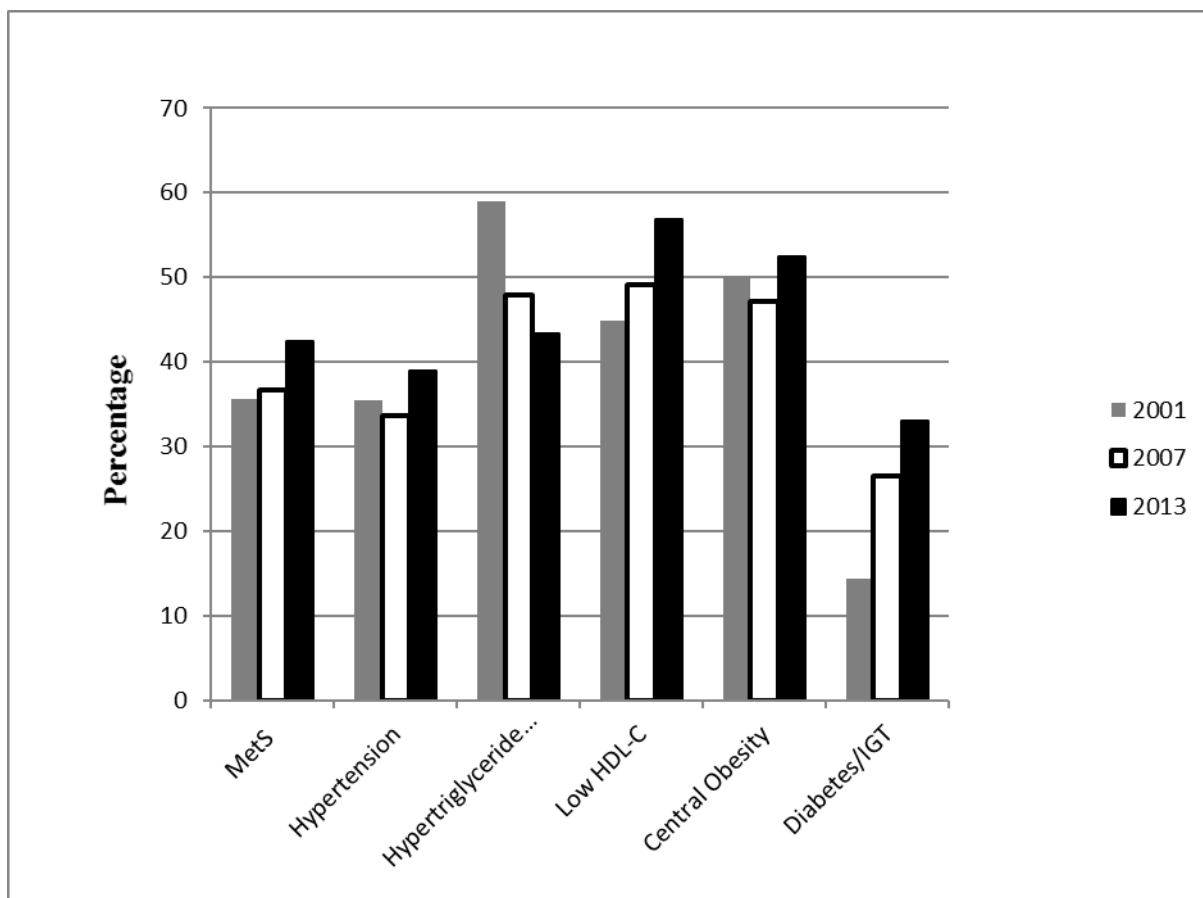
Figure 4.3.2: Age-standardized prevalence of MetS from 2001 to 2013
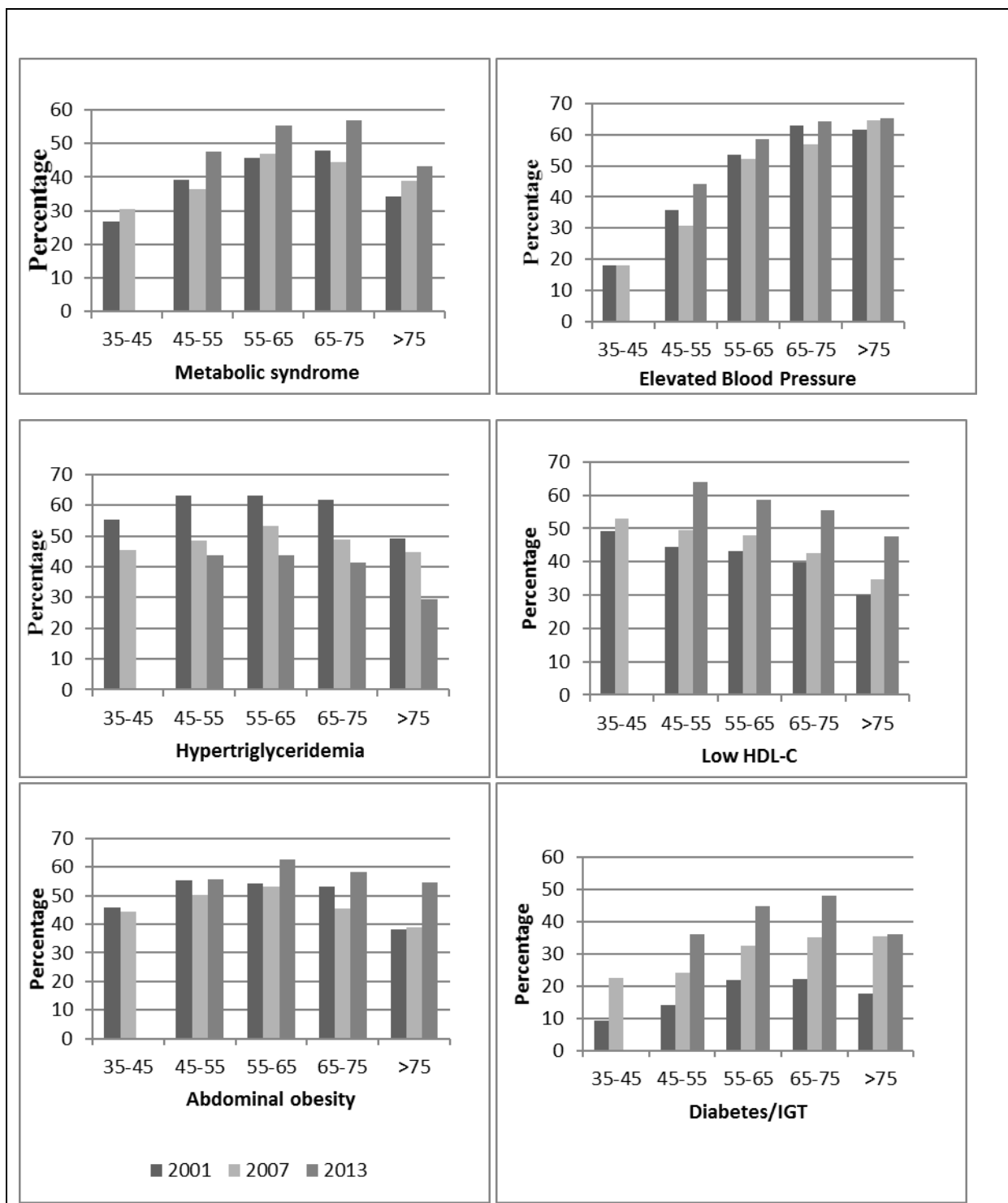
Figure 4.3.3: Prevalence of MetS and its components from 2001 to 2013 by age groups

4.4 Factors influencing the changes in metabolic syndrome components in a twelve-year cohort of Iranian adults

4.4.1 Preface

The previous section demonstrated that the prevalence of MetS and its components (except hypertriglyceridemia) have increased from 2001 to 2013. Earlier studies indicated different factors, including urbanization, socio-economic circumstances, unhealthy diet and physical inactivity as possible causes for these trends (Ntandou *et al.*, 2008; van Vliet-Ostaptchouk *et al.*, 2014). Identifying these potential risk factors is important to prevent the increasing trend of MetS and its components. Therefore, this is an incentive to explore the contributing factors to these trends in the study population. In this study, we studied the influence of various socio-demographic and lifestyle factors which might influence changes in MetS components. Figure 4.4.1 presents the theoretical framework of this study.
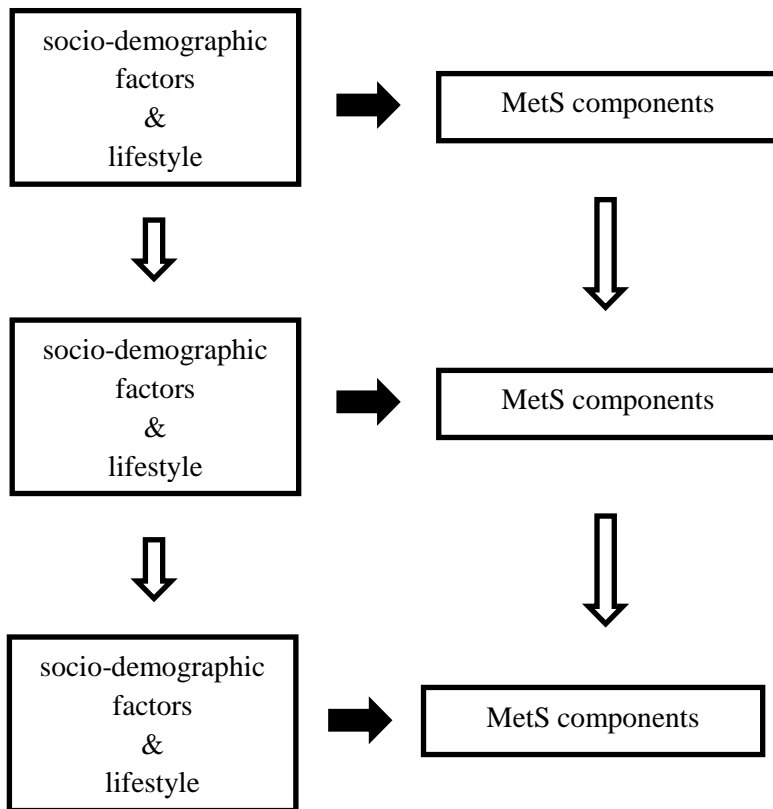
Figure 4.4.1: Theoretical framework of the study. The black arrows represent direct influence; the white arrows represent changes over time.

4.4.2 Statement of contribution to co-authored published paper:

This section includes a co-authored manuscript submitted in the journal, Diabetology and Metabolic Syndrome. The status of the co-authored paper, including all authors, is: Hossein Khosravi-Boroujeni, Nizal Sarrafzadegan, Masoumeh Sadeghi, Hamidreza Roohafza, Shu-Kay Ng, Ali Pourmogaddas, and Faruk Ahmed.

4.4.3 The research candidate has made the following contributions to this study:

- ✓ Developed the study design.
- ✓ Cleaned and prepared the variables for this study.
- ✓ Analysed the data and interpreted the findings.
- ✓ Prepared the manuscript and submitted to the journal.

4.4.4 Abstract:

Background: The prevalence of metabolic syndrome (MetS) has been increasing globally and it is known to be associated with increased risk of chronic diseases and related mortality. In order to prevent the increasing risk of chronic diseases, there is a need for better understanding of MetS components and their risk factors. This study was designed to determine the changes in the components of MetS in a cohort of Iranian adults from 2001 to 2013 and to examine the extent to which various socio-demographic and lifestyle factors might influence these changes.

Method: Study participants were followed up in an ongoing longitudinal population-based study over 12 years. The study population was selected from adults aged $\geq$ 35 years from central Iran in 2001. MetS components and its risk factors were measured using standard methods in 2001, 2007 and 2013. The Generalized Estimating Equation test was carried out to determine the changes during the follow-up and multivariate regression analysis was used to examine the association between various socio-demographic and lifestyle factors and changes in MetS components.

Results: A significant increase in systolic and diastolic blood pressure (SBP and DBP), fasting blood sugar (FBS), waist circumference (WC) and body mass index (BMI) were observed during the 12-year period, while serum cholesterol and triglyceride (TG), and physical activity levels decreased significantly. Age, gender, marriage status and education levels were found to have a significant independent association with the changes in MetS components.

Conclusion: The findings indicate that the components of MetS changed over a period of 12 years since 2001. In addition, lifestyle factors appear to have significant influences on the changes of the components of MetS and there is a need for changes in lifestyle factors to be included as a preventive strategy.

4.4.5 Introduction

Metabolic syndrome (MetS) is a group of interrelated metabolic risk factors, such as glucose intolerance, central obesity, dyslipidemia and hypertension (Alberti *et al.*, 2009a). Current evidence suggests that MetS is associated with the increased risk of chronic diseases and related mortality (Khosravi-Boroujeni *et al.*, 2015). Further, previous studies have shown that subjects with glucose abnormality, obesity, dyslipidemia or hypertension are at higher risk of cardiovascular disease (CVD) events (Bastien *et al.*, 2014; Colangelo *et al.*, 2015; González-Santos *et al.*, 2014; Li *et al.*, 2014). There has been a suggestion that each of the MetS components is also independently associated with chronic diseases and, thus, they need to be targeted for therapeutic interventions (Khosravi-Boroujeni *et al.*, 2015). The prevalence of MetS and its components has been increasing globally, and modern lifestyle, urbanization and ageing population have been blamed for this increasing prevalence of MetS (van Vliet-Ostaptchouk *et al.*, 2014). Moreover, behavioural and socioeconomic factors may also be associated with the risk of chronic diseases and MetS components, notably, lifestyle and social factors, including education level (Lee *et al.*, 2005b), physical inactivity (Rennie *et al.*, 2003), unhealthy dietary intake (Andersen & Fernandez, 2013), and smoking (Sun *et al.*, 2012).

As the risk of chronic diseases related to MetS has been increasing rapidly, prevention of MetS and controlling its components are currently recognised as one of the essential global

health goals (Grundy, 2008) and, thus, strategies to prevent the increasing trend of MetS and its components are warranted (Grundy *et al.*, 2004a). Therefore, in order to develop appropriate strategies to prevent the increasing trend of MetS and its components, it is crucial to identify the risk factors that might influence the development and/or changes of MetS components over time. The Isfahan Cohort Study (ICS) is a unique studies in the Eastern Mediterranean region, which considered metabolic and cardiovascular risk factors in a longitudinal study among Iranian adults. Recently we have demonstrated that the prevalence of MetS, obesity, hypertension, low high density lipoprotein cholesterol (HDL-C) and glucose abnormality increased from 2001 to 2013 in a population of Iranian adults (Khosravi-Boroujeni *et al.*, 2016b). To control this ascending trend and prevent the related diseases, it is of paramount importance to understand the underlying causes of this increase. Therefore, the current study aimed to determine the changes in the components of MetS in a cohort of Iranian adults from 2001 to 2013. In addition, we explored the extent to which various socio-demographic and lifestyle factors might influence these changes.

4.4.6 Method and Material

4.4.6.1 Study design

ICS is an ongoing longitudinal population-based study which began in 2001. The purpose of the study was to identify CVD risk factors by sampling 6504 adults aged $\geq$ 35 years, from three counties in central Iran (rural and urban areas) who participated at the baseline stage. More details about the ICS and the sampling method are accessible in previous publications (Sarrafzadegan *et al.*, 2006; Sarrafzadegan *et al.*, 2011).

4.4.6.2 Measurements

Blood pressure (BP) was measured twice, while sitting, at 5-min intervals with a random-zero sphygmomanometer. The mean of the measurements was used as the SBP and DBP. Weight, height and WC were measured by trained researchers using standard protocols (NIH & Obesity, 2000). Subjects were asked to fast before blood sampling. Serum total cholesterol (TC), TG and FBS levels were measured enzymatically (McNamara & Schaefer, 1987). Serum HDL-C was separated from very low-density lipoprotein (VLDL) and low density lipoprotein cholesterol (LDL-C) by combined ultracentrifugation. Serum LDL-C level was calculated using the Friedewald formula (Friedewald *et al.*, 1972b). AHA-NHLBI definition was used as the best definition for the studied population, based on our prior study (Khosravi-Boroujeni *et al.*, 2015). Family and medical history, as well as medications, smoking habits, and socioeconomic status were also assessed. Physical activity levels, including transport, leisure time activity and working at home were calculated based on total daily physical activity in MET (metabolic equivalent) per day (mets/d) (Sadeghi *et al.*, 2011). Dietary intake was assessed by a validated food frequency questionnaire (FFQ) and the dietary score was evaluated based on the average score of 29 questions regarding healthy and unhealthy dietary intake (Mohammadifard *et al.*, 2009a). ICS follow-ups which began in 2001 are carried out every two years by telephone, and complete structured follow-ups equal to the baseline were conducted in 2007 and 2013 (Sarrafzadegan *et al.*, 2011).

4.4.6.3 Statistical methods

The characteristics of the study population and loss-to-follow up participants were compared using the independent sample t-test and chi-square test. Differences in baseline biochemical

and socio-demographic characteristics between individuals with, and without, MetS were tested using Chi-square, Fisher exact test (when there are cells with the expected counts of less than 5) or an independent sample t-test, as appropriate. To examine the mean change in anthropometric and biochemical characteristics and physical activity levels from 2001 to 2013, the Generalized Estimating Equations test was applied with time as repeated measures. A multivariate regression model was applied to test the association between selected socioeconomic or lifestyle characteristics and changes in MetS components. For each variable, based on the univariate significance and clinical importance, the model was adjusted for other variables including age, sex, physical activity, education levels, diet, marital status and smoking habits. All statistical analyses were performed using SPSS 22 (IBM, Chicago, IL, USA), and $p<0.05$ was considered as a significant level.

4.4.7 Results

Comparing the characteristics of individuals with and without MetS presented significant differences in their characteristics. Participants with MetS were older and had lower levels of physical activity and were more likely to be female. They had lower education levels and were more often urban citizens compared to those who were free of MetS. Additionally, prevalence of smoking and marriage were lower in individuals with MetS (Table 4.4.1). Moreover, there were no significant differences in sex, age and MetS between available participants and loss-to-follow up participants (Table 4.4.2).

The mean change of biochemical and anthropometric characteristics of the participants from 2001 to 2013 revealed a significant increase in systolic and diastolic BP, FBS, WC and BMI levels, while there was a significant decrease in TC, HDL-C, LDL-C, TG and physical activity levels (Table 4.4.3). However, the trends of changes were slightly different between

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2001-2007 and 2007-2013. From 2001 to 2007, DBP decreased and total cholesterol increased, but changes in WC were not significant. On the other hand, there were no significant changes in TG and physical activity levels from 2007 to 2013.

The multivariate adjusted association of socioeconomic and lifestyle characteristics with changes in MetS components in the study population is presented in Table 4.4.4. Results indicated that, with increasing age, changes in SBP, DBP and FBS (r =-0.25, -0.07 and -0.47 respectively), and TG (r =-0.83 and -0.79) were lower from 2007-2013, while changes in TG (r =-0.83 and -0.79) and BMI (r =-0.04 and -0.03) were lower in both time periods. Moreover, in females from 2001 to 2007, changes in SBP and DBP were fewer (r =-3.00 and -2.01, respectively) but changes in HDL-C (r =3.74) were more than that of the males. Individuals with higher education levels were subjected to higher changes in FBS and HDL during the period 2001-2007 (r =2.92 and 1.40 respectively) and changes in TG from 2007-2013 (r =3.25); however, changes in FBS and HDL from 2007-2013 (r =-7.73 and -3.33 respectively), TG in the period 2001-2007 (r =-11.2) and BMI for both time periods (r =-0.63 and -0.41) were lower in individuals with higher education levels. In addition, the results showed that married individuals experienced lower changes in SBP, FBS and HDL-C from 2007-2013 (r =-5.57, -10.43 and 7.97 respectively) compared to singles.

4.4.8 Discussion

The present study demonstrated that the components of MetS among Iranian adults changed during the 12-year period since 2001. We have also reported the magnitude to which various socio-demographic and lifestyle factors have influenced the changes of the components of MetS. Our results showed that SBP, DBP, FBS, WC and BMI increased, while TC, HDL-C, LDL-C, TG and physical activity decreased from 2001 to 2013. The results also indicated

that age, gender, marriage status and education level were the most significant determinants of the changes in MetS components.

Previous studies also showed an increasing trend in most of the cardiovascular risk factors including hyperglycemia and abdominal obesity in recent years, which might be the result of modern lifestyle, civilization and an ageing population (Beltrán-Sánchez *et al.*, 2013). It has also been proposed that the decreasing trend in some of the risk factors including TC and TG might be the result of a better and earlier diagnosis and treatment of hyperlipidemia in recent years (Carroll *et al.*, 2012).

In the present study, the increases in the mean of BP and WC in the first period (2001-2007) were considerably less than in the second period (2007-2013). The first phase of the study coincided with the Isfahan Healthy Heart Program (IHHP), which was a comprehensive, community-based intervention program for non-communicable disease prevention and health promotion. The interventions were performed at community level using different strategies, including mass media, community activities and involvement of governmental or non-governmental organizations to reduce CVDs risk factors (Sarraf-Zadegan *et al.*, 2003a). The results of the IHHP study indicated that community-based interventions were effective in improving lifestyle behaviours and controlling risk factors such as the trend of increases in BP (Khosravi *et al.*, 2012; Khosravi *et al.*, 2010; Sarrafzadegan *et al.*, 2009b; Sarrafzadegan *et al.*, 2013b). The impact of these community interventions might be a possible reason for the different patterns in some of the cardiovascular risk factors in the two periods. A recent study in Alberta also reported positive effects of short term community-based interventions on some clinical risk factors; however, the authors suggested that more intense and long term interventions will be required to make these positive effects sustainable (Lytvyak *et al.*, 2016).

The results of our multivariable analysis revealed a negative association between age and changes in the components of MetS over the 12-year period; however, the association could not reach significant levels for TG and the first phase of BP. Although previous studies have shown positive association between age and MetS components (Ford *et al.*, 2002; Hildrum *et al.*, 2007), the patterns of increase in MetS component levels were not always the same. For example, rapid increase in the components of MetS, such as obesity and impaired glucose tolerance in susceptible adults, may occur until midlife and, thereafter, the increase is generally modest or there is a decreasing trend (Kuzuya *et al.*, 2007). The possible explanation for this changing pattern could be due to increased medical check-ups and the diagnosis of these abnormalities among older adults, which may lead to treatment and changes in lifestyles behaviours.

The results of our study also showed changes in BP and HDL in females in the first period; however, we failed to find the same association in the period from 2007-2013. In the second period, the study population became older. Therefore, in 2007 the median age was 51 years, and the likelihood of menopause was higher in females and the protective effect of sexual hormones was eliminated. Previous studies reported the positive effect of estrogen on CVD risk in women and reported that CVD was lower among women less than 45 years of age (Lobo, 2008). Others reported that from six years before to six years after the final menstrual period, the incidence of MetS increased significantly (Joylene, 2015). On the other hand, another hypothesis for the lower increase in MetS components in females might be related to the greater influence of community-based intervention on females compared to males (Mozumdar & Liguori, 2010; Sarrafzadegan *et al.*, 2013b).

Another important finding in our study was that higher education levels could moderate the increase in some of the MetS components in the second phase. Previous studies also indicated that education levels were independently associated with MetS and some of its components

including WC, BP, TG, HDL-C (Lee *et al.*, 2005b), and found that education is a good indicator of socio-economic status (Santos *et al.*, 2008). Moreover, it has been assumed that individuals with higher education levels, who usually have a higher level of health literacy, have a tendency to follow healthy lifestyle recommendations (Ngo *et al.*, 2013). The lower increase in some of the MetS components among less educated individuals in the initial time period might be explained by IHHP interventions. In this study, we failed to find any significant association between physical activity or dietary score and changes in MetS components after controlling for possible confounders. However, the mean physical activity level in our study population was very low and, therefore, it might have influenced the results.

The main strength of this study is the design, which was a population-based longitudinal design with a large study population to diminish the effect of diversity in the population. Another strength of the current study was that anthropometric variables were assessed by trained researchers and were not self-reported. Moreover, in this study, we assessed the determinants of changes in the components of MetS, which was innovative to some extent. However, our limitations include loss to follow-up individuals, which is usual in cohort studies. Furthermore, dietary intake and physical activity were self-reported, which might be a potential for reporting bias.

4.4.9 Conclusion

This study, on a cohort of Iranian adults, indicated that BP, FBS and BMI increased over a period of 12 years from 2001, while TG and HDL-C decreased during this period. Additionally, while socio-demographic factors such as age and gender are non-modifiable factors, the findings of the present study emphasize the need for changes in lifestyle factors as

a part of preventive strategies. Further research is warranted on the association of dietary factors and changes in MetS components.

4.4.10 Acknowledgment

4.4.11 Author's contribution:

H K-B contributed to the design of the study, analyzed the data and wrote the manuscript; MS, HR and AP collected the data. S-K N provided guidance in performing statistical analysis, contributed to the interpretation of results and critical revision of the manuscript; NS contributed to the design of the study and critical revision of the manuscript. FA provided guidance on the overall research plan and design of the study, contributed to the interpretation of results and critical revision of the manuscript. All authors have read and approved the final manuscript.

Table 4.4.1: Baseline socio-demographic and lifestyle characteristics of participants with or without metabolic syndrome

	Metabolic syndrome				P value
	Yes n=2350		No n=4154		
Age (years) mean (SD)	53.47	11.60	49.61	11.58	<0.001
Physical activity mean (SD)	719.7	500.7	944.7	555.0	<0.001
Gender (male) (%)	28.0		60.4		<0.001
Education (%)					<0.001
illiterate	34.8		44.7		
primary school	33.9		34.3		
more than primary school	31.3		21.0		
Residency area (%)					<0.001
Urban	77.0		69.9		
Rural	23.0		30.1		
Married (%)	86.2		94.2		<0.001
Smoking (%)	13.4		27.6		<0.001

Table 4.4.2: Characteristics of available participants and loss-to-follow up individuals

	loss-to-follow-up subjects	available subjects	P value
Gender (male) **(%)**	48.7	48.6	0.12
Age [mean(SD)]	51.3 (12.47)	49.2 (10.45)	0.06
BMI	26.42 (4.47)	27.47 (4.45)	0.18
MetS **(%)**	35.8	37.4	0.37
Hypertension **(%)**	36.8	31.7	0.01
Diabetes/glucose intolerance **(%)**	14.9	13.5	0.21
Hypertriglyceridemia **(%)**	58.5	62.2	0.14
Low HDL **(%)**	45.2	45.3	0.97
Smoking **(%)**	22.8	21.0	0.15

Table 4.4.3: Mean changes and 95% CI in metabolic syndrome components and other cardio-metabolic risk factors from 2001 to 2013

	Change (2001-2007)		Change (2007-2013)		Change (2001-2013)	
	mean changes	95% CI	mean changes	95% CI	mean changes	95% CI
Systolic blood pressure (mmhg)	1.22	0.37, 2.07*	5.56	4.29, 6.84*	6.79	5.60, 7.96*
Diastolic blood pressure (mmhg)	-1.90	-2.38, -1.41*	6.23	5.50, 6.96*	4.33	3.66, 5.01*
Fasting blood sugar (mg/dl)	11.27	9.84, 12.69*	6.72	4.58, 8.87*	17.99	16.00, 19.98*
Total cholesterol (mg/dl)	0.18	1.85, 2.21*	-14.52	11.46, -17.58*	-14.34	-17.17, -11.50*
HDL cholesterol (mg/dl)	-1.14	-1.67, -0.62*	-1.16	-1.95, -0.38*	-2.31	-3.04, -1.58*
LDL cholesterol (mg/dl)	-4.93	-6.51, -3.34*	-12.44	-14.83, -10.06*	-17.37	-19.58, -15.16*
Triglyceride (mg/dl)	-18.56	-22.89, -14.22*	-18.31	-24.82, 11.79	-36.87	-42.90, -30.83*
Waist circumference	-0.13	-0.63, 0.36	3.36	2.61, 4.12*	3.23	2.53, 3.93*
BMI	0.77	0.59, 0.96*	0.82	0.54, 1.10*	1.59	1.33, 1.85 *
Physical activity (mets/d)	-97.08	-121.72, -72.44*	-2.76	-37.04, 31.53	-99.84	-131.17, -68.51*

*P<0.05

Table 4.4.4: Multivariable-adjusted associations between socioeconomic, behavioural characteristics and changes in MetS components in participants

	SBP Change		DBP Change		FBS Change		TG Change		HDL Change		BMI Change	
	2001-2007	2007-2013	2001-2007	2007-2013	2001-2007	2007-2013	2001-2007	2007-2013	2001-2007	2007-2013	2001-2007	2007-2013
	r	r	r	R	r	r	r	r	r	r	r	r
Age	-0.04	-0.25**	-0.08	-0.07*	0.01	-0.47**	-0.83**	-0.79*	0.001	0.04	-0.04**	-0.03**
Gender (female)	-3.00**	1.98	-2.01**	0.48	2.65	1.84	0.53	9.41	3.74**	-0.39	0.02	-0.14
Physical Activity (Mets/d)	0.001	0.001	0.001	0.001	0.001	0.001	0.005	-0.006	0.006	0.003	-0.006	0.001
Higher education level	-0.48	-.026	-0.36	0.69	2.92*	-7.73*	-11.2*	3.25	1.40*	-3.33*	-0.63**	-0.41*
Dietary score	-0.24	-0.25	-0.10	-0.23	0.31	0.14	-1.30	-1.69	-0.03	-0.16	-0.03	0.03
Marriage	2.04	-5.57*	0.49	0.96	-2.42	-10.43*	-5.17	-13.98	1.12	-7.97*	-0.39	-0.02
smoking	0.40	-1.16	-0.81	1.41	0.96	1.89	-9.02	-5.39	-0.73	-1.75	-0.13	-0.28
Change in Physical Activity	0.01	0.01	0.01	-0.01	0.01	0.01	-0.004	0.005	0.001	-0.003	-0.0008	0.004
Change in dietary score	0.22	0.19	0.08	0.21	-0.31	-0.13	1.11	1.79	0.02	0.07	0.02	-0.02

*P<0.05, ** P<0.001

Higher education compared to lower education levels (primary school and lower), Dietary score compared with lower categories (<4)

r: Coefficient of association

4.5 Prevalence and trends of vitamin D deficiency among Iranian adults: A longitudinal study

4.5.1 Preface

In previous sections, we reported a high prevalence of MetS among Iranian adults and also indicated the effect of some socio-demographic factors on changes in MetS components. It is important to note that vitamin D deficiency has been considered as a re-emerging public health problem globally (Nadir *et al.*, 2010) and some studies have linked vitamin D deficiency to chronic diseases, including MetS (Lu *et al.*, 2009). Considering the possible association between MetS and vitamin D deficiency based on the available literature, we also explored whether vitamin D deficiency is the reason for these changes in MetS and its components. Therefore, we decided to ascertain the current prevalence of vitamin D deficiency in the study population, and also examined the association of vitamin D status with MetS and its components. In this section, the current prevalence of vitamin D deficiency in the study population is reported. Further, we examined the trends of vitamin D deficiency in a longitudinal study from 2001 to 2013. Figure 4.5.1 presents the Framework of this study from 2001 to 2013.
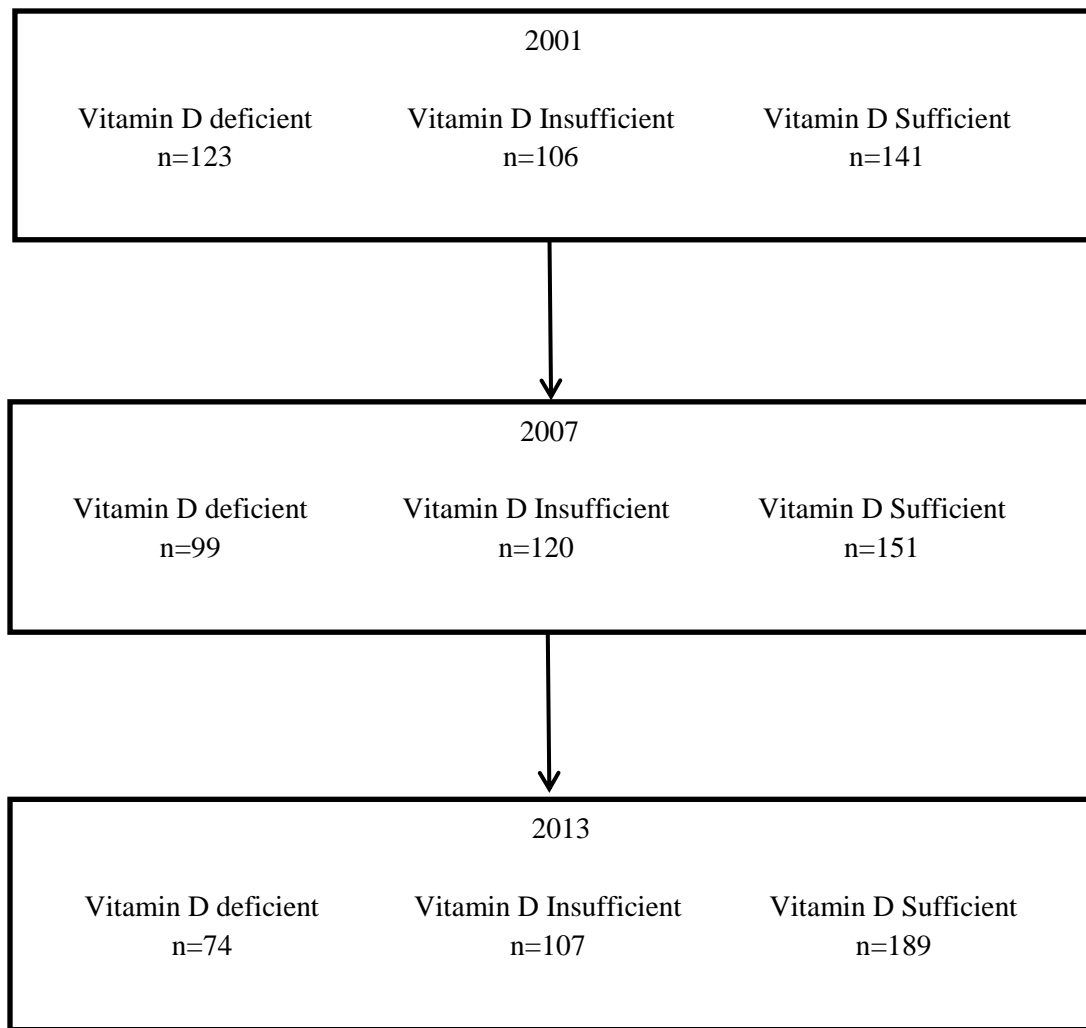
Figure 4.5.1: Framework of this study from 2001 to 2013

4.5.2 Statement of contribution to co-authored published paper:

This section includes a co-authored manuscript which has been submitted in the "Journal of Nutritional Science and Vitaminology".

The status of the co-authored paper, including all authors, is: Hossein Khosravi-Boroujeni, Nizal Sarrafzadegan, Masoumeh Sadeghi, Hamidreza Roohafza, Shu-Kay Ng, Ali Pourmogaddas, and Faruk Ahmed.

4.5.3 The research candidate has made the following contributions to this study:

- ✓ Developed the study design.
- ✓ Selected samples and assessed serum 25(OH)D,
- ✓ Analysed the data and interpreted the findings.
- ✓ Prepared the manuscript and submitted to the journal.

4.5.4 Abstract:

Background: Vitamin D deficiency/insufficiency is currently considered to be a re-emerging public health problem globally.

Aim: This study was designed to determine the prevalence of vitamin D deficiency and insufficiency and to investigate its trend from 2001 to 2013 in a longitudinal study of Iranian adults.

Method: This study was part of a population-based, longitudinal ongoing study of Iranian healthy adults aged 35 years and older at baseline. Vitamin D was assessed in a sub-sample of 370 subjects, who were apparently healthy at the time of recruitment in 2001 and were free from MetS, in three phases (2001, 2007 and 2013) during the 12-year study period. Adjusted prevalence and trend of vitamin D deficiency were calculated.

Results: Mean serum vitamin D levels increased over the time of the study (52.12, 54.27 and 62.28 nmol/L, respectively) and the prevalence of vitamin D deficiency decreased (30.5, 27.0 and 24.4, respectively). However, the prevalence of vitamin D insufficiency did not change during these time periods. The risk of vitamin D deficiency decreased significantly in 2007 [OR: 0.73 (95% CI: 0.53, 0.99)] and 2013 [OR: 0.50 (95% CI: 0.36, 0.70)] compared to the baseline.

Conclusion: The present study demonstrated some improvement in serum vitamin D levels, while the prevalence of vitamin D inadequacy was still high. Considering the possible health consequences of vitamin D deficiency, there is an urgent need for developing population-wide strategies, such as supplementation and fortification, to prevent or control vitamin D deficiency.

Keywords: Vitamin D deficiency, Vitamin D insufficiency, Prevalence, Longitudinal study.

4.5.5 Introduction

Vitamin D deficiency was described as a public health problem more than two hundred years ago during the industrial revolution (Shaw, 2004). The problem declined as a result of the discovery of vitamin D and increasing intake of vitamin D. However, as a consequence of modern lifestyles, more indoor activities and because of an inadequate amount of vitamin D in most people's diets (Vieth *et al.*, 2004), the number of individuals with low serum 25-hydroxyvitamin D (25(OH)D) levels has been increasing (Nadir *et al.*, 2010).

Vitamin D has long been recognized for its role in the regulation of calcium and phosphorus homeostasis and bone health (Holick, 2002). Moreover, there is also an increasing body of evidence that has found an association between vitamin D deficiency and other health problems (Holick, 2010b). For example, the risk of all-cause and cardiovascular mortality were reported higher in people with a lower level of serum 25(OH)D (Dobnig *et al.*, 2008; Melamed *et al.*, 2008; Zittermann *et al.*, 2012). Although the optimal serum level of 25(OH)D in humans is not fully recognised, several expert groups have considered levels of less than 25 nmol/L to be a 'deficiency' and 25-50 nmol/L to be an 'insufficiency' (Food and Nutrition Board. Institute of Medicine, 2011; Gallagher & Sai, 2010).

Due to the potential health problems related to vitamin D deficiency and its increasing prevalence, vitamin D deficiency/insufficiency is currently recognized as a re-emerging public health problem at the global level (Mithal *et al.*, 2009). It is estimated that more than one billion people are vitamin D deficient/insufficient (Holick, 2007). However, the situation seems to be worse in Middle-Eastern countries, despite the sunny climate throughout the year, compared to European countries (Lips, 2007). The prevalence of vitamin D deficiency/insufficiency in adults was reported as 61.4% and 87.8% in Kuwait and Saudi Arabia respectively (Ardawi *et al.*, 2012; Gaafar & Badr, 2013). Although there is no national level data on vitamin D deficiency in Iran, several small scale studies reported a prevalence

of 44.2% to 72.3% in adults (Heshmat *et al.*, 2008; Hossein-Nezhad *et al.*, 2009). Further, there are few longitudinal studies which have investigated the trends in the prevalence of vitamin D deficiency (Hovsepian *et al.*, 2011). Given the importance of adequate 25(OH)D levels and chronic disease prevention, this study aimed to determine the current prevalence of vitamin D deficiency and insufficiency, and to examine its trend in a longitudinal study of Iranian adults for the period 2001 to 2013.

4.5.6 Method and Materials

4.5.6.1 Study design

This study was completed as part of a population-based, longitudinal, ongoing study of healthy Iranian adults aged 35 years and older at the time of recruitment in 2001, known as the Isfahan Cohort Study (ICS) (Sarrafzadegan *et al.*, 2011). Individuals were selected from three provinces in central Iran, using the multistage random sampling method. ICS aimed to determine the impact of various risk factors, including individual and environmental risk factors on the incidence of cardiovascular diseases. Detailed information regarding ICS, including sampling, rationale and results was presented elsewhere (Sarrafzadegan *et al.*, 2013c; Sarrafzadegan *et al.*, 2011). In the current study, vitamin D levels were assessed in subjects who were free of MetS in 2001 and if their frozen serum samples were available in 2001, 2007 and 2013. Thus, using the above criteria, 370 subjects met the selection criteria for the current study. The ethics committees of the Isfahan Cardiovascular Research Institute (ICRI) and Griffith University approved the current study.

4.5.6.2 Measurements

After obtaining informed written consent, an interview was conducted by trained interviewers. A validated questionnaire, comprising questions on socio-demographic characteristics and related lifestyle behaviours, including smoking, physical activity, and nutritional habits, was applied. Anthropometric parameters were measured using standard protocols. Body mass index (BMI) was calculated by dividing the weight in kilograms by height in meters squared. Blood samples were collected after 12 hours of fasting and were immediately frozen at -80°C for future studies. The serum 25(OH)D levels, as the best measure of body vitamin D status (Wang *et al.*, 2008), was measured using the Enzyme Linked Immune Sorbent Assay (ELISA; Euroimmun AG, Luebeck, Germany). For the current study, the frozen serum samples were used to measure 25(OH)D, as it is very stable in serum when kept frozen (Hollis, 2008).

The dietary intake of participants was assessed using a validated 49-item food frequency questionnaire (FFQ) (Khosravi-Boroujeni *et al.*, 2013; Sarraf-Zadegan *et al.*, 2003b) that determined the consumption of each food item during the last year. The main dietary sources of vitamin D, including oily fish, butter, margarine, eggs, beef and liver were considered as dietary intake of vitamin D, which was assessed by summing up the frequency of consumption of those foods. Based on the median intake, individuals with a three or more times per week consumption of vitamin D sources were defined as high consumers. Serum vitamin D levels were categorized based on the most accepted cut off points for deficiency, insufficiency and sufficiency (<25 nmol/L, 25-50nmol/L and >50nmol/L respectively).

4.5.6.3 Statistical analysis

Variables were presented as the mean (standard deviation) or percentage. The frequency of distribution of participants among these three groups was examined using Chi-square or the Fisher Exact test, where appropriate. Changes in serum vitamin D levels from 2001 to 2013 were tested using repeated measures ANOVA. Analyses were stratified by gender, age, BMI, region, education levels, occupation and dietary intake of vitamin D. The Generalized Estimating Equation (GEE) (Hanley *et al.*, 2003) was used to investigate the age-adjusted odds ratios for the prevalence and trend of vitamin D deficiency and insufficiency from 2001 to 2013. In this model, the logistic regression and the AR(1) working correlation matrix have been used. To control the effect of potential confounders on the trends of vitamin D deficiency and insufficiency, age, BMI, region, season of blood collection and dietary intake of vitamin D were adjusted in the model. The predicted value was determined in GEE and the prevalence for each period was calculated. The SPSS statistical software package version 22 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

4.5.7 Results

Examining the serum vitamin D levels from 2001 to 2013 found that mean serum 25(OH)D levels were 52.1 nmol/L, 54.3 nmol/L and 62.3 nmol/L in 2001, 2007 and 2013, respectively (Table 4.5.1). The results identified an increasing trend in serum vitamin D levels over the 12-year period, even when classified according to gender, age, BMI, region, education levels, marital and smoking status, occupation and dietary intake of vitamin D. When comparing serum vitamin D levels separately at different points in time of data collection, there were no statistically significant differences in serum vitamin D levels by various socio-demographic groups, such age, BMI and gender. The frequency distribution of serum vitamin D

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concentration also showed a positive shift and increasing trend from 2001 to 2013 (Figure 4.5.2). The prevalence of vitamin D insufficiency or deficiency at baseline did not differ significantly between different categories of various socio-demographic factors. Further, it was not significantly different between groups with low and high vitamin D intake (Table 4.5.2).

The prevalence of vitamin D deficiency, insufficiency and adequacy were adjusted for age, BMI, region, season of blood collection and dietary intake of vitamin D (Table 4.5.3). The results identified a significant decreasing trend in the prevalence of vitamin D deficiency in both males and females from 2001 to 2013 (6.1%, p value <0.001), while vitamin D sufficiency increased significantly during this period (8.2%, p value < 0.001) from 2001 to 2013. However, the decreasing trend in the prevalence of vitamin D insufficiency approached a significant level. The overall prevalence of vitamin D deficiency/insufficiency also decreased by 8.1% (7.6% in males and 8.8% in females). Although the prevalence of vitamin D inadequacy (combined insufficiency and deficiency) decreased over the 12-year period, the prevalence of vitamin D deficiency and insufficiency were still high in 2001, 2007 and 2013 (62.0%, 57.9% and 53.9%, respectively).

Table 4.5.4 compares the risk of being vitamin D deficient and insufficient in the years 2007 and 2013 with the risk in 2001. For the studied population, the risk of vitamin D deficiency decreased significantly in 2007 [OR: 0.73 (95% CI: 0.53, 0.99)] and 2013 [OR: 0.50 (95% CI: 0.36, 0.70)], while the risk of vitamin D insufficiency decreased significantly only in 2013 [OR: 0.58 (95% CI: 0.44, 0.78)]. In females, the risk of vitamin D deficiency also decreased in 2007 and 2013, while in males, the reduced risk was significant only in 2013. Although the risk of vitamin D insufficiency decreased significantly in females in 2007 [OR: 0.61 (95% CI: 0.38,0.96)] and 2013 [OR: 0.44 (95% CI: 0.28-0.68)], no significant risk was

found in males. Analysing the association between dietary intake of vitamin D and serum vitamin D levels failed to find any significant relationship (data not shown).

4.5.8 Discussion

This study provided an insight into the prevalence and trends of vitamin D deficiency and insufficiency among Iranian adults from 2001 to 2013. The present longitudinal study revealed some important findings. First, this study identified a decreasing trend in vitamin D deficiency and an increasing trend in vitamin D adequacy over the 12-year period since 2001. Moreover, when examining the distribution of serum vitamin D levels by different points in time, there was a positive shift from 2001 to 2013. Furthermore, the results also indicated that vitamin D deficiency and insufficiency are still highly prevalent among the studied population. These results add to the expanding data suggesting that vitamin D inadequacy is a highly prevalent situation globally, as well as in the Middle Eastern countries (Ardawi *et al.*, 2012; Baroncelli *et al.*, 2008; Gaafar & Badr, 2013; Heshmat *et al.*, 2008; Hossein-Nezhad *et al.*, 2009). Previous studies in Iran also reported a high prevalence of vitamin D deficiency and insufficiency (Hovsepian *et al.*, 2011; Rahnavard *et al.*, 2010; Shahla *et al.*, 2015), however, to the best of our knowledge, no cohort study has reported changes in vitamin D levels over time in Iran or in any of the Middle East countries.

The present study also reported a decreased risk of vitamin D deficiency over the last 12 years. This apparent improvement in vitamin D status could be the result of several possible factors. First, in recent years, the general population and medical professionals are becoming more aware of the problem of vitamin D deficiency and its health consequences, including skeletal disorders and chronic diseases. Moreover, medical doctors are more likely to screen for vitamin D status, and there is a higher possibility for diagnosis of vitamin D deficiency

and a greater likelihood to recommend the use of vitamin D supplementation. A study in the same district of Iran compared results on vitamin D deficiency with previous studies in Iran and claimed that the prevalence of vitamin D deficiency was increasing (Hovsepian *et al.*, 2011). However, because of the cross-sectional design of the compared studies, different methods of serum 25(OH)D assessment, different population and different definitions for vitamin D deficiency among studies, this assumption may not be accurate.

Vitamin D deficiency could be attributable to low dietary intake of vitamin D and low sun exposure. However, the present study showed no association between serum vitamin D levels and dietary intake of vitamin D. This result is expected because there are limited foods that naturally contain vitamin D, and almost all foods have less than 400 IU of vitamin D per 100g edible portion. The best sources of vitamin D are oily fish, Cod liver oil, beef liver and egg yolk (Meerza *et al.*, 2010). Alternatively, during sunlight exposure, ultraviolet (UV) photons are absorbed by the epidermal and dermal cells and produce vitamin D_3 from its pre-vitamin (Holick, 2004b).

Despite ample sunshine, Iran is among the countries with the highest prevalence of vitamin D deficiency and insufficiency (Mithal *et al.*, 2009). The present study showed that over half of the studied population were vitamin D deficient or insufficient. This is, in large part, elucidated by restricted sun exposure, skin pigmentation and social factors (Baroncelli *et al.*, 2008). Skin pigmentation prevents UV absorption and thus the skin produces lower vitamin D in the body (Hagenau *et al.*, 2009). Likewise, more popular use of sunscreen may also reduce the cutaneous synthesis of vitamin D (Faurschou *et al.*, 2012). On the other hand, sun-seeking behaviour is not popular among the Iranian population because of the hot and dry climate and to prevent pigmentation, as fair skin is considered an aspect of beauty in this society (Mithal *et al.*, 2009). Moreover, many people avoid UV irradiation because of the growing awareness that sunshine may lead to premature aging and cancers (Hiom,

2006). Skin coverage is another factor affecting vitamin D synthesis and leading to vitamin D deficiency (Aguado *et al.*, 2000), and is common in Iranian women because of cultural and religious considerations. However, in this study there is no data to support this statement.

Generally, urbanization is another reason for low serum vitamin D levels, as people spend most of their time indoors, compared to rural citizens. As a result of increased population, a higher percentage of the population live in apartments, also diminishing exposure to sunshine (Puri *et al.*, 2008). Air pollution and high levels of air particles in big cities in Iran also diminish vitamin D synthesis because UV irradiation is absorbed by these particles and cannot reach the skin (Hosseinpanah *et al.*, 2010). However, in the current study we did not find any significant difference in vitamin D levels between rural and urban populations, which might be because of skin coverage in rural areas, or higher availability of vitamin D supplementation in cities.

The current study also reported a high prevalence of vitamin D deficiency independent of age. However, it is assumed that older individuals are more likely to be vitamin D deficient, not only because they may mostly stay indoors, but also because production of vitamin D in skin decreases with age (Holick *et al.*, 1989). Nevertheless, there are other studies which reported that vitamin D deficiency was highly prevalent in adolescents (up to 70%) (Moussavi *et al.*, 2005) and even more in younger individuals (Hovsepian *et al.*, 2011). Female gender is another causal factor for vitamin D deficiency due to likelihood of pregnancy and lactation. Furthermore, greater body fat composition, which can lead to more vitamin D storage and consequently lower serum vitamin D, can increase vitamin D deficiency in women (Dijkstra *et al.*, 2007; Hagenau *et al.*, 2009). The present study did not find any significant difference between males and females. Considering the higher levels of concern held by women regarding diet and health in women (Kiefer *et al.*, 2005), higher

consumption of vitamin D supplementation in women is conceivable. This could also explain a significantly reduced risk of vitamin D in females but not in males over the 12-year period of our study. Another possible reason for not finding any significant difference in the prevalence of vitamin D deficiency between characteristics of study population might be due to the limited number of individuals in each sub-group, which limits the power to detect significant differences; however, the sample size was calculated to be large enough to determine if there were any significant changes in the prevalence of vitamin D deficiency from 2001 to 2013.

Vitamin D deficiency may be worsened as a result of secondary hyperparathyroidism caused by calcium inadequacy (DeLucia *et al.*, 2003). Moreover, it has been shown that lower serum vitamin D levels were associated with specific genotypes (Baroncelli *et al.*, 2008). A well designed genome-wide association study revealed that different genes related to cholesterol metabolism, hydroxylation, and vitamin D transport, affect vitamin D status and play roles in the etiology of vitamin D deficiency and insufficiency (Wang *et al.*, 2010). This could be another possible reason for a higher prevalence of vitamin D deficiency in some populations, such as Iranians.

This study has a number of strengths. The first was its longitudinal design. There is limited information about the trend of vitamin D deficiency in longitudinal studies and thus this study is among the first that could assess this trend. This information is indispensable for health policies to prevent nutritional deficiencies and related diseases. Another strength is controlling for the possible confounders related to the prevalence and trend of vitamin D deficiency, including age, gender, BMI, region, season of blood collection and dietary intake of vitamin D. However, the current study also has some limitations. First, some of the characteristics of the study subjects differed significantly from that of the subjects in the total cohort. Overall, the study subjects were relatively younger and had better health conditions

than the total cohort and, therefore, the study subjects may not be representative of the wider population. Second, although all study subjects were free from MetS in 2001, some developed the condition over the 12-year period and this may have influenced the present findings. However, after adjusting for the effect of the presence of MetS in the multivariate model the results remained unaltered. Third, we do not have any data on vitamin D supplementation, PTH and calcium levels, which may have some influence on vitamin D levels and, therefore, the findings of the present study should be interpreted with caution.

In conclusion, the present study revealed that there is significant improvement in vitamin D status among Iranian adults over the 12-year period. However, the current prevalence of this nutrient deficiency is still high enough to be considered as being a significant public health issue. Considering the possible health consequences of vitamin D deficiency, there is an urgent need to develop population-wide strategies, such as supplementation and fortification, to prevent or control of vitamin D deficiency. Further studies with representative samples are required to confirm the downward trends of vitamin D deficiency among Iranian adults, and to investigate the determinants of this deficiency in this population.

4.5.9 Author's contribution:

H K-B contributed to the design of the study, analyzed the data and wrote the manuscript; MS, HR and AP collected the data. S-K N provided guidance in performing statistical analysis, contributed to the interpretation of results and critical revision of the manuscript; NS contributed to the design of the study and critical revision of the manuscript. FA provided guidance on study design, contributed to the interpretation of results and critical revision of the manuscript. All authors have read and approved the final manuscript.

Table 4.5.1: Serum 25(OH)D levels, based on individual characteristics, from 2001 to 2013

	n (2001)	2001 mean	(SD)	2007 mean	(SD)	2013 mean	(SD)	P value
Total	370	52.12	(44.81)	54.27	(42.93)	62.28	(46.26)	<0.05
Sex								
Male	216	50.66	(40.12)	51.38	(40.71)	57.99	(41.00)	<0.05
Female	154	54.19	(51.12)	56.34	(45.89)	68.28	(51.99)	
		NS		NS		NS		
Age Categories								
35-45	198	48.88	(39.96)	50.48	(39.21)	56.26	(39.78)	<0.05
45-55	109	51.85	(42.70)	55.14	(29.02)	56.26	(29.44)	
>65	63	62.63	(82.94)	64.58	(80.26)	77.80	(78.10)	
		NS		NS		NS		
BMI								
<25	141	48.91	(34.10)	52.30	(45.95)	62.35	(58.16)	<0.05
25-30	152	53.81	(53.01)	56.79	(43.02)	59.75	(42.38)	
>30	77	50.57	(45.27)	52.27	(38.91)	62.65	(48.27)	
		NS		NS		NS		
Region								
Rural	79	45.95	(39.73)	54.56	(36.44)	64.92	(46.75)	<0.05
Urban	291	53.78	(46.05)	54.19	(44.69)	60.55	(46.05)	
		NS		NS		NS		
Education Level								
Illiterate	67	60.53	(56.15)	65.48	(51.98)	73.13	(54.35)	<0.05
Primary school	135	49.61	(44.50)	52.61	(39.15)	65.64	(45.43)	
More than primary school	168	50.83	(39.27)	51.29	(41.62)	55.06	(41.92)	
		NS		NS		NS		
Marriage								
Single	16	58.92	(48.82)	72.13	(52.21)	79.38	(42.76)	<0.05
Married	354	51.84	(44.77)	53.50	(42.52)	61.53	(46.09)	
		NS		NS		NS		
Smoking								
Smoker	93	47.17	(36.54)	51.48	(42.23)	59.13	(46.48)	<0.05
Non-smokers	277	53.75	(47.43)	55.32	(43.02)	63.47	(46.12)	
		NS		NS		NS		
Occupation								
Out door	96	58.19	(42.13)	62.39	(52.51)	67.76	(33.97)	<0.05
In door	274	56.00	(98.65)	56.95	(90.37)	66.01	(90.93)	
		NS		NS		NS		
Dietary sources of vitamin D*								
low	223	57.99	(68.39)	59.60	(84.82)	61.11	(54.80)	<0.05
High	147	54.49	(64.62)	58.23	(60.98)	72.91	(69.23)	
		NS		NS		NS		

Data are means (standard deviation) of serum 25(OH)vitamin D

*butter, cream, high fat dairies, fish, liver and whole egg

Table 4.5.2: Baseline prevalence of vitamin D classifications according to the characteristics of the study population

	Serum 25(OH)vitamin D categories						P value
	Deficient <25nmol/L		Insufficient 25-50nmol/L		Sufficient >50nmol/L		
	n	(%)	n	%	n	(%)	
Total	123	(33.4%)	106	(28.8%)	141	(37.8%)	
Sex							
Male	69	(31.9%)	62	(28.7%)	85	(39.4%)	0.50
Female	54	(35.1%)	44	(28.6%)	56	(36.4%)	
Age Categories							
35-45	74	(36.4%)	54	(26.8%)	73	(36.9%)	0.99
45-55	32	(28.4%)	42	(37.6%)	39	(33.9%)	
>55	21	(38.5%)	13	(23.0%)	22	(38.5%)	
BMI							
<25	40	(28.4%)	48	(34.0%)	53	(37.6%)	0.26
25-30	59	(38.8%)	37	(24.3%)	56	(36.8%)	
>30	24	(31.2%)	21	(27.3%)	32	(41.6%)	
Region							
Rural	89	(30.6%)	86	(29.6%)	116	(39.9%)	0.11
Urban	34	(43.0%)	20	(25.3%)	25	(31.6%)	
Education Level							
Illiterate	22	(32.8%)	16	(23.9%)	29	(43.3%)	0.48
Primary School	52	(38.5%)	39	(28.9%)	44	(32.6%)	
More Than Primary School	49	(29.2%)	51	(30.4%)	68	(40.5%)	
Marriage							
Single	4	(28.6%)	3	(21.4%)	7	(50.0%)	0.11
Married	119	(33.4%)	103	(28.9%)	134	(37.6%)	
Smoking							
Smoker	32	(34.8%)	23	(25.0%)	37	(40.2%)	0.29
Non-smokers	92	(32.9%)	82	(29.6%)	104	(37.5%)	
Occupation*							
Out door	44	(33.6%)	34	(26.0%)	53	(40.5%)	0.34
In door	79	(33.1%)	72	(30.1%)	88	(36.8%)	
Dietary sources of vitamin D							
low	77	(35.6%)	53	(24.5%)	86	(39.8%)	0.11
High	46	(29.9%)	53	(34.4%)	55	(35.7%)	

*Governmental job and house work were considered indoor and self-employed and not working and retired were considered as outdoor occupation.

Table 4.5.3: Prevalence of Vitamin D deficiency and insufficiency from 2001 to 2013 according to sex

	Total	Male	Female	P value*
Vitamin D deficiency				
2001	30.5%	29.9%	31.2%	<0.001
2007	27.0%	26.7%	27.5%	
2013	24.4%	24.5%	24.2%	
Vitamin D insufficiency				
2001	31.7%	31.7%	31.7%	0.05
2007	30.7%	30.9%	30.5%	
2013	30.2%	30.8%	29.5%	
Vitamin D deficiency/ insufficiency				
2001	62.0%	61.6%	62.6%	<0.001
2007	57.9%	57.7%	58.2%	
2013	53.9%	53.9%	53.9%	
Vitamin D sufficiency				
2001	37.9%	38.4%	37.3%	<0.001
2007	42.1%	42.3%	41.8%	
2013	46.1%	46.0%	46.1%	

* Results adjusted for age, BMI, region, education levels, occupation, season of blood collection and dietary intake of vitamin D

Table 4.5.4: Odds ratios of Vitamin D deficiency and insufficiency from 2001 to 2013*

	Total		Male		Female	
	Odd ratio	95% CI	Odd ratio	95% CI	Odd ratio	95% CI
Vitamin D deficiency						
2001	1:00	-	1:00	-	1:00	-
2007	0.73	0.53, 0.99	0.76	0.50, 1.16	0.68	0.42, 0.98
2013	0.50	0.36, 0.70	0.45	0.28, 0.71	0.56	0.34, 0.93
Vitamin D insufficiency						
2001	1:00	-	1:00	-	1:00	-
2007	0.89	0.66, 1.19	1.17	0.79, 1.74	0.61	0.38, 0.96
2013	0.58	0.44, 0.78	0.72	0.48, 1.05	0.44	0.28, 0.69

* Adjusted for age, BMI, region and vitamin D intake

**In total model gender was also adjusted
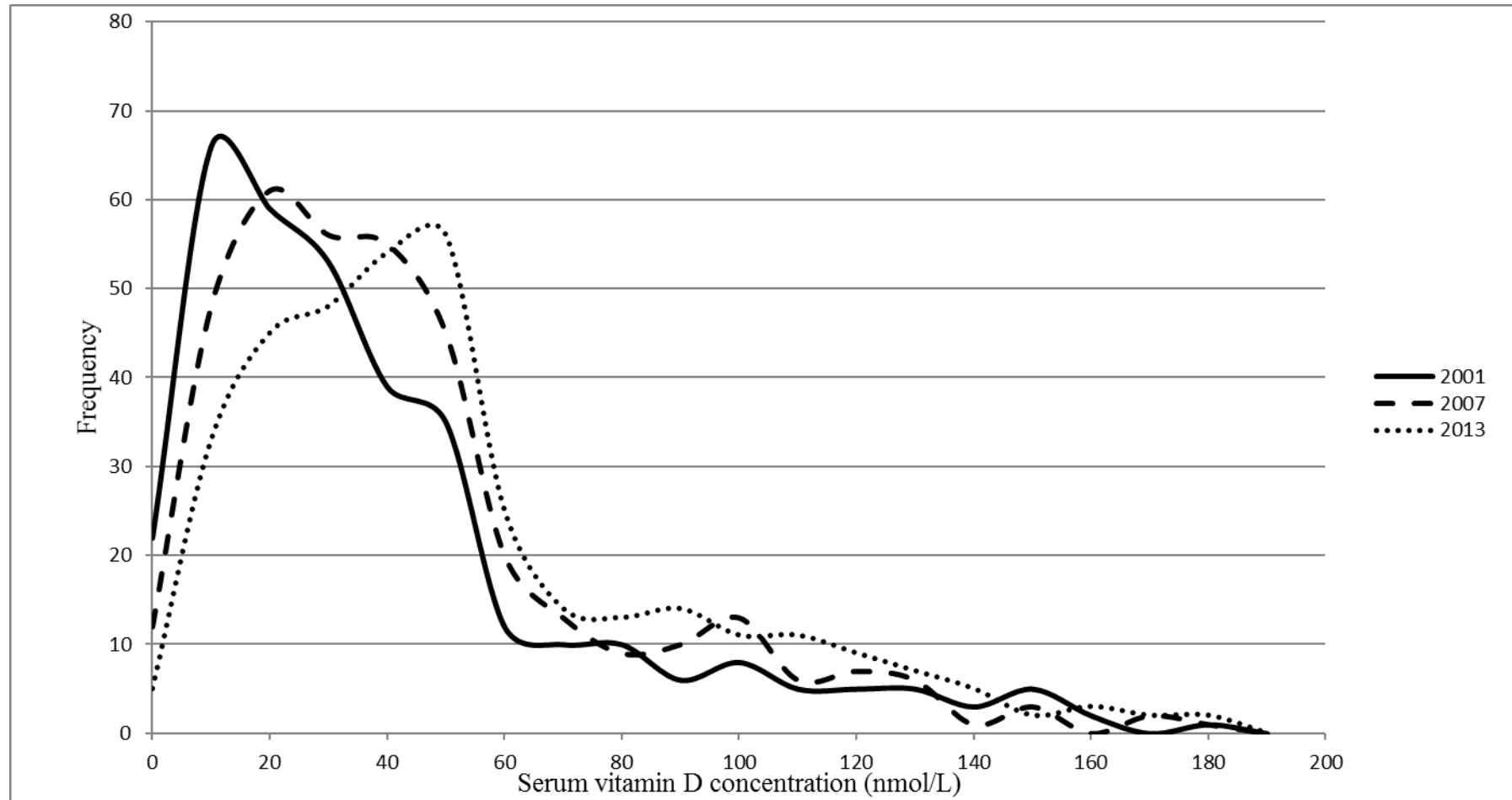
Figure 4.5.2: Frequency of serum vitamin D distribution in 2001, 2007 and 2013

4.6 Association between metabolic syndrome and vitamin D status in a longitudinal study of Iranian adults

4.6.1 Preface

In the previous section we reported a high prevalence of vitamin D deficiency among the Iranian adult population over a 12-year follow-up. We also found a high prevalence of MetS among the studied population. As mentioned earlier, there are studies that have reported an association between vitamin D deficiency and MetS (Lu et al., 2009), while others did not find any association (Amirbaigloo et al., 2013). However, the large majority of these studies were based on a cross-sectional study design. Because of the inconsistency in the findings of the current literature, we decided to examine this association in this longitudinal study. Therefore, in this section, we report the findings of the association between MetS and vitamin D status based on a longitudinal study of Iranian population. The selection criteria for this nested case-control study have been presented in Figure 4.6.1.

```
                    ┌─────────────────────────────────────────┐
                    │          Total samples 2013             │
                    │              n=1356                     │
                    └─────────────────────────────────────────┘

    ┌───────────────────────┐
    │   No serum sample     │ ◄──────┐
    │       n=874           │        │
    └───────────────────────┘        │

                    ┌─────────────────────────────────────────┐
                    │ Available frozen serum samples in 2001,  │
                    │       2007 and 2013                      │
                    │              n=482                       │
                    └─────────────────────────────────────────┘

                    ┌─────────────────────┬───────────────────┐
                    │   No MetS 2013      │  With MetS 2013    │
                    │     n=261           │      n=221         │
                    └─────────────────────┴───────────────────┘

    ┌───────────────────────┐
    │     MetS 2001         │ ◄──────┐
    │       n=112           │        │
    └───────────────────────┘        │

                    ┌─────────────────────────────────────────┐
                    │       No MetS samples 2001               │
                    │              n=370                       │
                    └─────────────────────────────────────────┘
```
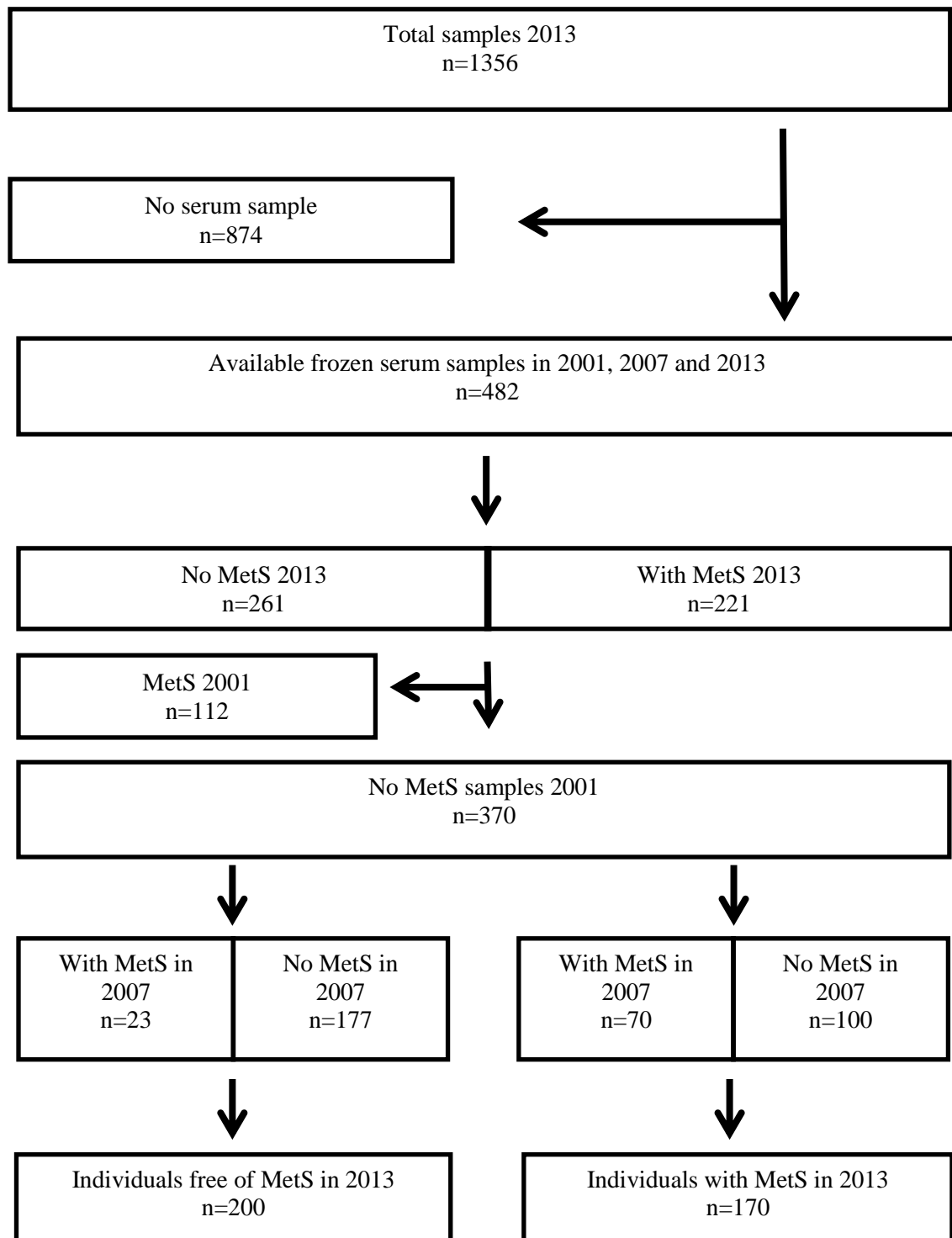
Figure 4.6.1: The selection criteria for the study using a nested case control design

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4.6.2 Statement of contribution to co-authored published paper:

This section includes a co-authored manuscript submitted in the "Metabolic Syndrome and Related Disorders" journal. The status of the co-authored paper, including all authors, is: Hossein Khosravi-Boroujeni, Nizal Sarrafzadegan, Masoumeh Sadeghi, Hamidreza Roohafza, Shu-Kay Ng, Ali Pourmogaddas, and Faruk Ahmed.

4.6.3 The research candidate has made the following contributions to this study:

- ✓ Developed the study design.
- ✓ Selected cases and controls and assessed serum 25(OH)D,
- ✓ Analysed the data and interpreted the findings.
- ✓ Prepared the manuscript and submitted to the journal.

4.6.4 Abstract:

Background and aim: Both metabolic syndrome (MetS) and vitamin D deficiency have been considered as public health problems. Therefore, the present study investigated the association between vitamin D status and MetS incidence in a nested case-control study from 2001 to 2013.

Method: This nested case-control study was designed based on the Isfahan cohort study which is an ongoing cohort of Iranian adults. In this study, all available cases (n=170) who developed MetS in 2013 but were free of MetS in 2001 were included. Similarly, all controls (n=200) who were free of MetS throughout the study period were included. Various biochemical parameters, including serum vitamin D concentrations, and socio-economic information were assessed in 2001, 2007 and 2013. GEE was used to analyse the longitudinal association between serum vitamin D and MetS.

Results: MetS cases had higher levels of serum 25(OH)D, total cholesterol, triglyceride, body mass index, waist circumference, systolic and diastolic blood pressure than that of the control subjects in 2001, while they had lower physical activity levels. Family history of hyperlipidemia was also more prevalent in the case group members. This study failed to find any association between MetS incidence and serum vitamin D levels after controlling for potential confounders.

Conclusion: In spite of the high prevalence of vitamin D deficiency and MetS among the studied population, this study could not find any evidence for the association between vitamin D status and MetS and/or its components. Randomized controlled trials are warranted to investigate the potential association more meticulously.

4.6.5 Introduction

Metabolic syndrome (MetS) has been recognized as a major public health problem, because a noticeable growth in the number of people with MetS has taken place all around the world (Grundy, 2008; van Vliet-Ostaptchouk *et al.*, 2014). MetS is a major risk factor for several disorders, including fatty liver, diabetes mellitus, cardiovascular disease (CVD), some cancers, and all-causes mortality (Browning *et al.*, 2004; Grundy, 2007). Moreover, it is diagnosed by a constellation of several cardio-metabolic risk factors, including impaired glucose metabolism, central obesity, dyslipidemia, and hypertension (Alberti *et al.*, 2009a). Although the etiology of MetS is not clearly recognized, previous reports showed that several risk factors, including inactive lifestyle and inappropriate diet, are associated with the higher incidence of this condition (Cameron *et al.*, 2004). Among the dietary factors, vitamin D has drawn great attention from researchers during the past decade.

Vitamin D has been widely known for its role in serum calcium and phosphate homeostasis and bone health (Holick, 2002). Further, it is reported that individuals with a low level of exposure to sunlight (as a source for vitamin D synthesis in body), have a higher risk of many chronic diseases such as hypertension (Rostand, 1997), common cancers (Ahonen *et al.*, 2000; Grant, 2002) and related mortality (Apperly, 1941). More recently the identification of the vitamin D receptor (VDR) in most of the body's tissues, revealed the non-calcemic roles of vitamin D (Nagpal *et al.*, 2005). Further studies have found possible associations between serum vitamin D concentrations and the components of MetS, including obesity (Caron-Jobin *et al.*, 2011), hypertension (Scragg *et al.*, 2007), dislipidemia (Smotkin-Tangorra *et al.*, 2007) and glucose intolerance (Scragg *et al.*, 2004).

As a consequence of the increasing prevalence of MetS and vitamin D deficiency in recent years, several investigators have examined the association between MetS and serum vitamin D levels; however, the results were inconclusive. For example, there are studies which have

shown an inverse association between the incidence of MetS and serum vitamin D levels (Cheng *et al.*, 2010; Ford *et al.*, 2005; Hyppönen *et al.*, 2008; Lu *et al.*, 2009), while a number of other studies did not find such an association (Bonakdaran & Varasteh, 2009; Liu *et al.*, 2009; Reis *et al.*, 2007; Rueda *et al.*, 2008; Tai *et al.*, 2008). Furthermore, other studies found that the association between vitamin D and MetS might exist only in cross-sectional studies, but not in longitudinal studies (Amirbaigloo *et al.*, 2013; Ju *et al.*, 2013).

Both vitamin D deficiency and MetS are highly prevalent among the Iranian population (49.1% and 42.5%, respectively). Consequently, there is a need to investigate the association between these public health problems to prevent and control their consequences. Previous reports on this association in the region of the Middle East were based on cross-sectional data and limited evidence is available regarding the association between MetS and vitamin D deficiency based on longitudinal data. Therefore, this study was designed to investigate the association between serum vitamin D levels and the incidence of MetS by controlling for the possible confounders in a nested case-control study within the frame of the Isfahan Cohort study (ICS) conducted on Iranian adults from 2001 to 2013.

4.6.6 Method and Material

4.6.6.1 Study design

This nested case-control study was conducted on ICS participants. ICS is an ongoing population-based cohort study of adults, aged 35 and older, from three counties in central Iran. It was designed to investigate the incidence of CVD and its risk factors. The cohort study commenced in 2001 and was followed up in 2007 and 2013. Detailed information regarding ICS, including sampling, rationale and results, has been presented elsewhere (Sarrafzadegan *et al.*, 2011). For the current study, all available cases (n=170) who were free

of MetS in 2001 but developed MetS in 2013 were included. Similarly, all controls (n=200) who were free of MetS throughout the study period were included. The study protocol was approved by the Isfahan Cardiovascular Research Institute (ICRI) and the Griffith University ethics committees.

4.6.6.2 Measurements

Following the informed written consent process, fasting blood sampling, blood pressure measurement and physical examinations were completed. Weight, height and waist circumference (WC) were measured using standard methods and body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m^2) (World Health Organization, 2000). Collected serum samples were frozen at -20°C until analysed in the central laboratory of ICRI and the extra serum samples were frozen at -80°C for future studies. Serum triglycerides (TG), fasting blood sugar (FBS) and cholesterol were assessed using the enzymatic method (McNamara & Schaefer, 1987). Serum High Density Lipoprotein (HDL) was measured, after separation of non HDL lipoproteins (Warnick *et al.*, 1985), by enzyme-linked immunosorbent assay (ELISA; BioVendor, Günzburg, Germany). For the present study, the frozen samples were used to assess serum 25-hydroxy vitamin D (25(OH)D), which is the best measure of body vitamin D status (Wang *et al.*, 2008) and it is very stable in frozen samples for a long time (Hollis, 2008). Serum 25(OH)D levels were measured using ELISA (Euroimmun AG, Luebeck, Germany) and through standard methods by expert technicians. A 49-item food frequency questionnaire (FFQ) was used to assess individuals' dietary intake. The global dietary index (GDI) was computed by calculating the average of the mean of 29 frequency questions in seven categories (Mohammadifard *et al.*, 2009b). The principal sources of vitamin D, including oily fish, margarine, butter, egg yolk,

and liver were considered for dietary sources of vitamin D. All measurements were repeated with the same methods in 2007 and 2013 for all participants (Sarrafzadegan *et al.*, 2011).

4.6.6.3 Definitions and criteria

MetS was defined based on the AHA-NHLBI definition, which was shown to be the best predictor of CVD in the studied population (Khosravi-Boroujeni *et al.*, 2015). Based on this definition, having three or more components from the following list identified MetS (Grundy *et al.*, 2005): 1) Impaired glucose: FBS ≥ 100 mg/ dl or using hypoglycemic medication; 2) Central obesity: WC > 102 cm or > 88 cm in men and women, respectively; 3) Hypertension: systolic blood pressure (SBP)/Diastolic blood pressure (DBP)≥130/85 mmHg or using blood pressure medication; 4) Hypertriglyceridemia: TG ≥150 mg/ dl or treatment; 5) Low HDL: HDL< 40 mg/ dl or < 50 mg/ dl in men and women, respectively.

4.6.6.4 Statistical analysis

Variables were presented as the mean (standard deviation) or percentage, where appropriate. Serum vitamin D levels were categorized as <25 nmol/L, 25-50nmol/L, 50-75nmol/L, 75-100nmol/L and >100nmol/L. To compare the means of continuous variables across categories of serum vitamin D, one-way ANOVA was applied and, for the categorical variables, Chi square or the Fisher exact test was used. The two independent sample T test was used to compare the means of continuous variables between cases and controls. Considering that MetS incidence was the dependent response in 2007 and 2013, and it was a repeated observation in a longitudinal data, Generalized Estimating Equations (GEE) were applied. In the model, the covariates were age, sex, physical activity, dietary score, dietary

sources of vitamin D, family history of diseases, baseline metabolic risk factors, smoking, and season of blood draw. Development of the model was based on univariate analysis and clinical significance. All statistical analyses were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was considered as the significance level.

4.6.7 Results

Comparing the baseline characteristics of cases and controls showed a lower percentage of females in the control group compared to the case group. Moreover, individuals who developed MetS in 2013 had higher levels of serum 25(OH)D, total cholesterol, TG, BMI, WC, systolic and diastolic blood pressure in 2001, while they had lower physical activity levels. Family history of hyperlipidemia was also more prevalent in the case-group members (Table 4.6.1). Assessing the characteristics of the study population based on categories of serum vitamin D levels indicated that there was no significant difference between categories of serum vitamin D based on individual characteristics, including gender, age, MetS components, physical activity, dietary score, and smoking (Table 4.6.2).

An adjusted association between MetS incidence and serum vitamin D levels could not predict the risk of MetS related to serum 25(OH)D categories during the 12-year follow up period. The association did not reach significance level, even in different categories of gender, BMI and age groups (Table 4.6.3). Moreover, MetS occurrence was not associated with variation in serum vitamin D levels.

The associations between the occurrence of MetS or its components and serum 25(OH)D levels were adjusted for age, sex, physical activity, dietary sources of vitamin D, family history of diseases, baseline level of metabolic components, smoking, and season of blood draw (Table 4.6.4). The model showed no association between continuous serum 25(OH)D

levels or its categories with MetS or its components (hypertension, diabetes/glucose intolerance, hypertriglyceridemia, low HDL and Obesity).

4.6.8 Discussion

Vitamin D deficiency has been attributed to a variety of health problems from bone diseases to chronic diseases, such as MetS. In the current longitudinal study among Iranian adults, we found no association between vitamin D status and MetS development after the 12-year follow up of Iranian adults. This study also did not find any significant association between vitamin D deficiency and the presence of MetS components. While, some longitudinal studies found an association between baseline vitamin D deficiency and MetS (Kayaniyil *et al.*, 2014; Li *et al.*, 2013), there are other longitudinal studies (Amirbaigloo *et al.*, 2013; Forouhi *et al.*, 2008; Gagnon *et al.*, 2012) which reported that serum 25(OH)D levels were not associated with increased MetS incidence and, thus, support our findings. Further, a meta-analysis of cross-sectional and longitudinal studies indicated that lower serum 25(OH)D concentrations were associated with a higher risk of MetS in cross-sectional but not in longitudinal studies (Ju *et al.*, 2013). Moreover, sub-group analysis showed that the robustness of the association varied based on the vitamin D assessment methods, regions and latitudes. Differences in the outcomes of previous studies were also related to the different characteristics of the studies, including participants' ages, adjusted covariates, and definitions of MetS (Ju *et al.*, 2013). Finding no association between vitamin D and MetS or its components in our study is also confirmed by scientists who recently declared that the association reported by some studies between vitamin D and health issues might be limited by reverse causation, possible confounders, as well as publication and classification bias (Autier *et al.*, 2014; Glendenning & Chew, 2015). Moreover, it has been recently stated that

low serum vitamin D levels might be a marker, rather than a cause, for health problems, because existing evidence does not support the significant effect of vitamin D supplementation on cardio-metabolic risk factors (Glendenning & Chew, 2015; Pilz *et al.*, 2016).

The discrepancy between different studies may be partly explained by the different concentrations of serum 25(OH)D in different populations (Vitezova *et al.*, 2015). In our study, we found a high prevalence of vitamin D deficiency and insufficiency in the studied population, which is similar to other studies in this region (Ardawi *et al.*, 2012; Hossein-Nezhad *et al.*, 2009; Hovsepian *et al.*, 2011; Neyestani *et al.*, 2012; Salekzamani *et al.*, 2010). In addition, lower serum 25(OH)D levels were reported in some ethnicities and specific genotypes (Baroncelli *et al.*, 2008). These genes can influence cholesterol metabolism, hydroxylation, and transportation of vitamin D (Wang *et al.*, 2010), which can affect vitamin D levels and its association with MetS. The ethnic effect might be a reason for finding similar results in several studies in our region. For instance, two case-control studies in Iran did not find any association between different serum vitamin D concentrations and MetS (Amirbaigloo *et al.*, 2013; Salekzamani *et al.*, 2010). Another study in Iran showed no association between vitamin D deficiency and MetS for all sub-groups (Hossein-Nezhad *et al.*, 2009) and another report from Jordan made the same findings (Khader *et al.*, 2011).

Different definitions of MetS and different thresholds for vitamin D deficiency (from 25nmol/L to 57nmol/L) might be other reasons for the diverse results (Gagnon *et al.*, 2012; Hyppönen *et al.*, 2008; Reis *et al.*, 2008). Although, the current study used the revised version of the ATP III in the analysis as a better predictor of CVD in the studied population (Khosravi-Boroujeni *et al.*, 2015), other definitions were also examined, but did not change our results (data not shown). Furthermore, we studied the association between concentrations of serum vitamin D and MetS using different thresholds of vitamin D levels as well as

continuous levels of vitamin D, but we still did not find any significant association. Moreover, it is important to consider the effect of various confounding factors when examining the association between a particular exposure and an outcome variable. There are some studies which reported an association between vitamin D and MetS, but after adjustment for possible confounders, the association disappeared (Forouhi *et al.*, 2008; Gagnon *et al.*, 2012). We also considered covariates affecting serum vitamin D levels, like dietary sources of vitamin D and the season, as well as factors affecting the development of MetS, such as age, sex, physical activity, family history of diseases, baseline metabolic risk factors, and smoking in our analysis. Of note, in a recently published review article, we inferred that there might be an interaction between other micronutrients, such as vitamin A, zinc and magnesium and vitamin D, as well as MetS which may affect the association between vitamin D status and MetS (Khosravi-Boroujeni *et al.*, 2016a). Unfortunately, in the current study we do not have any data on other micronutrient status. Thus, we could not adjust for any possible effect of other micronutrients while examining the association between MetS and vitamin D status.

This study has some strengths and limitations which should be considered. The first strength of this study is the use of longitudinal study design with a 12-year follow up, where vitamin D levels were assessed in 2001, 2007 and 2013. To our knowledge, this is the first study that analyzed the association based on several assessments of vitamin D and MetS. This allowed us to consider the possible effect of changes in serum vitamin D levels and its dynamic effect on the association. The limitation of this study was that, like most similar studies, we did not have data on vitamin D supplementation, duration of sun exposure, closing style and serum parathyroid hormone.

In conclusion, this nested case-control study among Iranian adults within the longitudinal cohort study failed to find any association between vitamin D deficiency and MetS, despite

the high prevalence of vitamin D deficiency and MetS. Similarly, it could not find any association between vitamin D concentration and the components of MetS. Further studies, particularly randomized controlled trials, are required to investigate the potential association more comprehensively and may explore whether vitamin D supplementation is effective for the prevention and treatment of MetS and its components. Nevertheless, it is recommended that serum vitamin D levels in vitamin D deficient individuals be improved for many other reasons.

4.6.9 Author's contribution:

H K-B designed the study, analyzed the data, interpreted the results and prepared the manuscript; MS, HR and AP collected the data. S-K N provided guidance in performing statistical analysis and contributed to the interpretation of results; NS contributed to the design of the study and critical revision of the manuscript. FA provided guidance on the overall research plan, contributed to the interpretation of results and critical revision of the manuscript. All authors have read and approved the final manuscript.

Table 4.6.1: Baseline characteristics of the study population by case and control *

	Controls n=200		Cases n=170		P value**
	mean or %	SD	mean or %	SD	
Age (years)	46.33	(9.26)	46.10	(8.45)	0.09
Gender (female)	36.6	-	45.8	-	<0.05
Serum 25(OH)D (nmol/L)	49.36	(52.49)	66.87	(78.04)	<0.05
BMI (kg/m^2)	25.32	(3.91)	27.14	(4.19)	<0.001
Waist circumference (cm)	92.18	(10.23)	96.43	(10.42)	<0.001
FBS (mmol/l)	79.26	(14.19)	83.56	(27.19)	<0.05
HDL cholesterol (mmol/l)	49.25	(10.19)	48.05	(10.66)	0.10
Total cholesterol (mmol/l)	205.28	(45.27)	220.09	(55.41)	<0.001
Triglycerides (mmol/l)	151.50	(78.16)	212.27	(128.01)	<0.001
Systolic blood pressure (mmHg)	112.93	(15.49)	116.01	(15.60)	<0.05
Diastolic blood pressure (mmHg)	72.59	(10.69)	74.93	(9.47)	<0.05
Family history of Diabetes (yes)	15.9	-	20.9	-	0.07
Family history of hyperlipidemia (yes)	14.9	-	20.3	-	<0.05
Family history of hypertension (yes)	24.6	-	23.7	-	0.80
Lipid lowering medication (yes)	41.5	-	38.7	-	0.71
Blood pressure lowering medication (yes)	75.0	-	73.7	-	0.57
Diabetes medication (yes)	82.	-	78.9	-	0.72
Education (low)	15.6	-	16.6	-	
Physical activity (min/week)	1023.58	(577.06)	937.32	(525.02)	<0.05
Dietary score	4.89	(1.76)	5.12	(1.83)	0.06
Smoking (current)	29.1	-	25.4	-	0.27

*Data presented as mean (Standard deviation) or percent
** P value computed using two independent sample T test, Chi square or Fisher exact test

Table 4.6.2: Baseline characteristics of the study population according to serum 25(OH)D

	Vitamin D categories								P ** value
	<25 nmol/L n=119		25-50 nmol/L n=106		50–75 nmol/L n=58		75<nmol/L n=69		
	mean or %	SD	mean or %	SD	mean or %	SD	mean or %	SD	
Age (years)	46.11	(8.94)	45.89	(7.51)	47.07	(9.82)	47.44	(9.62)	0.62
Gender (female) (%)	42.9%		41.5%		31.0%		43.5%		0.44
FBS (mmol/l)	80.59	(14.51)	81.72	(14.30)	81.15	(13.41)	81.44	(13.11)	0.98
HDL cholesterol (mmol/l)	50.72	(15.81)	50.24	(14.92)	47.00	(11.50)	48.68	(10.29)	0.60
Total cholesterol (mmol/l)	222.46	(79.60)	220.20	(67.09)	227.19	(73.47)	216.82	(54.03)	0.74
Triglycerides (mmol/l)	193.72	(99.63)	153.83	(85.63)	196.84	(120.33)	196.36	(112.32)	0.34
Systolic blood pressure (mmHg)	114.88	(19.30)	114.85	(20.27)	118.57	(27.50)	116.64	(17.01)	0.56
Diastolic blood pressure (mmHg)	72.64	(15.21)	73.91	(12.86)	75.64	(15.84)	74.50	(9.62)	0.98
BMI (kg/m^2)	26.94	(5.56)	26.25	(5.75)	26.37	(5.64)	27.06	(4.39)	0.35
Waist circumference (cm)	96.55	(13.32)	94.43	(15.94)	96.42	(10.89)	95.09	(11.70)	0.94
Physical activity (min/week)	970.7	(822.2)	880.2	(720.2)	1197.9	(950.6)	908.7	(551.6)	0.36
Dietary score (higher)*	6.7%		7.5%		6.9%		9.0%		0.95
Smoking (current) (%)	25.2%		21.9%		34.5%		21.7%		0.30
Education (low) (%)	16.8%		15.1%		19.1%		20.3%		0.82

Data presented as mean (Standard deviation) or percent

* higher score indicating higher total and saturated fat intakes

** P value calculated using one way ANOVA, Chi square or Fisher exact test

Table 4.6.3: Multivariable-adjusted associations between MetS and serum 25(OH)D separated by gender, BMI and age categories

| | Serum 25(OH)vitamin D categories | | | | | | | |
| | 75< nmol/L n= | | 50–75 nmol/L n=58 | | 25-50 nmol/L n=106 | | <25 nmol/L n=123 | |
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Men	1:00	-	0.92	0.41, 2.05	1.32	0.64, 2.77	1.07	0.45, 2.56
Women	1:00	-	1.22	0.56, 2.66	0.56	0.27, 1.16	0.47	0.17, 1.05
BMI								
<25	1:00	-	0.54	0.13, 2.19	0.46	0.10, 2.06	1.72	0.35, 8.58
25-30	1:00	-	0.49	0.20, 1.21	0.67	0.29, 1.49	0.38	0.18, 1.16
>30	1:00	-	1.01	0.85, 2.99	1.38	0.74, 2.59	1.49	0.56, 3.95
Age								
<50	1:00	-	0.97	0.57, 1.65	0.83	0.52, 1.34	0.59	0.44, 1.16
>50	1:00	-	1.83	0.48, 7.02	0.18	0.30, 1.11	1.26	0.27, 5.77

Model was adjusted for, physical activity, dietary score, dietary sources of vitamin D, family history of diseases, baseline metabolic risk factors, smoking, and season of blood draw.
The association was analyzed using Generalized Estimating Equations

Table 4.6.4: Multivariable-adjusted associations between MetS or its components and serum 25(OH)D

	Serum 25(OH)vitamin D categories							
	75< nmol/L n=		50–75 nmol/L n=58		25-50 nmol/L n=106		<25 nmol/L n=123	
	OR	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**	**OR**	**(95% CI)**
Metabolic syndrome	1:00	-	0.97	0.56, 1.67	0.87	0.52, 1.44	0.57	0.32, 1.11
Hypertension	1:00	-	0.82	0.48, 1.39	0.77	0.47, 1.25	0.81	0.47, 1.40
Diabetes/ glucose intolerance	1:00	-	0.88	0.49, 1.59	1.30	0.75, 2.24	0.76	0.41, 1.38
Hypertriglyceridemia	1:00	-	0.94	0.53, 1.69	0.48	0.89, 1.79	0.70	0.42, 1.16
Low HDL	1:00	-	0.87	0.50, 1.53	0.89	0.54, 1.46	0.62	0.38, 1.02
Obesity	1:00	-	0.75	0.43, 1.29	0.55	0.35, 1.39	0.75	0.44, 1.26

Model was adjusted for age, sex, physical activity, dietary score, dietary sources of vitamin D, family history of diseases, baseline metabolic risk factors, smoking, and season of blood draw.
The association was investigated using Generalized Estimating Equations

CHAPTER FIVE: DISCUSSION

5.1 Preface

This final chapter draws together the main findings of the study, which have been presented in chapter four. Furthermore, the clinical and public health implications of the study are described and the strengths and limitations of the research are expressed. The recommendations for future research are also discussed and, lastly, an overall conclusion of the research is presented.

5.2 Overview of findings regarding the research questions

The current research consists of a series of studies. The first study was designed to identify the best possible definition of MetS that can predict the CVD incidence among the study population as there has been a suggestion by the expert group that population specific best criteria of MetS need to be used for predicting the risk of CVD (Alberti *et al.*, 2009b). The second study explored the trends in MetS prevalence and its components using the definition that was identified in the first study. The third study examined the effect of changes in MetS risk factors on these trends. Then, in the fourth study, the trends in the prevalence of vitamin D deficiency were examined using a sub-sample based on inclusion criteria. Finally, the fifth study explored the association between serum vitamin D status and MetS and its components using the definition that was identified in the first study. For these research studies, longitudinal data related to a cohort of Iranian adults from 2001 to 2013 were used. Due to the nature of longitudinal data, there was a substantial loss to follow-up in subjects. For example, in the first 7 years, from 2001 to 2007, the attrition rate was about 48 percent. A major reason for the high number of loss to follow-up in this period was due to the complete

change in phone numbers in the whole country by the government. Changes in telephone numbers were part of the network capacity development and were not limited to special populations. Thus, the research team could not track a significant number of subjects in 2007, resulting in substantial loss to-follow-up. Moreover, another 31% percent dropped-out in the second follow-up in 2013. The reason for the loss to follow-up in this period was that ICS data collection was not continued in Arak in 2013. Originally, the ICS cohort study was designed to be followed-up for a 10-year period in three cities in central Iran (Isfahan, Najafabad and Arak). After 10 years and when the study was finished, the investigators extended the follow-up study in two cities (Isfahan and Najafabad cohort) for another 10 years. In addition, there was another round of attrition while conducting the vitamin D studies (fourth and fifth studies). The reason for this was that some subjects did not meet the study selection criteria. In these studies, we needed frozen serum samples to assess serum vitamin D levels. Some of the frozen serum samples had been used for other studies during this 12-year period and we could not include them in the study. As a result, only 482 frozen serum samples were available in 2001, 2007 and 2013. Furthermore, we needed individuals free of MetS in 2001 and, by these criteria, only 370 subjects were eligible for this study. Therefore, the results of these studies should be interpreted with caution. The research questions and an overview of the main results are outlined here:

5.2.1 Are the prevalence of MetS and the effect of MetS on CVD events influenced by different definitions?

As mentioned earlier, using different definitions of MetS can classify an individual from a normal to a diseased condition or vice-versa and, therefore, prevalence and incidence of MetS, as well as its association with an increased risk of CVD, depends on the criteria used (Brown *et al.*, 2008; Cameron *et al.*, 2007). In the first study, different definitions of MetS were used to examine their influence on the prevalence of MetS. In addition, we intended to identify the best definition of MetS for the prediction of CVD events over 10 years in a cohort of the Iranian adult population.

This study revealed that the prevalence of MetS varied widely when different definitions were used. Of the six definitions used in the present study, the AHA-NHLBI definition was able to predict the highest prevalence of MetS in the studied population. The results of the current study indicated that regardless of the definitions, except for the WHO and EGIR definitions, MetS was highly prevalent among the studied population. These findings are similar to that reported in earlier studies (Azizi *et al.*, 2003; Delavari *et al.*, 2009), and thus our results confirm the findings of previous studies.

This study compared the prevalence of MetS observed using different definitions, which possibly have different sensitivities for diagnosing MetS. Sensitivity is the ability of a test to truly diagnose individuals with the disease or syndrome. On the other hand, specificity is the ability of the test to truly recognize those individuals without the condition. Consequently, a highly sensitive test hardly overlooks a case (identifies more cases) and a highly specific test hardly diagnoses a healthy individual as diseased (identifies fewer cases). Therefore, a test with high sensitivity and specificity will be the best test to make a diagnosis (Akobeng, 2007). But by increasing sensitivity, specificity decreases and vice versa. In our study, a higher sensitivity test means it can diagnose more individuals with MetS and it may reduce

the probability that subjects diagnosed with MetS are truly at a high risk for CVD (lower positive predictive value). However, in the present study the predictive ability was not affected by the increased sensitivity, as the AHA-NHLBI definition diagnosed more individuals with MetS without decreasing the predicted risk of CVD (HRs). On the other hand, by increasing specificity, some individuals who are at risk for CVD may not be diagnosed (low negative predictive value) and may not be aware of their health condition and not receive the treatment for MetS. Therefore, the above explanation led us to reinforce the validity of study findings and, thus, we conclude that the AHA-NHLBI definition, which diagnoses more cases, is the best definition for diagnosing MetS.

Moreover, regardless of the definitions, the risk of developing CVD was significantly higher in MetS individuals compared to normal individuals. Nevertheless, this definition is limited by using the universal cut-off point for WC to define central obesity. The reason for this is that ethnic differences have been stated to be a factor which influences the development of MetS, even in individuals with a WC below the general cut-off points. Therefore, it has been proposed that ethnic specified cut-off points should be used to identify central obesity as a risk factor for MetS (International Diabetes Federation, 2006). Finally, significant associations were found between the risk of CVD and every component of MetS, which suggests that it is necessary to manage each component of MetS, along with MetS. It is noteworthy that in the subsequent studies, the AHA-NHLBI definition was used to define MetS.

5.2.2 What is the secular trend of MetS and its components over a 12-year time period?

The second study investigated the trend of MetS prevalence and its components in a cohort of Iranian adults from 2001 to 2013. As aging is an intrinsic part of cohort studies, the effect of

aging was statistically adjusted while evaluating the prevalence of MetS and its components. Moreover, this study calculated the effect of aging on the prevalence of MetS and its components.

The results demonstrated that the prevalence of MetS increased during this 12-year period. Increasing trends were also found for the prevalence of MetS components, except hypertriglyceridemia, which had a decreasing trend. The increasing trend for MetS and its components remained significant even after controlling for the effect of age. In this study the prevalence of smoking decreased from 16.7% to 4.8% over a period of 12 years and the mean frequency of consumption of bread and rice also decreased during this period. There is evidence that the decreasing trend in triglyceride levels is attributable to a reduction in cigarette smoking and carbohydrate intake (Carroll *et al.*, 2012). On the other hand, smoking has been associated with reduced weight due to increased metabolic rate and thermogenesis (Wack & Rodin, 1982) and, therefore, the decreasing prevalence of smoking may be partly associated with the increasing prevalence of obesity. Moreover, with the decreasing consumption of carbohydrates, the consumption of high fat foods increased in the studied population. High-fat, low-carbohydrate diets have been found to be associated with the development of diabetes mellitus (Marshall *et al.*, 1991). Furthermore, comparing characteristics of individuals who were retained in the study with the individuals who were lost indicated only that hypertension was lower in those who remained in the study, but there were no significant differences in the other components of MetS. Therefore, the increasing trend in MetS and its components were mostly because of changes over time.

This study also found that the prevalence of MetS increased as age increased to the age range of 65-75 years, then decreased. It is difficult to compare our findings as there were no previous studies in Iran that have used longitudinal data. However, one study from Japan also reported an increasing trend of MetS prevalence and the effect of aging on the increasing

prevalence of MetS and its components (Kuzuya *et al.*, 2007). It is believed that socio-economic growth and changes in lifestyle behaviours, including a sedentary lifestyle and embracing a western dietary pattern, are responsible for the increasing prevalence of MetS and most of its components (Ramachandran *et al.*, 2012). The current study also found that low physical activity, obesity and impaired glucose tolerance were the principal determinants for the increasing prevalence of MetS and should be monitored to prevent health related disorders. In addition, there is an urgent need to improve the lifestyle of this population by implementing appropriate interventions including education, screening and treatment of abnormalities and thereby reducing/preventing the cardiovascular risk factors.

5.2.3 Are changes in socio-demographic and lifestyle factors associated with MetS components?

The prevalence of MetS has been increasing globally and it is known to be associated with increased risk of chronic diseases and related mortality (Grundy, 2007). Thus, to prevent the increasing risk of chronic diseases, there is a need for better understanding of MetS components and their risk factors. Therefore, this study was designed to determine the changes in the components of MetS in a cohort of Iranian adults from 2001 to 2013 and to examine the extent to which various socio-demographic and lifestyle factors might influence these changes.

Using a longitudinal data with three time-point assessments of MetS risk factors as well as socio-demographic and lifestyle factors, enabled us to examine which socio-demographic and lifestyle factors may affect the MetS risk components. In addition, we examined whether the changes in these factors over the 12-year study influenced changes in MetS risk factors.

The current investigation observed that mean levels of SBP, DBP, FBS, WC and BMI increased, while cholesterol, HDL-C, TG and physical activity levels decreased during the study period from 2001 to 2013. This study also found a decrease in smoking and carbohydrate intake, while consumption of high fat foods increased in this period, which may affect some of the risk factors. The increasing pattern in most of the cardiovascular risk factors has been attributed to a more modern lifestyle and an aging population (Beltrán-Sánchez *et al.*, 2013). It was also stated that the reported improvement in cholesterol and TG might be a consequence of more developed methods of diagnosis and treatment of hyperlipidemia over the years (Carroll *et al.*, 2012). This study also demonstrated that individuals with MetS had significantly lower education levels and were more likely to be urban citizens than individuals without MetS. Furthermore, some of the socio-demographic factors, such as age and gender, were significantly associated with mean changes in MetS components. While socio-demographic factors such as age and gender are non-modifiable factors, the findings of the present study emphasize the need for changes in lifestyle factors as a part of preventive strategies.

5.2.4 What is the prevalence and trend of vitamin D deficiency in Iranian adults from 2001 to 2013?

Vitamin D deficiency, which has been recognized as a re-emerging public health problem globally, has been suggested as a cause for several chronic diseases (Holick, 2010b). In the previous studies, we demonstrated an increasing trend in the prevalence of MetS and most of its components and how various socio-demographic and lifestyle factors influence the changes in MetS. Since vitamin D deficiency has been linked to various chronic diseases, it is of paramount importance to investigate how vitamin D status influenced MetS in the study

population; consequently, it was necessary to have a clearer understanding of the prevalence and trend of vitamin D deficiency among the studied population. Therefore, the fourth study examined the current prevalence of vitamin D deficiency and insufficiency, and their trends for the period from 2001 to 2013 in the studied population before examining the association between vitamin D status and incidence of MetS.

Over the period of the current study, mean serum vitamin D concentrations increased and the prevalence of vitamin D deficiency decreased. A previous study, based on cross-sectional analysis, compared its results with those of earlier ones and reported an increasing trend in the prevalence of vitamin D deficiency (Hovsepian *et al.*, 2011). However, the current study was the first study that examined the prevalence of vitamin D deficiency based on a cohort study and, therefore, we can expect it to achieve more accurate results. When assessing the risk of vitamin D deficiency/insufficiency, the results revealed that the adjusted risk of vitamin D deficiency/insufficiency decreased from 2001 to 2007 and 2013. The improvement in vitamin D status might be due to the increasing knowledge regarding the importance of vitamin D status, and treating its deficiency. However, we have no data on the consumption of vitamin D supplements. Moreover, some of the characteristics of the study subjects differed significantly from that of the subjects in the total cohort, therefore, the findings of this study should be interpreted with caution. Finally, this study could not find any differences in the prevalence of vitamin D deficiency between different genders, age groups, and area of residence. Overall, the current prevalence of vitamin D deficiency was very high among the studied population, which is in line with previous surveys on vitamin D deficiency in Iran and other Middle Eastern countries (Ardawi *et al.*, 2012; Gaafar & Badr, 2013; Hossein-Nezhad *et al.*, 2009). Considering the possible health consequences of vitamin D deficiency, there is a need to prevent and control vitamin D deficiency.

5.2.5 Is vitamin D deficiency associated with MetS and/or its components and are these associations influenced by potential confounders, different MetS definition, and severity of vitamin D deficiency?

The fifth study examined the association between serum vitamin D status and the incidence of MetS based on a longitudinal study. In this study, MetS components and serum vitamin D levels were considered at three time-points, namely, 2001, 2007 and 2013. In order to examine this association, all possible confounders and different definitions of MetS, as well as different cut-off points for vitamin D deficiency, were considered.

This study failed to find any evidence regarding the association between vitamin D deficiency and the incidence of MetS. Also, investigating the association between serum vitamin D concentrations (as a continuous variable) and the incidence of MetS did not find any association. Our results are in line with other longitudinal studies which reported no association between serum vitamin D concentration and incidence of MetS (Amirbaigloo *et al.*, 2013; Forouhi *et al.*, 2008; Gagnon *et al.*, 2012). However, there are some studies that found an association between vitamin D deficiency and MetS (Kayaniyil *et al.*, 2014; Li *et al.*, 2013). Examining the association in different age groups, BMI categories and gender, revealed that the association between vitamin D and MetS is not affected by these factors. Moreover, there were no significant associations between serum vitamin D status and the components of MetS. Further, the available evidence does not suggest any beneficial effects of vitamin D supplementation on cardio-metabolic risk factors (Glendenning & Chew, 2015; Pilz *et al.*, 2016). Finally, using different definitions of MetS, different cut-off points for vitamin D deficiency did not change the results regarding the association between serum vitamin D and MetS. Nevertheless, it is recommended that serum vitamin D levels in vitamin D deficient individuals be improved for many other reasons. Further studies with representative sample size are warranted to confirm these findings.

5.3 Implications of the research

The study findings have numerous clinical and health implications. The results of this large population-based cohort study are important for many reasons and have an absolute clinical relevance in the prevention and control of CVD for high-risk individuals, as well as for the Iranian population in general.

Firstly, this study presented the best definition of MetS based on its ability to identify more individuals with MetS and better predicting ability for cardiovascular events among the Iranian population. As MetS has been assigned as a criterion to predict the risk of CVDs, it is important to diagnose MetS individuals accurately. The suggested definition can be used by health professionals to identify high-risk individuals and help them to prevent future health problems. Moreover, this study showed that individual components of MetS could also significantly increase the risk of CVD occurrence. Therefore, it could be a basis for motivating individuals and health professionals to control abnormalities in any component of MetS.

Secondly, this study has presented a comprehensive image of MetS and its components among Iranian adults. Considering the consequences of MetS, including the increased risk of developing CVD, diabetes, and some cancers, the present study has provided extremely important information with regard to the current prevalence and trend of MetS, which can help the Iranian Government and policy makers to comprehend the seriousness of the problem in the country. To our knowledge, the current study is the only study in this region which has presented the prevalence of MetS and its components using longitudinal data. Previous studies have used cross-sectional data at different points in time to examine the trend and compared the prevalence between different population groups/samples which might reflect the true situation. As the present study followed the same individuals over a period of 12 years, the results of the prevalence of MetS are likely to be more accurate and, thus, may

reflect the true picture of the trend of MetS prevalence. This could help policy makers in developing appropriate measures to control the prevalence of MetS and predict the health consequences of MetS. Moreover, identifying the trend in the prevalence of MetS components could also identify the most critical contributor to health problems. In the current study, the increasing trend in the prevalence of diabetes or impaired glucose was greater than other components, indicating that diabetes is the most important health problem in this population. Additionally, this study, based on the longitudinal design, highlighted the possible contributing factors which influenced the incidence of MetS and its components.

Thirdly, the findings of this research can contribute to the current knowledge about the situation of vitamin D status in the Iranian adult population. Vitamin D deficiency is now recognized as a public health problem globally, and is also a serious health problem in Iran where more than 60% of the studied population were vitamin D deficient or insufficient. When looking at the trend of vitamin D deficiency in the studied population, a decreasing trend was observed; however, the current prevalence is still very high. Considering the possible consequences of vitamin D deficiency, including bone diseases and other possible health problems, there is a pressing need for interventions and strategies to decrease vitamin D deficiency. Policy makers and health professionals may use these results in developing policies and programmes to control vitamin D deficiency and its possible consequences. Public health and other intervention policies should also be developed to enable proper sun exposure and vitamin D supplementation in this population.

Finally, the findings of this study regarding the association between vitamin D deficiency and incidence of MetS, based on a longitudinal study and several assessments of serum vitamin D and CVD risk factors, could influence future studies. Using a longitudinal study design may enable researchers to examine the causality in the association between vitamin D deficiency and chronic diseases by taking into account other potential confounders.

5.4 Limitation of the research

There are several limitations which need to be considered when interpreting the results of this study. Firstly, this study used a 12-year follow-up data set from a cohort of Iranian adults (ICS) which had a significant loss of subjects to follow-up. However, when we compared the characteristics (such as gender, age and metabolic syndrome) between the available participants and those who were lost to follow-up, no significant differences were observed (Supplemental Table 1 in Appendix B). Moreover, earlier publications from the same Isfahan Cohort Study data also reported that "the baseline characteristics and the prevalence of CVD risk factors were not significantly different among those participants lost to follow-up compared with those remained in the follow-up surveys" (Sadeghi *et al.*, 2014b; Sarrafzadegan *et al.*, 2011; Talaei *et al.*, 2014). Therefore, the available subjects in the present study are representative of the wider population and it is unlikely that the true situation will be different from the findings of the study.

In addition, when we investigated the trends and prevalence of vitamin D deficiency and its association with MetS, we could use only a sub-sample of 370 subjects who met the selection criteria. When compared with the total cohort, the subjects available in the current study were relatively younger and had better health conditions in terms of various components of MetS. Therefore, the present findings on the trends and prevalence of vitamin D deficiency may not be a true reflection of the general population. However, there is no evidence in the current literature that components of MetS influence the vitamin D status except for obesity. Obesity may affect serum vitamin D levels because fat tissue may trap vitamin D and decrease its bioavailability and transportation in the body (Wortsman et al., 2000). Further, when we adjusted the effect of MetS in the multivariate analysis model, it did not change the results. Nevertheless, given the high loss to follow-up subjects, the findings of this study should be interpreted with caution.

Secondly, vitamin D assessment was based on frozen samples from 2001, 2007 and 2013 which were kept frozen in -80ºC for a long time. However, it has been confirmed that long-time storage of serum samples does not affect serum 25(OH)D concentration (Hollis, 2008). Further, because of the number of samples for vitamin D assessment and limited time, serum vitamin D was assessed based on ELISA kits and not on the High Performance Liquid Chromatography (HPLC) method which is a gold standard for vitamin D assessment. However, Hollis indicated that for large samples, the ELISA method would be more appropriate (Hollis & Horst, 2007).

Finally, although in this study we adjusted for the effect of various confounding factors while examining the association between serum vitamin D and MetS, we were not able to include all potential confounders. Previous studies reported a negative association between serum vitamin D and PTH (Kota *et al.*, 2013), both of which are responsible for extracellular calcium homeostasis which is one of the possible mechanisms linking vitamin D with a variety of health problems (Reis *et al.*, 2007). Vitamin D increases the absorption of calcium in the intestine, and PTH, in response to low serum calcium levels, increases calcium resorption from the skeleton (Lips, 2001). Furthermore, it is reported that elevated PTH levels increase the risk of MetS and not low serum vitamin D levels (Reis *et al.*, 2007). On the other hand, some micronutrients, including calcium, vitamin A, zinc and magnesium play important roles in the activation and function of vitamin D and are also related to several components of MetS, including glucose intolerance, dyslipidemia and obesity (explained in more detail in section 2.5). As the current study was based on the ICS data, there were some variables, such as serum parathyroid hormone, calcium, magnesium, zinc and vitamin A, which had not been assessed during the first phases of the study. They were also not stable in frozen samples and, consequently, they could not be assessed. The data on other variables, including sun exposure, clothing style and vitamin D supplementation, were also not

collected at data collection time. Controlling for the effect of these variables may modify the association between serum vitamin D levels and MetS. Nevertheless, in this study, other possible confounders, including age, sex, physical activity, dietary score, dietary sources of vitamin D, family history of diseases, baseline metabolic risk factors, smoking, and season of blood draw were controlled to eliminate their effects.

5.5 Strengths of the research

The main strength of the current study is the study design, which was based on a longitudinal population-based study called Isfahan Cohort Study with a large study population. Socio-demographic variables were collected by trained interviewers and assistants. Other data were also collected via standard methods and experienced researchers. Furthermore, to the best of my knowledge, this study was the first that compared different definitions of MetS for the prediction of CVD events in a longitudinal study in Eastern Mediterranean countries.

Given the study design, this study assessed serum vitamin D concentrations, MetS and its components, three times over a period of 12 years. Multiple assessments at different points in time reduced the influence of diversity in the population. Three time data points over the study period provided the capacity to examine the changes in MetS, its components and risk factors. Moreover, this design allowed for the longitudinal association between vitamin D deficiency and the incidence of MetS.

5.6 Recommendations for future research

Based on the results of the presented studies in this thesis, some recommendations for future research are suggested. Firstly, the most appropriate definition of MetS for the prediction of

CVD was determined from all available definitions. However, this definition was not based on the WC cut-off point of the study population. Therefore, it is recommended that the most appropriate cut-off point for WC is examined in the studied population to provide a potentially better predictor of CVD events. Secondly, further studies with representative samples are required to confirm the downward trends of vitamin D deficiency among Iranian adults and to investigate the determinants of this deficiency in this population.

Thirdly, with regard to the potential association of vitamin D with MetS, it is recommended that other nutrients, including vitamin A, zinc and magnesium, be assessed to determine their influence on this potential association. Moreover, other possible confounders, including vitamin D intake, sun exposure and serum parathyroid hormone should be assessed.

Finally, most of the available studies on the association between vitamin D and MetS used a cross-sectional design which is unable to demonstrate a causation effect. High-quality designed studies, such as other longitudinal studies and double-blind randomized-controlled-trials are required to further explore whether vitamin D supplementation is efficient in the prevention and treatment of MetS and its components. The effects of different doses of vitamin D, long term vitamin D supplementation and fortification, or sun exposure on MetS or its components in individuals with low or adequate serum vitamin D are also needed to verify the effect of vitamin D supplementation.

5.7 Conclusion

The results of the current research extend the understanding of the current prevalence and the trends of MetS, its components and vitamin D deficiency/inadequacy among Iranian adults. The prevalence of MetS and its components have been increasing as a result of modern lifestyles and an aging population, thereby increasing the risk of CVD events and other

related diseases, while the prevalence of vitamin D deficiency has been decreasing. Moreover, there was no association between vitamin D status and MetS and its components. Thus, the present findings do not support the assumption that serum vitamin D deficiency is a risk factor for the development of MetS and its components. However, vitamin D deficiency was highly prevalent among the studied population. As inadequate vitamin D status has been found to be associated with some health problems and is a risk factor for bone-related diseases, there is a need to improve the vitamin D status of the Iranian population. In addition, strategies, such as changes in dietary behaviour and physical activity, need to be developed for the prevention and control of MetS and its related diseases.

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Appendix A: Ethical approval letters

GRIFFITH UNIVERSITY HUMAN RESEARCH ETHICS COMMITTEE

27-Oct-2014

Dear Mr Khosravi Boroujeni

I write further to the additional information provided in relation to
the conditional approval granted to your application for ethical
clearance for your project "NR: Prevalence of metabolic syndrome and
its components and their association with Vitamin D status among Iran
adults." (GU Ref No: MED/53/14/HREC).

This is to confirm receipt of the remaining required information,
assurances or amendments to this protocol.

Consequently, I reconfirm my earlier advice that you are authorised to
immediately commence this research on this basis.

The standard conditions of approval attached to our previous
correspondence about this protocol continue to apply.

Regards

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Cc:

Researchers are reminded that the Griffith University Code for the
Responsible Conduct of Research provides guidance to researchers in
areas such as conflict of interest, authorship, storage of data, & the
training of research students.
You can find further information, resources and a link to the
University's Code by visiting
http://policies.griffith.edu.au/pdf/Code%20for%20the%20Responsible%20Co
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Date: 20 OCT 2014

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To whom it may concern

It is my pleasure to inform you that as per Mr. Hossein Khosravi Boroujeni's request, I am happy to give access /permission to use the Isfahan Cohort Study (ICS) data set by Mr. Khosravi for doing his PhD research work.
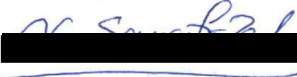
We also assure you that the ICS data set are de-identified and blood samples obtained from the samples were kept frozen using established laboratory/ biosafety protocols.

Informed written consent was obtained from participants and ethical approval was achieved from the Ethics Committee of Isfahan Cardiovascular Research Centre.

I wish Mr. Khosravi successfully complete his PhD program.

Nizal Sarrafzadegan MD
Professor of Medicine/Cardiology
Director of Isfahan Cardiovascular Research Institute
WHO Collaborating Center in EMR

Affliate Faculty
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Appendix B: Supplemental Tables

Supplemental Table 1: Characteristics of available participants and loss-to-follow up individuals

	loss-to-follow-up subjects	available subjects	P value
Gender (male)	48.7%	48.6%	0.12
Age [mean(SD)]	51.3 (12.47)	49.2 (10.45)	0.06
BMI [mean(SD)]	26.42 (4.47)	27.47 (4.45)	0.18
MetS	35.8%	37.4%	0.37
Hypertension	36.8%	31.7%	0.01
Diabetes/glucose intolerance	14.9%	13.5%	0.21
Hypertriglyceridemia	58.5%	62.2%	0.14
Low HDL	45.2%	45.3%	0.97
Smoking	22.8%	21.0%	0.15

Supplemental Table 2: Characteristics of participants in vitamin D studies and cohort samples

	Study samples	All 2001 samples	P value
Gender (male)	52.2%	48.7%	0.06
Age [mean(SD)]	46.3 (8.9)	50.2 (11.8)	0.00
BMI	26.3(4.2)	26.6 (4.5)	0.26
Hypertension	17.7%	35.7%	0.00
Diabetes/glucose intolerance	0.9%	14.6%	0.00
hypertriglyceridemia	50.2%	59.3%	0.01
Low HDL	28.9%	45.2%	0.00
smoking	26.1%	22.7%	0.21
Family history of diseases	95.1%	94.2%	0.28
married	95.1%	93.6%	0.12
Region (rural)	23.5%	27.5%	0.08