MICRONUTRIENT STATUS AND METABOLIC SYNDROME BIOMARKERS IN POST MENOPAUSAL WOMEN

A THESIS SUBMITTED TO
BHARATI VIDYAPEETH DEEMED UNIVERSITY, PUNE
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DOCTOR OF PHILOSOPHY IN PHYSIOLOGY

UNDER THE FACULTY OF MEDICAL SCIENCES
SUBMITTED BY

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UNDER THE GUIDANCE OF

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CERTIFICATE

This is to certify that the work incorporated in the thesis entitled, **"Micronutrient status and Metabolic syndrome biomarkers in Postmenopausal women"** for the degree of ***'Doctor of Philosophy'*** in the subject of Physiology under the faculty of Medical Sciences has been carried out by **Dr. Pranita Hiraji Kevale** in the Department of Physiology at Bharati Vidyapeeth Deemed University, Medical College, Pune, during the period from November 2011 to February 2017.

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I hereby declare that the thesis entitled, **"Micronutrient status and Metabolic syndrome biomarkers in Postmenopausal women"** submitted by me to the Bharati Vidyapeeth Deemed University, Pune for the degree of ***'Doctor of Philosophy'*** **(Ph.D.)** in the subject of Physiology under the faculty of Medical Sciences is original piece of work carried out by me under the supervision of **Dr. B Balsubramanian** and **Dr. Sadhana R. Joshi**. I further declare that it has not been submitted to this or any other university or Institution for the award of any degree or Diploma.

I also confirm that all the material which I have borrowed from other sources and incorporated in this thesis is duly acknowledged. If any material is not duly acknowledged and found incorporated in this thesis, it is entirely my responsibility. I am fully aware of the implications of any such act which might have been committed by me advertently or inadvertently.

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*.....**Pranita***

Abbreviations

Mets	Metabolic Syndrome
HDL	High Density Lipoprotein
CHD	Coronary Heart Disease
MI	Myocardial Infarction
LCPUFA	Long Chain Polyunsaturated Fatty Acids
WC	Waist Circumference
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
NGT	Normal Glucose Tolerance
BMI	Body Mass Index
FBG	Fasting Blood Glucose
HTN	Hypertension
TGs	Triglycerides
FFA	Free Fatty Acid
ATII	Angiotensin II
PAI-1	Plasminogen Activator Inhibitor-1

RAAS	Rennin Angiotensin Aldosterone System
SNS	Sympathetic Nervous System
HDL-c	High Density Lipoprotein-C
VO_2 max	Maximal Oxygen Consumption
apo B	ApolipoproteinB
HL	Hepatic Lipase
HOMA	Homeostasis Model Assessment
HOMA-IR	Homeostasis Model Assessment-Insulin Resistance
IR	Insulin Resistance
SAFA	Saturated Fatty Acids
MUFA	Monounsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
ALA	Alpha Linolenic Acid
EPA	Eicosapentaenoic Acid
DHA	Decosahexanoic Acid
LA	Linoleic Acid
GLA	Gamma Linolenic Acid
AA	Arachidonic Acid
TXA	Thromboxane

EFA	Essential Fatty Acids
DASH	Dietary Approaches To Stop Hypertension
SCD	Sudden Cardiac Death
CβS	Cystathionine β- Synthase
SAM	S-Adenosyl Methionine
5MTHF	5 Methyl Tetrahydrofolate
PEMT	Phosphatidyl Ethanolamine N Methyltransferase
DHA	Docosahexaenoic acid
PC	Phosphatidyl Choline
Hcy	Homocysteine
VT	Ventricular Tachycardia
VF	Ventricular Fibrillation
EPA	Eicosapentaenoicacid
BMD	Bone Mineral Density
WHR	Waist To Hip Ratio
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
GOD-POD	Glucose oxidase peroxidase

TMB	Tetramethylbenzidine
IQR	Inter Quartile Range
ANOVA	Analysis Of Variance
CETP	Cholesteryl Ester Transfer Protein
MTFR	Methylene tetrahydrofolatereductase
MM-CoA	Methyl Malonyl CoA
CPT-1	CarnitinePalmitoyl Transferase-1
SREBP-1	Sterol Regulatory Element-Binding Protein 1
PPARs	Peroxisome Proliferator-Activated Receptors
w3	Omega 3 fatty acids
w 6	Omega 6 fatty acids
w-6 : w-3	Omega 6 fatty acids: Omega 3 fatty acids

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PUBLICATIONS

1. Pranita Ashok, BhawaniBalsubramanian, Sadhana Joshi, Jayashree S Kharche, Savita M Vaidya. Associations of vitamin D with metabolic syndrome components in Indian urban middle-aged women .Natl J Physiol Pharm Pharmacol. 2017: Vol. 7 (5) ; 497-500.

2. Pranita Ashok, BhawaniBalsubramanian, Sadhana Joshi, GirijaWagh ,Savita M Vaidya. Prevalence of metabolic syndrome in urban middle aged women. International Journal of Scientific Research .2017 :Vol. 6 (7) ;570-572.

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2. The 5th Seoul International Congress of Endocrinology and Metabolism 2017. April 27 to 30, 2017, Walker hill Seoul Hotel, Seoul, Korea on April 27 to 30, 2017.SICEM2017-ABS-1142 - "Correlation of central obesity & insulin resistance in middle aged women" has been accepted for Poster Presentation with travel award of 600 dollars.

INTRODUCTION

Metabolic syndrome (MetS) is defined as a 'clustering' of metabolic abnormalities like elevated blood sugar level, an abnormal lipid profile, high blood pressure and abdominal obesity which make the person prone to cardiovascular diseases(CVDs) .[1] Prevalence of metabolic syndrome is increasing in developing countries, especially in the South Asian countries. [2,3] The recent data shows that one fourth to one third of urban population of India has metabolic syndrome.[4] Furthermore, the prevalence is 1.5–2 times higher in women compared with men at middle age [2] as they are more prone to obesity, impaired fasting glucose, low high density lipoprotein (HDL) and high triglycerides (TGs). The alternative name for metabolic syndrome is 'Insulin Resistance Syndrome'. [2,5,6]

Obesity is more in urban middle aged women than in men due to socioeconomic transition causing significant shifts in dietary and physical activity patterns. In addition middle aged women includes postmenopausal women who have deficiency of estrogen that triggers central obesity.[7]Central obesity leads to abdominal adiposity, dyslipidemia and insulin resistance in postmenopausal women.[8]

It is known that people with metabolic syndrome have more morbidity and mortality for coronary artery disease or stroke. In addition, people with metabolic syndrome have a fivefold greater risk of developing type 2 diabetes.[9] which further raises susceptibility to cardiovascular diseases.

According to Statistical Fact Sheet 2013 - Update of American Heart Association, incidence of sudden deaths has shown steep rise with age in females than males. A worse prognosis for females than males with coronary heart disease (CHD) was reported. In a cardiac rehabilitation

program after Myocardial Infarction, females were found to have less enrolment, poor program attendance and more dropouts. The fact remains that females have a worse prognosis and they die more often than males after a MI or bypass surgery in middle age.[4]

Various studies [10-14] indicate that all the above mentioned factors like obesity, physical inactivity, faulty dietary pattern, metabolic syndrome and estrogen deficiency in postmenopausal women lead to increase in the levels of homocysteine.

Homocysteine is considered as an independent risk factor for the development of cardiovascular diseases. Raised levels of Homocysteine results in increased production of free radicals leading to enhanced oxidative injury to endothelial cells precipitating atherosclerosis and hence CVDs. [15]

Micronutrients like folic acid and vitamin B_{12} play important role in homocysteine metabolism. Folic acid acts as a methyl donor and vitamin B_{12} acts as a co factor in the metabolism of homocysteine. Hyperhomocysteinemia is more common among Indians due to deficiencies of folic acid and vitamin B_{12} perhaps due to faulty cooking habits and inadequate consumption of animal origin foods. [15,16,17] In case of deficiency of these vitamins, homocysteine is not converted into methionine and hence homocysteine levels increases.

Vitamin D deficiency also increases homocysteine levels in addition to increasing insulin resistance. [18,19.] It also increases the risk for CVDs by influencing renin angiotensin system. Further, lack of sun exposure and high body fat are considered as major causes for vitamin D deficiency in Indians.[18]

Deficiency of Long chain polyunsaturated fatty acids (LCPUFA) diverts the methyl groups towards DNA which will trigger altered gene

expression of vital genes involved in various metabolic pathways ultimately leading to insulin resistance and metabolic syndrome.[16]

Interventional trials [19-23] with these nutrients found inconsistent results .This may be due to associations that are not causal or intervening too late in the history of disease .Very few studies have been done in India in this context.

Limited studies reported lower levels of either vitamin B_{12}[24] or vitamin D [22] among the middle aged women with metabolic syndrome. A negative association of vitamin B_{12} levels with body mass index[24] and adverse lipid profile has been reported.[25] Similarly inverse relationship of vitamin D with risk for metabolic syndrome has also been reported.[26] However, to the best of our knowledge no study has been conducted to examine the basal levels of all the above nutrients with various components of metabolic syndrome.

India is now facing an epidemic of increased risk of these non communicable diseases like CVDs in urban middle aged women. [27] It is therefore of high priority and urgency to understand the association of components of metabolic syndrome with micronutrients such as vitamin B_{12}, folic acid, vitamin D and LCPUFA especially in postmenopausal women. This would reinforce the role of micronutrients as a cheap viable option in the prevention of CVDs in urban middle aged women.

Hypothesis

It is hypothesized that micronutrients like vitamin B_{12}, folic acid, vitamin D and LCPUFA may be associated with components of metabolic syndrome in post menopausal women.

In order to test the above hypothesis, a human study was carried out to examine the levels of micronutrients like vitamin B_{12}, folic acid , vitamin D and LCPUFA. To find all mentioned micronutrients association with components of metabolic syndrome in postmenopausal women.

The next chapter describes the review of literature studied before deciding the present thesis work.

REVIEW OF LITERATURE

Definition of metabolic syndrome

Metabolic syndrome is defined as a cluster of interlinked physiological, biochemical, clinical and metabolic factors that directly aggravate the risk of cardiovascular diseases, type 2 diabetes mellitus and all-cause mortality. [28,29]

History

Metabolic syndrome (MetS) was first described by Kylin [30] in the 1920s as the clustering of hypertension, hyperglycaemia and gout. Two decades later, Vague [31] noted that upper body adiposity or male-type obesity) was most often associated with the metabolic abnormalities seen with diabetes and cardiovascular disease (CVD). During the 1988 Banting lecture, Reaven [32] used the term 'Syndrome X' and firmly established the clinical importance of this syndrome, although obesity was not included.In 1989, Kaplan [33] renamed it 'The Deadly Quartet' and others then coined the term 'The Insulin Resistance Syndrome'. Thus metabolic syndrome is now considered as group of metabolically related cardiovascular risk factors which also determine the risk of development of diabetes in future.[6]

Components of the metabolic syndrome:[6, 34]

Abdominal obesity, blood pressure, fasting blood glucose, triglycerides and HDL are the metabolic risk factors. These are described as follows:

Abdominal obesity represented by waist circumference (WC) is a strong component of metabolic syndrome. All expert groups add obesity to definition of metabolic syndrome due to its higher prevalence.

Elevated blood pressure strongly associates with obesity and common in insulin-resistant persons. Hypertension thus commonly is listed

among metabolic risk factors. But some studies observed that hypertension is less important component for metabolic-syndrome. Certainly, hypertension is multifactorial in origin. For example, increasing arterial stiffness contributes significantly to systolic hypertension in the elderly. Even so, most participants attending National Heart, Lung, and Blood Institute/American Heart Association Conference (NHLBI/AHA) Conference in 2004[6] favoured inclusion of elevated blood pressure as one component of the metabolic syndrome. Urbanisation and wrong diet pattern increase the risk of hypertension has been reported.

Third component is hyperglycaemia. Bo Isomaa et al 2001 [35] observed prevalence of metabolic syndrome increasing with Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT) as compared to those with Normal Glucose Tolerance (NGT). As regards this, hyperglycemia component increases with increasing obesity i.e waist circumference. Insulin resistance present in the majority of people with the metabolic syndrome, account for the alternative term as insulin resistance syndrome. Last component of MetS is atherogenic dyslipidemia manifested as raised triglycerides and low concentrations of HDL cholesterol.

Other Contributing Factors: [36,6]

Prevalence of the metabolic syndrome increases with aging by affecting pathogenesis. Insulin resistance is caused due to proinflammatory state and atherogenesis. Various endocrine factors have been linked to abnormalities in body-fat distribution and hence indirectly to metabolic syndrome. As metabolic syndrome pathogenesis is tangled, there is lot of scope for research.

Criteria for clinical diagnosis of metabolic syndrome[34]

There are various criteria for diagnosis of MetS by different organizations as follows: WHO the European Group for the study of insulin resistance (EGIR), the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), American Association of Clinical Endocrinologists(AACE) and the International Diabetes Federation(IDF). [37]

Table 1: Diagnostic criteria proposed for clinical diagnosis of Metabolic syndrome

TABLE I: Diagnostic criteria proposed for the clinical diagnosis of the MetS.

Clinical measures	WHO (1998) [5]	EGIR (1999) [6]	ATPIII (2001) [7]	AACE (2003) [8]	IDF (2005) [9]
Insulin resistance	IGT, IFG, T2DM, or lowered insulin Sensitivity[a] **plus any 2 of the following**	Plasma insulin >75th percentile **plus any 2 of the following**	**None, but any 3 of the following 5 features**	IGT or IFG **plus any of thefollowing based on the clinical judgment**	None
Body weight	Men: waist-to-hip ratio >0.90; women: waist-to-hip ratio >0.85 and/or BMI > 30 kg/m^2	WC ≥94 cm in men or ≥80 cm in women	WC ≥102 cm in men or ≥88 cm in women	BMI ≥ 25 kg/m^2	Increased WC (population specific) **plus any 2 of the following**
Lipids	TGs ≥150 mg/dL and/or HDL-C <35 mg/dL in men or <39 mg/dL in women	TGs ≥150 mg/dL and/or HDL-C <39 mg/dL in men or women	TGs ≥150 mg/dL HDL-C <40 mg/dL in men or <50 mg/dL in women	TGs ≥150 mg/dL and HDL-C <40 mg/dL in men or <50 mg/dL in women	TGs ≥150 mg/dL or on TGs Rx. HDL-C <40 mg/dL in men or <50 mg/dL in women or on HDL-C Rx
Blood pressure	≥140/90 mm Hg	≥140/90 mm Hg or on hypertension Rx	≥130/85 mm Hg	≥130/85 mm Hg	≥130 mm Hg systolic or ≥85 mm Hg diastolic or on hypertension Rx
Glucose	IGT, IFG, or T2DM	IGT or IFG (but not diabetes)	>110 mg/dL (includes diabetes)	IGT or IFG (but not diabetes)	≥100 mg/dL (includes diabetes)[b]
Other	Microalbuminuria: Urinary excretion rate of >20 mg/min or albumin: creatinine ratio of >30 mg/g.			Other features of insulin resistance[c]	

[a]Insulin sensitivity measured under hyperinsulinemic euglycemic conditions, glucose uptake below lowest quartile for background population under investigation.
[b]In 2003, the American Diabetes Association (ADA) changed the criteria for IFG tolerance from >110 mg/dl to >100 mg/dl [10].
[c]Includes family history of type 2 diabetes mellitus, polycystic ovary syndrome, sedentary lifestyle, advancing age, and ethnic groups susceptible to type 2 diabetes mellitus.
BMI: body mass index; HDL-C: high density lipoprotein cholesterol; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; Rx: receiving treatment; TGs: triglycerides; T2DM: type 2 diabetes mellitus; WC: waist circumference.

Source: Modified from Grundy et al [28] 2005, Circulation. 2005;112: 2735-2752.

But in 2009, a joint interim statement of the IDF Task Force suggested the IDF global consensus definition where central obesity was not considered as an obligatory parameter. It was also proposed that the presence of three or more of the five parameters could be considered as diagnostic of MetS. This joint statement also suggested that, the IDF-recommended race and gender specific cutoffs be used until WC cutoffs could be further evaluated based on data from various regions. The WC cutoffs recommended by various researchers from Japan, Korea, Iran, Iraq, and other regions were considered for the definition of MetS. Thus, there are now numerous race and gender specific WC cut-offs as follows:

Table 2: IDF recommended Race and Gender specific cut-offs

Table 2. Current Recommended Waist Circumference Thresholds for Abdominal Obesity by Organization

Population	Organization (Reference)	Recommended Waist Circumference Threshold for Abdominal Obesity	
		Men	Women
Europid	IDF (4)	≥94 cm	≥80 cm
Caucasian	WHO (7)	≥94 cm (increased risk)	≥80 cm (increased risk)
		≥102 cm (still higher risk)	≥88 cm (still higher risk)
United States	AHA/NHLBI (ATP III)* (5)	≥102 cm	≥88 cm
Canada	Health Canada (8,9)	≥102 cm	≥88 cm
European	European Cardiovascular Societies (10)	≥102 cm	≥88 cm
Asian (including Japanese)	IDF (4)	≥90 cm	≥80 cm
Asian	WHO (11)	≥90 cm	≥80 cm
Japanese	Japanese Obesity Society (12)	≥85 cm	≥90 cm
China	Cooperative Task Force (13)	≥85 cm	≥80 cm
Middle East, Mediterranean	IDF (4)	≥94 cm	≥80 cm
Sub-Saharan African	IDF (4)	≥94 cm	≥80 cm
Ethnic Central and South American	IDF (4)	≥90 cm	≥80 cm

*Recent AHA/NHLBI guidelines for metabolic syndrome recognize an increased risk for CVD and diabetes at waist-circumference thresholds of ≥94 cm in men and ≥80 cm in women and identify these as optional cut points for individuals or populations with increased insulin resistance.

Source : Modified from Alberti et al [34] 2009, Circulation;120:1640-1645

Table 3 : Metabolic syndrome criteria accepted by the joint interim statement of the IDF Task Force

1. Abdominal obesity by waist circumference
Men ≥ 90 cm Women ≥ 80 cm
2. Triglyceride ≥ 150 mg/dL
3. High-density lipoprotein cholesterol
Men < 40 mg/dL Women < 50 mg/dL
4. Blood pressure ≥ 130/85 mm Hg
5. Fasting glucose ≥100 mg/dL

Source : Modified from Alberti et al [34] 2009, Circulation;120:1640-1645

Indicators of metabolic syndrome

Genetic indicators includes sex, race , ethnicity and family history while environmental indicators includes age, socioeconomic status, sedentarism, BMI , stress, type of diet, physical activity and education. Thus, genetic and environmental factors affects Mets prevalence.[38]

Prevalence of Metabolic syndrome

MetS prevalence varies from <10% - 84% as it depend on region i.e urban or rural , sex , age, ethnicity, race and criteria used [39]. Around one-quarter of adult population of the world is suffering from MetS.[37]

Prevalence of MetS was reported to be 5% , 22%, and 60% in normal, overweight and obese individuals respectively by the National Health and Nutrition Examination Survey.[40]This risk increases with aging. 10% , 20% 45% for age between 20-29, 40-49 and 60-67yrs respectively.[41] MetS prevalence was reported to be highest i.e 32.6 - 41.5% in postmenopausal women by Ponholzer et al.[41] It was also observed increase in weight $\geq$ 2.25kg over 16yrs increases the MetS risk by 45 % in Framingham study.[42] Increase in waist circumference by 11cm, leads to the risk of Mets by 80% within five years was observed by Palaniappan et al.[43]

Prevalence of metabolic syndrome is increasing in developing countries, especially in the South Asian countries. The recent data shows that one fourth to one third of urban population of India has metabolic syndrome. Furthermore, the prevalence is 1.5–2 times higher in urban middle aged women[27] with moderate to higher socio economic status as compared with men. Because, obesity is more in middle aged women than in men due to socioeconomic transition causing significant shifts in dietary and physical activity patterns. These changes lead to significant effects on body composition and metabolism, often resulting in increase in BMI, excess generalized and abdominal adiposity, and dyslipidemia. This increases risk for metabolic syndrome and cardiovascular diseases whereas in a study by Park YW [40] in 2003 it was found that, postmenopausal status was associated with a 60% increased risk of the metabolic syndrome, even after adjusting for confounding variables such as household income.

Carr MC et al [8] in his study suggested that the presence of estrogen has a genetic masking effect, rather than a primary role in MetS control in spite of absence of central obesity.

A hallmark of the menopausal transition is the dramatic reduction in estradiol levels. [44] With this reduction, there is a progressive shift toward androgen dominance in the hormonal milieu.[45,46] Although little is known about how this hormonal shift influences. CVD risk, various studies suggested a link between androgenicity and CVD risk factors.[47,48,49]

Table 4 : Indian studies on metabolic syndrome conducted on middle aged women

	Age	Prevalence	Imp component involved
Gupta A [50] et al 2003	> 20 yrs	20.4%	WC, hypertension, high triglycerides and low HDL
Ramachandran A [51] et al 2009	20-75 years.	41.1%.(ATP III)	WC, hypertension, high triglycerides and low HDL
Sapna Goyal et al [52] ;2013	36 to 65 years	10 % Pre, 41.67 % peri and 46 % post menopausal	FBG, hypertension, high triglycerides and low HDL
Shefali Pandey et al [53] 2010	> 35 years	Pre 45% and post - menopausal 55%	WC, hypertension, high triglycerides and low HDL

Table 5 : International studies in middle aged women on metabolic syndrome in other countries of Asia, Africa and Latin America

	Nationality	Age	Prevalence	Imp risk factors/ remark
Fareed K N A et al [54] ;2013	Ghanaian	Pre and post menopausal	18 % and 43% (Pre and post menopausal IDF criteria)	WC, hypertension, FBG
Marjani et al; [55] 2012	Iranian	Age group between 20-40yrs	20.62%.	WC, low HDL
Ainy E et al [56] 2007	Tehranian	Pre , peri and post menopausal women	53%, 54% and 69% respt.	Low HDL and high diastolic blood pressure in postmenopausal women.
José Albuquerque et al [57] 2010	Brazil	pre and post-menopausal	37% (Pre) 61.5% (post) (IDF criteria)	Along with age , low HDL, hypertension , WC, high TGs
Karina Giane et al [58] 2013	Brazil	40 to 65 years	56.1 %	Hypertension, WC and low HDL cholesterol.
Petri Nahas EA et al [59] 2009	Brazil	40—75 yrs postmenopausal women.	39.6%	WC, affecting 62.5% of women
Zahra Jouyandeh et al [60] 2013	Iran.	postmenopausal women	30.1%.	1.WC 2.Number of components increases with increase in WC

Ruan X et al [61] 2010	Chinese	postmenopausal women	33.7%.	WC
Samir Ben Ali et al [62] 2014	Tunisian	35-70 years postmenopausal	45.7%	WC and hypertension significantly higher in postmenopausal
Katulanda P et al [63] 2012	Shrilanka	46.1±15.1years	28.3 %	Female gender, increasing age, urban living, higher socio-economic status and physical inactivity were all important factors associated with the occurrence of Metabolic Syndrome
Saira A et al [64] 2008	Pakistan	Pre and post menopausal	7% in Pre and 21% in post	----------
MS Jahan et al [65] 2016	Bangladesh	Postmenopausal	50%	Low HDL followed by high TG , obesity , high fasting blood sugar and hypertension .

As per the above references, prevalence was more in postmenopausal women. There was an ethnic variation as it was shown that there was minimum prevalence in Chinese population as compared to South Asians. Prevalence was maximum in developing economise like Iran, Brazil and India; intermediate in African countries (Ghana ,Tunisia) and minimum in Chinese. Even among premenopausal women, the prevalence increased from 20 % in 2003 to 45 % in 2013 according to Indian studies.

The above studies indicated that the most common risk factor was WC.More the WC more the number of MetS components present. WC was followed by hypertension and dyslipidemia as risk factors . Whereas IFG was a less common risk factor.

Pathophysiology of metabolic syndrome

The underlying mechanisms of MetS are not well explained but the role of sedentary lifestyle habits in the development of MetS is well known. Adiposity results as a consequence to sedentary life style. [66]

Adipose tissue is not only a storage tissue , but also produces free fatty acids and various molecules like inflammatory cytokines and reduces production of anti-inflammatory adipokines. In case of obesity, particularly abdominal obesity, the release of free fatty acids is increased. Elevated levels of circulating free fatty acids contribute to the development of insulin resistance by inhibiting insulin signalling.[67,68]

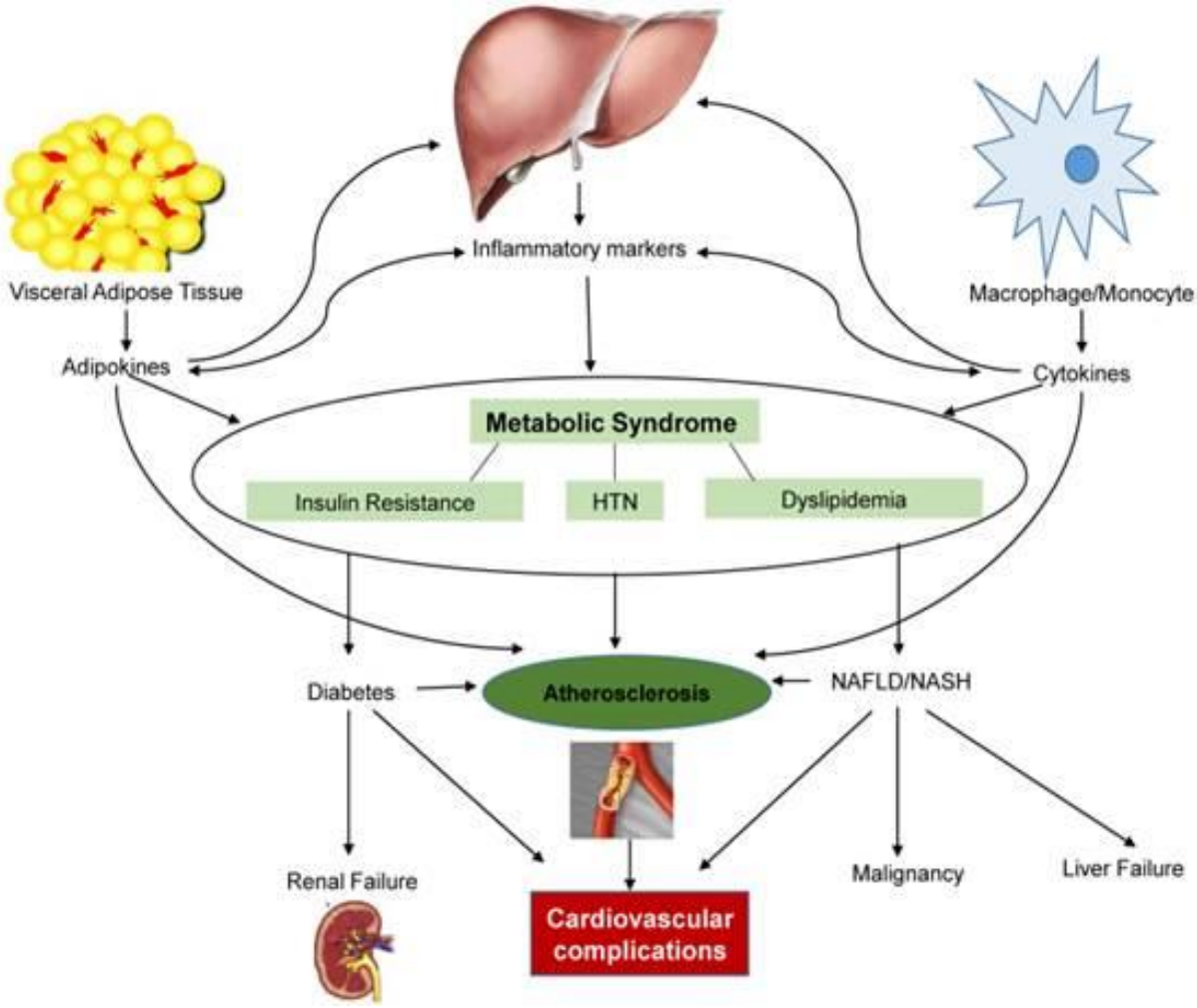
Insulin resistance can be defined as an insufficient insulin action in the liver, skeletal muscle and adipose tissue. When there is insulin resistance, it gives rise to increased gluconeogenesis in the liver, decreased glucose disposal in the muscle, endothelial dysfunction in the arteries and increased release of free fatty acids from the adipose tissue. Hyperglycemia occurs if the compensatory mechanism of production of insulin leading, to hyperinsulinemia to maintain euglycemic fails. [66]

When insulin resistance develops the inhibitory effect of insulin on lipolysis is supressed, further increasing free fatty acid circulation. Excess free fatty acids are converted to cholesterol, phospholipids lipoproteins and triglycerides giving rise to dyslipidaemia.[69]

Hypertension has been related to insulin resistance by several mechanisms.[68] For example, free fatty acids produced by the adipose tissue may directly mediate vasoconstriction. [70] Adipocytes give rise to different vasoactive peptides which may alter the vasodilatory effect of insulin. Apparently, the association between insulin resistance and hypertension is more obvious in obesity cases and the effect may be mediated by adipose tissue. In addition, it has been suggested that low-density lipoprotein and triglycerides may damage the arterial epithelium, inhibit nitric oxide release and cause endothelial dysfunction. Thus, dyslipidemia characterized by raised levels of apolipoprotein B (apo B) containing lipoproteins could lead to hypertension by mechanisms only partly related to obesity and insulin resistance.[71]

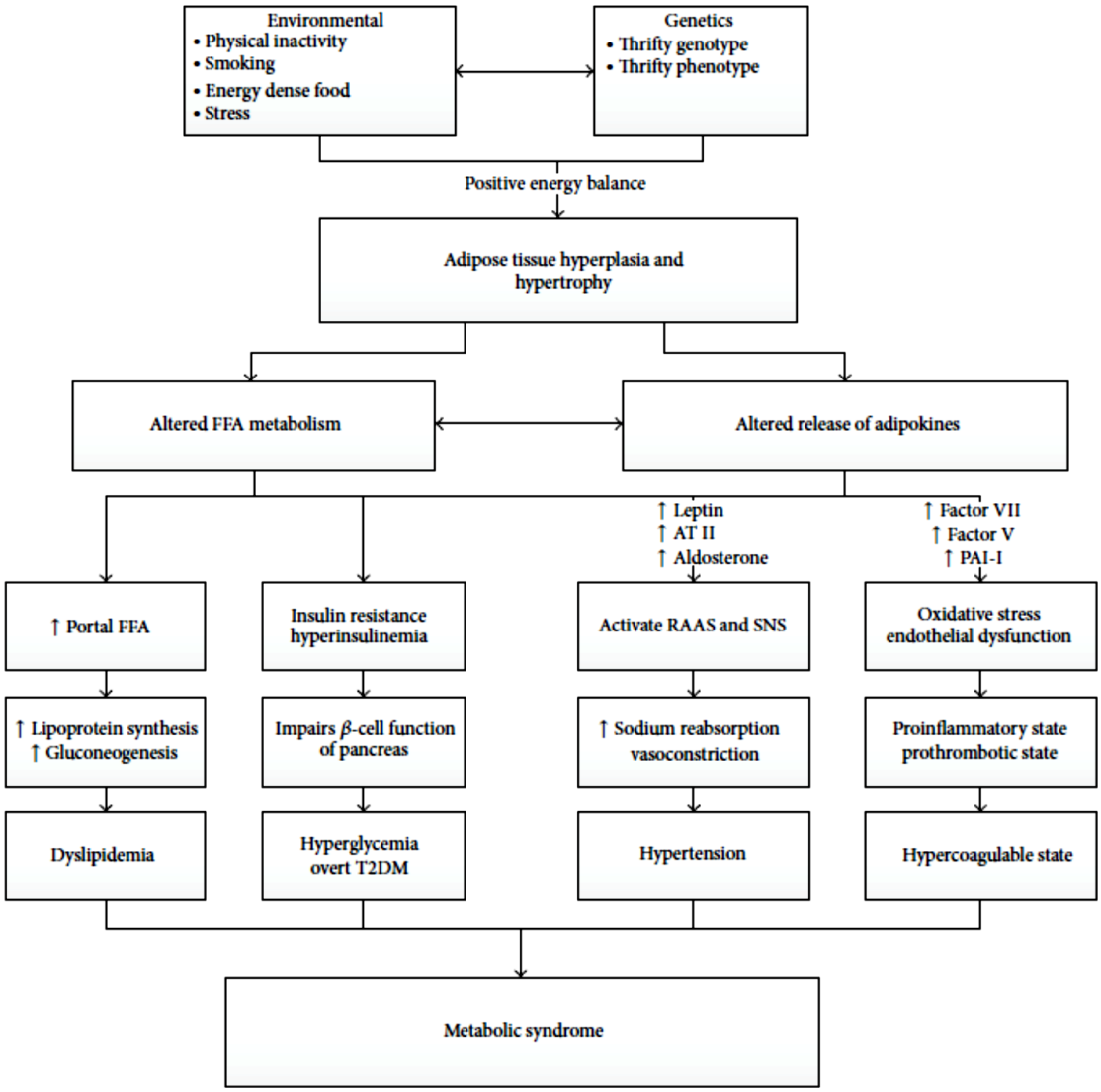
Role of genetics factors in the pathogenesis of MetS have weak effects when observed alone as explained by multiple genome-wide studies but very powerful when combined with environmental factors like diet, physical activity, alcohol intake and smoking. [72] The risk for MetS also increases due to intrauterine malnutrition explained by low birth weight at birth with reduced insulin sensitivity.[73]

Figure 1: Pathophysiology of Metabolic syndrome



Source : Modified from Krithika et al [74]2016, Int J Med Sci ; 13(1):25-38.

Figure 2 : Schematic presentation of Metabolic syndrome



Source : Modified from J Kaur. [75] 2014 , Cardiology Research and Practice. 2014, 1-22.

Outcome of Metabolic syndrome:

Cardiovascular disease (CVD) is considered as primary clinical indicator of the metabolic syndrome along with insulin resistance and type 2 diabetes. CVD risk increases with severity of type 2 diabetes. Individuals with metabolic syndrome are also at risk to develop polycystic ovary syndrome, sleep disorders, fatty liver, gallstones, asthma and cancer.[28] It is estimated that around a quarter of the world's adult population have metabolic syndrome [76] and they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without the syndrome.[9]

Deaths due to MetS represents 30% of all global deaths according to WHO fact sheets.[77] Low and middle-income countries contribute to more than 80% of these cardiovascular deaths.[78]

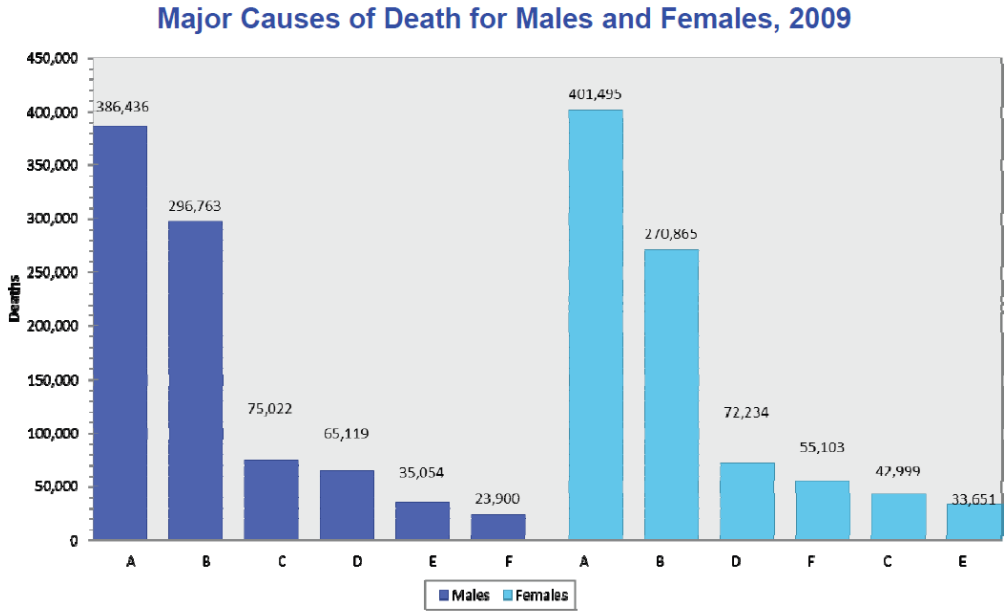
The prevalence of coronary heart disease (CHD) is known to be high in people of south asian descent.[2] It was also evident that among the Asian Indian women, postmenopausal women were more susceptible to diabetes and cardiovascular diseases than premenopausal women.[79]

After natural menopause, over the period of 10 yr the risk of CVDs increases 4-fold as reported by Framingham study. Estrogen deficiency contribute to an increased prevalence of the metabolic syndrome in postmenopausal women as compared to premenopausal women and in the postmenopausal women worsening of the metabolic profile may contribute to the future risk of CVD. It is estimated that half of all cardiovascular events in women are related to the metabolic syndrome.[80]

The number of people who die from CVDs are mainly from heart disease and stroke.16.5% of all deaths can be attributed to high blood pressure. According to a WHO report, the current age standardised CVD mortality rates are more among women than men in India.[81]

According to statistical fact sheet 2013 - update of American Heart Association[82], incidence of sudden deaths has shown a steep rise with age in females than males. A worse prognosis for females than in males with coronary heart disease was reported. In a cardiac rehabilitation program after myocardial infarction, females were found to have less enrolment, poor program attendance and more dropouts. The fact remains that females have a worse prognosis and they die more often than males after a MI or bypass surgery.

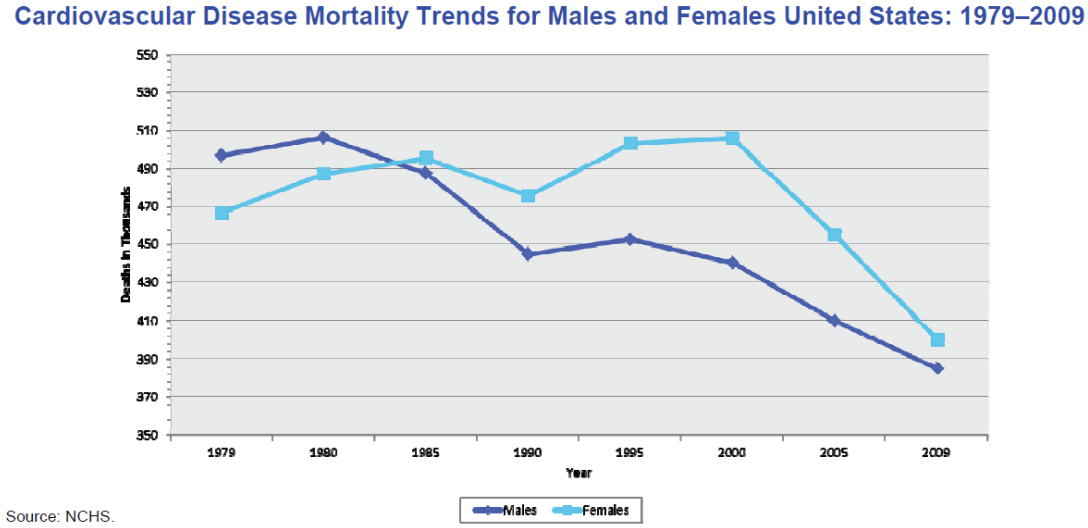
Figure 3: Major cause of deaths for male female in 2009



A indicates cardiovascular disease plus congenital cardiovascular disease (ICD-10 I00-I99, Q20-Q28); **B**, cancer (C00-C97); **C**, accidents (V01-X59,Y85-Y86); **D**, chronic lower respiratory disease (J40-J47); **E**, diabetes mellitus (E10-E14); **F**, Alzheimer disease (G30). Source: NCHS.

Source : Statistical fact sheet 2013- update of American Heart Association 82

Figure 4: CVD Mortality trends for male and females



Source: Statistical fact sheet 2013- update of American Heart Association[82]

There is fivefold risk of developing type 2 diabetes in people with metabolic syndrome. Type 2 diabetes, which accounts for 90 per cent of all diabetes, has become one of the major causes of premature illness and death, mainly through the increased risk of cardiovascular disease (CVD) which is responsible for up to 80 per cent of these deaths.[83] Hyperglycemia and dyslipidaemia are two important risk factors for CVDs even before developments of type 2 diabetes.[84]

Metabolic syndrome and Menopause

Menopause is defined as the absence of menses for 12 consecutive months. It is associated with significant fall in estrogen levels. The perimenopause has been defined as a period of menstrual irregularity and hormonal variability, beginning when menstrual cycle length changes from an established pattern into longer, shorter, or more variable cycles, with an average duration of 4 yr. It is commonly believed that estrogen levels fall gradually throughout the perimenopause.[8] Menopause is one of the crucial

stages in women's life which leads to various physiological and psychological changes. Menopausal status is an important factor that influences metabolic changes and hence Mets biomarkers.[85]

Menopause and body composition:

Menopause may lead to increase in body fat mass and redistribution of fat mass from the limbs to a more central or android location in women. Deficiency of estrogen during menopause leads to accumulation of central fat i.e visceral fat or android fat. However estrogen promotes the accumulation of gluteo-femoral fat and not visceral fat. It was reported that accumulation of visceral fat increases the risk of development of type 2 DM , hypertension and CVDs.[86,87]

Though middle-aged women gained approximately 0.55 kg /yr weight but its not true always.[8] However, during menopause body fat distribution changes though there is no weight gain. Various studies [88,89] showed that the menopausal transition was associated with a preferential increase in abdominal adiposity, independent of the effect of age and total body adiposity.

It was observed that lack of physical activity during menopause leads reduced lean body mass. In sedentary postmenopausal women inverse relationship between visceral adiposity and maximal oxygen consumption was found when compared with age-matched sedentary premenopausal women by Lynch et al.[90] Thus menopause is associated with adiposity and reduced lean body mass due to decrease in exercise capacity.[89]

Menopause and lipid metabolism

Menopause is associated with abdominal adiposity which is known to increase insulin resistance, free fatty acid (FFA) levels and decreased adiponectin along with increased levels of apolipoprotein B (apo B) particles. Apolipoprotein B results into hypertriglyceridemia and high hepatic lipase (HL) activity. This leads to higher levels of small dense LDL particles and decrease in large antiatherogenic HDL 2 particles.[8]

High hepatic lipase activity results into high TG and increased smaller, dense atherogenic lipoproteins. This generates FFA that acts as energy source or can be stored in adipocytes leads abdominal obesity. [91] It was also observed that endogenous estrogen levels are negatively associated with lipase activity.

Menopause and HDL

Most studies showed that total HDL levels fell slightly with menopause, whereas others revealed no changes. Regarding the athero protection, it is of importance to notify that HDL particles are highly heterogeneous in their size, structure, metabolism and biological function. Emerging evidence suggests that small, dense HDL 3 subspecies possess a higher capacity to protect LDL against oxidation than large, light HDL 2 particles. Menopausal changes in HDL metabolism are more complex than the measurement of total HDL reveals. Though the level of antiatherogenic HDL 3 is more than the atherogenic HDL 2, HDL 3 perhaps is not able to exerts its antioxidative capacity due to low level of total HDL in menopause.[8,92]

Asian Indian women have higher frequency of low HDL-C than Asian Indian men, even when not obese or overweight. These results are independent of age, BMI, smoking and menopause status. HDL cholesterol level decreases with menopause [93]. However, not all studies [94,95,8] agree with this conclusion.

Mechanisms of low HDL-C in South Asian women were not examined due to lack of data on dietary or physical activity profile , estrogen use in postmenopausal women, and evaluation of other potential mechanisms such as insulin resistance or inflammation. However, lower HDL-C observed in Asian Indian women seems to be only partially dependent on environmental factors such as living in urban areas in westernized lifestyle. [96]

According to Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III,2001), modification of the HDL-cholesterol cut point may be required in women in such populations.[97]

No association was found between HDL-C with age, type of diet, body mass index, exercise by Bhalodkar et al [98]. Genetic factors or environmental factors rather than the lifestyle factors are important determinants of HDL cholesterol.[99,100,84] Although it is possible that lower dietary fat intake may have different effect on lipid profile in women compared with men, a meta-analysis of sex differences in plasma lipid response to dietary fat did not find a consistent result. Therefore, the lower dietary fat intake in asian Indians cannot entirely explain the lipid profile .It is reported that Asian Indians have higher total body fat content for the same BMI of whites possibly due to differences in total and regional body fat for a given BMI. [96,101]

Menopause and TGs

Men generally have higher TG levels than women but in middle-aged (between 40–69 yrs) women TG levels start increasing. Various studies. [50,53] observed that TG levels increase with menopause transition and also in early menopausal period.[102] A study by Poehlman et al [103] found 16% increase in TG during menopause transition. Increased in TG

levels are associated with accumulation of abdominal fat and insulin resistance thus act as risk factor for metabolic syndrome.

Menopause and Insulin resistance

Irrespective of total body fat content abdominal obesity is directly related to increased insulin resistance, hyperinsulinemia, and risk of type 2 diabetes. This leads to increased levels of FFA which affects peripheral glucose uptake, hepatic gluconeogenesis and reduce hepatic clearance of insulin.[91]

Postmenopausal women showed increased fasting insulin and glucose levels than premenopausal women thus responsible for increased insulin resistance [102,8] Thus abdominal obesity and aging during menopause lead to decrease insulin sensitivity make postmenopausal women more prone for development of Mets.

In addition many studies have indicated that IFG as a less common risk factor in the development of metabolic syndrome in postmenopausal women as compared to other components. [50, 53]

Guthrie et al. [48] in the prospective study on 267 healthy women reported that women with high BMI over 5 yrs developed impaired fasting glucose, high fasting insulin, high TGs and low HDL when compared with women who maintained normal fasting glucose irrespective of menopausal status.[103] Thus it was concluded that weight gain is a stronger risk factor than menopause for development of impaired fasting glucose.

Insulin resistance, which represents a reduced physiological response of the peripheral tissues to the action of the normal levels of insulin, is a major finding in several metabolic disorders, including type 2 diabetes and metabolic syndrome. Therefore, a reliable measure of insulin resistance is important for investigating the link between insulin resistance and metabolic syndrome.

Insulin resistance and progressive pancreatic β-cell dysfunction have been identified as the two fundamental features in the pathogenesis of type 2 diabetes. Homeostasis model assessment (HOMA) is a validated clinical and epidemiological tool for estimation of insulin resistance and β-cell function.[104] The homeostasis model assessment (HOMA) calculated from hepatic glucose output and insulin secretion from fasting glucose and insulin levels is simple and reliable option for large-scale epidemiologic studies as compared to Euglycemic clamp which is considered as "gold standard" method but its an expensive and invasive methods.[105]

HOMA-IR was calculated as fasting insulin (U/l) × fasting glucose (mg/dl) /405, as described by Matthews et al [104] is regarded as a simple, inexpensive, and reliable surrogate measure of insulin resistance.

Insulin resistance:

Insulin sensitivity and glucose intolerance are not entirely explained by a woman's menopausal status. Paul et al [106] also reported that weight gain is a stronger risk factor than menopause for impaired fasting glucose. Jaber et al.[107] observed that decreased HDL cholesterol and increased fasting glucose were important risk factors for MetS in Arab American women which might be due to different genetics and environmental factors. Optimal cut-off of the homeostatic model assessment for the diagnosis of insulin resistance (IR) and metabolic syndrome (MetS) is 1.775 for non-diabetic individuals and 3.875 in diabetic individuals. [108]

Sandeep S et al [109] assessed the association of Insulin Resistance assessed by Homeostasis Assessment model (HOMA-IR) with cardiovascular risk factors in subjects with normal glucose tolerance [NGT] in Asian Indians. It was reported that HOMA- IR was correlated with blood pressure, triglycerides and HDL cholesterol even after adjustment for age, gender and BMI. People with family history of type 2

diabetes, low grade physical activity, generalized obesity and abdominal obesity also had higher HOMA- IR values. Thus, HOMA IR values increases with increase in number of components of MetS.

Nutrition and Menopause:

Some studies have been conducted on nutritional status of menopausal women that emphasize the need for special attention on this group.

Nutrition is defined as intake of food as per the body's dietary needs. An adequate, well balanced diet along with regular physical activity is very essential for good health. Poor nutrition leads to less immunity, person become more prone for the diseases, hampered physical and mental development. [110]

A rapid increase in the size of a population, demographic changes, urbanization and traditional habits are important for certain faulty dietary practices that lead to diet-related chronic diseases. Infants, children and adolescents, pregnant lactating women and the elderly are most important where special attention of dietary guidelines is needed for promotion of health and prevention of disease.

Macro-nutrients and micronutrients are the two important types of nutrients.[111] Macro-nutrients are needed in relatively large amounts. Carbohydrates, fats and protein are the some examples of macronutrients. Proteins are required for the growth and development. Carbohydrates and fats provide energy. In addition, lipids are needed to form cell membrane, hormones and cell signaling molecules.

Micronutrients are nutrients required in very small amounts and their deficiency leads to major health problems because, various physiological functions of the organisms throughout their life depend on

small quantities of micronutrients. These substances function as co-enzymes, hormones and cell signaling molecules essential for proper growth and development. Deficiency of some of the micronutrients like vitamin B_{12}, folic acid and vitamin D in low income countries leads to CVD. [112,113]

Menopause is one of the crucial stages in women's life which leads to various physiological & psychological changes. These changes can increase the risk of diabetes and cardiovascular diseases where genetic and other environmental factors are also playing important role. In menopausal women, all these, psychological and physiological changes have an impact on food intake and food choices.[114] It is an established fact that balanced diet is necessary to reduce some of the complications of menopause. Therefore, to study the nutritional status of menopausal women specially related to cardiovascular diseases is important.

Macronutrients like fatty acids are also important predictors for CVDs. Fats consist of a wide group of compounds that are generally soluble in organic solvents and insoluble in water. [115]

Chemically fats are triglycerides: triesters of glycerol and any of several fatty acids. Fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. Fatty acids present in the body are either free or fatty acyl esters like triacylglycerol. Plasma free fatty acids bound to serum albumin are transported from triacylglycerol of adipose tissue to most of the tissues in the body for utilisation. [115]

Functions of free fatty acids:

Free fatty acids can be oxidized by many tissues particularly liver and muscle to provide energy. Fatty acids form structural components of membrane lipids, such as phospholipids and glycolipids as well as

cholesterol. Fatty acids are connected to specific intracellular proteins and also act as precursors of prostaglandins. Esterified fatty acids, like triacylglycerols act as reserve for energy.

Classification of fatty acids

Depending on their degree of saturation/unsaturation in the carbon chain, they can be divided into three classes:

- Saturated fatty acids (SFA): No double bond is present; are popular with manufacturers of processed foods because they are less vulnerable to rancidity and are in general more solid at room temperature than unsaturated fats. e.g : myristic acid, palmitic acid and stearic acids

- Unsaturated fatty acids: There are one or more double bonds in the carbon chain. They undergo lipid peroxidation (rancidity) which is directly proportional to the degree of unsaturation. Antioxidants can protect unsaturated fat from lipid peroxidation. Unsaturated chains have a lower melting point, hence increasing fluidity of the cell membranes.

  - Monounsaturated fatty acids (MUFA): Only one double bond is present.
    eg. myristoleic, palmitoleic, oleic acid and nervonic acids
  - Polyunsaturated fatty acids (PUFA): Two or more double bonds are present.
    e.g omega 3 fatty acids includes alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), decosahexanoic acid (DHA)and Omega 6 fatty acids includes linoleic acid (LA), gamma linolenic acid (GLA).

Essential fatty acids:

Any fatty acid that cannot be synthesized by the body is called an essential fatty acid e.g Linoleic acid (LA), alpha linolenic acid (ALA). Associations of risk of coronary heart disease (CHD) with dietary fat and specific types of fat among women (aged 40-58 years) was examined by Kyungwon et al.[116] It was reported that polyunsaturated fat intake was inversely correlated. In younger overweight women, transfat intake was associated with CHD risk. Chisato et al[117] found that high intake of monounsaturated fat was associated with higher serum levels of estrone and dehydro epiandrosterone sulfate (DHEAS) in post-menopausal women.

Polyunsaturated fatty acids:

Omega-6 fatty acids: Linoleic acid (LA) is a polyunsaturated omega-6 fatty acid. It is a colorless liquid at room temperature. Linoleic acid is a carboxylic acid with an 18-carbon chain and two cis double bonds; with the first double bond located at the sixth carbon from the methyl end. Linoleic acid is an essential fatty acids as it can't be synthesized in the human body.

LA is a polyunsaturated fatty acid used in the biosynthesis of arachidonic acid (AA) from which some prostaglandins, leukotrienes (LTA, LTB, LTC), and thromboxane (TXA) are synthesized in the body. It is found in the lipids of cell membranes. It is abundant in many nuts, fatty seeds (flax seeds, hemp seeds, poppy seeds, sesame seeds, etc.) and their derived vegetable oils; poppy seed, safflower, sunflower, corn and soybean oils. [118] Reduced LA intakes are based on the assumption that because CHD has an inflammatory component and because the omega-6 fatty acid, AA, is the substrate for the synthesis of a variety of proinflammatory molecules, reducing LA intakes should reduce tissue AA

content, which should reduce the inflammatory potential and therefore lower the risk for CHD.[119]

Omega 3 fatty acids:

It is a essential polyunsaturated fatty acids (PUFAs) . Common types are Alpha-linolenic acid (ALA), Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA). ALA is only found in plant source e.g leafy green vegetables, nuts and vegetable oils such as canola, soy and especially flaxseed. EPA and DHA are found in fish and fish oils. Recommended intake is 1 g per day.[120] Vegetarians and vegans have substantially lower levels of DHA. DHA is important to regulate cell activity and healthy cardiovascular function. Epidemiologic data suggest that DHA derived from fish oil reduce cardiovascular disease by reducing the level of blood triglycerides in humans. [121,122,123]

EPA and DHA are called as essential fatty acids because only less than 5 % of ALA is converted to EPA and DHA in human being. The main sources of ALA are leafy vegetables, nuts and vegetable oils like canola, soybean oils, and flaxseed oil. EPA and DHA are also found in seafood, like fatty fish (e.g., salmon, tuna, and trout) and shellfish (e.g., crab, mussels, and oysters). Vegetarian source of DHA is Algae oil. Conversion of ALA to EPA is much better in premenopausal women than in postmenopausal women. [124]

Functions:

They are incorporated in many parts of the body including cell membranes play a role in anti-inflammatory processes and in the viscosity of cell membranes. [122] They are important for growth and development.

Omega 3 fatty acids have the ability to respond to inflammation in atherogenesis through direct and indirect mechanisms. A direct mechanism

decreases inflammation by regulation of transcription factors. Indirect mechanism include production of ecosanoids and inflammation resolving lipid mediators. [125]

EPA and DHA are essential for proper fetal development and healthy aging. DHA is a key component of all cell membranes and is found in abundance in the brain and retina. EPA and DHA are also the precursors of several metabolites that are potent lipid mediators to be beneficial in the prevention or treatment of several diseases. [121,126] Eicosanoids produced from DHA and EPA are generally less inflammatory. They serve as vasodilators and inhibit platelet aggregation . Low intake of dietary EPA and DHA is associated with increased inflammatory processes as well as poor fetal development, general cardiovascular health and risk of development of Alzheimer's disease. [127,128]

Omega 6 fatty acids such as arachnoid acid give rise to eicosanoid such as throbaxane A2 and leucotrines that are vasoconstrictors, platelet aggregators and proinflammatory respectively. Omega 3 fatty acids can reduce the production of arachidonic acid (AA) derived eicosanoids by competing with AA for incorporation into cell membrane phospholipids. This would shift the production of inflammatory eicosanoids derived from omega 6 fatty acids to omega 3 fatty acids which is beneficial to cardiovascular system. [127,128]

An imbalance of dietary omega 6: omega 3 PUFA ratio may also result in altered gene regulation and expression in downstream pathways. This results in altered protein expression and activity that can negatively affect cell membrane composition and fluidity and organ function. [129]

It was reported that lowering of omega-6:omega-3 ratio to 4:1 was associated with 70% decrease in total mortality due to CVDs. Omega 3 fatty acids can be useful in the secondary prevention of cardiovascular disease due to their vasodilatory and antiplatelet activity.[129] For reduction of risk of many chronic diseases lower ratio of omega-6/omega-3 fatty acids is recommended . [130]

Micronutrients are required in minute amounts, regulate physiological functions , play imp role in metabolism, growth, development eg . Vitamins, trace elements etc. Important vitamins are A,B,C, D,E,K and some of the important trace elements are Cu, Fe, Zn, Co, Se etc. [131]

Folic acid, vitamin B_{12} play imp role in maintaining the homocysteine levels.[132] Vitamin B_{12} is a water-soluble vitamin. Its active forms is Methylcobalamine. It has several function such as RBC maturation, neurological growth, acts as a cofactor for methionine synthase required for synthesis of DNA, RNA, hormones, proteins and lipids. Requirement for women is 1 mg/per day. Values < 170–250 pg/ml for adults indicate a vitamin B_{12} deficiency . Dietary Sources: naturally found in animal products, including fish, meat, poultry, eggs, milk and milk products. Vitamin B_{12} is generally not present in plant foods. [131]

Folic acid:

Folate is a naturally occurring water-soluble B vitamin, Folic acid is the synthetic form. It plays significant role in the conversion of homocysteine to methionine as an important methyl donor. Methylation in the formation of DNA is required for proper cell division. Values above 3 (ng)/ml indicating adequacy. Requirement for women is 100 mg/per day.Vegetables (especially dark green leafy vegetables), fruits, nuts, beans,

peas, dairy products, poultry and meat, eggs, seafood and grains. Spinach, liver, yeast, sprouts are among the rich sources of folate .[131]

Bertoia et al [133] studied mediterranean dietary patterns and risk of sudden cardiac death (SCD) in postmenopausal women and this Dietary approaches to Stop Hypertension (DASH) diet was recommended that includes higher intake of fruits, vegetables, whole grains, and unsaturated fatty acids. It may be useful for prevention of high cholesterol, inflammation, the development of atherosclerosis, and therefore risk of SCD. Also DASH diet is known to lower blood pressure and hypertension which is a major risk factor for SCD. However, sodium intake, an important component of the DASH dietary pattern, was not well documented. It was observed that in post-menopausal women mediterranean dietary pattern may be associated with a lower risk of SCD .

Relation between dietary folate and vitamin B_{12} intakes with risk of cardiovascular disease was studied by Cui R et al. [134] In this 14-year follow-up study it was found that there was inverse association of dietary folate and vitamin B_{12} with stroke, coronary heart disease and total cardiovascular disease mortality in women. Christin Heidemann et al [135] reported that 28% lower risk of cardiovascular mortality was associated with a prudent diet and also observed that western dietary pattern was associated with a higher risk of mortality from cardiovascular diseases among healthy women without any disease initially. Kozue Nakamura et al [136] reported that intake of vegetables is associated with reduced risk of death from CVD for women.

Vitamin D:

Recent studies showed that vitamin D deficiency may be a risk factor for the metabolic syndrome and cardiovascular diseases. [137 -141] It is a fat-soluble vitamin and produced endogenously. 7-Dehydrocholesterol converts into cholecalcifereol in presence of ultraviolet rays from sunlight. Cholecalciferol hydroxylates in liver into 25-hydroxy vitamin D_3 which is a important indicator of the total of vitamin D in a human body. [137,138]

Vitamin D is needed for bone growth and bone remodelling by osteoblasts and osteoclasts by absorption of calcium. It has other functions like modulation of cell growth, neuromuscular and immune function and reduction of inflammation. Dietary sources are cod liver oil, shrimp, liver, butter, yolk, cheese, milk, spinach and cabbage. Recommended levels in diet is 400 μg/day . Levels below 20 ng/ml are labelled as insufficiency & below 10 ng/ml is considered as deficiency. Hypovitaminosis D is associated with glucose intolerance, impaired insulin secretion, homocysteine & negative endocrine regulation of the renin-angiotensin system .[137 -142]

Schierbeck reported [143] that women with vitamin D deficiency had more cardiovascular risk factors like higher BMI and triglycerides and lower HDL. Thus, it was concluded that healthy women with vitamin D deficiency had increased risk of adverse cardiovascular outcome.

Facts of Indian food pattern:

It was found that there is dietary deficiency of folic acid and vitamin B_{12} due to vegetarian diet and faulty cooking habits in Indian diet. Deficiency of omega3 fatty acids along with vitamin D is also reported in Indian diet. All these deficiencies lead to increase in the levels of homocysteine.[137 -139, 144 -147]

Homocysteine and metabolic syndrome - Role of folic acid, vitamin 12, folic acid, omega 3 fatty acids and vitamin D:

Homocysteine is considered to be the risk factor for the development of cardiovascular diseases and type 2 Diabetes. Hyperhomocysteinemia results from deficiency of, folic acid, vitamin B_{12} and omega 3 fatty acids due to involvement of one carbon cycle.[148] Plasma concentrations of folate and vitamin B_{12} were reported to be inversely associated with homocysteine concentrations in middle aged women by Saw et al. [149] Recently it was found that vitamin D also plays an important role in maintenance of homocysteine levels by acting on cystathionine β-synthase enzyme. [150]

Following mechanisms are described by various studies for development of cardiovascular diseases due to hyperhomocysteinemia. [151-154]

Oxidation of homocysteine to homocystine is accompanied with production of hydrogen peroxide inducing damage of endothelium through oxidative stress. [155]

Decreases the level of glutathione peroxidase in the endothelial cells, and inhibits its activation leading to the impairment of oxidative defensive mechanism, and the free radical-induced NO-inactivation. [155]

Endothelial cytotoxicity & lipid peroxidation leads to atherosclerosis [151]

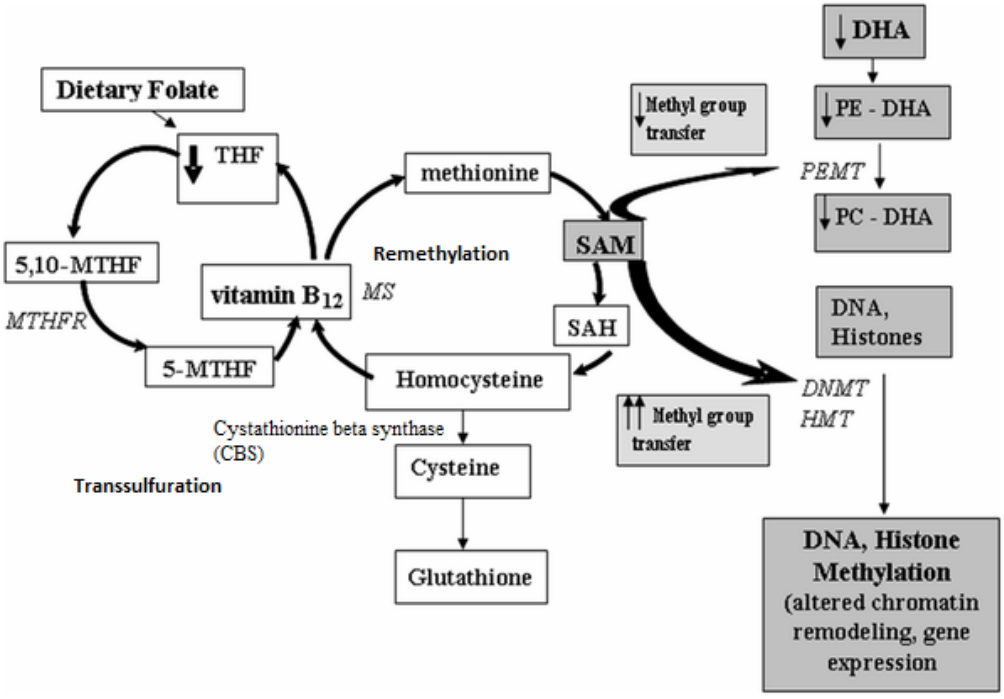
- Increased platelet adhesiveness and activation of the coagulation system.[152]
- Inhibition of the thrombomodulin-protein C and glycosaminoglycan-antithrombin-III anticoagulant system. [153]
- Vascular smooth muscle cell proliferation [153] Insulin resistance. [154]

- The above mechanisms lead to atherosclerotic vascular disease of the arteries of the limbs, the coronary arteries and the cerebrovascular system.

Homocysteine metabolism:

Micronutrients like folic acid, vitamin B_{12} play an important role in maintaining the homocysteine levels.[18]

Figure 5 One-Carbon Cycle: Interactions of folic acid, vitamin B_{12} and DHA.



MTHFR : Methylene tetrahydrofolate reductase. DHA :Docosahexaenoic acid. SAM : S - adenosyl methionine , SAH: S - adenosyl homocysteine.

Source : Kulkarni A et al. [18] PLoS ONE .2011 6(3): e17706.

Synthesis :

Homocysteine is a sulphur containing amino acid. Homocysteine is bio synthesized from methionine via a multistep process.[156,157] First, methionine receives an adenosine group from ATP, a reaction catalyzed by S-adenosyl-methionine synthetase, to give S-adenosyl methionine (SAM). SAM then transfers the methyl group to an acceptor molecule, (e.g norepinephrine as an acceptor during epinephrine synthesis, DNA methyltransferase as an intermediate acceptor in the process of DNA methylation). The adenosine is then hydrolyzed to yield L-homocysteine.

Fate of homocysteine :

Homocysteine is metabolised in two pathways:

1) Conversion to methionine by remethylation to methionine, which requires folate and vitamin B_{12} .
2) Conversion to L-cysteine by transsulfuration to cystathionine, which requires vitamin B 6 and vitamin D.

Role of folic and vitamin B $_{12}$: [156,157]

Dietary folate is converted to 5 methyl tetrahydrofolate (5 MTHF) by 5-methyl tetrahydrofolate reductase enzyme. 5 MTHF act as methyl donor in presence of cobalamin (vitamin B_{12}) related enzymes for conversion of homocysteine to methionine.

Role of vitamin D

Conversion of homocysteine to L-cysteine involves CBS enzyme which is activated by vitamin D. Deficiency of vitamin D has been linked with hyperhomocysteinemia .[150 ,158] A significant increase in the lower basal levels of CBS mRNA and protein was seen after incubation with activated vitamin D in murine preosteoblasts. This suggests that CBS is a target gene for vitamin D receptors. Thus, vitamin D may modulate homocysteine metabolism and may affect its serum and cellular homocysteine levels in by direct regulation of cystathionine synthase.[159]

Role of omega 3 fatty acids

Methionine is the precursor for SAM. Methyl groups from SAM are transferred by phosphatidyl ethanolamine N methyltransferase (PEMT) to docosahexaenoic acid (DHA) and to DNA and histones by the respective methyltransferases. Phosphatidylcholine (PC) plays important role for transport of PUFA from the liver to the plasma and peripheral tissues.

It was observed that low DHA levels resulted in excess methyl group availability for other transmethylation reactions such as DNA and histone methylation culminating in altered chromatin remodeling and gene expression.[18]

Metabolic Syndrome and Homocysteine:

Pradhan AD [160] found significant correlation of metabolic syndrome to body fat distribution among men and women. Vayá et al[161] reported that increased Hcy levels were related to abdominal obesity with insulin resistance in men and women and it was also observed that insulin resistance and homocysteine were significantly associated with coronary artery disease risk.

Karatela et al [162] reported that among the hypertensives, homocysteine was positively correlated with obesity. Hajer et al [149] found that Homocysteine levels were higher in metabolic syndrome patients compared to patients without metabolic syndrome. Shai et al[163] reported that Homocysteine is an independent risk predictor of coronary heart diseases. Dankner et al [164] found that higher level of physical activity was found to be an independently associated with a lower Hcy level in an elderly population.

Güven et al [165] observed that the risk of cardiovascular disease is higher in patients with metabolic syndrome & high homocysteine levels in both middle aged males and females. Pergola et al[166] reported that Homocysteine plasma levels were independently associated with insulin resistance in pre-menopausal women**.**

Studies on Homocysteine, Obesity and Diabetes:

Studies suggested that insulin resistance and obesity are associated with Hyperhomocysteinemia. Although the exact cause of hyperhomocysteinemia in diabetic patients is not known. However reduced glomerular filtration and latent nephropathy frequently present in diabetic patients might contribute to hyperhomocysteinemia . [167 ,154 ,168]

Homocysteine and Hypertension:

Homocysteine was regarded as an indicator of oxidative stress.[155] Mechanisms that could explain the relationship between homocysteine and blood pressure include increased arterial stiffness, endothelial dysfunction with decreased availability of nitric oxide, low folate status, and insulin resistance. [169] A clinical study came to the same conclusion, that Hcy was a determinant of vascular thickness and increased the risk of cerebrovascular

complications and glomerular sclerosis . [170] The Hcy-related vascular lesion should be further investigated.

Homocysteine and Menopause:

There are indications that plasma homocysteine may also be influenced by sex steroid hormones.[53] Homocysteine levels are generally lower in women than in men . Menopause, which is associated with a decrease in estrogen levels, is thought to be a major determinant of the rising incidence of cardiovascular disease in women after middle age . Only a few studies are available on the effect of menopause on plasma homocysteine levels . Results of these studies were inconsistent. Several studies reported an increase of homocysteine with menopause. The mechanisms through which estrogens may modulate plasma homocysteine levels are largely unknown. Possibly, lower homocysteine levels in premenopausal women may be due to higher methionine transamination. Decrease in estrogen levels after menopause possibly, increases the homocysteine levels. [171, 172]

Upto one-third of Indians have a genetic defect which predisposes them to decreased activity of methylene tetrahydrofolate reductase (MTHFR) enzyme. Indian population exhibits two polymorphisms of the MTHFR enzyme such as C677T and A1298C resulting in deficiency of MTHFR and hence a very high incidence of hyperhomocysteinemia in the Indian population. [173 ,174,175]

Various interventional trials - for folic acid ,vitamin B_{12} ,omega 3 fatty acids and vitamin D :

Van Der Griend R [176] et all determined the homocysteine lowering effect of different treatment regimens found that monotherapy of folic acid

(0.5 mg daily) was the lowest effective therapy for reducing total Homocystine concentrations, with the same results as high-dose folic acid (5 mg daily) and pyridoxine had no additional value.

P Katre[177] et al reported that pregnant women receiving no supplementation of plasma vitamin B_{12} and folate did not show change in homocysteine levels where as women who received a total dose of >1000 µg of vitamin B_{12} up to 34 weeks had lower concentrations of homocysteine . Thus increasing dose of vitamin B_{12} but not folic acid was associated with lower plasma total homocysteine concentration.

One of the trials observed lowering of homocysteine concentrations by supplementation of diet with folic acid and vitamins B_{12}. However suggested the need for further study to determine daily supplementation of folic acid and vitamin B 12 for reduction in the risk of vascular disease in high risk populations.[19,178]

A study by Pilar Galan [179] et al did not support the routine use of dietary supplements containing B vitamins or omega 3 fatty acids for prevention of cardiovascular disease in people with a history of ischaemic heart disease or ischaemic stroke, at least when supplementation was introduced after the acute phase of the initial event. Martí-Carvajal AJ et al [180] in his review of Homocysteine lowering interventions for preventing cardiovascular events reported that in available published trials there was no evidence to support the use of homocysteine lowering interventions to prevent cardiovascular events with cyanocobalamin (vitamin B_{12}, folic acid (vitamin B9) and pyridoxine (vitamin B6) supplementations.

Raitt MH et al[181] reported that among patients with a recent episode of sustained ventricular arrhythmia, fish oil supplementation, omega-3 fatty acids did not reduce the risk of ventricular tachycardia or ventricular fibrillation and may be proarrhythmic in some patients. Saito Y et al [182] in 2008 reported that multiple risk factors besides cholesterol are associated

with markedly increased incidence of coronary artery disease (CAD). Eicosapentaenoic acid (EPA) was effective in reducing the incidence of CAD events for patients with this dyslipidemic pattern, suggesting that EPA may be especially beneficial in patients with abnormal TG and HDL-C levels.

Forman, J.P et al[183] observed that within an unselected population of blacks, 3 months of oral vitamin D3 supplementation significantly, yet modestly, lowered systolic pressure. However suggested that future trials of vitamin D supplementation on blood pressure were needed to confirm these promising results, particularly among blacks, a population for whom vitamin D deficiency might play a more specific mechanistic role in the pathogenesis of hypertension.

Sokol et al[184] concluded that, supplementation of vitamin D in subjects with CAD failed to demonstrate any benefits on surrogate markers of cardiovascular health. These results question the role of vitamin D supplementation in modifying cardiovascular disease.

Failure of interventional trials [179-184] of folic acid, vitamin B_{12} and vitamin D may be due to associations that were not causals or intervening too late in the history of disease and very little studies have done in India in this context.

Menopause is one of the crucial stages in women's life which leads to various physiological and psychological changes. These changes can increase the risk of diabetes and cardiovascular diseases. Genetic and other environmental factors are also playing important role. In menopausal women, all these, psychological and physiological changes have an impact on food intake and food choices.[114] It is an established fact that for good health and to reduce some of the complications of menopause a well-balanced diet is important. Therefore, to study the micronutrient status of

menopausal women especially related to metabolic syndrome in order to decrease the future burden of CVDs is essential.

The next chapter describes the aims and objectives of the study needed to conduct the thesis work.

AIM AND OBJECTIVES

Aim: To study the micronutrient status and metabolic syndrome biomarkers in postmenopausal women

Objectives:

1. To assess the following in middle aged urban women:

   i) Waist circumference, blood pressure, fasting blood glucose, HDL, TGs as metabolic syndrome biomarkers.

   ii) Biochemical levels of Plasma vitamin B_{12}, folic acid, vitamin D, LCPUFA, homocysteine and insulin.

2. To examine the association of metabolic syndrome biomarkers with plasma levels of Vit $B_{12,}$ folic acid, Vit. D , LCPUFA and homocysteine.

3. To calculate insulin resistance using homeostasis model assessment ratio (HOMA-IR) in the above women.

The next chapter describes the methodology of the study conducted for the present work in this thesis.

MATERIALS & METHODS

Study design: This study was an observational cross-sectional study.

Experimental protocol:

Study area: The study was conducted in Department of Physiology, Department of Obstetrics and Gynaecology & Interactive Research School for Health Affairs, Bharati Vidyapeeth University Medical College and Hospital, Pune- 411043.

Work done from 18.11.2012 to 18.05.2014

Age group: 35-64years.

Sample size: 300 women volunteers between 35 - 64 yr were included in this period of the study

Sample size determination:[185]

P= 39.9% (Prevalence of Metabolic syndrome) [186]

Confidence level =95%

Absolute precision=E= 8%

Power = 80%

$$n = \{(Z_\alpha + Z_\beta)^2 P(1-P)\} / E^2$$

$$= 294 \text{ cases}$$

The sample size calculated from the above given formula is 294. Thus the sample size for the present study was considered as 300 by rounding up.

Recruitment of volunteers:

Volunteers were those women who were attending free camps organized by Department of Obstetrics and gynaecology , Bharati Hospital, Pune for measurement of Bone mineral density (BMD) , detection of anaemia and also those women who are attending Women Wellness Clinic conducted by them .Those who were in the age group of 35 to 64 yrs and were willing to participate were enrolled consecutively in the study. A written informed consent was taken after explaining all the information of the research project like advantage of the study, risk or hazard if any while taking blood sample, confidentiality of the information or data provided by them as well as co-operation needed from the volunteers from those who were willing to participate.

300 women volunteers were equally divided into pre, peri and post-menopausal groups according to their menstrual history for the sake of comparison. Volunteers were classified as premenopausal if they had regular menstrual periods, perimenopausal women were those who had irregular interval menstrual periods i.e more than 2-3 months and postmenopausal in case of absence of menstrual periods for 12 consecutive months and thereafter irrespective of surgical or natural menopause.[187]

Detailed history was taken regarding age, menopausal status, socioeconomic status, dietary pattern and physical exercise. Information on education was collected using the following categories Non graduates included Illiterates, HSC, SSC and Graduate included graduates and postgraduate or other occupation wise they were categorised as house wife and service women. Dietary pattern was noted according to the history of vegetarian or non-vegetarian food consumption.

Those women who achieved the recommended 150 minutes of moderate level physical activity a week were labelled as physical active & the rest physically inactive.

They were enrolled in the study on the basis of inclusion & exclusion criteria.

Inclusion criteria:

All non pregnant women volunteers between age group of 35 to 64 yrs were included in the study.

Exclusion criteria:

Consists of subjects with morbid conditions like diabetes, hypertension, ischemic heart disease, cancer , thyroid disease, or any other acute or chronic liver or kidney disease or subject who underwent hysterectomy surgery or any current infectious condition .Those taking treatment of anemia or taking hormonal supplementation or phytoestrogens were also excluded from the study.

Study parameters:

A joint interim statement of the IDF Task Force suggested IDF global consensus definition[34] without having central obesity as an obligatory parameter . It was proposed that the presence of three or more of the five parameters could be considered as diagnostic of metabolic syndrome . This joint statement also suggested that the IDF-recommended race- and gender-specific cutoffs be used until WC cutoffs could be further evaluated based on data from various regions. The WC cutoffs were recommended by various researchers from Japan, Korea, Iran, Iraq and other regions. Thus there are now numerous race- and gender-specific WC cutoffs.

Three or more of the following five risk factors were considered for clinical identification of the metabolic syndrome as per the joint interim statement is as follows : [34]

Table 6: Metabolic syndrome criteria [34]

1. Abdominal obesity by waist circumference
Men ≥ 90 cm Women ≥ 80 cm
2. Triglyceride ≥ 150 mg/dL
3. High-density lipoprotein cholesterol
Men < 40 mg/dL Women < 50 mg/dL
4. Blood pressure ≥ 130/85 mm Hg
5. Fasting glucose ≥100 mg/dL

Anthropometric measurements:

Body Mass Index (BMI) ,Waist circumference (WC) , Waist to Hip ratio (WHR) were included as key indicators of obesity .

Body Mass Index:

Body weight was measured with minimal clothing to the nearest 0.1 kg using a digital electronic scale and BMI was calculated as kg/m^2. Height was measured using a metal anthropometer to the nearest 0.1 cm with a standardized technique.

Following is the modified criteria of BMI for defining obesity in Asian Indians.

Table 7: Classification of Body Mass Index [188]

Category	BMI(kg/m2)
Normal BMI	18.0-22.9
Overweight	23.0-24.9
Obesity	>25

Waist circumference:

Waist circumference (WC) was obtained as the minimum value between the iliac crest and the lateral costal margin, and hip circumference as the maximum area of the buttocks and ratio was calculated as Waist to Hip ratio (WHR).[189]

BMI was used as markers of overall adiposity, whereas WHR and WC were used as markers of central obesity.

Blood pressure recording:

Systolic and diastolic blood pressure (SBP and DBP) was measured in the right arm in supine position by using a mercury- column sphygmomanometer positioned at heart level after 5 min rest . Two readings of SBP and DBP were recorded and the mean of each was considered.

Specimen collection and storage:

All women were asked to come to the hospital for blood sample collection 10-ml of fasting venous blood sample was taken in the morning (7:00–8:00 A.M.) after 12 to 14 hrs of overnight fast. Blood was collected by venipuncture of median cubital vein in the antecubital fossa. The area around intended puncture site was cleaned with prepackaged alcohol swab. The skin was allowed to dry as alcohol may cause hemolysis of collected blood. The plasma & serum were separated and frozen at -80°C for later analysis.

Biochemical Analysis:

Plasma levels of vitamin B_{12}, folic acid, vitamin D3 and homocysteine were assessed by Chemiluminescence method . [190] (Darwish 2006) Fasting plasma glucose was assessed by GOD -POD (mg/dl) [191] (Gowenlock 2006). Plasma levels omega 3 fatty acids were assessed by Gas chromatography.[192]

Estimation of Plasma Glucose and Insulin:[191,193]

Plasma glucose was analysed using the enzymatic method. [190] Glucose was phosphorylated by hexokinase in the presence of ATP and magnesium ions to produce glucose-6-phosphate and ADP. Glucose-6-phosphate dehydrogenase oxidises glucose-6-phosphate to gluconate-6-phosphate with the reduction of NAD+ to 70 NADH. The increase in absorbance at 340 nm is proportional to the glucose concentration in the sample. Glucose levels were expressed as mg/dL. Plasma insulin was analyzed using the Mercodia rat insulin ELISA kit [192] (Mercodia Developing Diagnostics, Mercodia AB, Uppsala, Sweden). It is a solid phase two-site enzyme immunoassay based on the sandwich technique. In this, two monoclonal antibodies are directed against separate antigenic

determinants on the insulin molecule. Insulin in the sample reacts with anti-insulin antibodies bound to microtitration wells and peroxidase-conjugated anti-insulin antibodies in the solution. The peroxidase-conjugated anti-insulin antibodies also bind to the insulin at the same time. After washing, 3,3',5,5'-tetramethylbenzidine (TMB) labelled substrate is added that binds to the conjugated antibodies. H_2SO_4 was added to stop the reaction and the color was read at 450 nm. Insulin levels were expressed as µg/L.

Estimation of Plasma TG and HDL: [194]

The estimation of plasma HDL and TG was carried out using enzymatic kit method (Siemens, Dimension RXL Max Integrated Chemistry System). Cholesterol in the plasma was analyzed enzymatically in a series of reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. H_2O_2 is formed as one of the byproducts, which was then measured quantitatively in a peroxidase catalyzed reaction. Absorbance was measured at 500 nm. The intensity of the color was proportional to cholesterol concentration in the sample. TG was measured enzymatically in which it hydrolyzed to produce glycerol. Glycerol was then oxidized using glycerol oxidase and H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction. Absorbance was measured at 500 nm. Levels of HDL and TG were expressed as mg/dL.

Estimations of plasma vitamin B_{12}, folate ,vitamin D and homocysteine: [190]

The chemiluminescent microparticle immunoassay technology was used for estimations of Plasma Vitamin B_{12}, Folate ,Vitamin D and Homocysteine (Abbott Laboratory, Chicago, IL, USA; Abbott Axsym

System 5F51–20) . Briefly, 100 µl of plasma was used for the analysis. The vitamin B_{12} and folate assay was a two-step assay with an automated sample pre-treatment for determining their presence in plasma. Vitamin B_{12} levels were expressed as pg/ml and folate levels as ng/ml. Homocysteine assay was performed by the chemiluminescent microparticle immunoassay technology (Abbott Laboratory, Chicago, IL, USA; Abbott Axsym System 5F51–20). Briefly, 150 µl of plasma was used for analysis of homocysteine.

It was an immunoassay method that utilizes the isolation of antigen/ antibody complex on a solid phase surface of small beads called microparticles. Latex microparticles were coated with the antibody to bind the specific analyte being measured. In this, phosphatase enzyme was conjugated to the antibody. Fluorescent 4-methylumbelliferone phosphate is formed in solution which reacts with the enzyme on the antibody. Homocysteine levels were expressed as µmol/ml.

The Gas Chromatography for fatty acid analysis: [192]

The fatty acid analysis was carried out using a Perkin-Elmer gas chromatograph (SD 2330, 30m capillary column, Supelco, PA, USA). Transesterification of plasma samples was carried out using a methylation reagent (hydrochloric acid and methanol). Individual fatty acids were identified from the sample by comparing with peaks of standard fatty acid methyl esters (Sigma, USA).

These were separated using a Perkin Elmer gas chromatograph (SP 2330, 30 m capillary Supelco column. Helium was used as carrier gas at 1 mL/min. Oven temperature was held at 150 ^{O}C for 10 min, programmed to

rise from 150 to 220 ^{O}C for 10 min, and at 220^{O} C for 10 min. The detector temperature was 275 ^{O} C and the injector temperature was 240 ^{O}C. Retention times and peak areas were automatically computed. The column was calibrated by injecting the standard fatty acid mixture in approximately equal proportion. The data was recorded and the peaks were identified as per the retention time of the standard fatty acids (Sigma) run under the identical conditions.

Fatty acids were expressed as g/100 g fatty acid. Total of 15 fatty acids were detected. Saturated fatty acids include myristic acid, palmitic acid and stearic acids, while total monounsaturated fatty acids include myristoleic, palmitoleic, oleic acid and nervonic acids. The omega 3 fatty acids included alpha linolenic acid, eicosapentaenoic acid and Docosahexaenoic acid while total omega 6 fatty acids included linoleic acid, gamma linolenic acid, di-homo-gammalinolenic acid, docosapentaenoic acid and arachidonic acid.

Homeostasis Model Assessment Ratio (HOMA-IR):

Insulin resistance was calculated using homeostasis model assessment ratio (HOMA-IR) Which is calculated using following formula :

$$\frac{\text{Fasting Glucose(mg/dl) x Fasting Insulin(µU/mL}}{405}$$

Statistical Analysis

Data is represented as mean (standard deviation). SPSS version 17.0 for Windows (SPSS Inc, Chicago) was used for the statistical analysis. Variables with skewed distribution were log transformed to satisfy the

assumptions of normality. In such cases, the data has been represented as median (inter quartile range, IQR). ANOVA (Analysis of Variance) and chi square test were used for comparison between three groups. Bonferroni correction for multiple comparisons was applied to identify significantly different group means. Pearson correlation coefficient was used to measure the linear correlation between two variables where 'r' and 'p'value were calculated. Odds ratios (ORs) was calculated to measure the association between an exposure and an outcome i.e metabolic syndrome . Multiple logistic regression analysis was used to find out determinants of metabolic syndrome. All the results were age adjusted. 'p'value of less than 0.05 was considered as statistically significant.

Ethical approval:

The procedure described in the study were approved by institutional ethics committee (BVDU/MC/42).

The next chapter describes the results of the study which is conducted in this thesis.

OBSERVATIONS AND RESULTS

Table 8: Demographic Profile in the three groups

N=100 in each group	Group I (Premenopausal) Mean (SD) in %	Group II (Perimenopausal) Mean (SD) in %	Group III (Postmenopausal) Mean (SD) in %	p for chi square
Age (y)	39.8 (3.5)	45. (3.74)	52.9 (5.8)	< 0.05
Income class **Lower** **middle** **Middle**	 41 59	 49 51	 70 30	0.000
Education **Non** **graduate** **Graduate**	 41 59	 56 44	 84 16	0.000
Family **Joint** **Nuclear**	 21 79	 42 58	 52 48	0.000
Occupation **Housewife** **Employed**	 80 20	 82 18	 90 10	0.12
Diet **Vegetarian** **Mixed**	 53 47	 47 53	 64 36	0.05
Physical Activity	129 (70.77)	137.1 (94.8)	111.6 (78.51)	> 0.05

All values are age adjusted .

Baseline characteristics of premenopausal, perimenopausal and postmenopausal women are shown in Table 8. In postmenopausal group maximum number of volunteers are in lower middle class income , non graduate , housewives , vegetarians and belonging to joint family.

Figure 6: Demographic Profile in the three groups

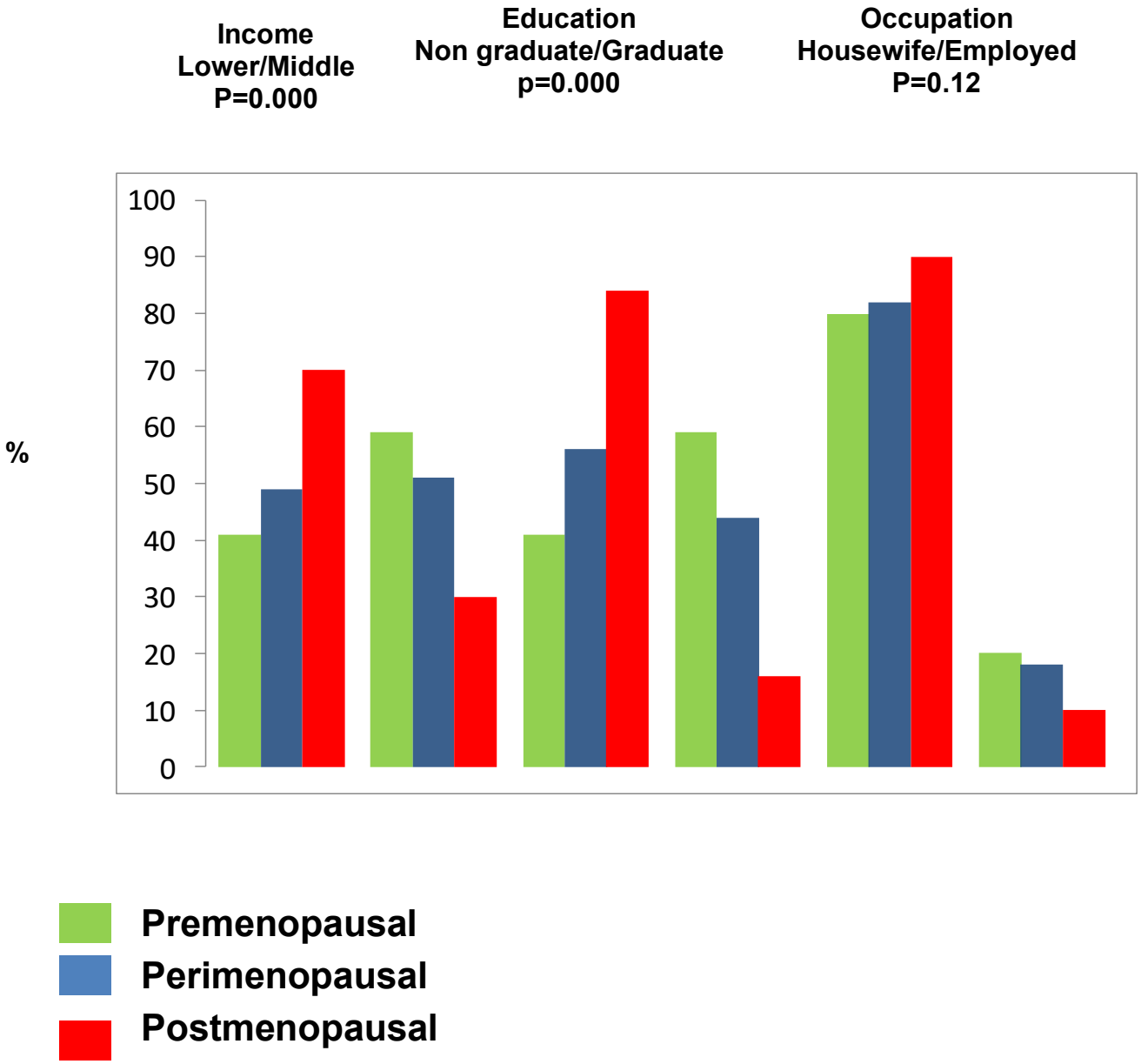


Figure 6: Demographic Profile in the three groups …..

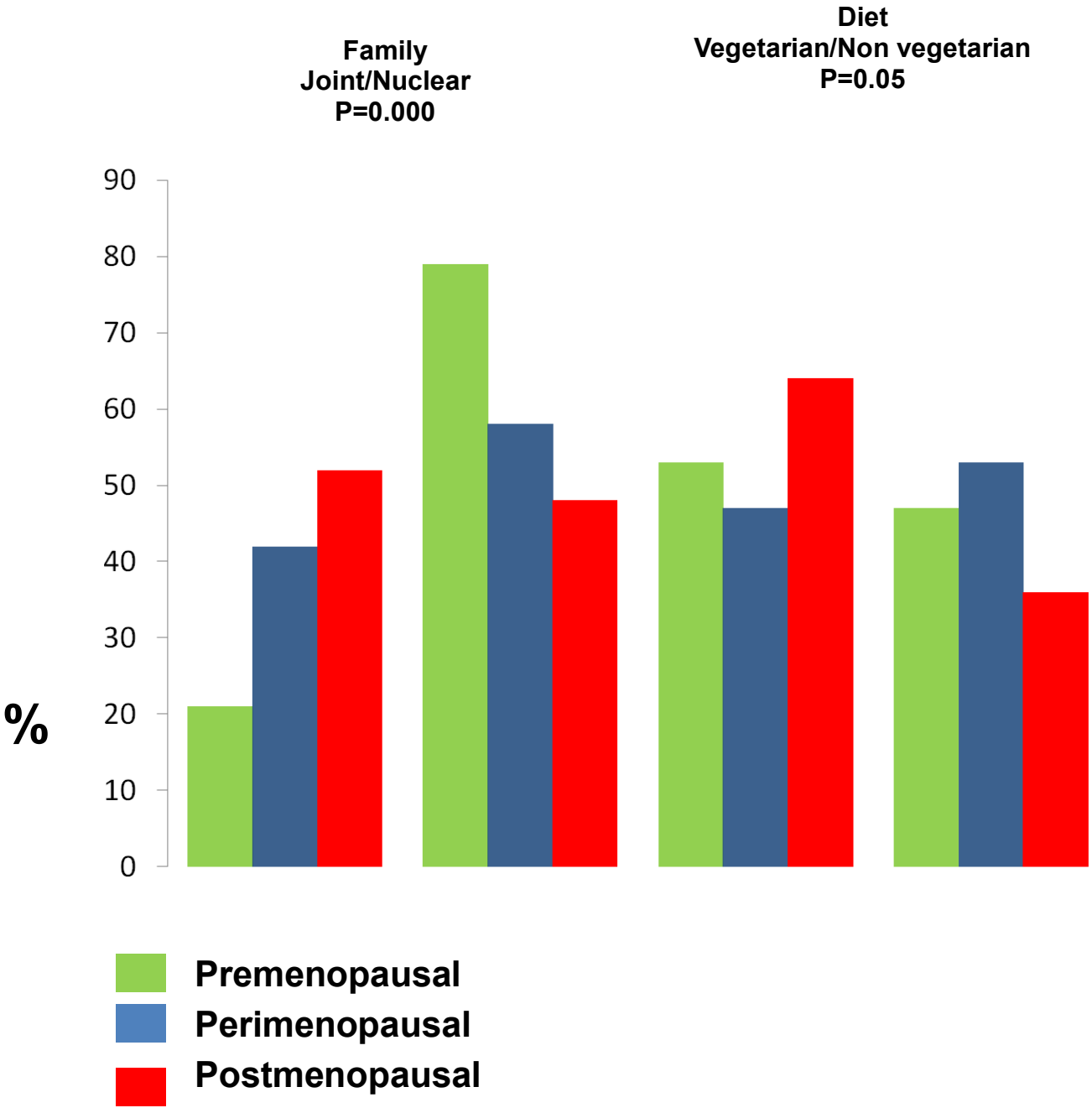


Table 9: Anthropometry measurements in the three groups

N=100 in each group	Group I (Premenopausal) Mean (SD)	Group II (Perimenopausal) Mean (SD)	Group III (Postmenopausal) Mean (SD)
Height (cm)	155.3 (5.5)	154.3 (5.6)	152.3 (5.5)
Weight (Kg)	62.3 (9.4)	64.1 (9.2)	63.8 (11.7)
BMI (Kg/m^2)	25.9 (3.7)	26.9 (3.6) [a]	27.5 (4.9)
Waist (cm)	80.1 (9.8)	82.6 (8.4) [a]	84.0 (13.20)
Hip (cm)	102.9 (9.8)	105.3 (10.0)	106.9 (12.5)
Waist-Hip ratio	0.78 (0.09)	0.79 (0.07)	0.79 (0.09)

All values are age adjusted .

[a] :Different from group I $p < 0.05$, [b] : Different from group II $p < 0.05$

Table 9 shows that the mean age of postmenopausal women was significantly higher ($p < 0.0001$ for both) than the mean age of the premenopausal and perimenopausal women. Postmenopausal women were shorter & perimenopausal women with significantly higher BMI and waist circumference than premenopausal women . There is no statistical difference for height among the groups.

Table 10: Components of metabolic syndrome , homocysteine and HOMA IR in the three groups

N=100 in each group	Group I (Premenopausal) Mean (SD)	Group II (Perimenopausal) Mean (SD)	Group III (Postmenopausal) Mean (SD)
Waist (cm)	80.1 (9.8)	82.6 (8.4)	84.0 (13.2)
Blood pressure (mmHg)			
Systolic	124 (13)	129 (14)	137 (17)
Diastolic	76 (9)	83 (12)[a]	82 (10)
Fasting glucose (mg%)	93 (80, 110)	102 (90,112) [a]	112 (102, 120)[a,b]
HDL cholesterol (mg%)	44 (2.5)	44 (2.5)	44 (2.5)
Triglycerides (mg%)	102.2 (33.4)	100.2 (41.3)	114.6 (39.6)[b]
Metabolic syndrome	29	65[a]	69[a,b]
Homocysteine (mM/L)	17.3 (7.7)	20.1 (7.9) [a]	22.9 (8.1) [a,b]
HOMA IR	3.11 (1.80)	4.04 (2.07) [a]	4.91 (2.05) [a,b]

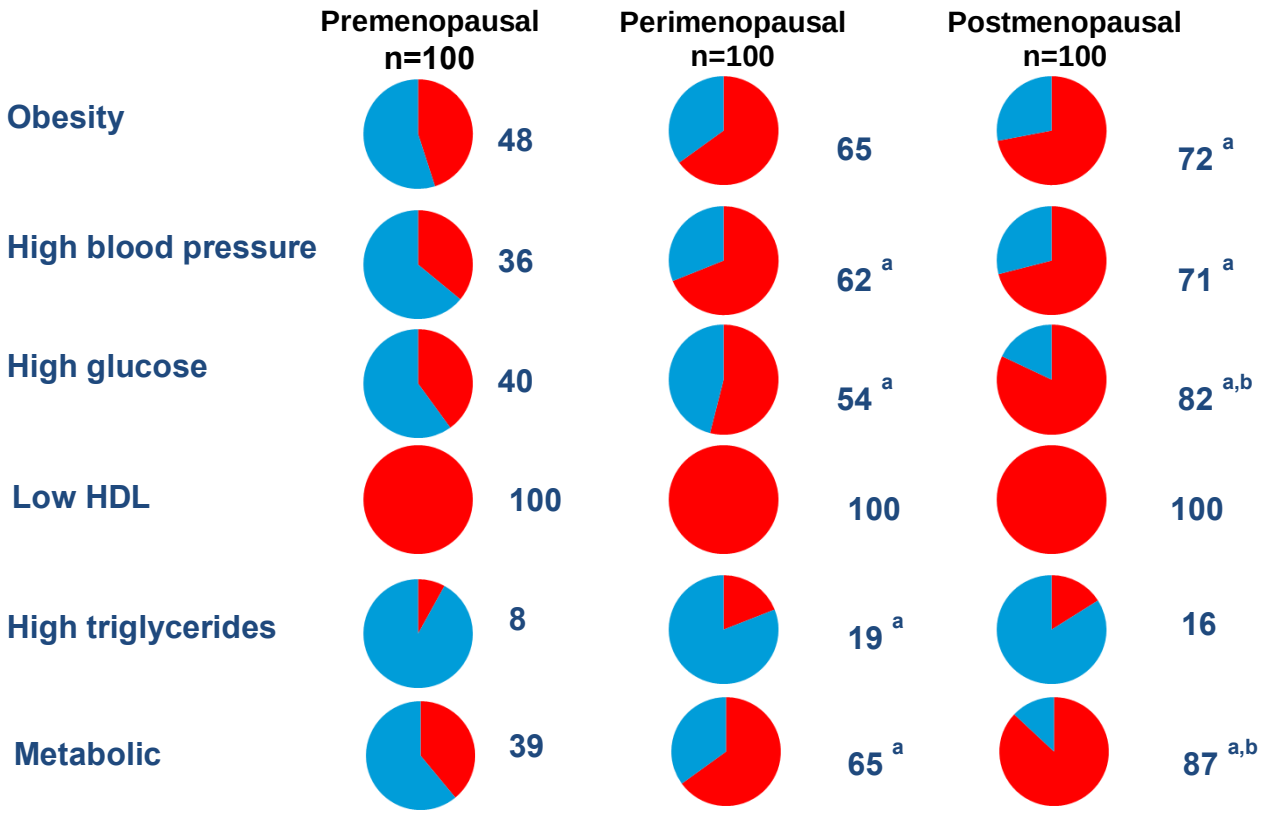
[a] :Different from group I p <0.05, [b] : Different from group II p <0.05 .

Mean (SD) for continuous variable, Median (IQR) for fasting glucose. All values are age adjusted .

Table 10 shows that mean levels of all metabolic syndrome components except HDL, are higher in peri & highest in postmenopausal women as compared to premenopausal women. HDL cholesterol levels are similar & lower than normal in all three groups. Homocysteine and HOMA IR levels

are also significantly higher in peri and postmenopausal women as compared to premenopausal women

Figure7: Percentage of women with abnormal levels of WC,BP, FBG, TGs, HDL and presence of metabolic syndrome



Percentage of women with normal levels of components of metabolic syndrome and absence of metabolic syndrome

Percentage of women with abnormal levels of components of metabolic syndrome and presence of metabolic syndrome

Table 11: Levels of micronutrients and fatty acids in the three groups

N=100 in each group	Group I (Premenopausal) Mean (SD)	Group II (Perimenopausal) Mean (SD)	Group III (Postmenopausal) Mean (SD)
Vitamin B_{12} (pg/mL)	347 (111)	277 (111)[a]	282 (106)[a]
Folic acid (ng/mL)	14.2 (5.1)	11.9 (5.3)[a]	11.0 (4.7)[a, b]
Vitamin D (ng/mL)	16 (5.5)	14 (5.5)	13 (5.6)
SFA (gm%)	33.2 (4.7)	32.7 (4.7)	32.2 (5.1)
MUFA	17.8 (4.2)	17.4 (4.8)	18.9 (3.6)
w-3	1.18 (0.42)	0.94 (0.39) [a]	0.93 (0.38) [a]
w-6	44.7 (7.3)	45.9 (7.4)	45.3 (5.9)
w-6:w-3	42.9 (19.2)	63.7 (40.1) [a]	58.4 (30.1) [a]

[a] : Different from group I p <0.05, ,[b] : Different from group II p <0.05 . All the values are age adjusted.

Table 11 shows that vitamin B_{12} , Folic acid & vitamin D concentrations are significantly lower in peri and post-menopausal women as compared to premenopausal women. Peri and postmenopausal group show significantly lower omega-3 fatty acid and higher omega-6/omega-3 ratio as compared to premenopausal group.

Figure 8 : Percentage of women with inadequate levels of vitamin B, folic acid and vitamin D in the three groups

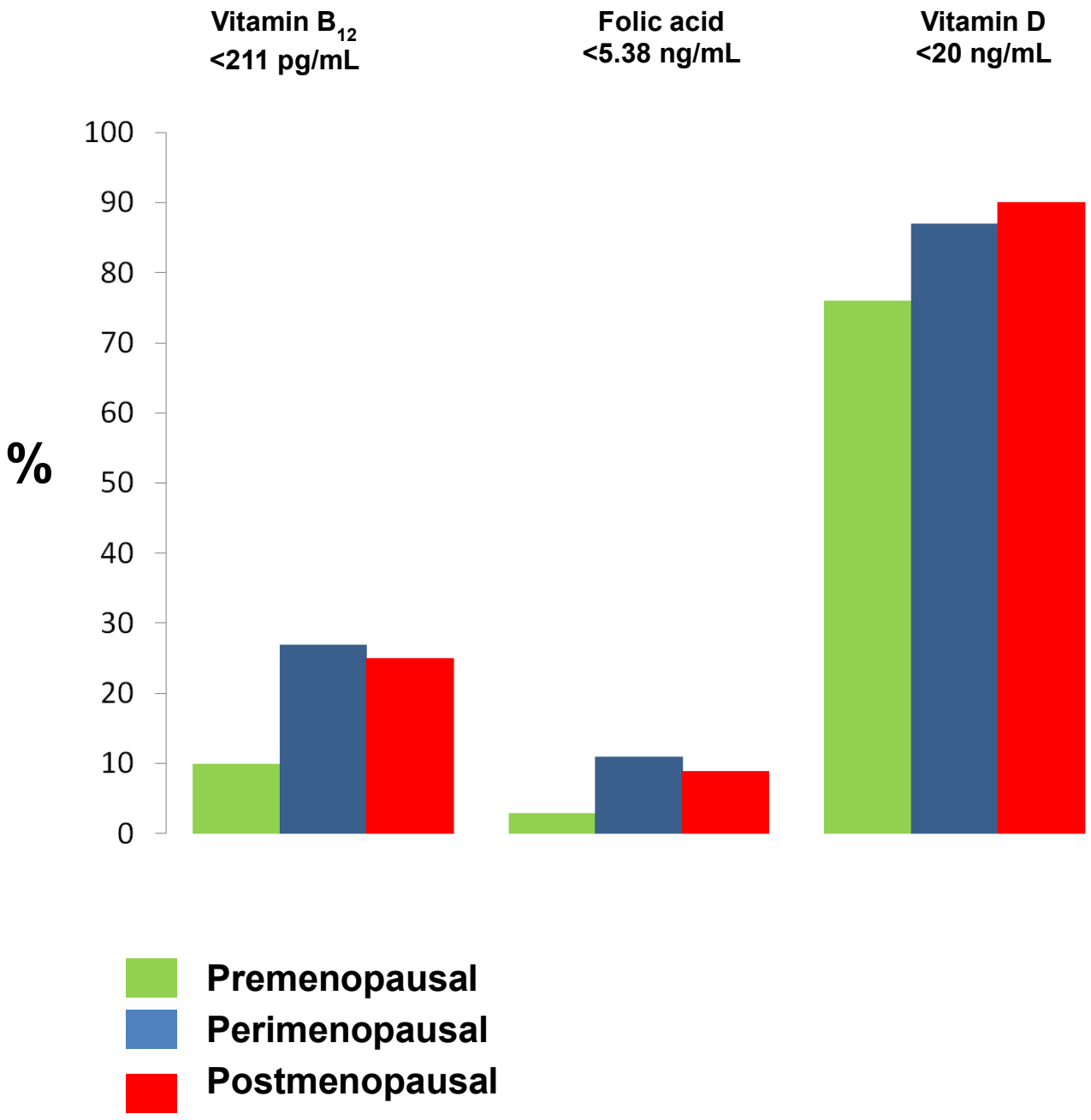


Table 12: Levels of micronutrients and fatty acids in women with and without metabolic syndrome

	Metabolic syndrome N=191 Mean (SD)	Normal N=109 Mean (SD)	p value
Vitamin B_{12} (pg/mL)	288 (113)	326 (111)	0.049
Folic acid (ng/mL)	11.6 (5.1)	13.6 (5.1)	0.008
Vitamin D (ng/mL)	13.3 (5.3)	17.0 (5.6)	0.000 *
Fatty acids (gm %)			
SFA	33.1 (4.9)	31.9 (4.8)	0.015 *
MUFA	18.3 (4.0)	17.7 (4.6)	NS
w-6	44.9 (6.5)	45.9 (7..6)	NS
w-3	0.97 (0.41)	1.09 (0.40)	NS
w-6:w-3	57.6 (39.5)	50.5 (28.5)	NS
Homocysteine (mM/L)	22.2 (8.2)	16.5 (7.0)	0.000 *
HOMA IR	4.49 (2.22)	3.48 (1.8)	0.000 *

*statistically significant, NS: Not Significant.

All the values are age adjusted.

There is a significant difference between levels of vitamin D, SAFA, homocysteine and HOMA IR in subjects with & without metabolic syndrome after adjustment for age.There is no significant difference between vitamin B 12, folic acid, w-3 fatty acids and w-6/w-3 ratio.

Figure 9: Levels of micronutrients in women with and without metabolic syndrome

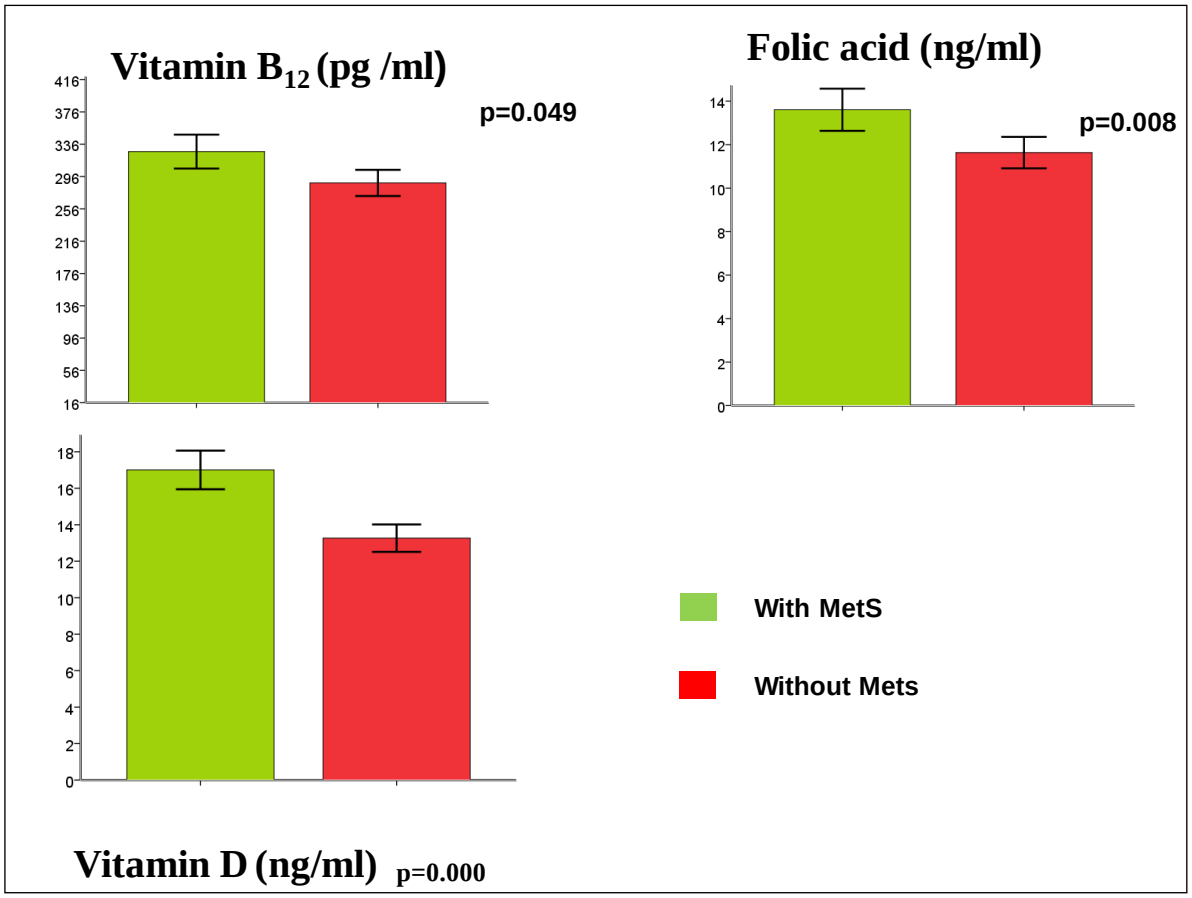


Table 13: Correlation of waist circumference with the levels of micronutrients and fatty acids

Micronutrient	Pre	Peri	Post
Vitamin B_{12} (pg/mL)	-0.07 0.46	-0.17 0.09	-0.11 0.30
Folic acid (ng/mL)	-0.08 0.42	-0.01 0.99	0.02 0.88
Vitamin D (ng/mL)	-0.20 0.04 *	-0.40 0.001**	-0.19 0.05*
Fatty Acids			
SAFA	0.17 0.08	-0.04 0.72	0.03 0.74
MUFA	0.02 0.82	0.11 0.28	-0.05 0.60
w3	0.14 0.16	-0.24 0.02 *	0.06 0.55
w 6	-0.15 0.13	0.03 0.79	-0.04 0.73
w-6:w-3	-0.19 0.06	0.19 0.06	-0.14 0.18

Figures in **Blue**: 'r' value Figures in Red: 'p' value

Underlined and * statistically significant ,** statistically highly significant

Table 13 shows a significant negative correlation of vitamin D in all three groups with WC. Negative correlation of omega 3 fatty acids with WC is present only in perimenopausal group.

Figure 10: Significant negative correlation of waist circumference with vitamin D

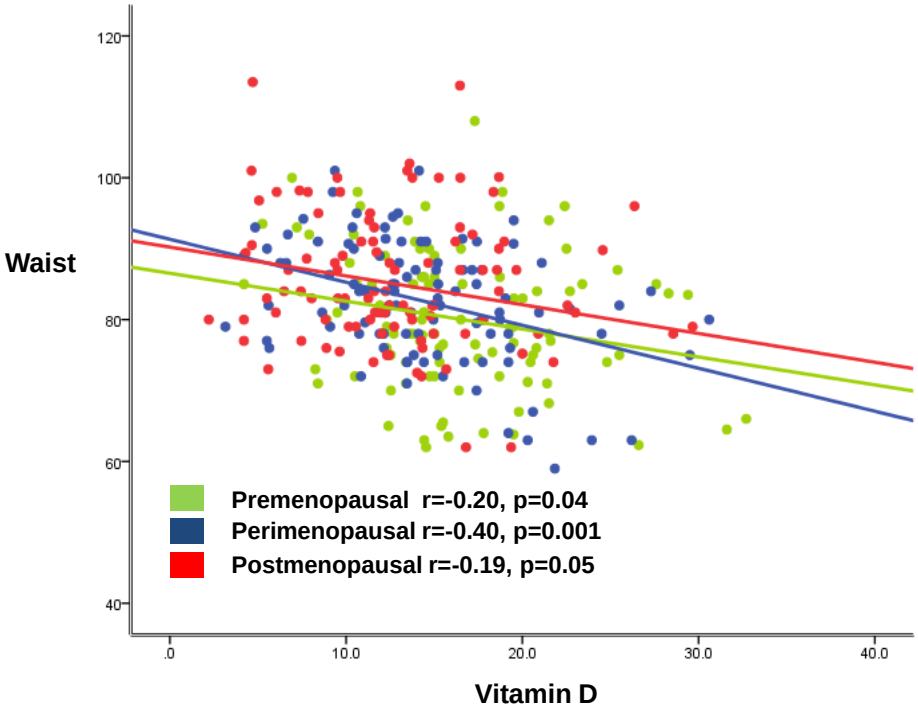


Figure 11: Significant negative correlation of waist circumference with omega 3 fatty acids

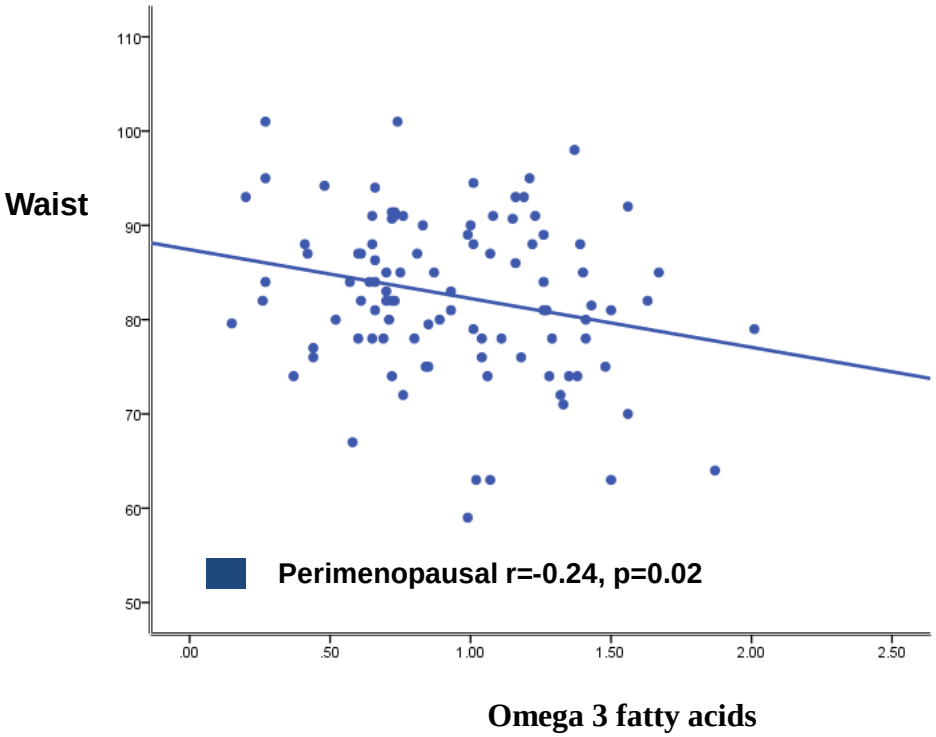


Table 14: Correlation of systolic BP with the levels of micronutrients and fatty acids

Micronutrient	Pre	Peri	Post
Vitamin B_{12} (pg/mL)	-0.001 0.99	-0.14 0.15	0.01 0.97
Folic acid (ng/mL)	-0.20 0.06	-0.07 0.51	0.06 0.58
Vitamin D (ng/mL)	-0.13 0.19	-0.36 0.001**	-0.01 0.96
Fatty Acids			
SAFA	0.11 0.26	0.02 0.80	0.19 0.06
MUFA	-0.12 0.25	0.04 0.73	0.03 0.75
w3	-0.09 0.36	-0.07 0.51	0.05 0.62
w 6	0.02 0.84	-0.03 0.77	-0.19 0.051
w-6:w-3	0.01 0.92	0.02 0.85	-0.13 0.20

Figures in **Blue**: 'r' value Figures in **Red** : 'p' value

**statistically highly significant

Table 14 shows significant negative correlation of vitamin D with systolic BP in peri menopausal group only.

Figure 12: Significant negative correlation of systolic BP with vitamin D

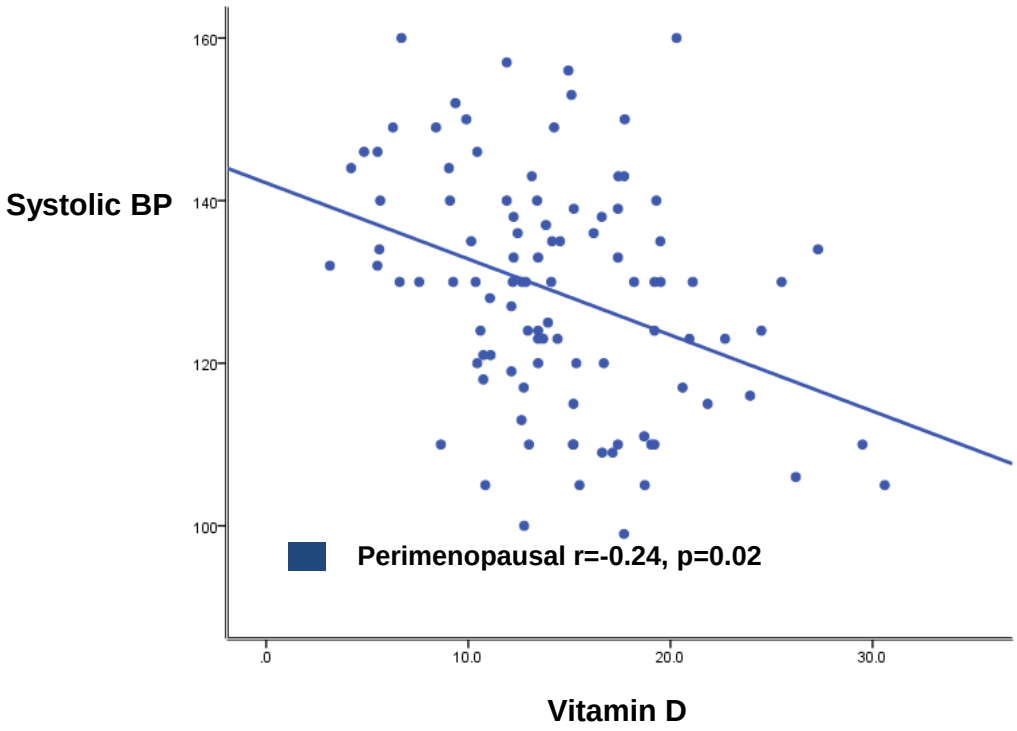


Table 15: Correlation of Diastolic BP with the levels of micronutrients and fatty acids

Micronutrient	Pre	Peri	Post
Vitamin B_{12} (pg/mL)	-0.02 0.86	-0.14 0.17	0.01 0.92
Folic acid (ng/mL)	-0.12 0.25	-0.03 0.76	0.02 0.83
Vitamin D (ng/mL)	-0.21 0.04*	-0.01 0.90	0.11 0.27
Fatty Acids			
SAFA	-0.06 0.54	-0.01 0.93	0.06 0.58
MUFA	-0.22 0.03*	-0.03 0.79	-0.07 0.48
w3	-0.08 0.45	0.002 0.98	0.02 0.88
w 6	0.19 0.055	0.002 0.98	-0.01 0.94
w-6:w-3	0.12 0.22	0.02 0.86	-0.013 0.89

Figures in **Blue**: 'r' value Figures in **Red**: 'p' value

*statistically significant

Table 15 shows a significant negative correlation of diastolic BP with vitamin D and MUFA levels in premenopausal group.

Figure 13 : Significant negative correlation of Diastolic BP with vitamin D

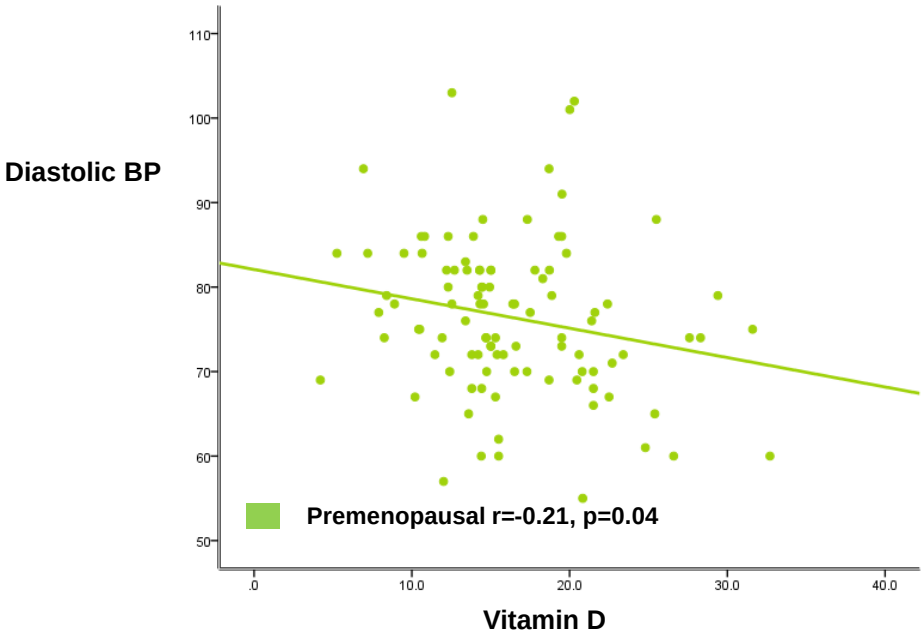


Figure 14: Significant negative correlation of Diastolic BP with MUFA

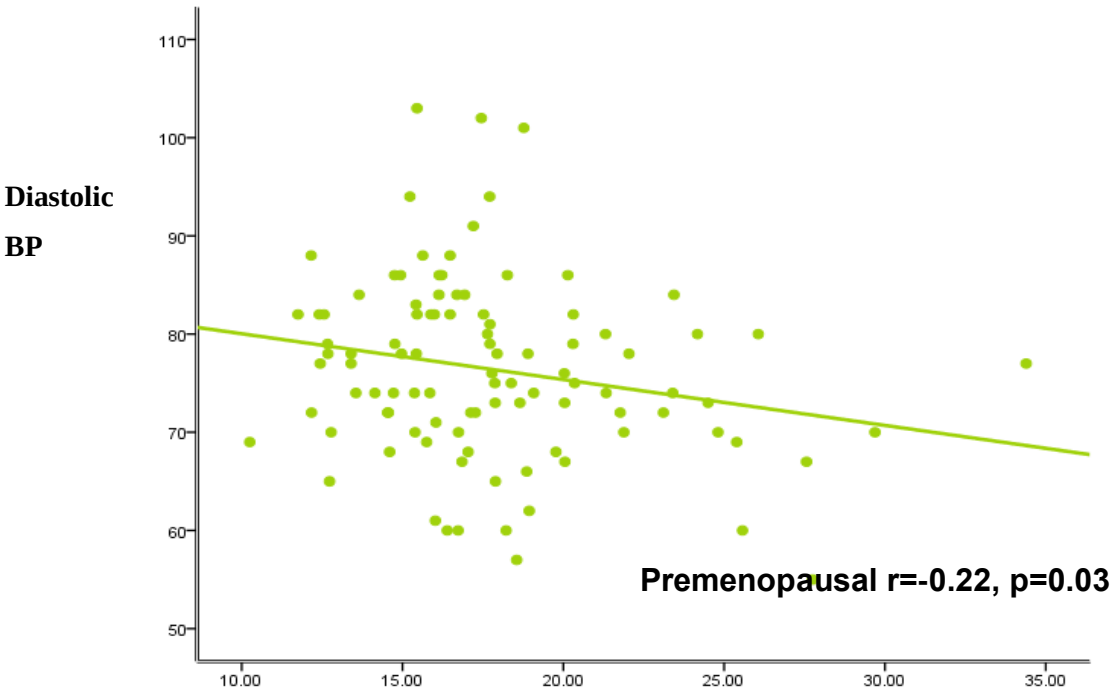


Table 16: Correlation of Fasting Blood glucose with the levels of micronutrients and fatty acids

Micronutrient	Pre	Peri	Post
Vitamin B_{12} (pg/mL)	0.02 0.86	-0.06 0.55	0.18 0.08
Folic acid (ng/mL)	-0.08 0.42	0.13 0.19	0.02 0.85
Vitamin D (ng/mL)	0.01 0.98	-0.17 0.09	0.10 0.32
Fatty Acids			
SAFA	0.09 0.36	0.10 0.30	0.08 0.45
MUFA	-0.08 0.42	0.11 0.27	0.20 0.051
w3	-0.05 0.59	-0.02 0.84	-0.24 0.04*
w 6	-0.014 0.89	-0.08 0.45	-0.16 0.12
w-6:w-3	0.003 0.98	0.03 0.78	0.09 0.36

Figures in **Blue**: 'r' value Figures in **Red** : 'p' value

*statistically significant

Table 16 shows significant negative correlation of fasting blood glucose with omega 3 fatty acid in postmenopausal group only.

Figure 15: Significant negative correlation of FBG with omega 3 fatty acids

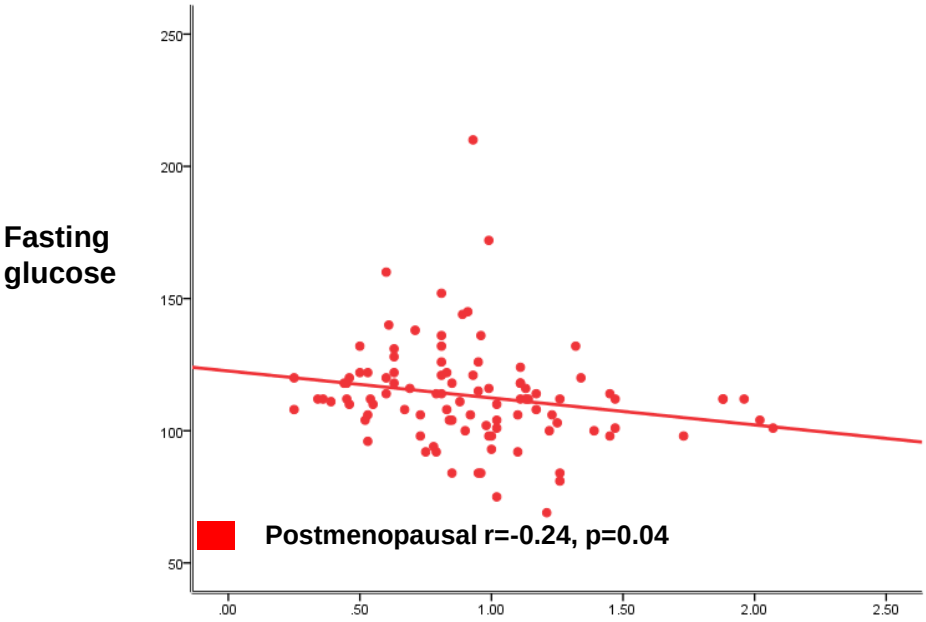


Table 17 : Correlation of triglycerides with the levels of micronutrients and fatty acids

Micronutrient	Pre	Peri	Post
Vitamin B_{12} (pg/mL)	-0.27 0.007 **	-0.11 0.29	-0.08 0.42
Folic acid (ng/mL)	-0.15 0.14	-0.05 0.60	0.17 0.10
Vitamin D (ng/mL)	-0.06 0.55	-0.11 0.03 *	-0.13 0.21
Fatty Acids			
SAFA	0.26 0.009 **	-0.05 0.60	-0.01 0.98
MUFA	-0.08 0.42	-0.11 0.29	-0.097 0.34
w3	0.03 0.77	-0.07 0.46	-0.08 0.44
w 6	-0.14 0.16	0.12 0.25	0.10 0.32
w-6:w-3	-0.11 0.29	0.11 0.29	0.03 0.72

Figures in **Blue**:'r' value, Figures in **Red** : 'p' value

*statistically significant **statistically highly significant

Table 17 shows significant negative correlation of triglycerides with vitamin B_{12} in premenopausal group and with vitamin D in perimenopausal women while in premenopausal group triglyceride levels are directly associated with SAFA levels.

Graph 16: Significant negative correlation of triglycerides with vitamin B_{12}

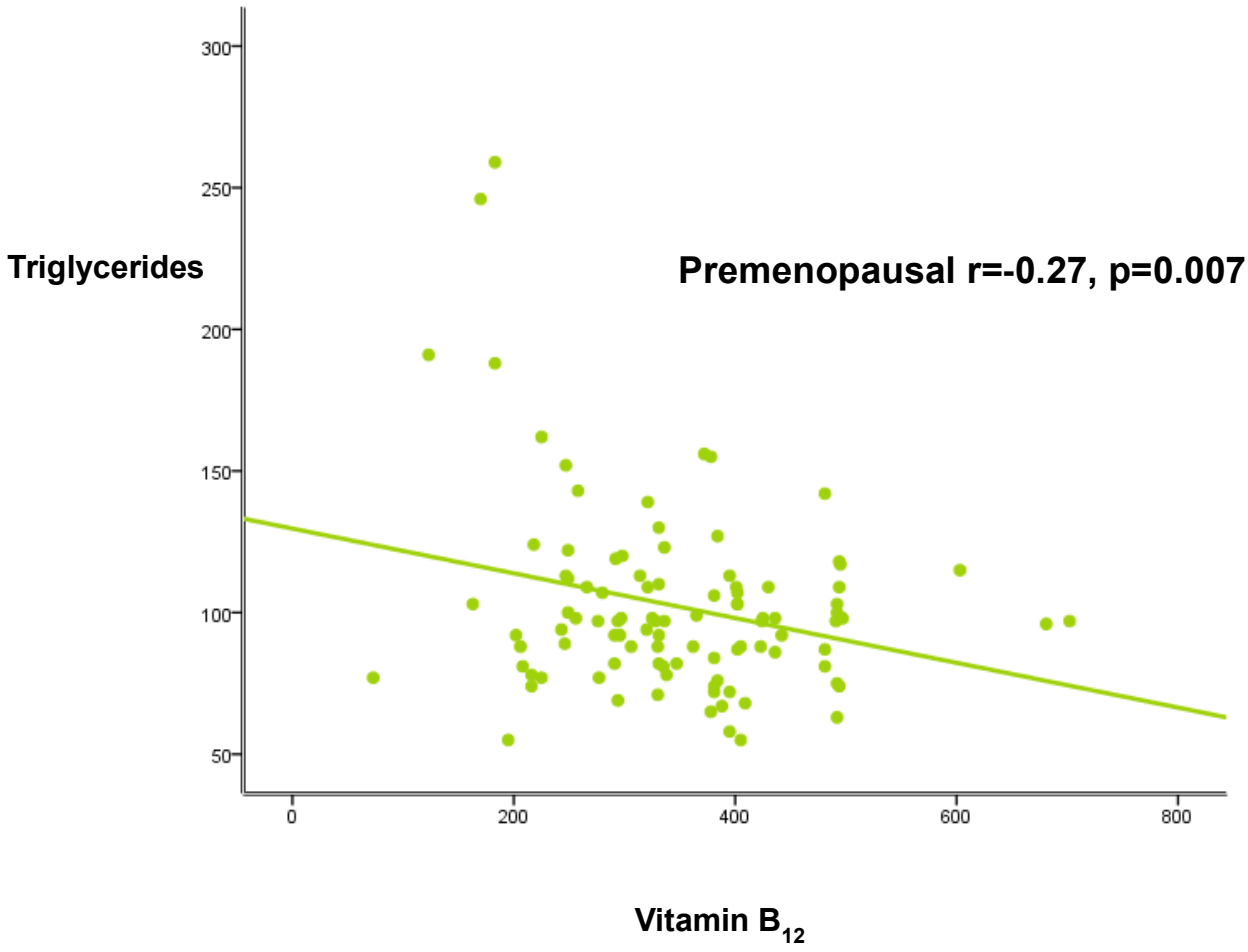


Figure 17: Significant negative correlation of triglycerides with vitamin D

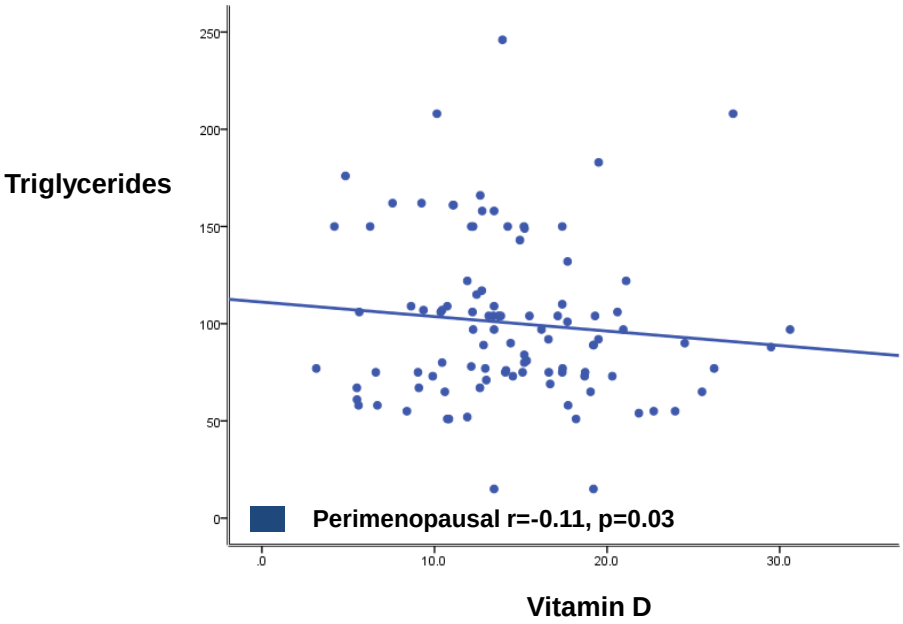


Figure 18: Significant positive correlation of triglycerides with SAFA

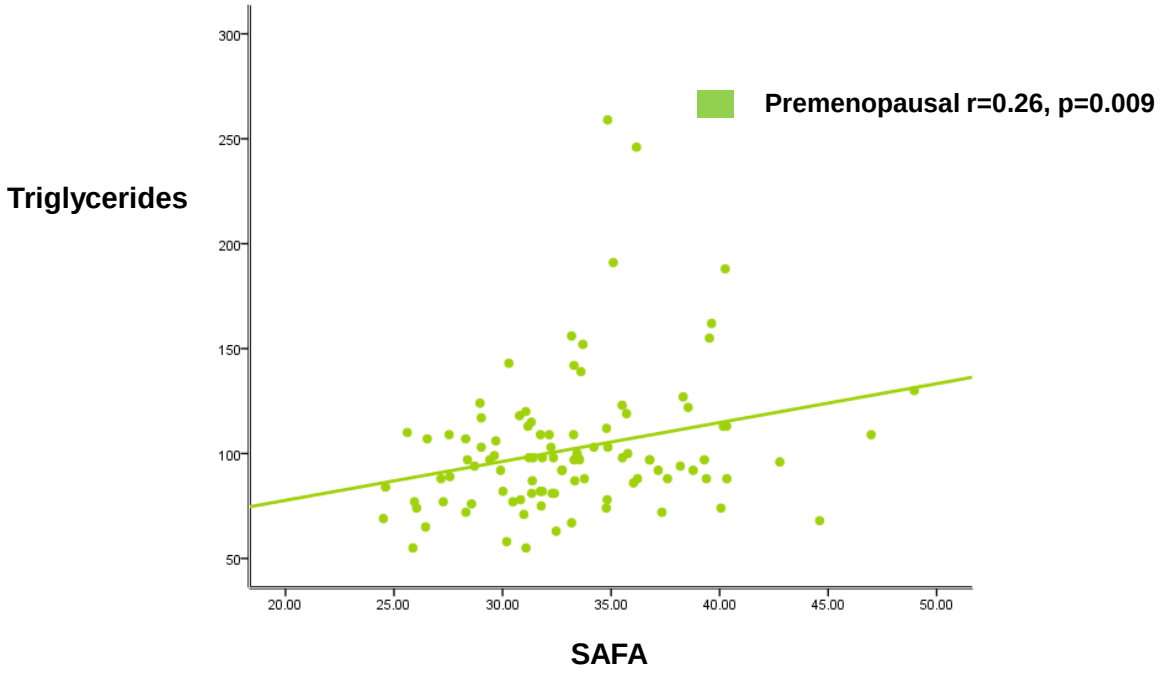


Table 18: Correlation of HDL (mg %) with the levels of micronutrients and fatty acids

Micronutrient	Pre	Peri	Post
Vitamin B_{12} (pg/mL)	-0.03 0.78	0.05 0.63	-0.04 0.70
Folic acid (ng/mL)	-0.01 0.89	-0.08 0.44	0.15 0.15
Vitamin D (ng/mL)	-0.07 0.47	-0.04 0.66	0.02 0.84
Fatty Acids			
SAFA	0.15 0.15	0.02 0.84	0.04 0.67
MUFA	0.14 0.16	-0.15 0.15	0.09 0.39
w3	0.06 0.53	-0.03 0.75	-0.05 0.61
w 6	0.10 0.32	-0.16 0.12	0.08 0.41
w-6:w-3	0.03 0.72	-0.13 0.20	0.13 0.20

Figures in **Blue**: 'r' value Figures in **Red**: 'p' value

In Table 18: HDL does not show any correlation with any of the micronutrients and fatty acids in any of the group.

Table 19: Multiple logistic regression analysis to find out determinants of metabolic syndrome

	Odds ratios	95% CI	P
Group Pre	1		
Peri	2.68	1.02,6.99	0.045*
Post	7.33	1.86, 28.9	0.004**
Age Q_1	1		
Q_2	1.66	0.72, 3.83	0.24
Q_3	1.05	0.35, 3.12	0.93
Q_4	1.49	0.39, 5.86	0.57
Vitamin B_{12} Q_1	1		
Q_2	1.69	0.70, 4.05	0.12
Q_3	1.08	0.46, 2.56	0.86
Q_4	0.83	0.34, 2.06	0.69
Folic acid Q_1	1		
Q_2	0.64	0.26, 1.58	0.33
Q_3	1.12	0.44, 2.82	0.82
Q_4	0.76	0.31, 1.90	0.56
Vitamin D Q_1	1		
Q_2	0.69	0.27, 1.75	0.43
Q_3	0.66	0.26, 1.67	0.38
Q_4	0.34	0.13, 0.90	0.03 *

Figures in **Red**: significant 'p' value

*statistically significant

**statistically highly significant

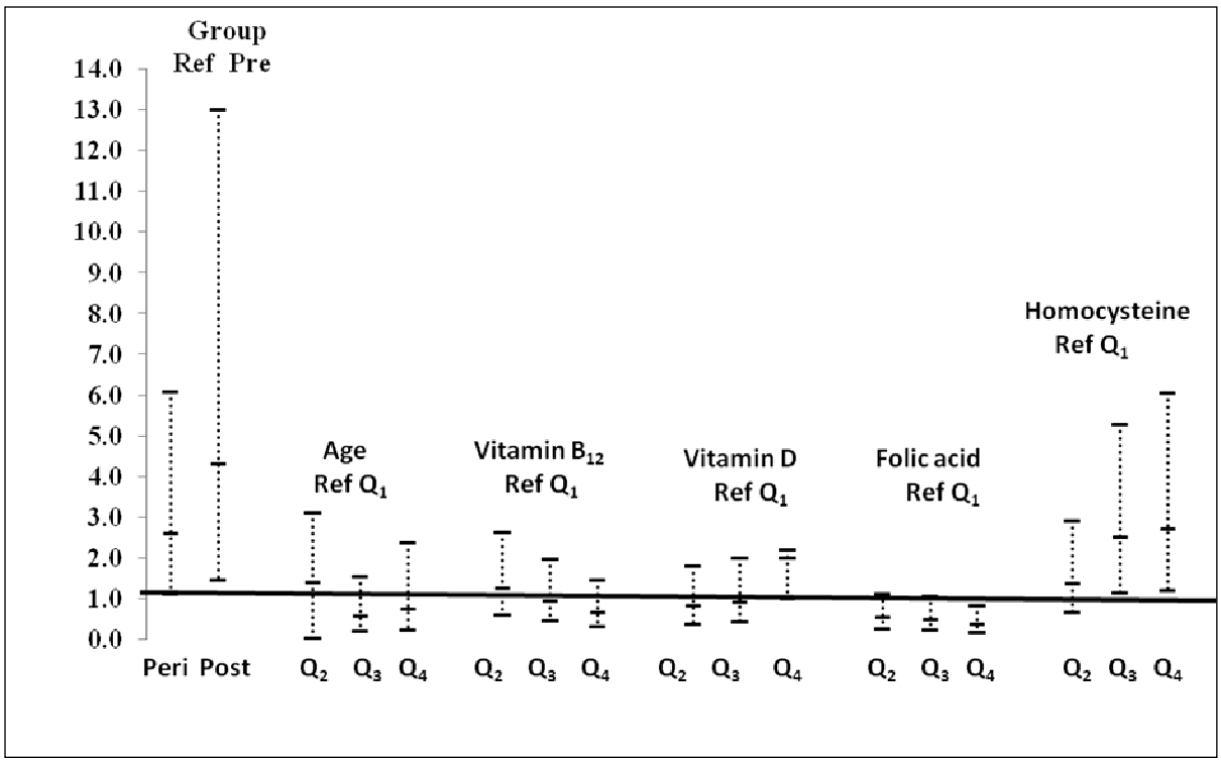
Table 19: Multiple logistic regression analysis to find out determinants of metabolic syndrome.....

	Odds ratios	95% CI	P
SAFA Q_1	1		
Q_2	1.70	0.68, 4.27	0.26
Q_3	1.70	0.64, 4.50	0.29
Q_4	1.64	0.50, 5.45	0.42
MUFA Q_1	1		
Q_2	1.11	0.48, 2.60	0.21
Q_3	1.18	0.45, 3.11	0.74
Q_4	1.16	0.37, 3.63	0.80
OMEGA 3			
Q_1	1		
Q_2	1.28	0.53, 3.15	0.58
Q_3	0.77	0.32, 1.82	0.55
Q_4	0.91	0.39, 2.12	0.84
OMEGA 6			
Q_1	1		
Q_2	1.28	0.53, 3.15	0.58
Q_3	0.77	0.32, 1.82	0.55
Q_4	0.91	0.39, 2.12	0.84
Income			
Middle	1		
Lower middle	0.96	0.51, 1.83	0.91
Education			
Graduate	1		
Non graduate	1.08	0.50, 2.08	0.82
Family			
Joint	1	0.96, 3.72	0.06
Nuclear	1.89		
Diet			
Veg	1	0.11, 1.61	0.89
Mixed	0.55		

Table 19 shows multiple logistic regression with metabolic syndrome as outcome. Each independent variable is converted into quartiles and used

the lowest quartile as reference quartiles and premenopausal group as the reference group. Menopausal group (Pre, Peri and Post) , quartiles of age, vitamin B_{12}, folic acid , vitamin D, homocysteine, SAFA, MUFA, omega-3 and omega-6 are entered as independent variables. Odds ratios is significant for postmenopausal status and lower quartile of vitamin D. Significance of odds ratio for postmenopausal women is more than ($p<0.005$) than that of low levels of vitamin D ($p<0.05$). This is true even after adjusting socioeconomic status.

Figure 19: Multiple Logistic Regression Analysis to find out determinants of metabolic syndrome



DISCUSSION

The present study was undertaken to examine the association of micronutrients like folic acid, vitamin $B_{12,}$ vitamin D and LCPUFA with metabolic syndrome components in middle aged urban women. The present study demonstrates the following important findings.

1. The proportion of women with lowest micronutrients levels was highest in postmenopausal women.
2. High prevalence of metabolic syndrome in postmenopausal women.
3. Less micronutrients levels was present in a group of women with metabolic syndrome than without metabolic syndrome.
4. Low vitamin D negatively correlated with more number of metabolic components such as WC, BP and TGs in pre and peri menopausal women whereas vit D varied inversely with only one metabolic component i.e WC in postmenopausal women.
5. Postmenopausal status as a stronger determinant followed by low vitamin D were important risk factors for development of metabolic syndrome.

Present study found a significant difference in the economic status between 3 groups. Maximum number of postmenopausal women were in lower middle class. (Table 8) A study by Almeida LC et al[195] reported that socio-demographic status influences predictive value for plasma levels of homocysteine, folic acid, and vitamins B_{12} among low-income women . Jean Dallongeville et al[196] in 2005 found that after adjustment of lifestyle variables, household income remained inversely associated with metabolic syndrome in women but not in men . It was reported that limited resources bring people to choose low-cost, energy-dense food, which favours the development of insulin resistance, hypertriglyceridemia, and body weight

gain.[197] Limited resources may also affect the ability to practice leisure activity, resulting in a higher risk of metabolic syndrome. But they do not clearly explain the sex difference from the same lower household income category. Therefore, another explanation could be that women in the lowest household income category are likely to be unemployed and bothered by limited resources. This in turn may favour body weight gain and insulin resistance.

There was a significant difference in the level of education among the three groups. Maximum number of volunteers were non graduate in postmenopausal group. (Table 8) The reasons for this association might be related to the influence of education in predicting food choices and healthy behaviours both of which are related to metabolic syndrome. Björntorp et al[198] argues that unfavorable socioeconomic circumstances i.e lower income and less education might be coupled with psychosocial stress may lead to a physiological defeat reaction, there by activating the hypothalamus-pituitary - adrenocortical (HPA) axis as indicated by elevation of the major components of the metabolic syndrome i.e obesity.

Present study found significant difference for type of family among three groups. Maximum number of volunteers were housewives in all group. (Table 8) Among female subjects, the age-adjusted prevalence of metabolic syndrome was higher in blue-collar (manual) than in white-collar workers (non manual), but this difference was not evident among male workers. [198] In general populations, low educational and income levels were related to metabolic syndrome in female subjects but not in male subjects. Women of high socioeconomic status tend to be more concerned about their fitness, consume healthy food, and practice regular

exercise.[199] No significant difference was observed between the three groups with regards to occupation

As per the type of diet, significant difference between the three groups was observed. Maximum number of volunteers consumed vegetarian type of diet.(Table 8) In Indian food pattern there is dietary deficiency of Folic acid & Vitamin B_{12}.[27,146] Vegetarians are deficient in folic acid and vitamin B_{12} throughout life [144] leading to increase in the levels of homocysteine and hence metabolic syndrome.[37]

As regards to physical activity no significant difference was found between the three groups. But in all three groups mean levels were below the recommended levels of physical activity i.e 150 min /wk. (Table 8) Reduced physical activity in turn may favours body weight gain and hence insulin resistance.

Various studies [200,201] support the findings that perimenopausal women had significantly higher BMI as well as waist circumference as compared to premenopausal women. These studies concluded that during middle age, women also have a tendency of weight gain associated with depression due to stressful life events. Psychosocial factors were associated with the dysregulation of the hypothalamo-pituitary-adrenal axis, resulting in an increased release of cortisol, decreased glucose uptake and elevated glucose levels.

Ainy E et al [56], Arthur FK et al [54] , Maharlouei N et al [202] support the present findings that WC was significantly higher in peri & postmenopausal women as compared to premenopausal women. This may be due to the fact as proved by various studies [203,103] that, in premenopausal women fat accumulates in lower extremities, to a greater extent, as a result

of oestrogen secretion.[15,16] However,during menopause the estrogen secretion falls and gradually causes fat accumulation in visceral tissues of abdomen which results in central obesity.[103] This may further be aggravated by reduced physical activity as observed in the present study. Sapna Goyal et al [52] didn't show difference in waist circumference because it was on the tribals of North East India who might have been physically more active .

Proportion of women with high systolic and diastolic blood pressure together as well as separately were significantly higher in post and perimenopausal women as compared to premenopausal women. (Figure 7)This observation matches with other studies by Ainy E et al [56], Arthur FK et al[54], Maharlouei N et al [202], Sapna Goyal et al [52] and Shefali Pandey et al [53].

Higher blood pressure especially among postmenopausal group may be due to presence of visceral fat as proved by various studies.[203-206] Visceral fat produces free fatty acids and inflammatory cytokines which directly drains into the portal vein, thus likely to have a direct signaling and metabolic changes in liver.[204, 205] Fat deposits in the liver are associated with the overproduction of very low-density lipoprotein predisposing women to atherogenic dyslipidaemia[205,206] i.e elevated triglyceride, low HDL-cholesterol level, and small dense LDL cholesterol particles . Elevated levels of small dense-LDL-cholesterol get entrapped in the endothelium of the arterial wall and are oxidized leading to arterial stiffness and atherosclerosis and these can culminate in high blood pressure and related conditions.[207]

In the present study, the mean fasting blood glucose levels and proportion of women with impaired fasting glucose were significantly higher in peri and post menopausal women compared to premenopausal women. 82% prevalence of raised fasting glucose was present in postmenopausal women (Table 10 and Figure 7) Similar results were obtained by various studies [54,54,56,62,202,]. Insulin sensitivity and glucose intolerance were not entirely explained by a woman's hormonal status. [50] In the present study, post menopausal women had higher WC than normal. More the WC more is the FBG. [200,201] Whereas studies by Sapana Goyal et al [52] and Haidari et al [208] which did not observe higher FBG in postmenopausal women as compared to premenopausal women suggesting that, high fasting blood glucose levels itself is not sufficient to diagnose metabolic syndrome. Rather, fasting glucose levels may be within normal range in presence of insulin resistance.[5]

Triglycerides (TG) levels were highest in postmenopausal group and there was a significant difference between peri and postmenopausal group. (Table 10 and Figure 7) Similar findings were reported by Ainy E et al [56] , Arthur FK et al[54] Maharlouei N et al [202] Sapna Goyal et al [52], Shefali Pandey et al [53] and Shruti Dasgupta et al.[209] But Samir Ben Ali et al[23] did not find high TG, in postmenopausal women after age adjustment.

Plasma TG and HDL-cholesterol are known to be inversely correlated as observed by various epidemiological studies.[210,211] The enzyme cholesteryl ester transfer protein (CETP) balances the levels of TG and HDL-cholesterol. Cholesteryl ester transfer protein mediates the exchange of cholesteryl ester for triglycerides between HDL, VLDL and LDL. It has been proved that patients who were lacking CETP had extremely high HDL-C levels, low LDL-C levels and a low incidence of Coronary heart disease. [212] It has been proposed that high CETP activity

explains some of the high TG levels and low HDL-C levels (dyslipidaemia), observed in Metaboilc syndrome .[213,214]

This concurs with the present study in which HDL cholesterol levels were similar and lower than normal in all pre, peri & post menopausal groups.(Table 10) Thus HDL levels were not influenced by menopausal status and age . Similar findings were reported by Manisha Chandalia et al,[95] Smith J et al [100], Kim et al[94], Maharlouei N et al [202] , Sapna Goyal et al, [52] Shruti Dasgupta et al[209] in 2010. Kamath SK et al [215] found low HDL-cholesterol values in South Asians (India and Pakistan).

Increased prevalence of low HDL-C independently of obesity or hypertriglyceridemia is observed in women but not in men of Asian Indian origin.[95] Higher prevalence of low plasma HDL-C concentrations in Asian Indian women compared with the men could contribute to decreased sex protection for CHD as previously reported in the Asian Indian population.[95,100]

Present study found the prevalence of Mets as 29 % , 65% & 69% in pre , peri and postmenopausal group respectively as showed in table 10. It was significantly higher in postmenopausal women as compared to pre and peri menopausal women. Similar results were obtained in a various studies on Indian population conducted by Shruti Dasgupta et al[214] in 2010 and Shefali Pandey et al [53] 2010 in Indian population and in Brazilian [57] and Korean [94] population as well.

Homocysteine and HOMA IR showed significant difference between pre peri and post menopausal women and it was maximum in post menopausal women. (table 10)Various studies reported similar

results.[145, 216] Age was positively correlated with homocysteine . Plasma homocysteine levels were also related to menopause even after adjustment for body mass index, thus indicating that plasma homocysteine was affected by menopause.[217]

Vitamin D concentrations was significantly lower and saturated fatty acid (SAFA), homocysteine and HOMA IR were significantly higher in subjects with metabolic syndrome. (Table 12)Whereas there were no significant difference for vitamin B_{12} , folic acid and omega 3 fatty acids in between two groups. This indicates that among the micronutrient vitamin D has an important role to play in the development of MetS. (Table 12)

Liu S [218] reported that dietary vitamin D was inversely associated with prevalence of metabolic syndrome. Narula et al[219] 2006 reported severe vitamin D deficiency in postmenopausal women. Klein-Platat et al [220] 2005 reported that saturated fatty acid composition was associated with the metabolic syndrome.

Cui R et al[133] reported no association of folic acid and vitamin $B_{12}$with risk of cardiovascular disease in Asian populations. However Baltaci et al[24] reported lower vitamin B_{12} levels in obese women with metabolic syndrome although there was no difference in the levels of folic acid. Thus the role of vit B_{12} and folic acid in MetS appears to be controversial. Higher HOMA IR in metabolic syndrome group may be due to high WC and FBG as observed in the present study. (Table 12)

In the present study higher homocysteine levels were reported in postmenopausal women. Low vitamin B_{12} and folic acid levels and Higher HOMA IR values might be cause of it. (Table 11)

Present study showed significant negative correlation of WC with vitamin D in all the three groups and with omega 3 fatty acids in

postmenopausal group. (Table 13) Similar results were reported by Song HR et al [22] and Gagnon C [21]. Vitamin D being fat soluble is readily stored in adipose tissue and gets sequestered in the fat depot of obese individuals In all the three groups WC was more than normal thereby possibly vitamin D levels were less than normal in all the three group.[161]

Vitamin D reduces PTH levels. PTH promotes calcium influx into the adipocytes and enhances adipogenesis.[221,222] Rosenstreich et al[223] supplemented rats with radiolabeled vitamin D where adipose tissue was found to be the major storage site . Similar results were obtained by Ross AC et al [224] in human tissues after injection of radioactive cholecalciferol. However no correlation of waist circumference with vitamin B_{12}, folic acid and SAFA, MUFA , omega 6 fatty acids and w-6:w-3 ratio were observed with any of the group in the present study.

Negative correlation of waist circumference with omega 3 fatty acids was also reported by Poudyal H et al. [225] This may be due to omega 3 fatty acids suppressing fat synthesis but increasing metabolism in adipose tissue via multiple mechanisms involving altered expression of nuclear transcription factors, viz. sterol regulatory element-binding protein 1 (SREBP-1) and peroxisome proliferator-activated receptors (PPARs). Both, SREBP-1 and PPARs when activated, stimulate lipid uptake and adipogenesis of fat cells. [226]

Significant negative correlation of systolic BP with vitamin D in perimenopausal group and diastolic BP with vitamin D and MUFA levels in premenopausal group were observed. (Table 14, 15) Similar results were documented by other studies.[227,228] Vitamin D receptors are distributed on vascular smooth muscle, endothelium and cardiomyocytes. 1,25(OH)D suppresses renin gene expression, regulating the growth and

proliferation of vascular smooth muscle cells and cardiomyocytes. Therefore, the absence of vitamin D receptor activation leads to tonic up regulation of the renin-angiotensin system, eventually leading to hypertension.[229,230] In addition in the present study, WC being more than normal. Vitamin D perhaps was not available for blood pressure regulation due to sequestration in visceral fat. [222] Whereas, vitamin B_{12} folic acid and SAFA, omega 6 fatty acids and w-6:w-3 ratio showed did not show any correlation in any of the group with systolic and diastolic BP.

Blood pressure was inversely associated with MUFA levels in the present study. (Figure 14)Similar results were reported by Lauszus et al [231] in which the diet rich in monounsaturated fat reduced systolic and diastolic blood pressure. Thus, concluded that a diet rich in monounsaturated fat had beneficial effects on blood pressure and could be useful as non-medication treatment.

Significant negative correlation of fasting blood glucose with omega 3 fatty acid in postmenopausal group was observed in the present study .(Figure 15) Similar results was reported by Carpentier et al[232] in 2008. In the present study postmenopausal women were obese indicative of consumption of high energy food such as saturated fats which is said to adversely affect insulin action . Supplementation of omega 3 fatty acids to rats fed with high fat prevented insulin resistance in muscle by reducing fat content. This was achieved by maintaining normal phosphatidylinositol-3 kinase activity. (PI 3 kinase) This enzyme promotes expression and translocation of GLUT4 receptors for glucose uptake and suppresses TG synthesis on activation of insulin receptors.[232,233] Delarue J et al [234] also reported that supplementation of omega 3 fatty acids prevented many alterations of insulin signaling. This explains elevation of FBG in presence of low omega 3 fatty acid in postmenopausal women. However there was no correlation of fasting blood glucose with vitamin B_{12} , folic acid,

vitamin D, SAFA, MUFA , omega 6 fatty acids and w-6/w-3 ratio in pre, peri and postmenopausal groups. (Table 16)

Significant negative correlation of Triglycerides with vitamin B_{12} in premenopausal group (Figure 16)and with vitamin D in perimenopausal groups (Figure 17)while positive correlation with SAFA levels (Figure 18) in premenopausal group were reported (Table 17). Similar results regarding TGs and vitamin B_{12}were obtained by Adaikalakoteswari, et al[25] 2014 .It was explained by many studies that vitamin B_{12} functions as a coenzyme in the conversion of methyl malonyl CoA (MM-CoA) to succinyl-CoA. [157,235] This reaction is blocked if there is vitamin B_{12} deficiency. As a result there is accumulation of MM-CoA which inhibits the rate-limiting enzyme of fatty acid oxidation i.e carnitine palmitoyl transferase (CPT1) thus, causing lipogenesis. This may be the likely mechanism for the link between B_{12} deficiency and adverse lipid parameters observed in this study.

Negative association of triglyceride with vitamin D is in accordance with Ling Lu[236] et al although, the exact mechanism is not well understood. Following mechanisms have been postulated for vitamin D mediated reduction in serum triglycerides.

1. Vitamin D reduces PTH levels. PTH promotes calcium influx into the adipocytes and enhances adipogenesis. Vitamin D suppress PTH and causes lipolysis.[221]
2. Vitamin D reduces hepatic triglyceride formation and secretion. [237]
3. Viamin D enhances insulin secretion and insulin sensitivity thereby indirectly influencing lipid metabolism. [238]

4. Vitamin D binds with nuclear receptor (VDR) and regulates gene expression at transcription level and increases lipid synthesis by regulating synthesis of the two lipases i.e lipoprotein lipase & hormone sensitive lipase.[224]

Triglyceride levels were directly associated with SAFA levels in the present study. Similar study was reported by Patty et al [239] which stated that replacement of saturated fat by carbohydrates, particularly refined carbohydrates and added sugars, increases levels of triglyceride and small LDL particles and reduces high-density lipoprotein cholesterol. However Triglycerides showed no correlation with folic acid, MUFA , omega 6 fatty acids, omega 3 fatty acids and w-6:w-3 ratio with any of the group. (Table 17)

No correlation of HDL with vitamin B_{12}, folic acid , vitamin D and omega 3 fatty acids was observed in pre ,peri and postmenopausal groups.(Table 18)

These results showed that there is a definite correlation between components of metabolic syndrome and micronutrients, although it is not the same between the groups. eg. FBG shows negative correlation with omega 3 fatty acid in postmenopausal women only. Blood pressure has a negative correlation with vitamin D in pre and peri but not in postmenopausal women. Whereas WC negatively correlates with vitamin D in all the three groups. Number of metabolic components that correlated with micronutrients and PUFA were maximum in premenopausal, minimum in postmenopausal and intermediate in perimenopausal group.

Vitamin D correlated with more number of metabolic syndrome components than other micronutrient. Therefore, multiple logistic regression analysis was done to determine the micronutrient, the deficiency of which plays an important role in development of metabolic syndrome. Considering age and menopausal status along with the micronutrients and homocysteine as independent variables and metabolic syndrome as outcome, it was found that perimenopausal women and postmenopausal women were more likely to develop metabolic syndrome as compared to premenopausal group. (Table 19) Women in the lower quartile of vitamin D concentrations were more likely to have metabolic syndrome compared to those in the upper quartile. Thus, metabolic syndrome was independently associated with vitamin D among the micronutrient studied.

Moreover postmenopausal status was found to be a stronger determinant ($p< 0.005$) than lower levels of vitamin D ($p< 0.05$). Perhaps, postmenopausal status caused stronger adverse metabolic changes than low levels of vitamin D in postmenopausal women. Hence only one metabolic component i.e waist circumference varied inversely with vitamin D levels, though the levels were lowest in the postmenopausal group. However, among premenopausal women low level of vitamin D was the only determinant of metabolic syndrome and varied inversely with three metabolic components such as waist circumference, blood pressure and triglycerides. Perimenopausal group was the intermediate group. Both perimenopausal status and vitamin D were equally significant as a determinant of Mets .This perimenopausal group showed negative correlation with low vitamin D for metabolic components such as waist circumference, blood pressure and triglycerides as in case of premenopausal group. Therefore, it is inferred that vitamin D plays an

important role in causation of metabolic syndrome in pre and perimenopausal women than in postmenopausal women.

SUMMARY

Metabolic syndrome (MetS) is defined as a 'clustering' of metabolic abnormalities like elevated blood sugar level, an abnormal lipid profile, high blood pressure and abdominal obesity which make the person prone to cardiovascular diseases and type 2 diabetes. Prevalence of metabolic syndrome is increasing in developing countries like India. Prevalence is higher in women compared with men at middle age as they are more prone to obesity due to faulty life style in urban area like wrong food choices, sedentary habits, physical inactivity and menopause.

Homocysteine is considered as an independent risk factor for the development of cardiovascular diseases. Micronutrients like vitamin B_{12}, folic acid, vitamin D, LCPUFA play important role in homocysteine metabolism. Hyperhomocysteinemia is more common among Indians and Indians are prone to deficiencies of folic acid and vitamin $B_{12,}$ vitamin D and LCPUFA.

Interventional trials with these nutrients found inconsistent results. This may be due to associations that are not causal or intervening too late in the history of disease .Very few studies have been done in India in this context. So it was hypothesized that micronutrients like vitamin B_{12},folic acid, vitamin D and LCPUFA may be associated with components of metabolic syndrome in post menopausal women.

The study was started after approval of institutional ethics committee. Sample was calculated to be 294 and rounded upto 300 women aged between 35 to 64 yrs. They were equally divided into three groups such as pre, peri and postmenopausal on the basis of inclusion and exclusion criteria.

Criteria of metabolic syndrome was considered according to a joint interim statement of the IDF Task Force in which WC, FBG, BP , TGs and

HDL were included as the components of Metabolic syndrome. Demographic profile such as age, income class, education, type of diet, type of family, occupation and physical activity were recorded. Anthropometric measurements like height, weight, BMI, WC, WHR were included as key indicators of obesity. Plasma levels of vitamin B_{12}, folic acid, vitamin D3, LCPUFA along with FBG ,TGs HDL , homocysteine and serum insulin were assessed as a biochemical parameters. HOMA–IR was calculated.

Plasma levels of the all above micronutrients were compared between those with and without metabolic syndrome and correlated with components of METs as well. Statistical analysis was done using ANOVA and chi square test were used for comparison between three groups. Pearson correlation coefficient was used to measure of the linear correlation between two variables where 'r'and 'p'value were calculated. Odds ratios (ORs) was calculated to measure the association between an exposure and an outcome i.e metabolic syndrome . Multiple logistic regression analysis was used to find out determinants of metabolic syndrome.

In present study maximum number of volunteers belonging to postmenopausal group were in lower middle class income group, non-graduate, housewives and vegetarians. Postmenopausal women were shorter and perimenopausal women were with significantly higher BMI and waist circumference than premenopausal women.

Mean levels and percentage of all metabolic syndrome components except HDL, were higher in perimenopausal women as compared to premenopausal women and highest in postmenopausal women. HDL cholesterol levels were lower than normal and the same in all the three groups. Homocysteine and HOMA IR levels were also significantly higher in peri and postmenopausal women as compared to premenopausal

women. Vitamin B_{12}, folic acid & vitamin D concentrations were significantly lower in peri and postmenopausal women as compared to premenopausal women. Peri and postmenopausal group showed significantly lower omega-3 fatty acid and higher omega-6: omega-3 ratio as compared to premenopausal group.

Vitamin D concentrations was significantly lower and saturated fatty acid (SAFA), homocysteine and HOMA IR were significantly higher in subjects with metabolic syndrome. Whereas there were no significant difference for vitamin B_{12}, folic acid and omega 3 fatty acids in between two groups. This indicates that among the micronutrient vitamin D has an important role to play in the development of MetS.

In premenopausal group, there were significant negative correlations of WC with vitamin D, diastolic BP with vitamin D and MUFA. Triglycerides showed negative correlations with vitamin B_{12}, vitamin D and positive correlations with SAFA levels.

In perimenopausal group there were significant negative correlations of WC, systolic BP and TGs with vitamin D.

In postmenopausal group there were significant negative correlation of WC with vitamin D and fasting blood glucose with omega 3 fatty acid. Thus, more number of metabolic components such as WC,BP and TGs showed negative correlation with vitamin D in pre and perimenopausal group as compared to postmenopausal group.

In the present study HDL did not show any correlation with any of the micronutrients and fatty acids in any of the group.

In order to find the determinant of Mets , multiple logistic regression analysis was undertaken. Considering age and menopausal status along with the micronutrients and homocysteine as independent variables and

metabolic syndrome as outcome, it was found that perimenopausal women and postmenopausal women were more likely to develop metabolic syndrome as compared to premenopausal group. Women in the lower quartile of vitamin D concentrations were more likely to have metabolic syndrome compared to those in the upper quartile. Thus metabolic syndrome was independently associated with vitamin D among the micronutrient studied.

CONCLUSION

The present study was undertaken to examine the association of micronutrients like folic acid, vitamin $B_{12,}$ vitamin D and LCPUFA with metabolic syndrome components in middle aged urban women. The present study demonstrates the following important findings.

6. The proportion of women with lowest micronutrients levels was highest in postmenopausal women.
7. High prevalence of metabolic syndrome in postmenopausal women.
8. Less micronutrient levels was present in a group of women with metabolic syndrome than without MetS.
9. Present study found low vitamin D negatively correlated with more number of metabolic components such as WC, BP and TGs in pre and peri menopausal women whereas vit D varied inversely with only one metabolic component i.e WC in postmenopausal women.
10. Postmenopausal status as a stronger determinant followed by low vitamin D are important risk factors for development of metabolic syndrome.

Vitamin D correlated with more number of metabolic syndrome components than other micronutrient. Therefore multiple logistic regression analysis was done to determine the micronutrient , the deficiency of which plays an important role in development of metabolic syndrome.

Considering age and menopausal status along with the micronutrients and homocysteine as independent variables and metabolic syndrome as outcome, it was found that perimenopausal women and postmenopausal women were more likely to develop metabolic syndrome as compared to premenopausal group. Women in the lower quartile of vitamin D concentrations were more likely to have metabolic syndrome

compared to those in the upper quartile. Thus metabolic syndrome was independently associated with vitamin D among the micronutrient studied.

Moreover postmenopausal status was found to be stronger determinant ($p< 0.005$) than lower levels of vitamin D ($p< 0.05$) . Perhaps, postmenopausal status caused stronger adverse metabolic changes than low levels of vitamin D in postmenopausal women. Hence only one metabolic component i.e waist circumference varied inversely with vitamin D levels, though the levels were lowest in the postmenopausal group. However among premenopausal women low levels of vitamin D was the only determinant of metabolic syndrome and varied inversely with three metabolic components such as waist circumference, blood pressure and triglycerides.

Perimenopausal group was the intermediate group. Both perimenopausal status and vitamin D were equally significant as a determinant of Mets. This perimenopausal group showed negative correlation with low vitamin D for metabolic components such as waist circumference, blood pressure and triglycerides as in case of premenopausal group, therefore it is inferred that vitamin D plays an important role in causation of metabolic syndrome in pre and perimenopausal women than in postmenopausal women.

Postmenopausal status was statistically stronger and lower levels of vitamin D were observed to be determinants of metabolic syndrome. Therefore perhaps, postmenopausal status causes stronger adverse metabolic changes than low levels of vitamin D in postmenopausal women. This was evident in the form of only metabolic component i.e waist circumference varying inversely with vitamin D levels, though the levels were lower in the postmenopausal group. However among premenopausal women low levels of vitamin D being the determinants of metabolic syndrome varied inversely with three metabolic components

such as waist circumference, blood pressure and triglycerides. Therefore it is inferred that vitamin D plays an important role in causation of metabolic syndrome in premenopausal women than in postmenopausal women.

Implications

Monitoring and improving vitamin D status will be an effective way in the prevention and treatment of MetS in pre and perimenopausal women so that, these women reach menopause in a better health to face metabolic challenges of menopause. Thus, the frequency of Mets may come down in postmenopausal women too.

Social relevance

India is now facing an epidemic of increased risk of Mets. It is therefore high priority and urgency to understand role of nutritional factors in the etiopathology of MetS. The present study indicates that among the micronutrients studied low vitamin D levels is a strong predictor of MetS. Therefore, regular monitoring of vitamin D level followed by oral supplementation when required will be cost effective strategy for its timely prevention rather that expensive interventional therapies needed in CVDs.

LIMITATIONS AND FUTURE SCOPE OF THE STUDY

The parathyroid hormone (PTH) was not measured in the present study. PTH should be included as a functional index of vitamin D status in future studies.

Besides vitamin B_{12},folic acid and vitamin D, another micronutrient i.e vitamin B_6 play important role in metabolism of homocysteine so there is a scope of inclusion of vitamin B_6 in future study.

The minimum level of micronutrients needed for prevention of MetS has not been found out in the present study.

The present study was conducted in a small part of Pune city so the finding cannot be extrapolated to urban area in general.

Majority of the women belong to lower middle class housewives and less educated so there is scope to expand this study to other urban area and other demographic group.

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