# Plasma Concentrations of Fat-soluble Vitamins in Metabolic Syndrome Subjects


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**Running title:** *Fat Soluble Vitamins in Metabolic Syndrome*


### Abstract

***Objectives:*** *Several studies have related deficient plasma levels of fat-soluble vitamins to increased prevalence of metabolic syndrome and suggested that optimal levels may reduce the risk and progression of metabolic syndrome. This study was designed to assess the plasma concentrations of vitamins A, D and E in Nigerians with metabolic syndrome.*

***Materials and Methods:*** *One hundred metabolic syndrome subjects were recruited into the study; 55% of them had type 2 diabetes. One hundred controls were age- and sex-matched. Blood pressure, body mass index, waist circumference, concentrations of plasma glucose, lipid profile, vitamins A, D and E were estimated.*

***Results:*** *The mean plasma vitamin E of metabolic syndrome subjects was significantly lower than that of controls (P=0.0001) and also lower in subjects with diabetes than in those without diabetes (P=0.006). The mean plasma vitamins A and D of subjects were similar to that of controls (P=0.231 and 0.391 respectively) and also similar in subjects with and without diabetes (P=0.134 and 0.061 respectively). Mean values of vitamins A, D and E in subjects and vitamins A and D in controls were suboptimal.*

***Conclusion:*** *Plasma vitamin E was lower in subjects with metabolic syndrome than in controls, and vitamins A and D were similar but suboptimal in both groups. This may be due to inadequate vitamin intake, increased oxidative stress and inflammation among other factors.*



**Keywords:** *Metabolic syndrome, Vitamin A, Vitamin D, Vitamin E*


### Introduction

Metabolic syndrome is a multiplex of metabolic risk factors that promote the development of cardiovascular disease and type 2 diabetes mellitus.[1,2] The primary components of metabolic syndrome include insulin resistance/ impaired glucose tolerance, abdominal obesity, hypertension and atherogenic dyslipidaemia. Other important components are prothrombotic and proinflammatory states[1,2]. Increasing trends in western diet and sedentary lifestyle, coupled with genetic susceptibility, contribute to the recent increase in the incidence of metabolic syndrome and type 2 diabetes[3]. Prevalence rates of metabolic syndrome among Nigerian type 2 diabetic patients vary between 25-59% according to previous studies[4,5]. Lifestyle modification which includes dietary modification, forms an integral part of the management of metabolic syndrome and type 2 diabetes[3,5].


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Fat-soluble vitamins include vitamins A, D, E and K[6]. Retinol, the predominant active form of vitamin A is transported in the circulation bound to retinol-binding protein (RBP) and transthyretin (TTR) as a 1:1:1 complex. RBP regulates vitamin A uptake and metabolism[6-8]. Vitamin A may promote differentiation of activated T cells to regulatory T cells, which can prevent autoimmunity. It has also been shown to play a role in the balance of proinflammatory and anti-inflammatory immunity, in lipid, glucose and energy homeostasis, and has been proposed to have antioxidant properties. [9-11]. In addition, it has recently been discovered that adipocyte-derived RBP (RBP 4) may act as an adipokine that increases hepatic gluconeogenesis and contribute to the development of obesity-induced insulin resistance through impaired fatty acid metabolism[8].

The main circulating form of vitamin D is 25-hydroxyvitamin D (25(OH)D), also known as calcidiol[1]. It has been shown to enhance pancreatic beta cell function, accelerate the conversion of pro-insulin to insulin, reduce insulin release from the pancreas and increase insulin sensitivity. Thus, it has been proposed to play a role in insulin and glucose metabolism [1,12,13]. It may also lower blood pressure by reducing activation of the renin-angiotensin system[13]. Insufficient vitamin D status has been linked with beta cell dysfunction, insulin resistance, hyperglycaemia, hypertension and increased total serum cholesterol levels. Hypovitaminosis D may therefore be a risk factor for metabolic syndrome and diabetes[1,13,14]. Evidence suggests that vitamin D can inhibit proinflammatory cytokines and reduce positive acute phase proteins like C-reactive protein (CRP)[1]. Vitamin D supplementation has been shown to prevent the development of type 1 diabetes primarily through immune regulation, and also indirectly through its inhibitory effects on inflammation[13].

The predominant and most potent form of vitamin E in plasma is alpha-tocopherol[7]. Vitamin E is the primary liposoluble antioxidant[6] and it enhances endothelial function by increasing endothelium dependent vasodilation and inhibiting the expression of adhesion molecules. It therefore protects against atherogenesis and cardiovascular disease[15]. Studies have shown that vitamin E may have a beneficial effect in improving insulin sensitivity and insulin-mediated glucose uptake[16-18].

Low status of vitamins A, D and E has been linked with metabolic syndrome and type 2 diabetes [2,14,17]. The aim of this study was to determine the plasma levels of vitamins A, D and E in Nigerians with metabolic syndrome and compare results obtained with those of age- and sex-matched controls.

## Materials and Methods

### Subjects

The study was conducted in a tertiary hospital in Port Harcourt, one of the major cities in Nigeria. After obtaining approval from the ethical committee of the hospital and an informed written consent from each participant, metabolic syndrome subjects were selected from patients attending the General Outpatient and Metabolic Clinics of the hospital. Control subjects were recruited from among the hospital workers.

Individuals that were acutely or chronically ill, those on vitamin or mineral supplements, pregnant women, alcoholics (consumption of 20 or more units of alcohol weekly), current smokers and those with a significant history of smoking (10 or more cigarettes daily) were excluded from the study[4,19]. Individuals that were aged twenty years and above, willing to participate and comply with the instructions of the study were recruited. Inclusion criteria for metabolic syndrome subjects included those with any three or more of the National Cholesterol Education Program – Adult Treatment Panel Three (NCEP-ATP III) 2001 diagnostic criteria defined as[1,2]:

1) Central/Abdominal obesity:
   Waist Circumference e" 102cm (40 in) in men and e" 88cm (35 in) in women
2) Hypertriglyceridaemia:

Fasting plasma triglycerides ³ 150mg/dl (1.7 mmol/L)

3) Low plasma HDL cholesterol: < 40mg/dl (1.0 mmol/L) in men and < 50mg/dl (1.3 mmol/L) in women
4) High blood pressure: ³ 130/85 mmHg
5) High fasting plasma glucose (Hyperglycaemia): ³ 110mg/dl (6.1 mmol/L)

One hundred consenting metabolic syndrome subjects and one hundred age- and sex-matched apparently healthy controls were selected to participate in this study.

**Physical Examination**

The blood pressure, waist circumference, weight and height of participants were measured and body mass index (BMI) was calculated ($kg/m^2$)[4].

**Sample Collection**

After an overnight fast 10ml of venous blood was drawn from each participant into an ethylenediaminetetraacetic acid (EDTA) bottle for fasting lipid analysis, fluoride oxalate bottle for fasting plasma glucose analysis and lithium heparin bottle for analysis of vitamins A, D and E. Plasma was separated from blood cells immediately after centrifugation at 2,500g for 15 minutes. Fasting plasma glucose was measured within 24 hours of collection of blood. Samples for lipid profile, vitamins A, D and E analysis were stored frozen and analysed within two weeks of collection.

**Laboratory Analysis**

Plasma glucose concentration was determined using the glucose oxidase method (Randox kit)[20]. Plasma triglyceride, total cholesterol and HDL were determined using the enzymatic method of analysis (Randox kit)[20]. The LDL concentration was calculated according to the equation by Friedewald et al[21]. Plasma vitamins A, D and E were determined using the reverse-phase high performance liquid chromatography (HPLC) method (Agilent HPLC 1100 series)[22]. Randox quality control sample was included in every batch during the analysis of samples[20].

**Definitions**

Type 2 diabetes was diagnosed in subjects who had a prior history of type 2 diabetes or had a fasting plasma glucose concentration e" 7.0mmol/L[23]. Low vitamin status was defined as a vitamin concentration below the reference interval[24,25]. Low and optimal vitamin A levels were defined as retinol levels <1.05 μmol/L and >2.5μmol/L respectively[24]. Low and optimal vitamin D levels were defined as <25nmol/L and >75nmol/L respectively[23]. Low and optimal vitamin E levels were defined as á-tocopherol levels <12μmol/L and >30μmol/L respectively[24].

**Statistical Analysis**

Statistical analysis of the data generated from the study was done using the Statistical Package for Social Sciences (SPSS) version 11.0. Values were expressed as mean ± standard deviation. The means of continuous variables were compared using unpaired students t test. P-values less than or equal to 0.05 were taken to be significant.

**Results**

One hundred metabolic syndrome subjects between 21 and 73 years with a mean age of 51.41 ± 10.88 years and one hundred controls between 22 and 78 years with a mean age of 48.57 ± 12.05 years were studied. There was no statistically significant difference between the mean ages of subjects and controls (P=0.082). Subjects had significantly higher waist circumference, body mass index, blood pressure, fasting plasma glucose, triglyceride, LDL and lower HDL concentrations than controls (P<0.001 for all).

Of the 200 participants, 94 metabolic syndrome subjects consisting of 40 males and 54 females, and 94 controls consisting of 44 males and 50 females had values for all the fat-soluble vitamins studied. Fifty-two (55%) of the metabolic syndrome subjects had type 2 diabetes and 42 (45%) did not have type 2 diabetes. Subjects with type 2 diabetes had a mean duration of diabetes of 6.94 ± 3.22 years. They were on dietary and oral hypoglycaemic therapy (Metformin and Glibenclamide). Seventy-two percent of them were known

hypertensive patients on various combinations of antihypertensive therapy (captopril, lisinopril, nifedipine, amlodipine, moduretic and methyldopa). Subjects with diabetes were older, less obese (P<0.01), had higher fasting plasma glucose (P=0.0001), lower blood pressure (P<0.01) and lower LDL (P=0.04) than subjects without diabetes (Tables 1 and 2 respectively).

The mean plasma vitamin A and vitamin D concentrations of metabolic syndrome subjects were not significantly lower than that of controls (P=0.231 and 0.391 respectively) (Table 3). They were also similar in subjects with and without type 2 diabetes (P=0.134 and 0.061 respectively) (Table 4). The mean concentrations of both

**Table 1**

**Physical Attributes of Metabolic Syndrome Subjects with and without Type 2 Diabetes**

| Parameter | Diabetic Subjects (n=52) Mean ± SD | Non-diabetic Subjects (n=42 Mean ± SD | P-Value |
|---|---|---|---|
| Age(years) | 54.32 ± 9.08 | 47.53 ±11.91 | 0.003* |
| Waist circumference(cm) | 99.61 ± 10.81 | 106.16 ±11.23 | 0.003* |
| Body mass index (Kg/m$^2$) | 28.75 ± 5.09 | 32.56 ±6.28 | 0.002* |
| Systolic blood pressure (mmHg) | 138.60 ±22.07 | 150.70 ± 20.98 | 0.007* |
| Diastolic blood pressure (mmHg) | 86.93 ± 12.09 | 100.00 ± 14.14 | 0.0001* |

** Statistically significant (P<0.05)*

**Table 2.**

Biochemical Parameters of Metabolic Syndrome Subjects with and without Type 2 Diabetes

| Parameter | Diabetic Subjects (n=52) Mean ± SD | Non-Diabetic Subjects (n=42) Mean ± SD | P-Value |
|---|---|---|---|
| Fasting plasma glucose(mmol/L) | 7.46 ±2.09 | 4.99 ±1.13 | 0.0001* |
| Triglyceride (mmol/L) | 1.24 ± 0.58 | 1.29 ± 0.63 | 0.739 |
| High density lipoprotein (mmol/L) | 1.37 ± 0.58 | 1.36 ± 0.45 | 0.915 |
| Low density lipoprotein (mmol/L) | 2.76 ± 0.91 | 3.16 ± 1.02 | 0.04* |
| Total cholesterol (mmol/L) | 4.66 ± 1.15 | 5.12 ± 1.04 | 0.067 |

** Statistically significant (P<0.05)*

**Table 3.**

Vitamin Levels of Metabolic Syndrome Subjects and Controls

| Vitamin | Subjects (n=94) Mean ± SD | Controls (n=94) Mean ± SD | P-Value |
|---|---|---|---|
| Vitamin A(mol/L) | 1.55 ± 0.73 | 1.68 ± 0.72 | 0.231 |
| Vitamin D (nmol/L) | 60.93±28.72 | 64.42 ± 25.2 | 0.391 |
| Vitamin E (ìmol/L) | 17.0 ± 4.63 | 30.89 ± 6.45 | 0.0001* |

** Statistically significant (P<0.05)*

vitamins were not up to optimal levels in subjects as well as controls, indicating some level of inadequacy. Twenty-one percent of subjects had low and 11% had optimal vitamin A levels. Thirteen percent of the controls had low and 10% had optimal vitamin A levels. None of the subjects or controls had low vitamin D levels. Twenty percent of subjects and 24% of controls had optimal levels of vitamin D respectively.

The mean plasma vitamin E of metabolic syndrome subjects was significantly lower (P=0.0001) than that of controls, which was in the optimal range (Table 3). Sixteen percent of subjects had low and 4% had optimal vitamin E levels. None of the controls had low vitamin E levels; 50% had levels in the optimal range. The mean plasma vitamin E of subjects with diabetes was significantly lower than those without diabetes (P=0.006) (Table 4).

There was no difference in the plasma concentrations of vitamins A, D and E between male and female subjects (P= 0.097, 0.106 and 0.795) and controls (P= 0.185, 0.630 and 0.934) respectively.

**Table 4:**

Vitamin Levels of Metabolic Syndrome Subjects with and without Type 2 Diabetes

| Vitamin | Diabetic Subjects (N=52) Mean ± SD | Non- Diabetic Subjects (N=42) Mean ± SD | P-value |
|---|---|---|---|
| Vitamin A(ìmol/L) | 1.65 ± 0.77 | 1.42 ± 0.66 | 0.134 |
| Vitamin D (nmol/L) | 65.82±31.65 | 54.63 ± 23.35 | 0.061 |
| Vitamin E (ìmol/L) | 15.33 ± 4.05 | 17.98 ± 5.12 | 0.006* |

** Statistically significant (P<0.05)*

## Discussion

Low levels of vitamins A, D and E have been found to increase the risk of metabolic syndrome and other insulin resistant conditions like hypertension, diabetes and cardiovascular disease, and supplementation with these vitamins has been found to improve insulin sensitivity and action.[14,17,18]

We observed that metabolic syndrome subjects had a much lower mean plasma vitamin E concentration than controls. Mean plasma vitamin E was also lower in metabolic syndrome subjects with diabetes compared to subjects without diabetes. Vitamins A and D concentrations were similar in metabolic syndrome subjects and in controls. However, most of the subjects and controls had suboptimal levels of vitamins A and D. Vitamins A and D levels were also similar in subjects with and without diabetes.

Previous studies have reported low concentrations of fat-soluble vitamins in individuals with metabolic syndrome.[2,13,14,16,17] and diabetes[11,13,14,17]. Optimal levels of fat-soluble vitamins have also been demonstrated to reduce the risk of developing these diseases, slow down their progression, and decrease morbidity and mortality associated with them[1,11,18,24]. Various components of metabolic syndrome have been independently associated with fat-soluble vitamins. Central obesity, hypertriglyceridaemia, high blood pressure and hyperglycaemia have been shown to have inverse associations with vitamins A, D and E[2,12,25].

It has been suggested that increased adiposity in obese metabolic syndrome subjects may be partly responsible for the decrease in plasma bioavailability of fat-soluble vitamins due to increased sequestration in adipose tissue[1,16,25]. Low levels of antioxidant vitamins have also

been associated with increased oxidative stress in metabolic syndrome[2,16,17] and type 2 diabetes[11,17]. Existing evidence suggests that inflammation may influence fat-soluble vitamin status[25]. Increased levels of C-reactive protein (CRP), an inflammatory marker, have been inversely associated with metabolic syndrome as well as independently with deficiencies of fat-soluble vitamins, particularly vitamins A and D[1,8,25]. Malaria, which is an endemic disease in Nigeria, has also been associated with reduced serum concentrations of vitamins A and E[10]. This has been attributed to malarial- induced oxidative stress and inflammatory response, resulting in increased utilization of these antioxidant vitamins in combating free radical damage. The inflammatory response may also result in reduced RBP and TTR concentrations and redistribution of vitamin A to the tissues, thereby reducing plasma levels[10,25].

Concentrations of fat-soluble vitamins in metabolic syndrome may also be related to intake. A low intake of vitamins resulting from poor dietary habits has been described in healthy individuals[6], in obese individuals[16,25], in the elderly[24], and in metabolic syndrome[2,13]. Forty-one percent of the subjects in this study were 60 years and above. Physiological age-related changes, morbidity and use of medication in the elderly may affect intake, absorption and metabolism of vitamins[24]. Some researchers have found concentrations of fat-soluble vitamins to correlate positively with dietary intake or intake of vitamin supplements[2,12,13].

Plasma concentration of vitamin D is regulated by parathyroid hormone, calcium and phosphate[1] and the close interrelationships between these factors, therefore, need to be taken into consideration in determining vitamin D status in metabolic syndrome. Plasma 25(OH)D also reflects the degree of sun exposure and geographic location[1,14]. Though living in a sunny climate alone does not eliminate low vitamin D levels, it is an important factor in vitamin D synthesis and thus in determining vitamin D status[1]. Inadequate cutaneous vitamin D synthesis may predispose to low vitamin D status because it is the major source of vitamin D and dietary sources of vitamin D are limited[1,24]. Race has also been shown to be an important factor[1]. Our study population consisted of dark-skinned individuals living in a city with a sunny climate all year round. It has been observed that the presence of increased melanin in blacks absorbed some of the ultraviolet radiation and decreased vitamin D synthesis[1]. Other factors that may decrease cutaneous vitamin D production include sunscreen use, type of clothing, sedentary lifestyle and social or religious practices that reduce outdoor activities[1,12]. Some of these factors may have contributed to the suboptimal levels of vitamin D observed in this study.

Body mass index, waist circumference, blood pressure and LDL of subjects with diabetes were observed to be lower than in subjects without diabetes. This could be explained by the fact that diabetic subjects were on dietary, oral hypoglycaemic and antihypertensive therapy. They had been previously counselled in the clinics to cut down on carbohydrates with high glycaemic index and to increase their intake of high fibre diets including fruits, vegetables and legumes. They had also been counselled about the importance of exercise, cessation of excessive alcohol intake and smoking. Angiotensin converting enzyme-inhibitors like captopril are well known for their renoprotective effects and efficacy in reducing blood pressure, cardiovascular morbidity and mortality in diabetics[3,5]. Metformin is known to improve insulin resistance, lower blood glucose and lipid levels, and promote weight loss[3]. Subjects without diabetes, on the other hand, were not on any form of therapy.

Lower vitamin E concentration in diabetic subjects compared to non-diabetic subjects could be due to a number of factors. Hyperglycaemia is strongly associated with insulin resistance and studies have suggested that as hyperglycaemia becomes more severe, insulin resistance increases with a

corresponding increase in oxidative stress[11,17,19]. Fasting plasma glucose of diabetic subjects was higher than that of non-diabetic subjects. This may have predisposed them to a greater degree of oxidative stress, higher depletion and subsequently lower levels of vitamin E.

Some limitations were present in this study. Parathyroid hormone was not measured and like several related studies, because of its cross-sectional design, causative nature of the association between metabolic syndrome and low levels of fat-soluble vitamins could not be established. Intervention studies using fat-soluble vitamin supplements are required to determine causality.

## Conclusion

This study showed that metabolic syndrome subjects in Port Harcourt had significantly lower plasma levels of vitamin E but similar concentrations of vitamins A and D compared to healthy control subjects. Apart from vitamin E levels in controls all other vitamin levels were in the suboptimal range. Vitamin E was also lower in subjects with type 2 diabetes than in subjects without type 2 diabetes. Low status of vitamins A, D and E may be due to several factors associated with metabolic syndrome which include increased adiposity, oxidative stress and inflammation, as well as inadequate intake and degree of sun exposure.

## Recommendation

Subjects with metabolic syndrome should be encouraged to increase their intake of foods rich in fat-soluble vitamins and to have adequate and appropriate sunlight exposure. Vitamin supplementation may be of benefit to those with low vitamin status.

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