Plasma concentrations of water-soluble vitamins in metabolic syndrome subjects

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Abstract

Context: Vitamins B1 (thiamine), B3 (niacin), B6 (pyridoxine), and C (ascorbic acid) are vital for energy, carbohydrate, lipid, and amino acid metabolism and in the regulation of the cellular redox state. Some studies have associated low levels of water-soluble vitamins with metabolic syndrome and its various components.

Aims: This study aims to determine the plasma concentrations of vitamins B1, B3, B6, and C in Nigerians with metabolic syndrome and in healthy controls.

Settings and Design: One-hundred subjects with metabolic syndrome were recruited into the study. One-hundred controls were age- and sex-matched.

Materials and Methods: Blood pressure, body mass index, waist circumference, concentrations of plasma glucose, lipid profile, and vitamins B1, B3, B6, and C were estimated.

Statistical analysis used: Statistical Package for Social Sciences (SPSS) version 11.0.

Results: The mean plasma vitamins B1, B3, B6, and C concentrations of subjects were significantly lower than that of controls ($P = 0.001, 0.05, 0.045, 0.001$ respectively). Fourteen percent and 32% of subjects had inadequate vitamins B1 and C status, respectively. Vitamin B6 was lower ($P = 0.001$) and vitamin C was higher ($P = 0.012$) in female than in male subjects.

Conclusions: Thiamine, niacin, pyridoxine, and ascorbic acid levels were lower in subjects than in controls. Pyridoxine was also lower and ascorbic acid was higher in female than in male subjects.

Key words: Ascorbic acid, metabolic syndrome, niacin, pyridoxine, thiamine

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Introduction

Water-soluble vitamins consist of vitamin C (ascorbic acid) and the B-complex group. The focus of this study include vitamins C, B1 (thiamine), B3 (niacin), and B6 (pyridoxine).\(^{[1,2]}\) Due to their water solubility, water-soluble vitamins are easily destroyed by heat and food-processing techniques, they are not stored in the body for long periods of time, and have a greater loss by way of urinary excretion. With the exception of vitamin B12, most other B-complex vitamins and vitamin C are stored for less than 2 months; thiamine is stored for only about 2–3 weeks.\(^{[1,2]}\) Thus, frequent and adequate dietary intake is essential to maintain optimal levels in the body.\(^{[1,2]}\) Low levels and deficiencies of water-soluble vitamins have been reported in association with several diseases in Nigeria\(^{[3-6]}\) and other countries.\(^{[7-12]}\)

Thiamine is important in energy generation in cells and in the metabolism of carbohydrate, branched-chain amino acids, fats, and alcohol as well as in nerve membrane conduction.\(^{[1,2]}\) The major transport form in plasma is thiamine, but TPP (thiamine pyrophosphate), which is the active form of thiamine, is predominantly found in tissues.\(^{[1]}\) In carbohydrate metabolism,
TPP is required as the cofactor in the glycolytic pathway for the formation of acetyl coenzyme A, which is essential in the tricarboxylic acid cycle for energy production in the form of adenosine triphosphate (ATP) required for cellular processes. TPP is also important in the pentose phosphate pathway (PPP), which supplies reduced nicotinamide adenine dinucleotide phosphate (NADPH) necessary for synthesis of reduced glutathione, an antioxidant that is essential for the body’s defense against oxidative stress. Therefore, thiamine has a role in reducing cellular oxidative stress. Low thiamine levels may lead to impaired energy metabolism and cell damage or death by way of oxidative stress, necrosis, or apoptosis.

Thiamine has also been shown to be essential for pancreatic function and its deficiency has been related to impaired insulin synthesis and secretion, increased metabolic dysfunction, endothelial dysfunction, and increased risk of atherosclerosis. Thiamine deficiency and suboptimal levels have been implicated in several disorders including diabetes, cardiovascular, and neurological disorders. Optimal levels appear to have a protective effect against tissue damage caused by hyperglycemia and in the prevention of diabetic retinopathy.

Vitamin B3 consists of nicotinic acid (niacin) and its amide, nicotinamide (niacinamide). Nicotinamide is the main circulating form of vitamin B3 in plasma and the precursor of the coenzyme forms, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are essential in many cellular oxidation-reduction reactions catalyzed by dehydrogenases, including glycolysis and oxidative phosphorylation. These active coenzyme forms can also be derived from tryptophan. Nicotinamide is also an essential factor for the activity of glutathione peroxidase antioxidant enzyme. Nicotinamide is important in enhancing antioxidant defense and ensuring a balanced redox state in the cell. It has been shown to have a protective effect on pancreatic beta-cell survival, probably by inhibiting the immune-mediated beta-cell destruction, by reducing production of reactive oxygen species, and increasing the energy metabolism of the cell. It may also prevent or delay clinical onset of diabetes.

NADPH is important to ensure a continuous supply of reduced glutathione in the body. Glutathione is an antioxidant that scavenges a variety of radical species and is also an essential factor for the activity of glutathione peroxidase antioxidant enzyme. Thus, nicotinamide is important in enhancing antioxidant defense and ensuring a balanced redox state in the cell. It has been shown to have a protective effect on pancreatic beta-cell survival, probably by inhibiting the immune-mediated beta-cell destruction, by reducing production of reactive oxygen species, and increasing the energy metabolism of the cell. It may also prevent or delay clinical onset of diabetes.

The three natural forms of vitamin B6 are pyridoxine, pyridoxal, and pyridoxamine. These dietary precursors of vitamin B6 are all converted in the liver to pyridoxal-5-phosphate (PLP), the biologically active cofactor. PLP functions in the metabolism of glucose, lipids, and amino acids and in the synthesis of neurotransmitters, histamine, hemoglobin, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). It has an important role in the metabolism of tryptophan to NAD and NADP and in the metabolism of homocysteine to cysteine through the trans-sulfuration pathway. Increased homocysteine levels are associated with an increased risk for cardiovascular disease. PLP is a coenzyme for cystathionine β-synthase and cystathionine γ-lyase, both required for the synthesis of cysteine from homocysteine. Glutathione is synthesized from cysteine. Vitamin B6 has also been demonstrated to prevent oxygen radical generation and lipid peroxidation caused by hydrogen peroxide. Therefore, vitamin B6 is involved in combating oxidative stress and low vitamin B6 status may impair the antioxidant defense system. Vitamin B6 also modulates the action of hormones and is important for optimal function of the immune system and maintaining the health of lymphoid organs.

Vitamin B6 has been found to have a role in enhancing vascular endothelial function and protecting against cardiovascular disease, apart from reduction of homocysteine levels, via inhibition of platelet aggregation and endothelial cell proliferation and decreased blood pressure and cholesterol levels. Vitamin B6 has been shown to improve glucose tolerance, restore pancreatic beta-cell function, and have therapeutic effects for diabetic neuropathy. Its deficiency decreases insulin synthesis and secretion, and causes degenerative changes in beta cells.

Ascorbic acid functions as a potent antioxidant, in the synthesis and stabilization of collagen and thus in connective tissue formation, and enhances the immune system. It also functions as a cofactor in the reductive processes necessary for maintaining metal ions (particularly iron and copper) in their reduced forms, in hormone synthesis, and in cholesterol catabolism. Ascorbic acid prevents oxidative modification of LDL primarily by scavenging free radicals and other reactive species in the aqueous milieu. It also regenerates other antioxidants like alpha-tocopherol from their respective radical species.

Ascorbic acid has been shown to improve endothelial function, insulin action and glucose metabolism, and to reduce blood pressure. It inhibits adhesions of leukocytes to the endothelium and enhances endothelium-dependent vasodilation and blood flow, thereby decreasing atherogenesis. Ascorbic acid inhibits the enzyme, aldose reductase, and reduces sorbitol accumulation in diabetics; it also has a protective effect against diabetic nephropathy. Ascorbic acid deficiency can result in capillary permeability,
poor wound healing, increased cholesterol levels, and immune suppression, and may contribute to diabetic complications.[1,14]

This study was designed to assess the plasma concentrations of thiamine, niacin, pyridoxine, and ascorbic acid in Nigerians with metabolic syndrome and in controls.

Materials and Methods

Subjects
The study was conducted in a tertiary hospital in one of the major cities in Nigeria. Approval was obtained from the ethical committee of the hospital. Metabolic syndrome subjects were selected from patients attending the General Outpatient and Metabolic Clinics of the hospital and control subjects were recruited from among the hospital workers.

An informed written consent was obtained from participants and questionnaires were administered to them. 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.[4,15] One hundred consenting subjects that satisfied the ATP (Adult Treatment Panel) III criteria for metabolic syndrome and 100 age- and sex-matched apparently healthy controls were selected to participate in this study.

Physical examination
Wearing light clothing, the waist circumferences of participants were measured with a flexible but non-stretchable tape just above the iliac crest to the nearest 0.5 cm.[10] Their weights and heights were measured with a bathroom weighing scale (Camry) and a stadiometer (Surgifriend). Wearing light clothing, the waist circumferences of participants were measured with a flexible but non-stretchable tape just above the iliac crest to the nearest 0.5 cm.[10]

The blood pressure was obtained from the right upper arm after ten minutes of rest and with the patient in a sitting position using a standard mercury sphygmomanometer (Accoson, England). Systolic blood pressure was taken to correspond to the appearance of Korotkoff sounds (Phase I) and diastolic blood pressure corresponded to the disappearance of Korotkoff sounds (Phase V). Two consecutive measurements were taken and the average of the two measurements was used.[15]

Sample collection
After an overnight fast, 10 mL 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 thiamine, nicotinamide, pyridoxine, and ascorbic acid. Plasma was separated from blood cells within 30 min of collection by centrifugation at 2500 rpm (revolutions per minute) for 15 min and transferred into plain bottles with Pasteur pipettes. Samples for all the assays were batch-analyzed; fasting plasma glucose was measured within 24 h of collection of blood. Samples for lipid profile, thiamine, nicotinamide, pyridoxine, and ascorbic acid analysis were stored frozen and analyzed within 2 weeks of collection.

Laboratory analysis
Plasma glucose concentration was determined using the glucose oxidase method (Randox kit).[16] Plasma triglyceride, total cholesterol, and HDL were determined using the enzymatic method of analysis (Randox kit).[16] The LDL concentration was calculated from the total cholesterol, the HDL, and the triglyceride concentrations according to the equation by Friedewald et al.[17] Plasma thiamine, nicotinamide, pyridoxine (PLP), and ascorbic acid were determined using reverse-phase high-performance liquid chromatography (HPLC) method (Agilent HPLC 1100 series). Randox quality control sample was included in every batch during the analysis of samples.[16]

Definitions
Metabolic syndrome was defined, according to the NCEP-ATP III (2001), by the presence of three or more of five criteria: blood pressure (BP) ≥130/85 mmHg, fasting plasma glucose (FPG) ≥6.1 mmol/L, waist circumference (WC) >102 cm in men and >88 cm in women, triglyceride (TG) ≥1.7 mmol/L, and HDL <1.0 mmol/L in men and <1.3 mmol/L in women.[10]

Vitamin B inadequacy (low status) was defined as vitamin concentrations below the lower limit of the reference intervals.[11] Vitamin B1 inadequacy was defined as thiamine levels <90 nmol/L,[10] vitamin B3 inadequacy as nicotinamide levels <0.9 µmol/L,[18] and vitamin B6 inadequacy as PLP levels <20 nmol/L.[9,12] Vitamin C deficiency, inadequacy, and optimal levels were defined as ascorbic acid levels <16.5 µmol/L, <23 µmol/L, and >50 µmol/L,[18] respectively.

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 and 100 controls between 22 and 78 years
were included in the study. Half of the subjects were businessmen and women, 30% were civil servants, and 20% were retired individuals. Controls were hospital workers who had more years of education and were better informed concerning healthy dietary and lifestyle habits. There was no statistically significant difference between the mean ages of metabolic syndrome subjects and controls (P = 0.082). Subjects were more obese, had higher blood pressure [Table 1], had higher fasting plasma glucose, triglyceride, LDL, and total cholesterol, and had lower HDL than controls [Table 2].

Of the 200 participants, 96 metabolic syndrome subjects consisting of 40 males and 56 females, and 96 controls consisting of 46 males and 50 females had values for all the water-soluble vitamins studied.

The mean plasma vitamin B1 of metabolic syndrome subjects was significantly lower (P = 0.001) than that of controls [Table 3]. Fourteen percent of subjects had inadequate vitamin B1 status [Figure 1]. None of the controls had vitamin B1 inadequacy.

The mean plasma vitamin B3 and vitamin B6 of metabolic syndrome subjects were significantly lower (P = 0.05 and 0.045) than that of controls, respectively [Table 3]. Concentrations of both vitamins were adequate in subjects as well as in controls.

The mean plasma vitamin C of metabolic syndrome subjects was significantly lower (P = 0.001) than that of controls. Vitamin C concentrations of subjects and controls were both below the optimal level [Table 3]. None of the subjects or controls had vitamin C deficiency, 32% of subjects had vitamin C inadequacy [Figure 1] but none of the controls had vitamin C inadequacy.

Mean vitamin C was higher (P = 0.012) and vitamin B6 lower (P < 0.001) in female subjects than in male subjects [Table 4], but their levels were similar in female and male controls. There were no gender differences in the concentrations of other vitamins.

**Discussion**

Recent studies have shown that low levels of thiamine, niacin, pyridoxine, and ascorbic acid are associated with increased insulin resistance, metabolic dysfunction, increased risk of atherosclerosis,[12] and conditions like obesity,[11,12,19] metabolic syndrome,[10,12] diabetes,[4,12,20] and cardiovascular disease.[11,12]

In this study, ascorbic acid, thiamine, niacin, and pyridoxine levels were lower in metabolic syndrome subjects than in controls. Ascorbic acid was also higher and pyridoxine lower in female than in male subjects but not in controls. There were no gender differences in the concentrations of thiamine and niacin in subjects or controls. There was no deficiency of any of the vitamins; however 14% and 32% of subjects had inadequate thiamine and ascorbic acid levels, respectively.

Inadequate dietary intake and lifestyle habits have been proposed as factors contributing to low status of water-soluble vitamins. Low intake of fruits and vegetables, important sources of water-soluble vitamins, has been described in metabolic syndrome.[10] A staple diet in Port Harcourt consists of gari and fufu, both derived from cassava (Manihot esculenta) roots, which are deficient in proteins, thiamine, niacin, and riboflavin.[5,6] Cassava also has a high content of toxic cyanogenic glycosides, most of which can be removed by a number of processing methods.[6] However, consumption of insufficiently processed cassava may result in chronic cyanide intoxication; thiamine can be inactivated by cyanide and its metabolites.[5] The average cyanide content of gari and fufu in Port Harcourt has been reported to be 25.4 mg/Kg and 20.0 mg/Kg, respectively, which is about twice the World Health Organization (WHO) safe level of 10 mg/Kg.[21]
Another staple diet in this area is rice, predominantly polished rice, which has a low thiamine and pyridoxine content, further reduced by washing the rice many times before cooking.[2] Most of the subjects consumed these staple diets at least five times in a week. They consumed fruits and vegetables on an average of three times in a week and in small quantities. Controls, on the other hand, consumed less of the staple diets and more fruits and vegetables. These dietary factors may have contributed to the low levels of these vitamins observed in this study.

These findings are similar to those from previous studies.[10,12] In a study by Ford et al., metabolic syndrome subjects consumed fewer fruits and vegetables and had lower concentrations of vitamin C than controls, though the use of vitamin and mineral supplements was similar between the two groups. However individuals using vitamin and mineral supplements were not included in this study.[10] Shen et al., observed that higher plasma PLP concentration was associated with higher intake of vegetables.[12]

Low concentrations of water-soluble vitamins could also be partly due to the metabolic abnormalities in metabolic syndrome.[4] The cellular uptake and renal reabsorption of ascorbic acid may be inhibited by hyperglycemia, resulting in a deficit of ascorbic acid in the cells and in plasma.[10]

Ascorbic acid is the most important aqueous phase chain-breaking antioxidant, and thiamine, niacin, and pyridoxine have their roles in reducing oxidative stress.[2,12-14] Oxidative stress has been shown to be increased in insulin-resistant conditions and is recognized to play a role in the pathophysiology of metabolic syndrome.[12,22] Measures of oxidative stress were not assessed in this study to determine if subjects with metabolic syndrome have higher levels of oxidative stress than those without metabolic syndrome, but previous studies have shown that hyperglycemia, advanced glycation end-products, hyperinsulinemia, and oxidized lipids, metabolic derangements that characterize metabolic syndrome, can generate reactive oxygen species.[22] Four of the five components of metabolic syndrome (obesity, hyperglycemia, hypertension, and hypertriglyceridemia) have been shown to be characterized by high oxidative stress.[10] Thus, it could be postulated that increased utilization of these vitamins in combating the increased burden of reactive oxygen species may have made some contribution to their low status in subjects with metabolic syndrome.

Inflammation is a component of metabolic syndrome. Markers of inflammation were not estimated in this study, but recent cross-sectional studies have shown that inflammation correlates inversely with reduced concentrations of water-soluble vitamins.[11,12] Asheim et al. and Shen et al. demonstrated that higher plasma CRP concentrations were associated with lower plasma vitamins B6 and C.[11,12] Inflammation may cause low levels of water-soluble vitamins as a result of decreased production of transport proteins like albumin, increased turnover of antioxidant vitamins or a shift in tissue distribution.[11] It has been suggested that inflammation may be the common link between low vitamin B6 and cardiovascular risk, independent of homocysteine.[12]

### Table 2: Fasting plasma glucose and lipid profile of subjects and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects (n=100) Mean±SD</th>
<th>Controls (n=100) Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>6.45±2.2</td>
<td>4.1±0.64</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.26±0.62</td>
<td>0.71±0.31</td>
<td>0.0001*</td>
</tr>
<tr>
<td>High density lipoprotein (mmol/L)</td>
<td>1.37±0.55</td>
<td>1.6±0.33</td>
<td>0.0003*</td>
</tr>
<tr>
<td>Low density lipoprotein (mmol/L)</td>
<td>2.93±0.97</td>
<td>2.28±0.82</td>
<td>0.01*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.87±1.14</td>
<td>4.21±0.91</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*Statistically significant (P<0.05)

### Table 3: Vitamin levels of subjects and controls

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Subjects (N=96) Mean±SD</th>
<th>Controls (N=96) Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 (nmol/L)</td>
<td>98.16±11.46</td>
<td>116.70±14.12</td>
<td>0.001*</td>
</tr>
<tr>
<td>B3 (µmol/L)</td>
<td>3.47±1.55</td>
<td>4.02±1.97</td>
<td>0.050*</td>
</tr>
<tr>
<td>B6 (nmol/L)</td>
<td>61.57±25.86</td>
<td>77.35±24.08</td>
<td>0.045*</td>
</tr>
<tr>
<td>C (µmol/L)</td>
<td>29.05±7.32</td>
<td>43.94±7.26</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Statistically significant (P<0.05)

### Table 4: Vitamin levels of male and female subjects

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Males (n=40) Mean±SD</th>
<th>Females (n=56) Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 (nmol/L)</td>
<td>120.09±15.79</td>
<td>114.04±12.29</td>
<td>0.147</td>
</tr>
<tr>
<td>B3 (µmol/L)</td>
<td>3.77±1.67</td>
<td>4.21±2.18</td>
<td>0.307</td>
</tr>
<tr>
<td>B6 (nmol/L)</td>
<td>71.08±25.86</td>
<td>49.20±28.60</td>
<td>0.001*</td>
</tr>
<tr>
<td>C (µmol/L)</td>
<td>27.48±7.36</td>
<td>44.26±7.30</td>
<td>0.012*</td>
</tr>
</tbody>
</table>

*Statistically significant (P<0.05)
Tissue distribution may also influence vitamin status. It has been hypothesized that obese patients may store a greater amount of body thiamine in cells with relatively lower plasma thiamine levels. Metabolic syndrome subjects in this study were more obese than controls. Aasheim et al. demonstrated that the greater the degree of obesity, the more pronounced the reductions in vitamins B6 and C concentrations. Increased total body water in obese patients may also lead to dilution of the extracellular compartment, further reducing the plasma vitamin concentration.

Gender differences in concentrations of water-soluble vitamins have been observed by some researchers. In one study that assessed the bioavailability of vitamin C from orange juice, baseline plasma levels of vitamin C were higher in women than in men. After daily ingestion of the orange juice for 7 days, the plasma levels of vitamin C were still higher in women. In this study, we also found baseline vitamin C levels to be higher in women than in men.

Some studies have reported lower levels of vitamin B6 in women than in men. In a study by Morris et al., plasma PLP was lower in women, particularly those of reproductive age, compared with men in a population that included both users and non-users of vitamin B6 supplements. It was also observed that most of the women who used oral contraceptives had biochemical deficiency of vitamin B6. They therefore suggested that estrogen may play a role in reducing plasma PLP concentrations in women. Findings from this study revealed higher PLP levels in male subjects. Eighty percent of body stores of vitamin B6 is in the muscles; therefore, higher muscle mass in men may have also contributed to the higher PLP levels in men.

Conclusion

This study established that ascorbic acid, thiamine, niacin, and pyridoxine levels were lower in metabolic syndrome subjects than in controls. Pyridoxine was also lower and ascorbic acid higher in female than in male subjects but not in controls. This low status of water-soluble vitamins could be as a result of metabolic abnormalities in the syndrome, coupled with dietary and other factors. On the other hand, low levels of these vitamins in the body may also worsen the metabolic abnormalities in metabolic syndrome and increase the risk of diabetes and cardiovascular disease.

Individuals with metabolic syndrome should be encouraged to increase their dietary intake of foods rich in water-soluble vitamins, particularly whole grains, fortified cereals, meat products, legumes (pulses, beans, and groundnuts), fruits, and vegetables. They should also be educated on how to improve food processing techniques in order to further reduce toxic substances and decrease loss of water soluble vitamins.

References


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