

## Research Article

# Glycemic control, micronutrients and some metabolic enzyme activity in type 2 diabetes

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## ABSTRACT

**Background:** Alterations in the metabolism of some essential micronutrients and activities of some metabolic enzymes have been reported in diabetes mellitus. These changes have been attributed to hyperglycemia and increased protein glycosylation associated with diabetes and seems to be more pronounced in poor glycemic states. The influence of glycemic control on serum levels of calcium (Ca), Iron (Fe), phosphorus (P), vitamin C, and lactate dehydrogenase (LDH) and amylase activity in type 2 diabetes were determined in this study.

**Methods:** Fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), serum calcium (Ca), Iron (Fe), phosphorus (P), vitamin C, LDH and amylase activity were determined in fifty type 2 diabetic subjects aged between 40-70 years and fifty age matched apparently healthy non-diabetic subjects in Calabar, Nigeria using colorimetric methods. Socio-demographic characteristics, anthropometric indices (body mass indices (BMI), waist to hip ratio (WHR)) and blood pressure were determined using standard methods. Data was analyzed using t-test, ANOVA and Pearson correlation analysis at  $p = 0.05$ .

**Results:** The FPG, HbA1c, serum vitamin C, Iron and calcium levels were significantly higher and serum phosphorus lower in diabetics than in non-diabetics. Diabetics with poor glycemic control (HbA1c >8.0%) have higher serum calcium and LDH activity compared to those with good glycemic control (HbA1c <8.0%).

**Conclusions:** Diabetes may alter the metabolism of vitamin C, Iron, calcium and phosphorus while poor glycemic control may be associated with changes in calcium and LDH activity.

**Keywords:** Diabetes, Micronutrients, Enzymes, Glycemic control

## INTRODUCTION

Micronutrients and minerals are known to play essential role in living systems, both in growth and in metabolism. Their deficiencies and excess in human physiologic system have been implicated in the aetiopathogenesis of several human diseases including diabetes. Impaired metabolism and alterations in the plasma concentrations of several essential elements has been reported in the diabetic state and may be involved in some of the metabolic dysfunctions associated with diabetes

mellitus.<sup>1</sup> Calcium ions play important role in glycemic control by affecting the biosynthesis and release of insulin from the beta cells of the pancreas. Hypocalcaemia has been associated with uncontrolled hyperglycaemia in patients with type 2 diabetes.<sup>2</sup> Abnormal regulation of intracellular calcium affecting both insulin sensitivity and release has been suggested as a possible mechanism to explain the association between calcium insufficiency and the risk of diabetes.<sup>3</sup> Studies have also shown that type 2 diabetics have higher phosphate levels than healthy controls with those with

poor glycemic control demonstrating elevated phosphate level with moderate increase in calcium levels compared to those with good control.<sup>4,5</sup> The actual mechanism underlying the relationship between phosphorus and carbohydrate metabolism and diabetes has not been elucidated, though lower levels of this element have been demonstrated in the diabetic state.<sup>6</sup> Iron has been shown to influence glucose metabolism, even in the absence of significant iron overload. Body iron stores have been positively associated with the development of glucose intolerance, type 2 diabetes, and gestational diabetes.<sup>7</sup> Pancreatic enzyme abnormalities have also been reported in diabetes mellitus. However, the molecular and cellular mechanisms of pancreatic amylase synthesis and release and the interaction between the diabetic endocrine and exocrine pancreas is less understood. Increases in the activity of amylase have been reported in poorly controlled diabetes.<sup>8</sup> LDH is a glycolytic enzyme predominantly found in the heart, liver and muscle tissues. Atherosclerosis, cardiomyopathy and liver dysfunction are frequently associated with diabetes; therefore changes in LDH activity may be seen in the diabetic state.

Hyperglycaemia is characterized by increased generation of glycosylation products which are associated with oxidative stress and subsequent development of various diabetic complications.<sup>9</sup> Interconnecting systems of antioxidant micronutrients (minerals) and enzymes are also present to accomplish the body's defence against oxidative stress. Vitamin C is structurally similar to glucose and can replace it in many chemical reactions, and thus is effective in prevention of non-enzymatic glycosylation of proteins. Studies have demonstrated increased oxidative stress, and decreased basal vitamin C and E levels in diabetic patients.<sup>10</sup> The interrelationship between micronutrients, antioxidant vitamins, pancreatic enzyme and the level of metabolic control is still uncertain, this work therefore assesses the influence of glycemic control on serum levels of calcium (Ca), Iron (Fe), phosphorus (P), vitamin C, and lactate dehydrogenase (LDH) and amylase activity in type 2 Diabetes.

## METHODS

### *Study design*

This case control study which examined the influence of metabolic control on serum levels of calcium, iron, phosphorus, vitamin C and lactate dehydrogenase (LDH) and amylase activities in Type 2 Diabetes was carried out at the Diabetic clinic of the University of Calabar Teaching Hospital (UCTH) Calabar, Cross River State Nigeria. This study was carried out in accordance with the Ethical Principles for Medical Research involving Human Subjects as outlined in the Helsinki Declaration in 1975 (revised in 2000). Subjects were selected based on the following criteria; age range between 40 and 70 years at the time of study, known type 2 diabetic patient

for the past one year, and non-diabetic according to the World Health Organisation diagnostic criteria for diabetes.<sup>11</sup> Subjects who were pregnant or hypertensive were excluded from the study. Diabetes in this study was defined based on laboratory findings as fasting plasma glucose levels greater than 7.00mmol/l in two or more occasions or 2-hour postprandial plasma glucose levels greater than 11.00mmol/l in two or more occasions.<sup>11</sup> Diabetes duration was defined as time since diagnosis of type 2 diabetes. Socio-demographic characteristics of the study population - family history, social history, past medical history, medication and gynaecological history, occupation, physical activity, life habit pattern such as smoking and alcohol consumption were obtained using a semi-structured questionnaire. Anthropometric indices - height, weight, hip and waist circumference were taken to calculate the body mass index and waist to hip ratio respectively. The blood pressure was also taken to rule out hypertension.

### *Selection of subjects*

Fifty consenting diabetic subjects aged between 40-70 years (32 males and 24 females) were consecutively recruited from known type 2 diabetic patients attending the Diabetic Clinic of University of Calabar Teaching Hospital and 50 age matched non-diabetic (28 males, 16 females) apparently healthy individuals living within Calabar and its environs who served as controls were recruited into this case control study. The objectives of the study were explained to the volunteers before recruitment into the study. Informed consent was sought and obtained from each subject before recruitment into the study. All subjects were advised to report to the clinic in the fasting state on the morning of the day for sample collection.

### *Sample collection*

Five millilitres of fasting venous blood samples were drawn from each subject aseptically by venepuncture. Two millilitres was dispensed into fluoride oxalate container for fasting plasma glucose estimation, 1ml was transferred into K<sub>2</sub>EDTA container for glycosylated haemoglobin estimation, the remaining samples were dispensed into plain sample bottles, allowed to clot and retract and then centrifuged at the rate of 3,000 revolution per minute to extract the sera for calcium (Ca), Iron (Fe), phosphorus (P), vitamin C, lactate dehydrogenase (LDH) and amylase activity estimation.

### *Methods*

#### *Blood pressure*

The systolic and diastolic blood pressures of subjects were taken at 3 intervals one month prior to sample collection. This is to rule out hypertension. The pressure was also taken on the day of the test in a seated relaxed position. Anthropometric data; body weight and height

were measured while subjects wore light indoor clothing but no shoes. This was used to calculate the body mass index (BMI), which is used as a measure of relative body weight. Normal body weight was defined as BMI between 18-25 kg/m<sup>2</sup> while generalised obesity was defined as BMI >30 kg/m<sup>2</sup>.<sup>12</sup>

#### *Glycemic control*

Measurement of glycated haemoglobin was used as index of glycaemic control. Good glycaemic control was defined as HbA1c <7.0% while poor glycaemic control was defined as HbA1c >8.0%.<sup>13</sup>

#### *Glucose estimation using Glucose Oxidase method of Barham and Trinder.*<sup>14</sup>

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye indicator. The absorbance of dye is proportional to the concentration of glucose in the sample.

#### *Glycohaemoglobin estimated using column chromatography method with cation exchange resin of Triveli et al.*<sup>15</sup>

Glycohaemoglobin was estimated using column chromatography method with cation exchange resin. A haemolysed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin during this time, HbA<sub>1c</sub> binds to the resin. After the mixing period, a filter is used to separate the supernatant containing the glycohemoglobin from the resin. The percent glycohemoglobin is determined by measuring the absorbance at 405nm of the glycohemoglobin fraction and the total haemoglobin fraction. The ratio of the two absorbances gives the percentage glycohemoglobin.

#### *Amylase estimation using the amyloclastic method of Caraway.*<sup>16</sup>

Amylase is allowed to act on a starch substrate to which iodine has been attached. As amylase hydrolyses the starch molecules into smaller units, the iodine is released, and a decrease in the initial dark blue colour intensity of the starch-iodine complex occurs. The decrease in colour is proportional to the amylase concentration in the sample.

#### *Calcium estimation using Modified O-cresolphthalein – complexone method by Stern and Lewis.*<sup>17</sup>

Calcium in serum reacts with O-cresolphthalein complexone in an alkaline medium to form a purple coloured complex. The absorbance of this complex is proportional to the calcium concentration in the sample.

#### *Iron estimation using modified Chromazurol B method of Alessandro et al.*<sup>18</sup>

Iron (iii) reacts with chromazurol B (CAB) and Cetyltrimethylammonium bromide (CTMA) to form a coloured complex with an absorbance maximum at 620nm. The intensity of the colour produced is directly proportional to the concentration of iron in the sample.

#### *Phosphorus estimation using ammonium molybdate method of Young.*<sup>19</sup>

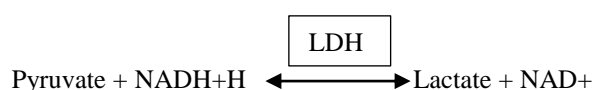
Phosphorus present in the sample combines with reagent Ammonium Molybdate in presence of strong acids to form phosphomolybdate. The absorbance of this complex is directly proportional to the phosphorus concentration in sample.

#### *Estimation of vitamin C using the modified reduction method of Roe and Kuether.*<sup>20</sup>

Ascorbic acid is converted to dehydro-ascorbic acid by shaking with Norit activated charcoal and this is then coupled with 2, 4 – dinitrophenyl hydrazine in presence of thiourea as a mild reducing agent. In the presence of sulphuric acid, dinitrophenyl hydrazone which is a red compound is assayed colorimetrically. The absorbance of coloured compound is proportional to Vitamin C concentration in the sample.

#### *Estimation of lactate dehydrogenase using modified method based on the recommendations of the Scandinavian Committee on enzyme.*<sup>21</sup>

Lactate dehydrogenase catalyses the interconversion of lactic and pyruvic acids, a hydrogen transfer enzyme that utilizes the coenzyme NAD<sup>+</sup>.



#### **Statistical analysis**

Data analysis was done using the statistical package for social sciences (SPSS version 20.0). Student's t-test analysis was used to determine mean differences between variables. Analysis of variance (ANOVA) was used to test significance of variations within and among group means and Fisher's least significant difference (LSD) post hoc test was used for comparison of multiple group means. Pearson correlation analysis was employed to determine relationship between variables. A two sided probability value p<0.05 was considered statistically significant.

#### **RESULTS**

The mean age, body mass index (BMI), systolic and diastolic blood pressure (Sys. & Diast. B.P), waist to hip

ratio (WHR ratio), fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), calcium (Ca), iron (Fe), phosphorus (Phos), Vit C, amylase (Amyl) and lactate dehydrogenase activity (LDH) in diabetics and non-diabetics. Significantly increased levels of FPG, HbA1c, Ca, Fe, Vit C and lower phosphorus levels were seen in type 2 diabetics compared to non-diabetic controls ( $p < 0.05$ ), were shown in Table 1. No significant differences were seen in the levels of other indices in both groups studied ( $p > 0.05$ ).

**Table 1: Mean Age, BMI, B.P (Sys. & Diast.), Waist/Hip Ratio (WHR), Fasting Plasma Glucose (FPG), Glycated Haemoglobin (Hba1c), Calcium (Ca), Iron (Fe), Phosphorus (Phos) and Vitamin C (Vit C) levels, amylase (Amyl) and lactate dehydrogenase (LDH) Activity in diabetics and Non-diabetics.**

Index	Diabetics (n = 50)	Non-Diabetics (n = 50)	p – value
Age (years)	54.54 ± 10.09	55.08 ± 10.78	0.789
BMI (kg/m <sup>2</sup> )	25.43 ± 4.79	24.34 ± 3.02	0.731
WHR ratio	0.83 ± 0.05	0.81 ± 0.05	0.092
Sys. B.P (mmHg)	128.60 ± 8.57	127.80 ± 9.10	0.652
Dias.B.P (mmHg)	82.80 ± 10.50	82.20 ± 11.11	0.782
FPG (mmol/l)	7.67 ± 3.25	4.52 ± 0.15	0.000*
HbA1c (%)	9.99 ± 3.33	5.05 ± 1.07	0.000*
Ca (mmol/l)	2.46 ± 0.02	2.32 ± 0.01	0.000*
Fe (µmol/l)	24.34 ± 1.77	16.44 ± 0.82	0.000*
Phos (mmol/l)	1.20 ± 0.05	1.82 ± 0.06	0.000*
Vit C (mg/dl)	9.39 ± 0.60	6.73 ± 0.25	0.000*
Amyl (IU/L)	239.44 ± 39.51	235.49 ± 29.64	0.231
LDH (IU/L)	267.98 ± 18.24	245.82 ± 18.86	0.520

\* = Significant at  $p < 0.05$ , BMI = body mass index, B.P = blood pressure, Sys. B.P = systemic blood pressure, Diast B.P = diastolic blood pressure

Table 2 shows the effect of gender and age on biochemical indices in all subjects studied. Male subjects have higher phosphorus levels compared to the female counterparts ( $p < 0.05$ ). No significant differences were observed in the levels of other indices studied in both gender ( $p > 0.05$ ). Increasing Ca levels and decreasing phosphorus levels were observed with increasing age in all subjects of the study ( $p < 0.05$ ). No significant

differences were seen in the levels of other indices with increasing age ( $p > 0.05$ ).

The effect of glycemic control and duration of diabetes on biochemical indices in type 2 diabetics studied were shown in table 3. Increased FPG, Ca and LDH activity was observed in diabetics with poor metabolic control compared to those with good control ( $p < 0.05$ ). No significant differences were observed in the levels of other indices in relation with metabolic control. The Vit C level was significantly higher in diabetics of  $\geq 5$  years duration compared to those of less than 5 years duration ( $p < 0.05$ ). No significant differences were observed in the levels of other indices in relation with duration of diabetes ( $p > 0.05$ ).

## DISCUSSION

Hyperglycaemia, increased protein glycosylation and poor metabolic control states have been implicated in alterations in the metabolism of various biochemical parameters in diabetes. In this study, diabetics had significantly increased levels of fasting plasma glucose, glycated haemoglobin, calcium, iron and vitamin C and decreased levels of phosphorus compared to non-diabetic controls. Higher FPG and HbA1c is the result of insulin deficiency, insulin resistance or both and increase protein glycation associated with diabetes mellitus.<sup>22,23</sup> Studies have shown that increased glycosylation of membrane protein is associated with hyperglycaemia, the rate of glycation being proportional to the concentration of glucose in the cell.<sup>23</sup> Protein glycation has been implicated in the development of various diabetic complications.

Increased calcium levels were observed in type 2 diabetics and those with poor metabolic control compared to non-diabetic controls. Previous studies have reported that serum total calcium levels are higher in individuals with diabetes than in those without. An increase in serum calcium concentrations have been associated with an increased risk of type 2 diabetes in individuals at high cardiovascular risk.<sup>24</sup> Insulin secretion is a calcium-dependent biological process as it is required for both the first and second phase of insulin secretion, increasing cytosolic calcium has been associated with an increase in the expression of GLUT4 transporters in the myocyte, which, in turn, increases the insulin-stimulated glucose transport activity in these cells.<sup>25</sup> Since hyperinsulinaemia is the predominant finding in type 2 diabetes, this therefore explains the increased calcium levels seen in the diabetic state. However, hyperglycaemias was not associated with changes in serum calcium concentration in a cross section of Sudanese diabetics.<sup>5</sup> Increased serum total calcium levels were seen in diabetics with poor metabolic control compared to those with good control. This observation was consistent with previous studies.<sup>26</sup> Contrary to our findings; Bellan et al did not demonstrate such differences in calcium levels in relation to metabolic control.<sup>27</sup>

Serum vitamin C levels of diabetics were significantly higher than those of non-diabetic population of the study. Data on medication shows that majority of diabetics (80%) recruited into this study take vitamin C supplements in combination with oral hypoglycaemic agents. Higher dietary vitamin intake and supplementation has been associated with higher serum levels.<sup>28</sup> High but physiologic concentrations of ascorbic acid can directly inhibit erythrocyte aldose reductase thus preventing intracellular accumulation of sorbitol, or its sequelae, thereby inhibiting the progression of chronic

diabetic complications; this is the rationale for the use of oral vitamin C supplements in diabetes.<sup>9</sup> Vitamin C as a result of its structural similarity to glucose can compete with it to reduce haemoglobin glycosylation in patients with type 2-diabetes. The reduction of glycosylated haemoglobin can reflect the decrease of total glycosylation of proteins.<sup>29</sup> Ascorbic acid supplementation for diabetic subjects may therefore provide a simple means of preventing and ameliorating the complications of diabetes.

**Table 2: Effect of gender and age on biochemical indices in all subjects.**

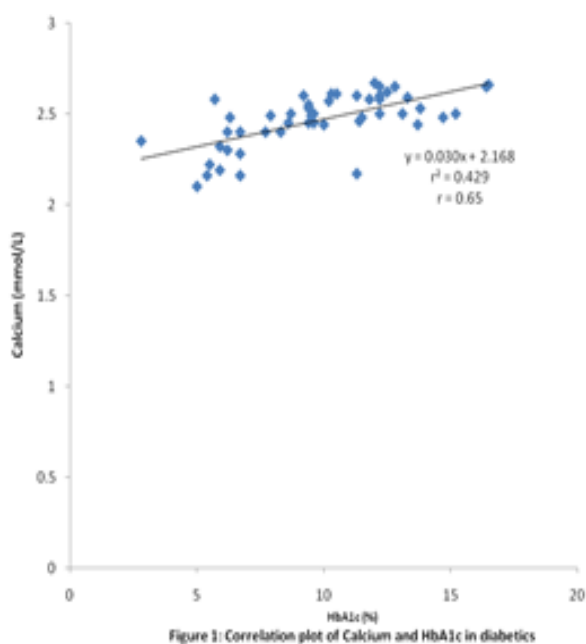
Factor	Sys. B.P (mmHg)	Diast.B.P (mmHg)	HbA1c (%)	Ca (mmol/l)	Fe (µmol/l)	Phos (mmol/l)	Vit C (mg/dl)	Amyl (U/l)	LDH (UI)
<b>Gender</b>									
Males n = 60	128.00 ±1.17	82.17 ±1.41	7.21 ±0.46	2.39 ±0.02	19.93 ±1.47	1.64 ±0.48	7.69 ±3.23	215.72 ±52.87	263.61 ±13.50
Females n = 40	128.50 ±1.36	83.00 ±1.68	7.97 ±0.53	2.41 ±0.02	21.07 ±1.45	1.30 ±0.46	8.60 ±3.90	204.76 ±61.02	246.83 ±11.12
p value	0.761	0.705	0.285	0.338	0.585	0.001*	0.220	0.387	0.340
<b>Age (yrs)</b>									
40-49 n = 46	127.60 ±1.36	81.30 ±1.26	6.49 ±0.45	2.35 ±0.12	21.50 ±1.75	1.74 ±0.08	7.48 ±0.48	208.27 ±54.96	277.94 ±16.91
50-59 n = 21	130.00 ±1.95	81.90 ±2.54	8.99 ±0.87	2.42 ±0.17	18.48 ±2.20	1.32 ±0.07	8.26 ±1.00	214.28 ±56.57	225.74 ±12.25
≥ 60 n = 33	127.87 ±1.43	84.54 ±2.22	8.01 ±0.57	2.45 ±0.12	20.04 ±1.52	1.27 ±0.07	8.97 ±0.53	212.49 ±59.17	247.39 ±11.75
F ratio	0.559	0.910	2.639	4.336	0.614	11.721	1.401	0.100	2.646
p value	0.573	0.406	±.077	0.016*	0.543	0.000*	0.251	0.905	0.076

\*= Significant at p<0.05

**Table 3: Effect of glycemic control and duration of diabetes on biochemical indices in type 2 diabetics.**

Glycemic State	FPG (mmol/l)	Ca (mmol/l)	Fe (µmol/l)	Phos (mmol/l)	Vit C (mg/dl)	Amylase (U/l)	LDH (UI)
<b>Good control</b>							
HbA1c <8.0% n = 15	6.33 ±1.49	2.32 ±0.13	26.05 ±2.12	1.21 ±0.10	8.35 ±4.88	190.96 ±60.72	223.59 ±18.85
<b>Poor control</b>							
HbA1c >8.0% n = 35	8.24 ±3.62	2.53 ±0.01	23.60 ±2.38	1.19 ±0.05	9.81 ±3.96	218.46 ±57.70	287.00 ±24.23
p value	0.010*	0.000*	0.446	0.876	0.317	0.149	0.045*
<b>Duration</b>							
<5 yrs n = 33	7.88 ±1.16	2.47 ±0.02	23.51 ±2.04	1.17 ±0.06	8.38 ±4.16	209.22 ±61.59	278.21 ±24.39
≥5 yrs n = 17	7.31 ±1.87	2.45 ±0.04	25.93 ±3.47	1.24 ±0.07	11.31 ±3.87	212.14 ±56.61	248.11 ±25.53
p value	0.28	0.578	0.553	0.502	0.019*	0.868	0.399

\*= Significant at p<0.05



**Figure 1: Correlation plot of Calcium and HbA1c in diabetics.**

The serum iron levels were significantly higher in diabetics when compared with those of non-diabetics studied. Increased serum iron levels have been reported in diabetes.<sup>30</sup> Insulin is known to cause a rapid and marked stimulation of iron uptake by fat cells, redistributing transferrin receptors from an intracellular membrane compartment to the cell surface. Insulin has also been shown to be responsible for the increased ferritin synthesis in cultured rat glioma cells. Transferrin receptors have been shown to colocalize with insulin-responsive glucose transporters and insulin-like growth factor II receptors in the microsomal membranes of cultured adipocytes, suggesting that regulation of iron uptake by insulin occurs in parallel with its effects on glucose transport.<sup>7</sup> Therefore hyperinsulinaemia associated with type 2 diabetes will therefore induce a corresponding increase in iron uptake leading to increased serum iron seen in diabetics studied. A causal relationship have been demonstrated between excess iron and increased diabetes risk, excess iron even within the “normal” range has important detrimental effects on insulin secretion, insulin sensitivity, adipokine levels, and metabolic flexibility.<sup>31</sup> Iron is intimately linked to oxidative stress. Iron in its free form i.e., in non-transferrin bound form is known to induce oxidation of biomolecules through Heber-Weiss and Fenton reactions by producing harmful hydroxyl radicals and superoxide anion, which are capable of inducing lipid peroxidation.<sup>30,7</sup> High iron levels has been linked to oxidative damage to DNA, lipids, and proteins that in turn has been implicated in the development of atherosclerosis and cardiovascular disease in diabetes.<sup>32,33</sup>

Lower phosphorus levels were seen in diabetics compared to the controls. Previous studies also reported

decreased phosphorus levels among diabetics in comparison to control group. Changes in serum phosphate level have been related with severity of diabetes mellitus.<sup>5,34</sup> Early in the progression of diabetes, a paradoxical metabolic imbalance in inorganic phosphate occurs that may lead to reduced high energy phosphate and tissue hypoxia.<sup>35</sup> These changes take place in the cells and tissues in which the entry of glucose is not controlled by insulin, particularly in poorly regulated diabetes patients in whom long-term vascular complications are more likely.<sup>34</sup> Mechanisms involved in this disturbance in inorganic phosphate metabolism in diabetes may include; alteration in the homeostatic function of the kidney as a result of hyperglycaemia, the ensuing glycosuria may induce depolarization of the brush border membrane for reabsorption of inorganic phosphate and lead to lack of intracellular phosphate and hyperphosphaturia.<sup>35</sup> Secondly, during hyperglycemic-hyperinsulinemic intervals, high amounts of glucose enter muscle and fat tissues, which are insulin sensitive. Intracellular glucose is metabolized by phosphorylation, increased phosphorylation during this interval, may lead to a reduction in plasma phosphate levels, and subsequent deleterious effects on glucose metabolism in insulin insensitive tissues.<sup>34</sup> Males seem to have higher inorganic phosphate levels compared to the females of the study population, while increasing age was associated with decreasing phosphate levels. It has been shown that the concentration of plasma ionized phosphates (Pi) and renal tubular reabsorption of Pi are similarly closely related to age and gender, with the highest values occurring in childhood.<sup>35</sup> Contrary to our findings, plasma Pi in men was shown to decline with age almost linearly to the eighties, whereas, in women under the age of 45, the values overlap those of men and then increase between 45 and 54 years before declining thereafter. Thus, during aging, hypophosphatemia may also be associated with a decreasing amount of intracellular tissue Adenosine triphosphate (ATP).<sup>36</sup>

Increased serum lactate dehydrogenase activity was seen in diabetics with poor metabolic control compared to those with good control. This agrees with the findings of who also reported same.<sup>37</sup> Diabetes is associated with  $\beta$  cells dysfunction and alteration in insulin signalling which results in inefficient glucose uptake by muscle and adipose tissue, insulin resistance and compensatory hyperinsulinemia.<sup>38</sup> Persistent hyperinsulinemia might trigger endothelial dysfunction, atherosclerosis and development of cardiac dysfunction. These dysfunctions are accelerated in poor metabolic control. Elevated LDH activity seen in diabetic with poor glycemic control may originate from the heart and may therefore herald the onset of diabetes associated cardiac dysfunction. Elevated LDH activity was demonstrated in diabetic patients compared to normal individual by Sreenivasan et al.<sup>39</sup> The increased enzyme activity seen diabetes has also been attributed to the influence of insulin on liver and muscle tissues. Muscle and liver dysfunction is frequently associated with diabetes, so serum enzyme activities

derived from muscle and liver may also be contributing to increased LDH activity in diabetes. However, decreased LDH activities have been reported in both elderly diabetics and non-diabetics.<sup>39</sup> Oliver et al.<sup>40</sup> did not observe any increases in LDH activities between diabetics and non-diabetic controls

The findings of this study has shown that the diabetic state may influence the metabolism of vitamin C, Iron, calcium and phosphorus while poor metabolic control may be associated with changes in calcium and LDH activity. Increasing duration of diabetes seems to be associated with corresponding increases in Vitamin C levels while ageing seems to associated with increasing Ca and decreasing phosphorus levels.

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*Ethical approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards*

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