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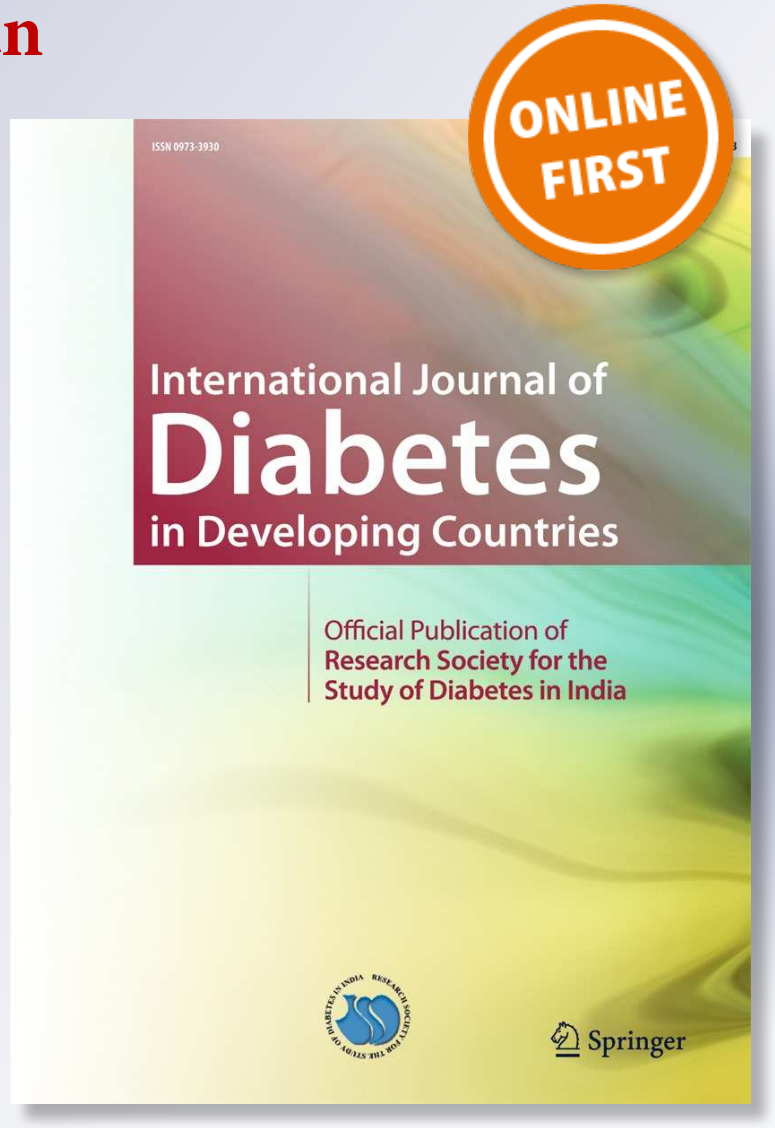

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ORIGINAL ARTICLE

Evaluation of serum metallothionein-1, selenium, zinc, and copper in Ghanaian type 2 diabetes mellitus patients

George Awuku Asare · Shiloh Osae · Ezekiel Nii Noye Nortey · Faisal King Yambire · Elsie Amedonu · Derick Doku · Yvonne Annan

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Abstract Metabolic disturbances of trace elements may be implicated in the complications of type 2 diabetes mellitus (T2DM). The aim of the study was to determine the level of Zinc (Zn), Selenium (Se), Copper (Cu) and the metal binding protein Metallothionein-1 (MT-1) in T2DM. Fifty-five (55) T2DM subjects and 30 Controls (C) were studied for, Se, Zn, Cu and MT-1. Zn, Se and Cu were analyzed using Flame Atomic Absorption Spectroscopy. Mean FBG in the T2DM and C groups were 183±5 mg/dl and 88±5 mg/dl, respectively. Mean Se, Zn and Cu levels in the T2DM group were 204±91 μg/l, 407±117 μg/l and 1,337±527 μg/l, respectively. The control group had Se, Zn and Cu levels of 123±25 μg/l, 750±190 μg/l and 989±197 μg/l, respectively. While Zn levels in T2DM were half that of the C, Se levels were≈2-fold. Se, Zn and Cu differences between the two groups were statistically significant (P=0.000; P=0.000, P=0.000, respectively). The metabolic derailment of MT-1 in the T2DM group showed a wide variation with the T2DM having significantly lower MT-1 values (P=0.000). A negative correlation was seen between Cu and Zn in the T2DM group (P=0.022). A standardized canonical discriminant function was obtained as D= 0.823*FBG−0.149*MT−0.457*Zn+0.172*Cu+0.362*Se with contributions of FBG>Zn>Se>Cu>MT-1. In conclusion, alterations in the levels of Zn, Se and Cu were observed in Ghanaian T2DM patients.

Keywords Diabetes · Oxidative stress · Trace elements · Ghanaian

Introduction

The prevalence of diabetes in Ghana was reported to increase from 2.3 % in the early 1990's to 6.3 % in the year 2002 [1]. Diabetes is accompanied by left ventricular (LV) hypertrophy (LVH), LV dysfunction, and coronary artery disease [2] by mechanisms still needing elucidation. The valence states of Zn, Cu and Se present them as perfect candidates for organ toxicity. Therefore, the homeostasis of these trace elements is a tightly coordinated and regulated system by different proteins involved in their uptake, utilization and excretion. Altered levels of Zn, Cu and Se are implicated in the pathogenesis of several diseases including DM [3]. Thus, Cu deficiency has been implicated in cardiac disease [4]. A chronic intracellular Cu overload, e.g., Wilson's disease may not be associated with heart disease [5].

Oxidative stress may be responsible for the development of diabetes and its complications such as Alzheimer's disease and Parkinson's disease and which occur in DM may also be related to reactive oxygen species (ROS) resulting from oxidative stress [6], cell death and necrosis [7]. ROS attack lipid biomembranes by lipid peroxidation and diabetes-induced-susceptibility to lipid peroxidation [8] which can lead to atherosclerosis [9].

To protect against ROS, humans have lead to evolve an antioxidant protection system (APS) which consists of a

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variety of endogenous and exogenous components [10] that includes metal binding proteins such as metallothionein (MT), ferritin; nutrient derived antioxidants like vitamins C, E, carotenoids; low molecular weight compounds such as glutathione, lipoic acid; and antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase.

Metallothioneins are a superfamily of small proteins which are potent antioxidants [11]. They are cysteine-rich, low molecular weight proteins that bind metals such as zinc (Zn), copper (Cu) and selenium (Se) [12]. Cysteine residues from MTs can capture oxidant radicals like the superoxide and hydroxyl free radicals [13]. In this reaction, cysteine is oxidized to cystine, and the metal ions bound to cysteine are liberated. This mechanism has been proposed to be an important mechanism in the control of oxidative stress by MTs. Furthermore, the role of MTs in oxidative stress has been confirmed by MT knockout mutant mice [14]. Finally, Zn can activate the synthesis of more MTs and Zn supplementation in diabetics can induce MT synthesis in the pancreas to reduce the extent of the disease and its complications [15].

Trace elements which are essential were shown to be for metabolism, growth, neurological and immune functions [15, 16] altered in DM [17, 18]. Cu, a cofactor of several metalloproteins is essential for oxidative metabolism, myelination and the metabolism of steroid hormones [19]. Superoxide dismutase (SOD) is a copper metalloenzyme. MT synthesis is also controlled by Cu-responsive transcription factors [20].

Se is a constituent of glutathione peroxidase (GPx) related to vitamin E in its functions [21]. Selenoprotein P, a major Se-containing protein in plasma, has both antioxidant and transport functions [22].

Zn, binds to SOD and ensures its structural stability [23, 24]. Also the binding of Zn to the "metal response element-binding transcription factor" (MTF1) activates MT expression and levels fall in zinc deficiency [24].

The aim of the study therefore was to determine the level of MT-1 (a member of the MT super family) in type 2 diabetes and any possible Zn, Se, Cu dysmetabolism.

Materials and methods

The study conformed to the Helsinki Declaration on Human Experimentation of 1975, revised in 1985 and 1989. The proposal was approved by the University of Ghana School of Allied Health Sciences Academic and Ethics Review Committee. Individual patient consent was sought before commencement.

The study was conducted at the National Diabetes Management and Research Centre (NDMRC), Korle-bu Teaching Hospital (KBTH) and the Ridge Hospital (Accra).

Subjects

85 subjects age range 40–65 years, took part in this study. Fifty-five (30 males and 25 females) had diabetes and 30 were non-diabetic, (18 males and 12 females). They had the disease for less than 12 months. The mean age of the diabetics in this study was 51.3±4.7 years and that of the controls was 49.3±5.4 years.

A commercial ELISA kit from USCN Life Sciences Inc., (Wuhan, China) was used according to the manufacturer's instructions. Microtiter plates pre-coated with human MT-1 antibodies was used. The principle behind the assay was the double sandwich technique. After 2 h of incubation at 37 °C with 100 µl of samples, standards and controls, the excess was removed without washing to bring the plate to near dryness. Plates were further treated with 100 µl MT-1 antibodies and incubated for 1 h at 37 °C. After washing, 100 µl of a detection solution was added and incubated further at 37 °C for 30 min. The plate was washed to dryness and 90 µl of substrate was added. This was incubated at 37 °C in the dark for 20 min. 100 µl of the stop solution was then added. The absorbance was read at 450 nm on an ELISA plate reader.

Trace elements determination

Microwave digestion of samples for Flame Atomic Absorption Spectroscopy (FAAS)

Digestion of the samples was done to only the trace elements to be analyzed. 1 ml of the serum was weighed into TFM Teflon Vessels of a microwave digester (Milestone ETHOS 900).

6 ml of 69 % HNO3 (no. 84385) (Fluka, Germany) and 1 ml of 30 % H_2O_2 (FW 34.1) (Sigma, Germany) were then added. The HNO_3 had density of 1.41 g/mL at 20 °C; presence of trace elements were minute and as follows: Cl <0.3 mg/kg, PO4≤0.01 mg/kg, SO_4≤50 mg/kg, Se 0.05 ug/kg, Cu< 0.05 ug/kg, Zn<1 ug/kg among other trace elements. The vessels were swirled gently to mix well and fitted vertically into the microwave digester and digested for 25 min following which they were cooled in a water bath for 10 min. The contents were transferred into a volumetric flask and diluted to 20 ml using distilled water. Distilled water blank and a reagent blank were prepared in a similar fashion with distilled water and reagent only, respectively, but without the analyte. All samples were analyzed at the same time using VARIAN AA240FS Flame Atomic Absorption Spectrometer along with standards of high purity metals from Teknolab AS (Kungsbacka, Sweden) and reference material Seronom™ Trace Elements Serum L-1, L-2 (Billingstad, Norway) to assay Zn, Se and Cu quantitatively after microwave digestion of the samples. Blank samples were also digested along with each set of samples and subsequently analyzed for appropriate elements

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through the same procedure. For Se, FAAS was used in conjunction with hydride generation.

The analytical conditions adopted for the analyses of the elements are listed in Table 1.

Method validation

Accuracy was validated through the analysis of laboratory matrix spikes, certified reference materials and blank samples. For Zn, three standards, 0.25, 0.50 and 1.00 mg/L were used. For Cu, 2.00, 5.00 and 10.00 mg/L standards were used. Se standards, 0.020, 0.040 and 0.060 mg/L were employed. These standards were added to samples in triplicates. The spiked samples were then treated similarly as the samples and the mean concentrations used for the recovery calculations. Linearity was also determined at the interval of 0.0–1.0 mg/L (Zn), 0.0–10.0 mg/L (Cu) and 0.0–0.06 mg/L (Se). The regression equations and correlation coefficients for Zn, Cu and Se were determined.

Statistical analyses

The statistical analyses of the data was done using SPSS (Statistical Package for Social Sciences) version 17.0. Means ± SD were determined for quantitative variables. The unpaired *t*-test was used for the comparison of serum FBG, MT-1, Zn, Cu and Se levels between the diabetics and non-diabetics. Analysis of variance (ANOVA) was used to determine the existence of statistical significance between variables possibly with more than two outcomes. Pearson's correlation was used to test the relationship between FBG, the trace elements and MT-1 levels. *P*-values ≤0.05 were considered statistically significant.

Results

Linear dependence absorbance at 213.9 nm on the concentration of Zn was obtained at the interval of 0.0–1.0 mg/L. Similarly, linearity for Cu and Se at 327.4 nm and 196.0 nm, respectively, were obtained at 0.0–10.0 mg/L and 0.0–0.06 mg/L, respectively. The regression equations are seen in Table 2. The good linearity of the calibration curve and minimum scatter of experimental points are evidenced by the high correlation coefficients in the order of 0.99–1 (Table 2).

The limit of quantification (LOQ) was determined by establishing the lowest concentration that could be measured with acceptable accuracy and precision. Zn, Cu and Se could therefore be quantified at a concentration of 0.00330 mg/L, 0.00332 mg/L and 0.00330 mg/L, respectively (Table 2).

The percentage recovery for Zn, Cu and Se was>97.8 %, > 99.75 % and>96.67, respectively. Furthermore, relative deviation for Zn ranged from 0.2 to 2.2 %. For Cu, the relative deviation ranged from 0.02 to 0.25 % and for Se, the relative deviation was 2.5–5 %. Good precision between 0.4 % and 0.6 % was obtained for all three elements. The repeatability of the method was fairly high as indicated by low values of relative deviation (Table 3).

The means of plasma fasting blood glucose (FBG), Zn, Se, Cu and serum MT-1 concentrations in the diabetics were 183± 5 mg/dL, 407±117 μg/L, 204±91 μg/L, 1,337±527 μg/L and 16.9±9.7 ng/mL, respectively. The control group had a mean FBG of 88±5 mg/dL, that was slightly less than half that of the diabetes. Zn, Se and Cu for controls were 750±190 μg/L and 123±25 μg/L, and 989±197 μg/L, respectively. The control group's MT-1 was 22.3±8.0 ng/ml.

The ages between the two groups were not significantly different ($P>0.05$). However, Cu, Zn, Se, FBG and MT-1 were significantly different between the diabetic group and the control group ($P=0.000$; $P=0.000$; $P=0.000$; $P=0.000$ and $P=0.000$, respectively) (Figs. 1, 2, 3, 4, and 5).

In finding out how well these analytes could discriminate between the test and the control groups, a standardized canonical discriminant function was obtained as

$$D = .823^{*}FBG - .149^{*}MT - .457^{*}Zinc + .172Copper + .362Selenium$$

Table 1 Analytical Conditions of Elements in flame atomic absorption spectrophotometry

Element	Lamp current (mA)	Wavelength (nm)	Slit width (nm)	Fuel gas	Oxidant
Zn	5	213.9	0.1	Acetylene	Air
Cu	4	327.4	0.1	Acetylene	Air
Se	10	196.0	1.0	Acetylene	Air

Table 2 The linearity of Zn, Cu and Se together with the limits of detection (LOD) are shown. Furthermore, the range of detection and the degree of correlation have been shown as spectrophotometrically determined

Trace element	Linear range (mg/L)	*Limit of detection (LOD) (mg/L)	*Limit of quantification (LOQ) (mg/L)	Calibration curve	R^2
Zn	0-1.0	0.001	0.0033	y=0.6648x +0.0061	0.9987
Cu	0-10	0.001	0.00332	y=0.1506x −0.0216	0.9981
Se	0-0.06	0.001	0.0033	y=1.3445x +0.001	1

*. The blank was measured 20x and the Standard Deviation (SD) was generated. LODs and LOQs were calculated according to the following formulae: LOD=3(SD/Gradient of Curve); LOQ=10(SD/Gradient of Curve)

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Table 3 Concentrations of Zn, Cu and Se standards with relative deviations are shown. The precision of the method used is reported ranged from 0.4 % to 0.6 %. The table further shows accepted recoveries of the trace elements

Trace element	Standards mg/L	Measured value	% Recovery	Relative deviation of recovery (%)	*Precision (%)
	0.25	0.245	98	2	
Zn	0.50	0.501	100.2	0.2	0–0.4
	1.00	0.978	97.8	2.2	
	2.00	1.995	99.75	0.25	
Cu	5.00	5.001	100.02	0.02	0–0.6
	10.00	9.978	99.78	0.22	
	0.020	0.021	105	5.00	
Se	0.040	0.039	97.5	2.50	0–0.47
	0.060	0.058	96.67	3.33	

*. Precision was determined as a read-out from the computer programme inter-phased with the Varian AA240FS

whose covariance structure matrix with each of the compounds is found in Table 4. From Table 4, it can be observed that FBG contributed the largest share to the discriminant function, followed by Zn, Se, Cu and MT-1 in that order. Both Zn and MT-1 have an inverse relationship with the discriminant function. However, FBG, Se and Cu are directly related with the discriminant function.

From the plot of Fig. 6, the control group encircled by a firm line was highly distinguishable from the test group (encircled by a broken line). The control group was

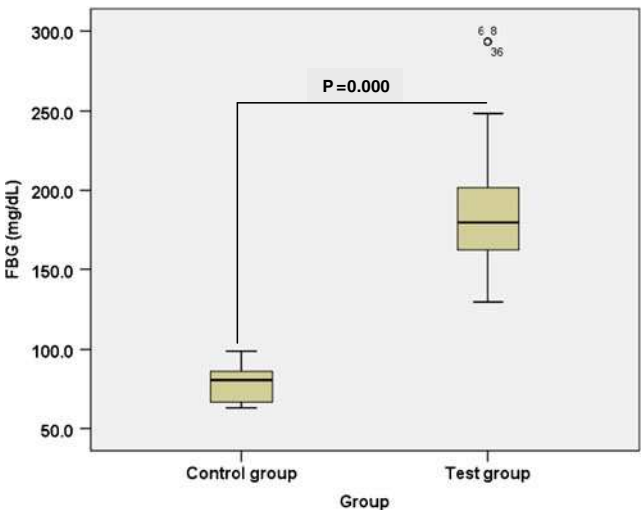

Fig. 1 The figure shows the box-and-whisker plot for both the test (T2DM) and control groups FBG levels. The control group has a skewed distribution but the test group has approximately a normal distribution. Mean FBG in the control and T2DM groups were 88±5 mg/dl and 183±5 mg/dl, respectively. Significant difference between the mean levels is depicted by horizontal bars between the boxes with significance P=0.000. Also the variations in the FBG levels are higher in the test group than the control group. (The numbers 6,8, and 36 above the test group are outliers)

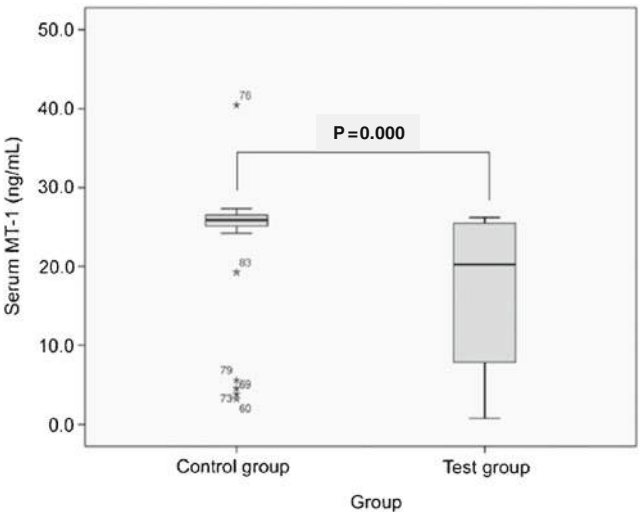

Fig. 2 The figure depicts the box-and-whisker plot for both control and test (T2DM) groups for Serum MT-1. The control group shows a relatively normal distribution compared to the test group which has a skewed distribution. Mean MT-1 values for the control and T2DM groups are 22.3±8.0 ng/mL and 16.9±9.7 ng/mL, respectively. A significant difference between the mean levels of the control and test groups is shown by the horizontal bar between the boxes with significance P=0.000. In addition, the variations in Serum MT-1 are higher in the test group than the control group. (The numbers 76, 83, 79, 69, 73, and 60 above and below the control group are outliers)

characterized by low values of FBG and high levels of Zinc, whereas high values of FBG and low values of Zn levels characterized the T2DM group with a dispersed range of values for MT-1 and Cu as seen in Fig. 7.

The contrast between the test group and the control group was distinct as seen in Fig. 8. Furthermore, a higher Zinc/MT-1 and lower Se was observed in the control group. Generally, this figure demonstrates a departure of T2DM subjects from the normal Zn/Se metabolism.

The cluster analysis on the three trace element variables is seen in Fig. 9. Levels of all three trace elements in the control group were closely "packed" compared to the widely scattered plots in the T2DM group. The T2DM group showed high Cu and Se levels coupled with low Zn levels. A negative correlation was established between Cu and Se (P=0.022).

Discussion

Validation of the analytical procedure showed good linearity of the calibration curves with little scatter of the experimental points. Furthermore, high correlation coefficients (r^2) in the order of 0.99–1 for the trace elements were obtained (Table 2). Accuracy of the method used by way of determining Zn, Cu and Se concentrations showed good recovery of spiked samples (98 %, 99.75 % and 105 %, respectively). Similar levels of recovery were obtained for Zn and Cu (97.77 % and 98.70 %, respectively) by Kazi et al. [25].

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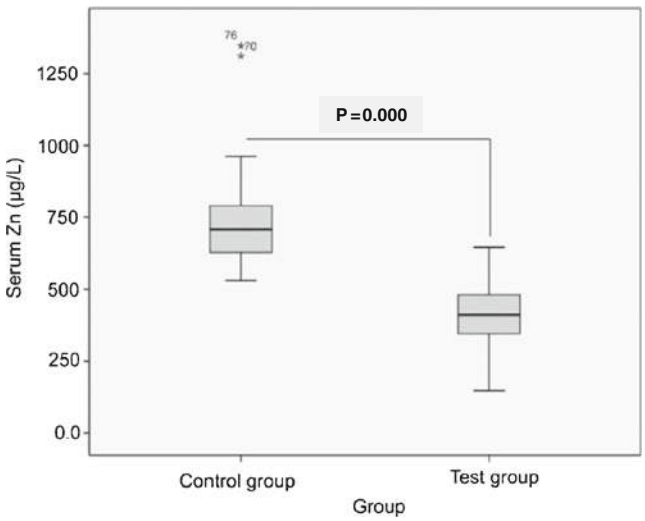

Fig. 3 This figure shows the box-and-whisker plot for Serum Zn for both test (T2DM) and control groups. Both groups have approximately normal distributions. Mean levels of Zn for control and T2DM groups are 750±190 µg/L and 407±117 µg/L, respectively. However, the significant difference between their mean levels is depicted by the horizontal bar between the boxes with significance P=0.000. Also, the two groups show approximately the same variations. (The numbers 76, and 70 above the control group are outliers)

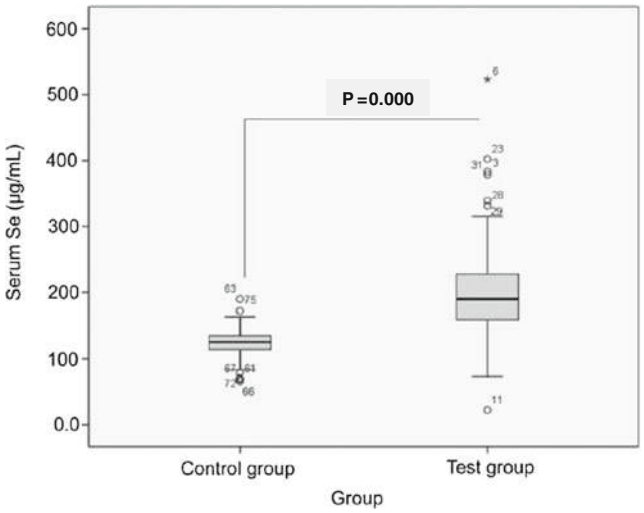

Fig. 5 This figure shows the box-and-whisker plot for Serum Se for control and test groups. The two groups have approximately normal distribution. Mean levels of control and T2DM Se are 123±25 µg/L and 204±91 µg/L, respectively. Significant differences between the mean levels is depicted by the horizontal bar between the boxes with significance P=0.000. Also, both groups showed relatively the same variation (The numbers above and below the control and test groups are outliers).

However, Błazewicz et al. [26] using ion chromatography (IC) for Zn and Cu determination among other trace elements, obtained percentage recoveries of 87.9 % to 102 % for Zn and 89.9 % to 100 % for Cu which is close to that obtained using FAAS in this study. Additionally, good precision between 0.4 % and 0.6 % was obtained for all three elements (Table 3). By the IC method of Blazewicz et al. [26] relative standard deviation (RSD) was 2.8 % for Zn and 5.20 % for Cu compared to≤2.2 % for Zn and≤0.25 % for Cu in this study. Thus, the FAAS method produced better relative deviation of recovery than the IC method. Finally, the repeatability of the method used in this study was very high as indicated by the low values of the relative deviation. The results obtained therefore by the procedure indicate that the method was precise for the determination of Zn, Cu and Se (Table 3).

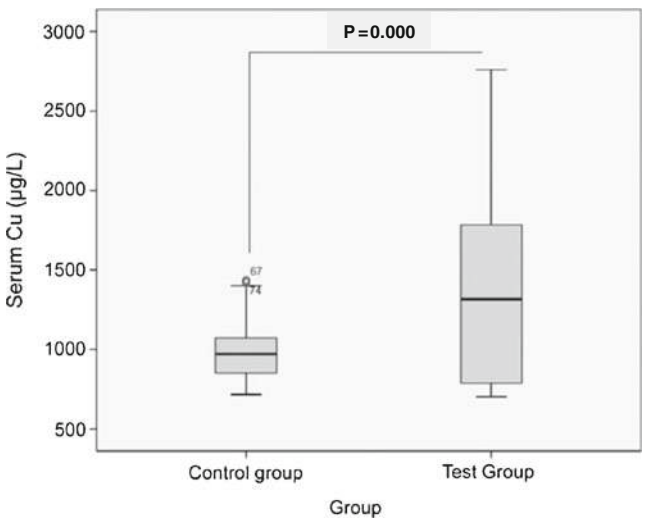

Fig. 4 The figure depicts the box-and-whisker plot for Serum Cu for both control and the test T2DM groups. Both groups showed skewed distributions. Mean values for control and T2DM groups were 989± 197 µg/L and 1,337±527 µg/L, respectively. Significant differences between the mean levels for both groups exist with a significance probability P=0.000. The variations in the Serum Cu are virtually the same for the groups. (The numbers 67, and 74 above the control group are outliers)

The Cu level in this study was raised in the DM group. Significantly raised copper levels in diabetics have been reported by Sedar et al. [27] who also found a strong correlation between Cu and glycated hemoglobin (HbAlc). Furthermore, excess copper was found to essentially increase the incidence of diabetes [28]. This was attributed to free radical production as one of the major mechanisms

Table 4 Standardized canonical discriminant function coefficients

Analyte	Function 1
FBG (mg/dL)	0.823
MT (ng/mL)	−0.149
ZINC (µg/mL)	−0.457
COPPER (µg/mL)	0.172
SELENIUM (ug/mL)	0.362

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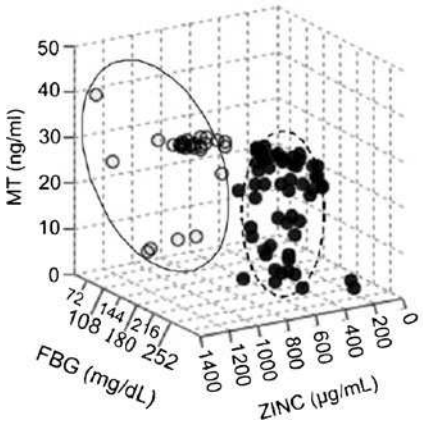

Fig. 6 A 3-Dimensional plot obtained from MT-1, FBG and Zn in distinguishing between the test group (T2DM) from the control group. From the above plot, the control group encircled by a firm line is highly distinguishable from the test group (encircled by a broken line). The control group is characterized by low values of FBG and high levels of Zinc whereas high values of FBG and low Zn levels characterize the T2DM group

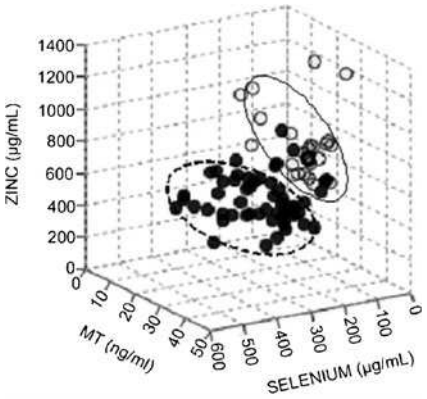

Fig. 8 The contrast between the test group and the control group is distinct in this figure. Furthermore, the figure shows higher Zn and MT-1 and lower Se in the control group. However, the T2DM group demonstrates distinctly lower levels of Zn and higher levels of Se. Generally this figure demonstrates a departure of T2DM subjects from the normal Zn/Se metabolism

through the Fenton reaction, responsible for the high copper levels. On the contrary, copper deficiency can potentially affect the synthesis of MTs; predisposing one to oxidative damage. Besides, copper/zinc superoxide dismutase function as an antioxidant cannot be over emphasized. Thus, maintaining copper within normal limits is vital in the management of diabetes.

Cu has a role in diabetic cardiomyopathy; administration of chelators in experimental diabetic rat models decreased sciatic motor nerve conduction velocity and systemic arterial pressure. Furthermore, chelators are said to cause hypernormal sciatic nutritive vascular conductance [4] and restore nutritive endoneurial blood flow. In a randomized placebo-controlled study a copper(II)-selective chelator ameliorated left-ventricular hypertrophy in type 2 diabetic patients [29]. The Cu level of the diabetic group was 1.4-fold that of controls, although that of the T2DM group still falls within the normal reference interval. Such Cu increases in diabetics have been reported in another study [30]. Viktorínová et al. [31] showed higher Cu levels in diabetics than controls. A positive correlation between Cu and FBG was reported unlike the present study. On the contrary, Basaki et al. [32] observed significantly lower Cu levels in 20 Iranians diabetics. Cu enhances amyloid cytotoxicity and mediates human islet amyloid polypeptide (hIAPP) oligomerization [33]. Co-existence of high levels of Cu and homocysteine inhibit microtubule formation and is a risk for cardiovascular diseases [34].

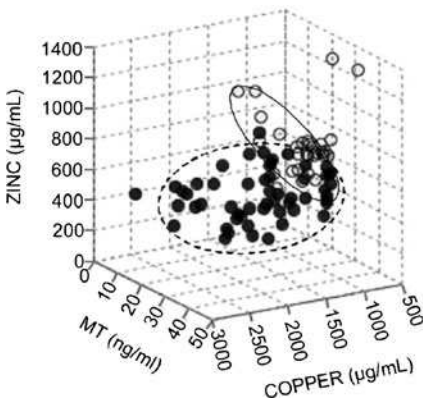

Fig. 7 A 3-Dimentional plot obtained for Zn, MT-1 and Cu. The contrast between the test group (T2DM) and the control group is seen by the broken and firm circles, respectively. Figure shows higher levels of Zn and MT-1 and lower levels of Copper for the control group. However, the test group (T2DM) is characterized with lower levels of Zn and a dispersed range of values for MT-1 and Cu

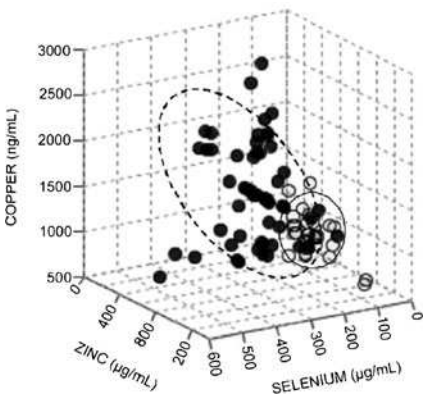

Fig. 9 Cluster analysis on the three variables: Serum Cu, Zn, and Se. The control group is characterized by lower levels of Cu and Se and a higher Zn level. Furthermore, levels of all three trace elements are closely "packed" compared to the T2DM group that is widely scattered. The T2DM group shows high Cu and Se levels coupled with low Zn levels. A negative correlation (P=0.022) was established between Cu and Se

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Another mechanism of increased Cu leading to myocardial damage is by increased advanced glycation end products and glycoxidation products in tandem [35]. Cooper et al. [3] suggest that diabetes might cause 2 to 3 fold increases in extracellular matrix (ECM) Cu. Glycation is reported to increase in vitro Cu binding to collagen [36]. Oral administration of the Cu chelator tetrathiomolybdate decreased neointimal thickening after balloon injury in the rat models [37]. It is proposed that other trace elements like molybdenum interact with protein-bound intra- and extra- cellular copper and remove Cu from tissues into the serum. Furthermore, copper and molybdenum concentrations in serum and copper concentration in urine have been demonstrated to be directly associated with T2DM complications. This molybdenum-copper antagonistic interaction is suggested to be involved in the disease progression [38].

In this study higher levels of Cu (Figs. 4 and 9) subsequently led to reduction in MT-1 and Zn (Figs. 2 and 3). This correlation has been demonstrated by administering drinking water enriched with Zn. Significant up-regulation of metallothionein production in pancreatic islets of mice prevented diabetes induced with STZ [39]. Lower Zn levels are seen in the DM group (Fig. 8). The development of diabetic cardiomyopathy has been prevented by zinc supplementation in a suggested mechanism that is predominantly mediated by an increase in cardiac MT [40].

In addition to the role played by zinc in glucose metabolism by its involvement in the physiological action of insulin [41, 42], zinc (and copper) which binds MT under physiological conditions, significantly induces MT synthesis in various organs [43]. Zinc levels on the contrary were about 50 % lower in diabetics compared to controls (407±117 μg/L and 750± 190 μg/L, respectively,) in this study. Similar results were obtained for Pakistani subjects [44]. A Saudi study also showed lower levels of Zinc in diabetes [45]. Finally in a Turkish study, Zn was 18.99 μmol/L (124.1 μg/L) for T2DM, compared to 14.44 μmol/L (94.4 μmol/L) for controls [46]. This phenomenon of reduced Zn levels in T2DM has been demonstrated in STZ-induced diabetic rats where not only Zn but other trace elements were 45–53 % less absorbed from the intestines as a result of DM [47]. Diabetes is a Zn-deficient condition [48]. Binding of zinc to insulin is important for the crystallization of the hormone, with two Zn ions lying at the center of each hexameric unit [49] and hence, it is believed that this ensures adequate insulin amounts to be stored in pancreatic β-cells to allow sufficient release after a meal [50]. Intraperitoneal Zn injections have been shown to mitigate the induction of diabetes in STZ mice [51–53]. Low Zn levels were attributed to low soil Zn content [54]. This is consistent with animal studies where Zinc supplementation was done via MT [28]. The human zinc transporter 8 (hZnT-8) is said to be potentially involved in the development and/or progression of DM because of its location in insulin secreting pancreatic vesicles [55]. The single nucleotide polymorphisms (SNP) within this gene, predisposes recipients to T2DM [56, 57]. However, 8 (hZnT-8) also functions as an autoantigen in T1DM [58]. The islet-restricted zinc transporter ZnT8 (SLC30A8) is a likely candidate in the control of insulin storage and secretion, ultimately leading to DM [59]. Possession of two copies of the at-risk allele corresponds to the polymorphic variant rs13266634, that encodes a non-synonymous mutant in which Arg replaces Trp at position 325 in the C terminus of the protein. A 53 % increased risk of developing DM is associated with this [50].

DM led to a higher level of Cu compared to controls (Figs. 4 and 9) with subsequent reduction in Zn and MT-1 (Figs. 2 and 3). This may perhaps account for the perturbation of other trace elements [60] such as Se (Fig. 5). In a large US study of 8,876 participants with age 20 years or more, mean serum Se in the control group was 125.7 μg/L. Although that of the diabetic group was slightly higher (126.5 μg/L), high serum Se levels were positively associated with the prevalence of diabetes [61]. However, in a smaller cross-sectional study of a representative sample of Asians residing in Singapore (126 participants with diabetes and 530 participants without diabetes) mean serum Se levels were 120.7 ng/ml and 121.4 ng/ml, respectively [62]. Similarly, Kornhauser et al. [63] observed a lower Se level in the Mexican diabetic group.

The high serum selenium levels in diabetics may result from its antioxidants which are assembled in deranged oxidant-antioxidant systems like in diabetes. Mueller and Pallauf [64] hypothesized that the anti-diabetic mechanism of selenium species is attributed to their insulin-like properties and this beneficial effect has been observed with selenate and to a lesser extent with selenite and selenomethionine. Recent studies have shown that C57BL/6 J mice fed Se supplemented diet developed hyperinsulinemia and had decreased insulin sensitivity. This elevation was accomplished by an increased expression of selenoproteins. However, reduced selenoprotein synthesis caused by an over expression of an i6A- mutant selenocysteine tRNA promotes glucose intolerance, leading to a diabetes-like phenotype. Thus, high and low expressions of selenoproteins lead to disturbances of glucose homeostasis (Vyacheslav et al.) [65].

Se offers antioxidant protection because of the formation of seleno-proteins and selenocysteine through a mechanism encoded by the UGA codon (6). Selenoproteins, including glutathione peroxidases, thioredoxin reductases, iodothyronine deiodinases, and selenoprotein P, are important for diverse biological functions, including protection against oxidative stress, immune function, and thyroid function [66]. The awareness of the antioxidant protection of Se by virtue of the formation of seleno-proteins seem to have over-shadowed the toxic potentials of this dietary supplement. At 70–90 ng/ml the dose dependent relations of Se intake and the formation of selenoproteins reaches a saturation point. At greater levels,

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additional Se intake further increases the plasma Se level because of nonspecific incorporation of selenomethionine into albumin and other proteins [67]. Few population studies, have evaluated the association between selenium and diabetes.

In a recent study the average Se intake at baseline was 55.7 μg/day. After a median follow-up in a study of 16 years, 253 women out of the 7,182 participants developed diabetes. Increased dietary Se intake was associated with an increased risk of type 2 diabetes [68].

Excess Se may also accumulate in pancreatic tissue as shown in animal models [69]. Under pro-oxidative stress conditions, ROS may increase insulin resistance and affect pancreatic β-cell function [70]. Excess Se therefore is paradoxically diabetogenic [71]. By taking Se supplements where dietary intake is adequate, the risk for the development of diabetes or its complications is heightened [71]. Although Cu has been implicated in T2DM cardiomyopathy, Se has not. Serum selenium levels did not differ between type 2 diabetic subjects with and without coronary artery disease in a study that determined serum Se levels by use of atomic mass spectrometry (Sotiropoulos et al.) [72]. On the contrary, Park et al. [73] have recently reported that at dietary levels of intake, individuals with higher toenail Se levels (assessed by neutron activation) were at lower risk for T2DM. Further research is certainly required to determine the involvement of Se in T2DM.

In conclusion, trace element metabolic derailment exists in T2DM Ghanaian patients. Zn is reduced inversely and correlates with Cu increases. Se levels are higher in T2DM while the metal binding protein MT-1 is significantly reduced.

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