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The variation of macro- and micro-minerals of tissues in diabetic and non-diabetic rats

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Abstract

This study determined the levels of Ca, Mg, Fe, Zn, Cu, and Na in various tissues samples (liver, brain, kidney, intestines, muscle and hair) of diabetic and non-diabetic rats by flame atomic absorption spectroscopy, in order to assess the role of element levels during T2DM. The ratios of Ca/Mg, Zn/Cu, Ca/Zn, and Mg/Zn in diabetic and non-diabetic rat tissues were also calculated. The determined element levels were further subjected to a student-*t* test statistical analysis and multiple-linear-regression in order to evaluate similarities, differences, and an inter-element association in tissues of diabetic and non-diabetic rats. The results of the study showed high variability in element levels and Ca/Mg Zn/Cu Mg/Zn Ca/Zn ratios in the tissues of diabetic and non-diabetic rats, but are tissue- and element-dependent, suggesting differences in the accumulation of the elements in tissues of diabetics and non-diabetics. The obtained significant differences in the levels of elements and Ca/Mg Zn/Cu Mg/Zn Ca/Zn ratios in several tissues of diabetic and non-diabetic rats in this study suggest that the investigated elements play considerable roles in the T2DM disease process. Strong inter-element associations ($R^2 \geq 0.9$) were observed for some elements in tissues of diabetic and non-diabetics rats. However, poor inter-elemental associations were obtained for some elements in the tissues of diabetic and non-diabetic rats.

Keywords

Type II diabetes mellitus; Macro- and micro-elements status; Calcium/Magnesium and Zinc/Copper ratios; Tissues; Rats

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Conflict of interest statement

None.

1. Introduction

Human health is highly dependent on vital exchanges that constantly occur in enzymatic systems activated by macro- and micro minerals or elements [1]. It is widely accepted that the human body's metabolic activities can be impacted by the presence or lack of trace or macro elements [1,2]. For instance, calcium (Ca) and magnesium (Mg) are essential macro-elements required for bone structure development and necessary for carbohydrate and protein metabolism [2,3]. Because, Mg and Ca aid to regulate both blood vessel dilation and heartbeat, a deficiency in either of these elements can negatively impact metabolism, and is often associated with hypertension [4,5]. An increased Ca/Mg ratio has been reported as a pathogenetic risk factor for the development of arteriosclerosis and hypertension [4,5]. Since type II diabetes mellitus (T2DM) is often accompanied by hypertension, the levels of both Ca and Mg directly impact the disease. Magnesium is important for glucose homeostasis, as it is a cofactor in the transport of glucose across cell membranes; it is the second most important intracellular cation after potassium [6]. Therefore, a deficiency in Mg has been linked to an increase in the complications associated with the metabolic T2DM disorder including diminished insulin secretion [6,7].

Iron (Fe) is also an element found in the heme proteins hemoglobin and myoglobin [8]. Iron deficiency may result in decreased immune function and an inhibition of hemoglobin synthesis, which leads to anemia, insomnia, and other health related complications [9,10]. Iron is important for cellular metabolism, and specifically affects glucose metabolism. Iron also impacts vascular dysfunction [11]. Copper (Cu) and Zinc (Zn) promote normal body metabolism, but are considered to be micro-elements due to their limited quantity in humans [8]. Copper is necessary in humans for the development of bone, connective tissue, and nerve coverings, while playing a key role in Fe metabolism [8]. The distributions and varying levels of Cu in different tissues, including the brain and liver have been reported [8]. Studies have also investigated the biomedical implications of Zn in human health. Zinc protects against oxidative stress and heart disease, and promotes normal cell growth. Zinc is also known to play an active role in muscular performance and endurance [12]. A deficiency in any of these micro elements can lead to undesirable pathological conditions. For instance, low Zn levels have been associated with both cancer patients and individuals who have T2DM [13,14]. Meanwhile, sodium (Na) is a macro-element, generally integrated into anatomic structures such as bone and nucleic acids.

T2DM is a chronic, metabolic disorder, resulting from insulin resistance and an abnormal increase in the blood glucose [15,16]. This disease can lead to a host of other health-related pathologies including atherosclerosis and hypertension, which affects millions across the globe [1]. Decreases in serum Mg levels have been observed in both T2DM, as well as in hypertension [7,17,18]. Increases in intracellular Ca/Mg ratios have also been suggested as a possible marker for hypertension, glucose intolerance, and insulin–resistant atherothrombogenic syndrome [17]. Research has demonstrated a close relationship between T2DM and hypertension due to the fact that T2DM is associated with advanced age, a person's lifestyle, and obesity [19,20]. Hypertension also contributes to diabetic retinopathy, which is a primary cause of blindness [20]. However, it has been concluded that plasma Zn and Mg levels were not altered by non-insulin diabetes mellitus [21,22], but Cu plasma

levels did increase, indicating that the antagonistic relationship between Zn and Cu needs further investigation. Zinc deficiency is a common factor of T2DM, mainly because this element controls the structural integrity of insulin. Furthermore, Zn functions to neutralize the prevalence of free radicals and reduces the probability of oxidative stress in T2DM; decreased serum Cu reduces free radical production in the disease [23–25].

A limited number of studies have investigated the role of micro elements in T2DM, and the results are variable [26–28]. However, a study investigating a comparative analysis of micro element levels, Ca/Mg ratio, Cu/Zn ratio, and inter-element associations in various tissues of diabetic and non-diabetic rats is sparse, and not readily available. To the best of our knowledge, there are no prior investigations of the relationship between the Ca/Zn and Zn/Mg ratios in the tissues of diabetic or non-diabetics rats. An accurate understanding of the micro element levels of Ca/Mg, Cu/Zn, Ca/Zn, and Zn/Mg ratios, as well as the knowledge of the inter-elemental associations in tissues of diabetic and non-diabetic rats, is necessary and may provide insight to the T2DM disease process for medical diagnosis. Accordingly, this study investigated a comprehensive comparative analysis of the macro- and micro- element levels in different tissues (brain, liver, muscles, intestines, kidney and hair samples) of diabetic and non-diabetic rats. Additionally, this study is the first to investigate a comparative analysis of Ca/Mg ratio and Zn/Cu ratio as well as inter-elemental associations in different tissues of diabetic and non-diabetic rats.

2. Experimental

2.1. Experimental animals, sample collection, sample preparation, and element analysis

The experimental protocol in this study was approved by the Winston-Salem State University Institutional Animal Care and Use Committee prior to the commencement of the study. All necessary protocols, involving the use of animals in research were strictly observed in this study. Eight week old, male type II diabetic Goto-Kakizaki (n=8) and non-diabetic Wistar rats (n=7) averaging 250 ± 50 g, were obtained from Charles River (Wilmington, MA). The diabetic and non-diabetic rats were fed with normal chow (Prolab®RMH 300, LabDiet, St. Louis, MO). The rodents were pair-fed where the amount of chow given was adjusted to approximately 5 g of chow/100 g body mass, with fluids ad libitum in identical pair-feeding. Daily, each rodent consumed ~14 g of chow based on an average body mass of ~280 ± 3.1 g. At day 0 of the experiment, the rats were divided into two groups (non-diabetic versus diabetic). After the 14day experimental period, each rodent was sacrificed. The brain, liver, muscles, intestines, kidneys, and hair samples were collected, placed in pre-nitric acid washed polyethylene bags and immediately refrigerated prior to the laboratory analysis.

A known weight of the diabetic and non-diabetic rat tissues and hair samples were digested, using official and standardized methods of analysis procedures [29] for approximately 6 h to ensure complete sample digestion. The digested samples were filtered (Whatman® filter paper, Fisher Scientific, USA) and diluted to the mark in a standard volumetric flask. Working range standard solutions used to construct the calibration curves for each element were prepared by serial dilution of 1000mg/L standard stock solutions of each element (Fisher Scientific, USA). The standard and the digested sample solutions were subjected to

element analysis using a flame atomic absorption spectrophotometer (Shimadzu, AA-6300), with a pre-mixed burner air-acetylene flame. The flow rates of the fuel and oxidant gases were always carefully optimized for each metal analysis. Other routine instrumental checks and calibrations were always performed on the spectrometer before use to ensure the accuracy, reliability and consistent performance of the spectrometer. The calibration curve for each element was constructed by plotting the absorbance obtained from FAAS analysis of the standard solution versus the element level. The constructed calibration curve for each element was subsequently utilized to determine the element level in the rat samples.

2.2. Statistical and regression analysis

The statistical and linear regression analysis and inter-element association in the tissues of diabetic and non-diabetic rats for pattern recognition samples were performed using chemometric software (The Unscrambler, CAMO Inc., 9.4).

3. Results and discussion

3.1. Calibration curves

Table 1 presents the results of the calibration curve parameters constructed for the investigation of elements in the diabetic and non-diabetic rats, showing the regression equation, square correlation coefficient (R^2), wavelength used for AAS elemental analysis, the limits of detection (LOD), and limits of quantification (LOQ) of the elements analyzed. The LOD, which is defined as the minimum detectable amount of metal, was calculated using the equation: $LOD = 3s/m$; where s is the signal of the blank and m is the slope of the calibration curve. The limit of quantitation, defined as the lowest measurable element level, was evaluated using the formula: $LOQ = 10s/m$. The parameters used to calculate LOQ were as previously defined for LOD. The high values of the square correlation coefficients (R^2) obtained in Table 1 demonstrate good linearity and high correlation of the absorbance with element levels.

3.2. Macro- and micro-element levels in diabetic and non-diabetic rats tissues

Table 2 shows an overall average element level in the liver, brain, muscles, kidneys, intestines, and hair samples in diabetic and non-diabetic rats. The element levels in diabetic and non-diabetic rats vary widely. The element levels in diabetic and non-diabetic rats were also tissues and element-dependent. An overall average Ca level of 30.3 μg/g (liver), 44.9 μg/g (brain), 29.0 μg/g (muscles), 57.5 μg/g (kidney), 178 μg/g (intestine), and 188 μg/g (hair) were obtained in diabetic rat samples. However, the Ca level of 21.2 μg/g (liver), 103 μg/g (brain), 37.5 μg/g (muscles), 47.8 μg/g (kidney), 125 μg/g (intestine), and 200 μg/g (hair) was obtained in non-diabetic rats. In general, the overall average Ca level increased in the liver (30.3 μg/g) of diabetic rats compared to a 21.2 μg/g Ca level in the non-diabetic rats. An average 103 μg/g Ca level measured in the brain of the non-diabetic rats was significantly higher than the 44.9 μg/g Ca level in the brain of the diabetic rats. On the contrary, a relatively greater Ca level of 178 μg/g was attained in the intestines of the diabetic rats, compared to a 125 μg/g Ca level in the non-diabetic rats. There was a comparative decrease in the Ca levels in hair of the diabetic rats (188 μg/g) versus the non-diabetic rats (200 μg/g).

An average level of Mg of 86.3 μg/g, 104 μg/g, 80.6 μg/g, 145 μg/g, 82.6 μg/g, 87 μg/g was obtained in the liver, brain, muscles, kidney, intestines, and hair samples of the diabetic rats, respectively. Similar Mg levels of 80.9 μg/g (liver), 106 μg/g (brain), 61 μg/g (muscles), 143 μg/g (kidney), and 92 μg/g (intestines) were observed in the non-diabetic rats. However, an overall Mg level of 87 μg/g that was found in the diabetic rat hair sample was 10 times higher than the corresponding average of the 8.3 μg/g Mg level in the non-diabetic rat hair samples.

In general, compared with Ca, Mg, Na, and Fe levels, relatively lower Cu levels were measured in the diabetic and non-diabetic rats. An overall average Cu level of 2.6 μg/g (liver), 0.8 μg/g (brain), 0.02 μg/g (muscles), 2.0 μg/g (kidney), 1.12 μg/g (intestines), and 5.66 μg/g (hair) were obtained in diabetic rat samples. The average Cu level seen in the non-diabetic rats was 4.3 μg/g (liver), 1.9 μg/g (brain), 0.51 μg/g (muscles), 7.3 μg/g (kidney), 1.34 μg/g (intestines), and 8.14 μg/g (hair). It is of considerable interest to note that the level of Cu in the kidney of the non-diabetic rats (7.3 μg/g) is approximately four times higher than the corresponding Cu level (2.0 μg/g) in the kidney of diabetic rats. The levels of Zn in the diabetic and non-diabetic rats are also organ-dependent. The highest Zn levels of 79.0 μg/g and 89.6 μg/g were found in the hair samples of the diabetic and non-diabetic rats, respectively. An overall average level of Zn in the muscles of the non-diabetic rats (12.3 μg/g) is three times higher than the Zn level (4.57 μg/g) in the muscles of diabetic rats. Relatively lower Zn levels of <0.1 μg/g and 0.9 μg/g, respectively, were observed in the kidney and intestines of diabetic rats. This can be compared to the higher, corresponding Zn levels of 3.45 μg/g in the kidneys and 8.84 μg/g in the intestines of the non-diabetic rats.

The overall average Fe level in the diabetic rat liver was 57.5 μg/g. An iron level of 24.5 μg/g (brain), 4.57 μg/g (muscles), 33.9 μg/g (kidney), 11.3 μg/g (intestines), and 27.9 μg/g (hair) samples were also acquired in the diabetic conditions. The highest Fe level in diabetic rats was obtained in the liver (57.5 μg/g). An overall average Fe level in the non-diabetic tissues is approximately twice the Fe level in the diabetic state as shown in Table 2. As expected, Na had the highest level compared with the level of other elements in the diabetic and non-diabetic tissues. Although, the 1674 μg/g Na level in the hair sample of the non-diabetic rats is notably higher, and nearly twice the Na level (904 μg/g) in the hair sample of the diabetic rats.

As previously discussed, macro and trace elements plays a considerable role in T2DM disease process and insulin sensitivity. For instance, Ca and Mg are macro-elements required for carbohydrates, and are also necessary to regulate both blood vessel dilation and heartbeat. Deficiencies in Ca and Mg can negatively impact metabolism, resulting in hypertension and complications associated with the metabolic T2DM disorder including diminished insulin secretion. Magnesium is also essential for glucose homeostasis, as it is a cofactor in the transport of glucose across cell membranes. A comprehensive roles and variations in macro and trace elements in progression of type 2 diabetes have been thoroughly reviewed [30]. The observed variations in macro and trace elements in diabetes rats and non-diabetes rats in this study are consistent with previously reported variations in macro and trace elements in type 2 diabetes patients and healthy individual. For instance, differences in variations in elements ($Fe^{3+,}$ Cu^{2+}, Zn^{2+}, Mn^{2+}) in the blood serum of patients

with type 2 diabetes patients and health control humans have been reported [31]. Previous studies have also highlighted the critical roles and direct relationships between the levels of macro and trace elements and diabetes mellitus [32]. Besides, the influence of macro and trace elements on insulin activity and its implications on blood glucose reduction has been reported [33]. Most macro and trace elements generally serves as co-factors for many enzymes. Accordingly, macro and trace elements can activate insulin receptors sites necessary for glucose utilization [34] that may potentially increase insulin sensitivity or act as antioxidants in order to prevent tissue peroxidation [35].

3.3. Quality assurance of the analytical data and sample analysis

Each sample was analyzed in triplicate and the averages of the element levels in the rat samples were calculated, and used for data analysis. All necessary precautions were observed to ensure the accuracy of the results of this study. For example, the samples were immediately refrigerated prior to sample analysis to prevent sample decomposition or microbial growth. The element grade nitric acid (99.999%) purity grade was used for tissue digestion and standard solution preparation. All glassware were pre-soaked in 6 M HNO_3 for three days and thoroughly rinsed with de-ionized water before use to remove impurities and contaminants. All element sample analyses were also blank subtracted.

Reference and certified standard rat material for elements is not available. Therefore, a recovery study was performed for each metal to further evaluate the accuracy and reliability of the results of the element levels obtained in the analysis. The recovery study was performed by randomly spiking ten previously analyzed tissue samples with a known level of metal standard solution. The spiked samples were then subjected to HNO_3 digestion and metal analysis procedures as previously described. The recovery of the metals in the spiked samples was evaluated by comparing the known level of the spiked metal with the level detected using the FAAS spectrometer. The recovery of the element analysis was within 80–120% acceptable range of spike recovery [36].

3.4. Evaluation of differences in element level in diabetic and non-diabetic rats tissues using student t-test statistical analysis

The average levels of the elements obtained in the diabetic and non-diabetic rat tissues were further subjected to a statistical student *t*-test analysis [36] to further evaluate whether there is a significant difference between the average element levels obtained in the tissues of diabetic and non-diabetic rat tissues. At 95% confidence level and 13° of freedom, there was a significant difference between the average level of Ca ($t_{\text{calculated}}$ 2.88, $t_{tabulated}$ 2.131–2.228) in the kidney of diabetic and non-diabetic rats. Significant differences were also observed for the average level of Mg in the muscles ($t_{\text{calculated}}$ 3.00, $t_{tabulated}$ 2.131–2.228) and hair samples ($t_{\text{calculated}}$ 2.78, $t_{tabulated}$ 2.131–2.228) of diabetic and non-diabetic rats. Interestingly, the average levels of Cu were significantly different in the liver ($t_{\text{calculated}}$ 3.85, $t_{tabulated}$ 2.131–2.228), brain ($t_{\text{calculated}}$ 5.61, $t_{tabulated}$ 2.131–2.228), muscles (*t*calculated 10.6, *t*tabulated 2.131–2.228), kidney, (*t*cal 5.86, *t*tabulated 2.131–2.228), and hair samples (t_{cal} 3.07, $t_{tabulated}$ 2.131–2.228) of diabetic and non-diabetic rats. At 95% confidence interval, the average levels of Zn were also significantly different in the muscles ($t_{\text{calculated}}$ 4.12, $t_{tabulated}$ 2.131–2.228), liver ($t_{\text{calculated}}$ 2.97, $t_{tabulated}$ 2.131–2.228), and intestines

($t_{calculated}$ 2.40, $t_{tabulated}$ 2.131–2.228) of diabetic and non-diabetic rats. Furthermore, the average levels of Fe were found to be significantly different in the liver ($t_{calculated}$ 7.3, $t_{tabulated}$ 2.131–2.228), muscles ($t_{calculated}$ 4.38, t_{tab} 2.131–2.228), kidney, ($t_{calculated}$ 3.56, $t_{tabulated}$ 2.131–2.228), hair samples ($t_{calculated}$ 3.90, $t_{tabulated}$ 2.131–2.228), and intestines ($t_{calculated}$ 5.20, $t_{tabulated}$ 2.131–2.228) of diabetic and non-diabetic rats. A significant difference in the average Na levels ($t_{calculated}$ 2.76, $t_{tabulated}$ 2.131–2.228) was also obtained in the hair samples of diabetic and non-diabetic rats. The observed significant differences in the levels of elements in several tissues of diabetic and non-diabetic rats further confirmed that the investigated elements play considerable roles in the T2DM disease process.

3.5. Ca/Mg, Zn/Cu, Ca/Zn and Zn/Mg ratio

As earlier discussed, the levels of Ca, Mg, Fe, Zn, and Cu have been widely used to evaluate the pathogenetic risk factors for the development of arteriosclerosis and hypertension in the diabetic disease process. For instance, Ca and Mg are essential macro-elements required for carbohydrates, and are required to regulate both blood vessel dilation and heartbeat. Deficiencies in Ca and Mg can negatively impact metabolism, resulting in hypertension and complications associated with the metabolic T2DM disorder including diminished insulin secretion. Magnesium is also essential for glucose homeostasis, as it is a cofactor in the transport of glucose across cell membranes. Furthermore, low Zn levels have been associated with both cancer patients and individuals who have T2DM. Calcium and Mg are group 2 elements in the periodic table, with similar physical and chemical reactivity, oxidation states, and charge/mass ratios. Cu and Zn are also transition elements, with similar charge/mass ratios and chemical reactivity.

The similarities in the physical and chemical reactivity of these elements can potentially result in one element mimicking another element in their chemical behavior or result in destructive competition and/or interfering with each other in their biochemical reactions. For example, high levels of Mg are known to hinder the utilization and absorption of Ca, affecting carbohydrate metabolism and reduction in insulin secretion [37]. More importantly, a high Ca/Mg level ratio has been associated with various health issues, including atherosclerosis, hypertension, glucose intolerance, insulin–resistant atherothrombogenic syndrome, low Ca absorption and utilization in humans [17,38,39]. Additionally, Ca/Mg imbalances may lead to other health related issues such as diabetes and alterations in insulin sensitivity [40], necessitating the need to critically evaluate the Ca/Mg ratio in humans. A reduced erythrocyte copper-zinc superoxide dismutase activity at high Zn levels has also been reported [41]. In addition, high levels of Zn have been implicated in low Cu plasma caeruloplasmin, serious anemia, and alterations in immune responses and serum lipids [42–44]. High Zn/Cu ratio may also affect the utilization of Cu, resulting in Cu deficiency in the body. For instance, an increase in the Zn/Cu ratio has been observed in pancreatic cancer patients compared to healthy humans [45]. Evaluations of the Ca/Zn and Zn/Mg ratio are therefore required to gain better insight into the T2DM metabolic disorder and disease process.

Consequently, the Ca/Mg and Zn/Cu ratios of diabetic and non-diabetic rat tissues were further calculated for a comparative analysis. Table 3 shows the average Ca/Mg and Zn/Cu

ratios in various diabetic and non-diabetic rat tissues. The Ca/Mg ratio of 0.35, 0.44, 0.36, 0.40, 2.17, and 2.16, were calculated in the liver, brain, muscles, kidney, intestines and hair samples of diabetic rats, respectively. It is of particular interest to note that the calculated Ca/Mg ratio of 24 in the hair sample of non-diabetic rats is eleven times larger than the corresponding calculated Ca/Mg ratio of 2.16 in the diabetic rat hair sample. The calculated Ca/Mg ratios of 0.96 and 0.70, respectively, in the brain and muscles of non-diabetic rats, are also twice the calculated Ca/Mg ratios for the brain (0.44) and muscles (0.36) of diabetic rats.

In general, the calculated Zn/Cu ratio in tissues of diabetic rats is higher than the corresponding Zn/Cu ratio in the tissues of non-diabetic rodents. The calculated Zn/Cu ratios for the diabetic liver, brain, muscles, kidney, intestines, and hair samples were: 7.2, 0.22, 229, 0.5, 9.7, and 14, respectively. Conversely, the Zn/Cu ratio of the equivalent tissues for the non-diabetic state were 5.0 (liver), 0.41 (brain), 24 (muscles), 0.47 (kidney), 6.6 (intestines), and 11 (hair). The calculated Zn/Cu ratio in the muscles for the diabetic rats is approximately 10 times larger than the corresponding Zn/Cu ratio in non-diabetic rats.

Previous studies have investigated the role of Ca/Mg and Zn/Cu ratios in functional health [17]. However, there are no studies that have examined the relationship between the Ca/Zn and Zn/Mg ratios in diabetic or non-diabetic rats to date. Accordingly, a comparative analysis of the Ca/Zn and Zn/Mg ratios was conducted for both diabetic and non-diabetic conditions. Table 3 shows the calculated Ca/Zn and Zn/Mg ratios in the diabetic and non-diabetic rat tissues. The highest Ca/Zn ratios were found in the tissues of diabetic rats. The Ca/Zn ratios of: 1.62, 204, 6.35, 575, 198, and 2.38 were found in the liver, brain, muscles, kidney, intestines and hair samples of diabetic rats, respectively. Significantly lower Ca/Zn ratios of 0.99 (liver), 134 (brain), 3.05 (muscles), 13.9 (kidney), 14.1 (intestines), and 2.53 (hair) were calculated in the non-diabetic rats. The results of Mg/Zn ratios in diabetic and non-diabetic rat tissues are very interesting. The highest Mg/Zn ratios were found in the kidney, brain, and intestines of both diabetic and non-diabetic rat tissues. The Mg/Zn ratios in all of the diabetic rat tissues were significantly larger than the corresponding Mg/Zn ratios in the tissues of non-diabetic rats. For example, the Mg/Zn ratio found in the diabetic kidney (1450) was 33 times larger than the Mg/Zn ratio in the absence of disease. Also, the Mg/Zn ratio in the diabetic intestines was 9 times larger than the non-diabetic Mg/Zn intestines ratio.

3.6. Correlations and inter-element associations in diabetic and non-diabetic rat tissues

To gain further insight of the inter-element association of elements in the tissues of diabetic and non-diabetic rats, the levels of elements were subjected to a linear regression. Tables 4 through 9 show the summary of the results of the regression analysis of the inter-element associations in diabetic and non-diabetic rats. Strong correlations between Ca and Na (R^2 =0.9938), Ca and Mg (R^2 =0.9403), Ca and Cu (R^2 = 0.8938), and Ca and Zn (R^2 = 0.8039) were obtained in diabetic liver (Table4). There were also strong correlations between Cu and Na (R^2 = 0.9183), Mg and Zn (R^2 = 0.8912), Mg and Na (R^2 = 0.8987), Zn and Fe (R^2 = 0.8854) observed in the diabetic liver. Slight correlations between Zn and Na (R^2 = 0.7676) and Mg and Cu (R^2 = 0.7306), were also seen in the liver of diabetic rats. In contrast, the

element levels were generally poorly correlated in the liver of non-diabetic rat samples (Table 4). However, strong correlations between Mg and Na (R^2 = 0.9978) were present; weak correlations between Ca and Mg (R^2 = 0.564), as well as Zn and Fe (R^2 = 0.6754) were observed in the liver of non-diabetic rats.

Poor correlations and inter-element associations were attained for most elements in the brain samples of diabetic and non-diabetic rats. Nevertheless, modest correlations were observed between Mg and Cu (R^2 = 0.8506), Cu and Fe (R^2 = 0.7328), Mg and Fe (R^2 = 0.7277), and Mg and Zn (R^2 = 0.648), in the diabetic brain (Table 5). On the contrary, only a strong inter-element association occurred between Cu and Fe (R^2 = 0.9674) in the non-diabetic rat brain samples (Table 5). A strong inter-element association between Mg and Zn (R^2 = 0.9731), and a moderate correlation between Ca and Mg (R^2 = 0.7716), Ca and Zn (R^2 = 0.6219), and Fe and Cu (R^2 = 0.5764) was found in the muscles of the diabetic rats (Table 6). However, the only observed inter-element associations in non-diabetic rat muscles occurred between Mg and Na (R^2 = 0.9397), and between Ca and Zn (R^2 = 0.5712) (Table 6).

Strong inter-element associations between Ca and Mg (R^2 = 0.9995), Ca and Zn (R^2 = 0.9042), and Ca and Na (R^2 = 0.9603) occurred in the intestines of the diabetic rats (Table 7). Strong inter-element associations between Mg and Na (R^2 = 0.9588), Mg and Zn (R^2 = 0.891), Mg and Na (R^2 = 0.9684), Zn and Cu (R^2 = 0.8534), Zn and Na (R^2 = 0.7572) were also found in the intestines of diabetic rats. However, weak correlations between Mg and Cu (R^2 = 0.5559), and between Ca and Cu (R^2 = 0.5775) were observed in the intestines of diabetic rats. Modest inter-element associations were observed between Ca and Fe (R^2 = 0.6406), Mg and Cu (R^2 = 0.777), Mg and Fe (R^2 = 0.6961), Cu and Fe (R^2 = 0.6401), Cu and Na (R^2 = 0.676), and Fe and Na (R^2 = 0.5646) in the diabetic intestines (Table 7). It is important to note that there was no correlation between Fe and any of the elements in the intestines of the non-diabetic state. Also, there was a strong correlation between Ca and Mg (R^2 =0.9995) in the intestines of diabetic rats. On the contrary, Ca was poorly associated with Mg (R^2 = 0.2702) in the non-diabetic intestines.

Strong correlations between Ca and Fe (R^2 = 0.9976), Mg and Cu (R^2 = 0.9276), Ca and Mg (R^2 = 0.8114), Mg and Fe (R^2 = 0.848), and Cu and Na (R^2 = 0.8633) were also observed in the kidney of the diabetic rats (Table 8). There were weak inter-element associations displayed between Cu and Fe (R^2 = 0.6115), Mg and Na (R^2 = 0.6326), and Ca and Cu (R^2 = 0.5635) in the diabetic kidney. The inter-element association in the kidney of the non-diabetic rats was significantly different from the corresponding inter-element association in the kidney of the diabetic rats. Apart from a weak association between Mg and Na (R^2 = 0.6768), all of the other elements were poorly correlated in the kidney of the non-diabetic rat samples (Table 8).

There were strong associations between Ca and Na (R^2 = 0.8657), and Cu and Na (R^2 = 0.828) in the hair samples of the diabetic rats (Table 9). Meanwhile, weak associations between Ca and Cu (R^2 = 0.5276), Mg and Cu (R^2 = 0.6514), Mg and Zn (R^2 = 0.5623), and Mg and Fe (R^2 = 0.5748) occurred in the diabetic hair samples. Mg is fairly associated with Fe (R^2 = 0.7363) and Na (R^2 = 0.5381) in the hair samples of the non-diabetic rats (Table 9). A weak correlation between Fe and Na (R^2 = 0.5225) was also observed in the hair samples

of non-diabetic rats. Copper and Zn had no association with any of the elements in the hair samples of the non-diabetic rats.

4. Conclusion

A comparative study to investigate macro- and micro- element levels in various tissues) and hair samples of diabetic and non-diabetic rats, by use of flame atomic absorption spectroscopy, was performed in order to evaluate the role of element levels in the T2DM disease process. Analysis of the Ca/Mg, Zn/Cu, Ca/Zn, and Mg/Zn ratios in the tissues of diabetic and non-diabetic rats was further determined. The element levels were further subjected to a regression analysis to evaluate the inter-element associations in the tissues of diabetic and non-diabetic rats. The results of the study showed a high variability in macro- and micro-element levels in the tissues of diabetic and non-diabetic rats. The element levels in diabetic and non-diabetic rats were found to be organ- and element-dependent, suggesting differences in the accumulation of the elements in tissues of diabetics and non-diabetics. However, the levels of Zn for all tissues were expected, as many diabetic individuals are deficient in this particular element. Significant differences in the levels of elements in several tissues of diabetic and non-diabetic rats were also observed in this study; this demonstrates that elements play considerable roles in the T2DM disease process. The Ca/Mg, Zn/Cu, Ca/Zn, and Mg/Zn ratios were considerably different in the tissues of diabetic and non-diabetic rats. The degree of inter-element associations in the tissues of diabetic and non-diabetic rats also varied. Strong correlations and inter-element associations were found for some elements. However, some elements are weakly or poorly associated in the tissues of diabetic and non-diabetic rats. The results of this study suggest differences in the macro- and micro element levels, Ca/Mg, Zn/Cu, Ca/Zn, and Mg/Zn ratios, and inter-element associations in various tissues of diabetic and non-diabetic rats. The results of this study are very interesting and clearly suggest that the macro- and micro-element levels, and Ca/Mg, Zn/Cu, Ca/Zn, and Mg/Zn ratios play a considerable role in T2DM. Knowing that T2DM leads to a host of other health-related concerns such as atherosclerosis, hypertension and oxidative stress, there is a need for a robust study to gain insight into the cause of T2DM in humans, both for preventive measures, medical diagnosis, and possible T2DM therapy. Thus, this study will serve as the basis for future investigations of the role of macro- and micro-element levels, Ca/Mg, Zn/Cu, Ca/Zn, and Mg/Zn ratios, and inter-element associations in human tissues in T2DM diabetic patients.

Unarguably, more studies are required to fully explain the variability observed. Further studies are also necessary to fully evaluate the influence of the element levels, Ca/Mg, Zn/Cu, Ca/Zn, and Mg/Zn ratios, and inter-element associations obtained in the tissues of the diabetic and non-diabetic rats. There is ongoing research towards this effort in our laboratory, and the outcomes will be communicated in future manuscripts.

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Table 1

Linear regression equation, linear correlation coefficient (R^2) of calibration curves, wavelength of detection (nm), limit of detection (LOD), and limit of quantitation (LOQ) obtained for each element:.

Element	Regression equation	R^2	Wavelength (nm)	LOD (μg/g)	LOQ(μg/g)
Ca	y = 0.0040 × + 0.0048	0.999	248.3	0.080	0.25
Mg	y = 0.087 × +0.29	0.973	228.8	0.030	0.11
Zn	y = 0.080 × +0.77	0.922	213.9	0.030	0.13
Cu	y = 0.092 × +0.055	0.999	309.93	0.030	0.12
Fe	y = 0.0097 × +0.0029	0.998	283.3	0.030	0.10
Na	y = 0.0005 × −0.0002	0.9914	588.21	0.050	0.16

Table 2

Macro- and Micro- Element Levels in Diabetic and Non-Diabetic Rat Tissues.

Organ		Ca (μg/g)		Mg (μg/g)		Cu (μg/g)		Zn (μg/g)		Fe (μg/g)		Na (μg/g)	
		[a]DBrat	[b]NDrat	DBrat	NDrat	DBrat	NDrat	DBrat	NDrat	DBrat	NDrat	DBrat	NDrat
Liver	Average	30.3	21.2	86.3	80.9	2.6	4.3	18.7	21.5	57.5	147	207	221
	Maximum	48.8	29.2	135	152	3.4	6.0	24.2	30.0	81.6	163	389	448
	Minimum	20.9	16.5	65.0	51.7	1.6	3.3	11.5	12.9	28.2	93.8	101	131
	STDEV	12.6	5.1	32.9	34.5	0.76	0.95	5.3	6.3	22.5	25.0	126	106
Brain	Average	44.9	103	104	106	0.8	1.9	0.22	0.77	24.5	27.1	282	315
	Maximum	60.7	426	113	110	1.2	2.9	0.22	0.77	33.0	35.8	330	344
	Minimum	35.6	26	95	102	0.3	1.7	<0.1	<0.1	3.8	1.7	174	296
	STDEV	11.6	149	7.8	2.3	0.35	0.41	2.5	1.5	14.4	3.9	72.2	16.6
Muscle	Average	29.0	37.5	80.6	61.0	0.02	0.51	4.57	12.3	6.1	13.2	207	167
	Maximum	33.1	110	96.9	72.0	0.09	0.68	8.99	17.4	10.6	14.7	291	215
	Minimum	25.9	18	69.1	42.8	0	0.33	<0.1	8.75	2.2	9.8	142	108
	STDEV	3.0	32.2	12.8	12.4	0.05	0.12	4.2	2.8	4.0	1.6	62.4	38.6
Kidney	Average	57.5	47.8	145	143	2.0	7.3	<0.1	3.45	33.9	70.2	427	442
	Maximum	66.7	55.5	161	158	3.8	9.7	<0.1	6.69	47.9	90.5	468	506
	Minimum	52.6	41.7	127	128	0.5	4.8	<0.1	<.01	6.1	49.1	345	404
	STDEV	8.0	4.1	16.9	10.0	1.7	1.8	<0.1	3.2	24.0	13.0	70.6	33.8
Intestine	Average	178	125	82.6	92.3	1.12	1.34	0.9	8.84	11.3	30.9	218	272
	Maximum	268	357	125	148	1.17	2.10	15.4	15.6	18.1	42.3	304	386
	Minimum	108	46.1	48.5	43.1	0.13	0.6	1.95	3.95	2.8	23.2	132	128
	STDEV	81.5	118	39.0	39.3	0.93	0.50	7.8	4.1	7.7	6.7	86.0	106
Hair	Average	188	200	87	8.34	5.66	8.14	79.0	89.6	27.9	53.1	904	1674
	Maximum	228	286	127	8.34	7.01	10.6	103	130	37.0	63.6	1072	2786
	Minimum	158	121	51.2	<0.1	4.73	6.02	33.8	68.2	12.3	31.5	761	652
	STDEV	29.4	63.0	31.0	73.0	1.1	1.97	30.9	25.3	10.9	14.1	132	779

[a]DBrat, diabetic rat (n=8).

[b]NDrat, non-diabetic rat (n=7).

Table 3

Average organ Ca/Mg, Zn/Cu, Ca/Zn and Mg/Zn ratios in diabetic and non-diabetic rats.

Organ	Ca/Mg		Zn/Cu		Ca/Zn		Mg/Zn	
	DBrat	NDrat	DBrat	NDrat	DBrat	NDrat	DBrat	NDrat
Liver	0.35	0.27	7.2	5.0	1.62	0.99	4.61	3.76
Brain	0.44	0.96	0.22	0.41	204	134	473	138
Muscle	0.36	0.70	229	24	6.35	3.05	17.8	4.95
Kidney	0.40	0.34	0.5	0.47	575	13.9	1450	44
Intestine	2.17	1.44	9.7	6.6	198	14.1	91.8	10.4
Hair	2.16	24	14	11	2.38	2.53	1.10	0.09

DBrat, diabetic rat (n=8);

*NDrat, non-diabetic rat (n=7).

Table 4

Inter-element associations (square of correlation coefficient, R^2) in the liver of diabetic and non-diabetic rats.

	Ca		Mg		Cu		Zn		Fe		Na	
	DBrat	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**
Ca			**0.9403**	**0.5640**	**0.8938**	0.0031	**0.8039**	0.0188	0.5401	0.1042	**0.9938**	0.4677
Mg	0.9403	0.5640			**0.7306**	0.0081	**0.8912**	0.1745	0.6063	0.0087	**0.8987**	**0.9978**
Cu	0.8938	0.0031	**0.7306**	0.0081			0.4965	0.4116	0.2491	0.2689	**0.9183**	0.0080
Zn	0.8039	0.0188	**0.8912**	0.1745	0.4965	0.4116			**0.8854**	**0.6754**	**0.7676**	0.1715
Fe	0.5401	0.1042	**0.6063**	0.0087	0.2491	0.2689	**0.8854**	**0.6754**			**0.5228**	0.0072
Na	0.9938	0.4677	**0.8987**	**0.9978**	**0.9183**	0.0080	**0.7676**	0.1715	0.5228	0.0072		

*DBrat, diabetic rat (n=8).

*NDrat, non-diabetic rat (n=7).

Table 5

Inter element associations (square of correlation coefficient, R^2) in brain of diabetic and non-diabetic rats.

	Ca		Mg		Cu		Zn		Fe		Na	
	DBrat	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**
Ca			0.0301	0.4255	0.0258	0.0447	0.2923	0.0114	0.0681	0.0045	0.0459	0.0286
Mg	0.0301	0.4255			**0.8506**	0.2647	**0.648**	0.4172	**0.7277**	0.4277	0.0062	0.0000
Cu	0.0258	0.0447	**0.8506**	0.2647			0.4898	0.1953	**0.7328**	**0.9674**	0.0030	0.0150
Zn	0.2923	0.0114	**0.648**	0.4172	0.4898	0.1953			0.1453	0.2986	0.2019	0.0040
Fe	0.0681	0.0045	**0.7277**	0.4277	**0.7328**	0.9674	0.1453	0.2986			0.2204	0.0095
Na	0.0459	0.0286	0.0062	0.0000	0.003	0.0150	0.2019	0.0040	0.2204	0.0095		

*DBrat, diabetic rat (n=8).

*NDrat, non-diabetic rat (n=7).

Table 6

Inter element associations (square of correlation coefficient, R^2) muscle of diabetic and non-dia in the betic rats.

	Ca		Mg		Cu		Zn		Fe		Na	
	DBrat	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**
Ca			**0.7716**	0.2821	0.4803	0.3629	**0.6219**	**0.5712**	**0.4314**	0.2317	0.4166	0.2205
Mg	**0.7716**	0.2821			0.2139	0.0878	**0.9731**	0.2128	0.0488	0.2303	0.3080	**0.9397**
Cu	0.4803	0.3629	0.2139	0.0878			0.1193	0.1312	**0.5764**	0.1105	0.0019	0.1124
Zn	**0.6219**	**0.5712**	**0.9731**	**0.2128**	0.1193	0.1312			0.0033	0.0889	0.2591	0.1255
Fe	0.4314	0.2317	0.0488	0.2303	**0.5764**	0.1105	0.0033	0.0889			0.1250	0.2848
Na	0.4166	0.2205	0.308	**0.9397**	0.0019	0.1124	0.2591	0.1255	0.1250	0.2848		

*DBrat, diabetic rat (n=8).

*NDrat, non-diabetic rat (n=7).

Table 7

Inter element associations (square of correlation coefficient, R^2) in the intestine of diabetic and non-diabetic rats.

	Ca		Mg		Cu		Zn		Fe		Na	
	DBrat	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**
Ca			**0.9995**	0.2702	**0.5775**	0.4237	**0.9042**	0.0804	0.0137	0.6406	**0.9603**	0.1177
Mg	**0.9995**	0.2702			**0.5559**	0.7770	**0.8910**	0.0198	0.0192	0.6961	**0.9684**	**0.9588**
Cu	**0.5775**	0.4237	**0.5559**	0.7770			**0.8534**	0.3309	0.3099	0.6401	0.3785	0.6760
Zn	**0.9042**	0.0804	**0.8910**	0.0198	**0.8534**	0.3309			0.0385	0.0166	**0.7572**	0.0105
Fe	0.0137	**0.6406**	0.0192	0.6961	0.3099	0.6401	0.0385	0.0166			0.0976	0.5646
Na	**0.9603**	0.1177	**0.9684**	**0.9588**	0.3785	0.6760	**0.7572**	0.0105	0.0976	0.5646		

*DBrat, diabetic rat (n=8).

*NDrat, non-diabetic rat (n=7).

Table 8

Inter element associations (square of correlation coefficient, R^2) in the kidneys of diabetic and non-diabetic rats.

	Ca		Mg		Cu		Zn		Fe		Na	
	DBrat	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**
Ca			**0.8114**	0.0583	0.5635	0.1648	n/a	0.0162	**0.9976**	0.0076	0.2053	0.0005
Mg	**0.8114**	0.0583			**0.9276**	0.0311	n/a	0.0065	**0.848**	0.2637	0.6326	**0.6768**
Cu	0.5635	0.1648	**0.9276**	0.0311			n/a	0.4343	**0.6115**	0.1914	**0.8633**	0.2418
Zn	n/a	0.0162	n/a	0.0065	n/a	0.4343			n/a	0.0158	n/a	0.0035
Fe	**0.9976**	0.0076	**0.8480**	0.2637	**0.6115**	0.1914	n/a	0.0158			0.2461	**0.6610**
Na	0.2053	0.0005	0.6326	**0.6768**	**0.8633**	0.2418	n/a	0.0035	0.2461	0.6610		

*DBrat, diabetic rat (n=8).

*NDrat, non-diabetic rat (n=7).

Table 9

Inter element associations (square of correlation coefficient, R^2) in the hair samples of diabetic and non-diabetic rats.

	Ca		Mg		Cu		Zn		Fe		Na	
	DBrat	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**	**DBrat**	**NDrat**
Ca			0.0932	0.4888	**0.5276**	0.2635	0.4448	0.0851	0.1385	0.2996	**0.8657**	**0.8793**
Mg	0.0932	0.4888			**0.6514**	0.3623	**0.5623**	**0.0100**	**0.5748**	**0.7363**	0.2669	**0.5381**
Cu	**0.5276**	0.2635	**0.6514**	0.3623			0.4772	0.2853	0.1152	0.4135	**0.828**	0.2024
Zn	0.4448	0.0851	**0.5623**	0.0100	0.4772	0.2853			0.0417	0.0000	0.3978	0.3978
Fe	0.1385	0.2996	**0.5748**	**0.7363**	0.1152	0.4135	0.0417	0.0000			0.0065	**0.5225**
Na	**0.8657**	**0.8793**	0.2669	**0.5381**	**0.828**	0.2024	0.3978	0.2032	0.0065	**0.5225**		

*DBrat, diabetic rat (n = 8).

*NDrat, non-diabetic rat (n = 7).