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Impact of Trace Element Changes on Dehydroepiandrosterone Sulfate in Healthy and Diabetic States among Middle-Age and Elderly Egyptians

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Abstract The aim of this study was to confirm if there is a link between the alteration in blood levels of trace elements (chromium, copper, lead, cadmium, and zinc) and dehydroepiandrosterone sulfate (DHEAS) in healthy and diabetic states. This study is the first study to test these parameters in Egyptians. The study included 150 subjects divided into the following four groups: healthy middle-aged, healthy elderly, middle-aged diabetics, and elderly diabetics. Our results revealed a statistically significant decrease in the level of DHEAS in the elderly compared to middle-aged healthy and diabetic groups ($p<0.05$). There was a significant difference between the middle-aged groups with respect to zinc, copper, chromium, and cadmium levels. Zinc and copper were lower in the diabetic subjects while chromium and cadmium were higher in the same group in comparison to healthy subjects. In the elderly groups, there were significant increases in chromium and cadmium levels in diabetic subjects rather than healthy ones. There was a significant increase in the thiobarbituric acid reactive substance level in the elderly healthy and diabetic groups and a significant decrease in the glutathione level in the elderly groups. There was no correlation between the levels of trace elements and DHEAS or between the levels of DHEAS, oxidants, and antioxidants in all of the tested groups. In conclusion, only the DHEAS level was correlated with age. There was no difference between the diabetic and healthy groups with respect to the levels of trace elements, with the exception of chromium and cadmium, which suggests the effect of pollution on the pathogenesis of diabetes in Egyptians. No correlation existed between the levels of DHEAS and trace elements, oxidants, and antioxidants. Finally, we believe that there is a large regional variation in the levels of trace elements due to different environmental exposure and

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nutritional factors which are responsible for contradictory results regarding the pathogenesis of diseases related to alterations in the levels of trace elements.

Keywords Trace elements · DHEAS · Diabetes mellitus · Oxidants · Antioxidants

Introduction

Trace elements, like copper and zinc, play an important role in the human body, and other trace elements are considered to be toxic, such as lead, hexavalent chromium, and cadmium [1].

The metabolism of several trace elements is altered in patients with diabetes mellitus, which might have specific roles in the pathogenesis and progression of the disease [2–5].

Data indicate the beneficial effect of serum dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) levels on diabetes [6, 7].

In males, hyperglycemia is postulated to result from a decline in the DHEAS level, and this decline is independent of the serum insulin level [8]. It has been reported that DHEA and its sulfate have a role in the regulation of insulin sensitivity, and therefore have the potential to attenuate age-related increases in insulin resistance [9].

Little has been mentioned about the relationship between the levels of trace elements and DHEAS. Copper deficiency decreases the serum DHEA level by approximately 50% in rats. Thus, people who associate higher serum concentrations of DHEA with health probably should eat a diet adequate in copper [7]

An advanced stage of diabetes is linked with an increase in oxidative substances [10]. DHEA has been considered as an antioxidant [11].

There are increased levels of thiobarbituric acid reactive substances (TBARS) in diabetics. The increased copper levels catalyze lipid peroxidation and may enhance oxidation of low density lipoprotein (LDL) causing an increased level of TBARS in diabetic patients, as reported by Heinecke et al. [12].

Glutathione (GSH) is involved in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates. Thus, the decrease in the GSH level might reflect a direct reaction between GSH and free radicals generated by hyperglycemia in patients with diabetes. This is consistent with GSH function in scavenging oxidants by covalent binding [13].

The relationship between the levels of trace elements and DHEAS has not been studied in diabetic and healthy states. The purpose of the current study was to demonstrate whether or not there is a link between the alteration in some trace elements (chromium, copper, lead, cadmium, and zinc) in the blood with the DHEAS level and oxidative status in healthy and diabetic states in middle-aged and elderly subjects. This study was the first study to test these parameters together with respect to the different environmental and nutritional factors involving Egyptians in comparison to previous studies.

Patients

The study had the approval of the ethical committee of the faculty of medicine, Cairo University, and written consent was obtained from all participants in the study.

The study included 150 male subjects selected from the Endocrinology and Diabetes Center of Kaser El Eini Hospital of Cairo University. The subjects were divided into the following four groups: middle-aged healthy group, 50 men (40–59 years of age) without endocrinopathies; elderly healthy group, 25 men (>60 years of age) without endocrinopathies; middle-aged diabetic group 50 men with non-insulin-dependent diabetes mellitus (NIDDM); elderly healthy group, 25 men (>60 years of age) with NIDDM.

All subjects were cigarette smokers. Healthy and diabetic subjects had no history of receiving antioxidant supplementation. None of the diabetic subjects were on chromium supplementation. The diabetic subjects did not have any other chronic illness or inflammatory condition and showed no signs or symptoms of diabetic complications. The duration of NIDDM was <5 years, and all patients were under treatment with oral hypoglycemic drugs.

Methods

Eight milliliters of venous blood was obtained from the antecubital veins for blood chemistry analyses. Each blood sample was collected in two types of test tubes (plain and sodium flouride tubes). Each blood sample in the plain and sodium flouride tubes was left in an ice bath for 1–2 h, then centrifuged at 3,000 rpm. The collected serum and plasma were kept at −20°C in a deep freezer pending assay. Blood glucose was estimated using a colorimetric method carried out using a commercial kit purchased from Bioanalytics (Palm City, FL, USA). Determination of the DHEAS level was estimated using a commercial kit (Immunotech, Beckman Coulter Company, France) utilizing a radioimmune assay procedure. Determination of zinc, copper, chromium, cadmium, and lead were carried out using an atomic absorption spectrophotometer (G.B.C., 902 DB), as described before [14].

Lipid peroxidation was determined by measuring the formation of TBARS using a colorimetric spectrophotometer at a wavelength of 532 nm. Serum glutathione was measured using 5,5′-dithiobis-2-nitrobenzoic acid in a spectrophotometer at a wavelength of 412 nm. Serum glutathione-S-transferase was measured using a spectrophotometer at a wavelength of 340 nm.

Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 12.0; SPSS, Inc., Chicago, IL, USA). The results are expressed as the mean±SD. For dual comparisons, a Student's *t* test was used to compare group results. Correlation analysis was performed using the Spearman's test. Differences and correlation were considered as significant at a $p<0.05$.

Results

There are 11 items compared in four tested groups. Table 1 demonstrates the clinical and laboratory data in the four tested groups. There was a significant decrease in the level of DHEAS in the elderly group in comparison to the middle-age group in healthy and diabetic subjects.

There was a significant difference between the middle-age groups of the diabetic and healthy subjects, specifically the zinc and copper levels were lower in diabetics, and the chromium and cadmium levels were higher in diabetics. For oxidants and antioxidants, there was a significant increase in TBARS and a decrease in GSH in the healthy and

Table 1 The laboratory data of tested subjects

Parameter	Healthy middle-aged	Healthy elderly	Middle-aged diabetics	Elderly diabetics
Age in years	50.2±0.93	65.7±0.65	50.6±0.77	68.4±1.17
FBG (mg/dL)	84.07±2.33	90.6±3.4	275.16±18.201	294.6±26.41
DHEAS (μmol/L)	3.04±0.385	1.6±0.19**	3.19±0.58****	1.71±0.458*
Zn (μmol/L)	220.4±14.85	149.3±8.85***	168.5±12.21******	144.1±11.88****
Cu (μmol/L)	19.5±1.32	22.5±2.16	15.06±0.72****	19.05±1.36*
Cr (μg/dL)	0.19±0.014	0.17±0.018	0.31±0.037****	0.46±0.061*,****
Pb (μmol/L)	0.79±0.092	1.13±0.127*	0.75±0.129	0.801±0.177
Cd (nmol/L)	1.43±0.181	1.94±0.248*	3.05±0.378 ****	4.61±0.598*,****
TBARS (mmol/dL)	4.57±0.244	6.21±0.351 ***	5.67±0.396	8.13±0.377**
GSH (ug/dL)	15.14±2.038	10.14±1.49*	14.2±1.42*****	8.9±1.25**
GST (U/dL)	0.46±0.007	0.43±0.008	0.44±0.006	0.41±0.012

Cd cadmium, *Cu* copper, *Cr* chromium, *DHEAS* dehydroepiandrosterone sulfate, *FBG* fasting blood glucose, *GSH* glutathione, *GST* glutathione-S-transferase, *Pb* lead, *TBARS* thiobarbituric acid reactive substance, *Zn* zinc

*$p<0.05$; **$p<0.01$; ***$p<0.001$, the difference between the two age groups in the same state (Student's *t* test); ****$p<0.05$; *****$p<0.01$; ******$p<0.001$, The difference between the two groups in the same age group

diabetic elderly groups in comparison to the middle-age groups. There were insignificant changes in GST activity in all groups.

Figures 1, 2, 3, 4, 5, and 6 demonstrate the serum levels of DHEAS, zinc, cadmium, copper, TBARS, and GSH in different groups.

Correlation analysis between the different parameters and DHEAS in the studied population revealed a significant negative correlation with age ($p<0.05$) in the middle-aged and elderly healthy and middle-aged diabetic groups, but not in the elderly diabetics.

There was also a positive correlation with copper in the healthy elderly group and a positive correlation with TBARS in elderly diabetics ($p<0.05$).

Fig. 1 Serum DHEAS in various groups

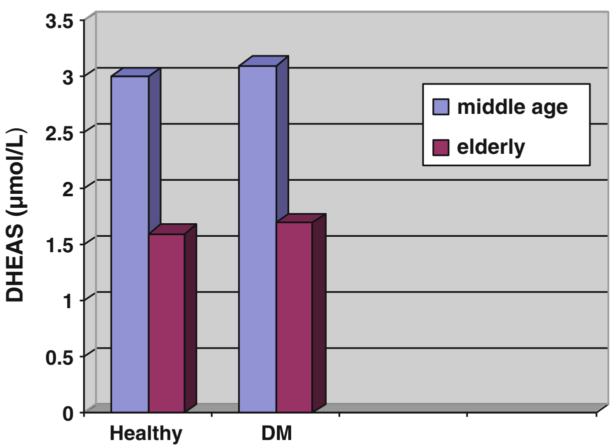
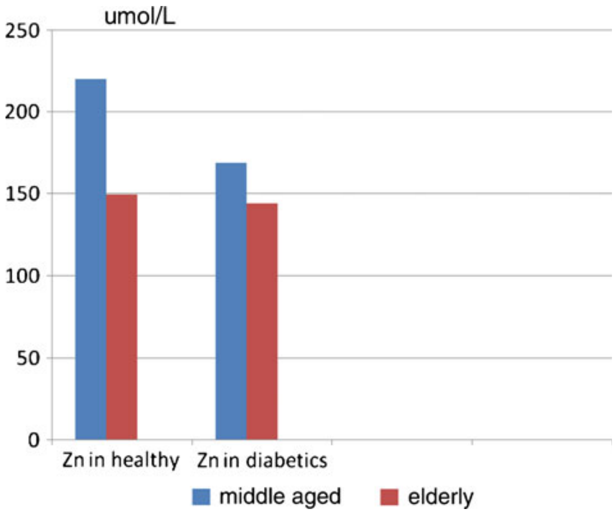


Fig. 2 Zn level in healthy and diabetics

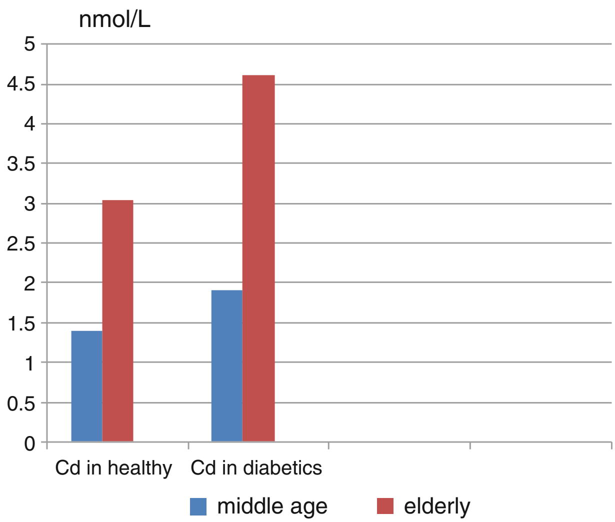


Discussion

This report on Egyptian male subjects is the first to assess the levels of trace elements and DHEAS in healthy and diabetic states in both middle-aged and elderly subjects. There are many reports of assays of trace elements in the blood with variable reference ranges that differ from one population to another depending on different environmental factors and dietary habits and measurement methods. Thus, we believe that this research conducted on an Egyptian population is of value to highlight these changes.

The level of zinc was shown to be significantly lower in diabetic patients in comparison to the healthy subjects in both age groups. The level of zinc also decreased significantly in the healthy elderly group in comparison to the healthy middle-aged group; however, no

Fig. 3 Serum cadmium in various groups



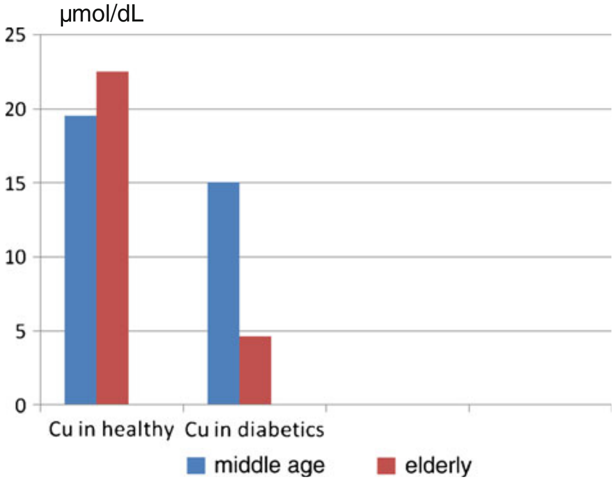


Fig. 4 Serum copper in various groups

statistical difference existed in the two age groups with NIDDM. This finding is in agreement with Zheng et al. [14] and Kazi et al. [15].The mechanism underlying the decreased zinc level in the elderly subjects is likely multifactorial and may be due to an internal redistribution of zinc and a redistribution of zinc in the liver [16]. Reduced serum levels of zinc in patients with NIDDM have been reported in a number of studies, while in other studies elevated levels were recorded, which is why plasma zinc varies under the influence of metabolic and hormonal interferences [17].

The copper level was significantly decreased in middle-aged diabetics in comparison to the healthy group and was generally higher in the elderly. Our results are consistent with Chen et al. [18] who reported low levels of copper in serum and erythrocytes in diabetics in comparison to healthy subjects, but most of the studies on copper assays in diabetics have reported normal or increased copper levels [15, 17, 19]; however, this

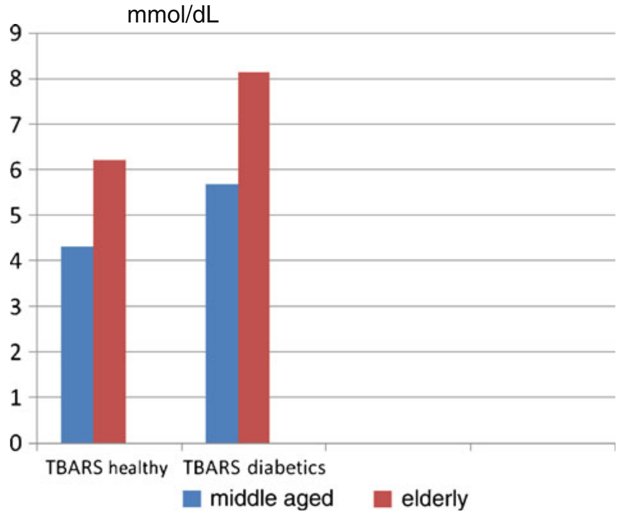


Fig. 5 Serum TBARS in various groups

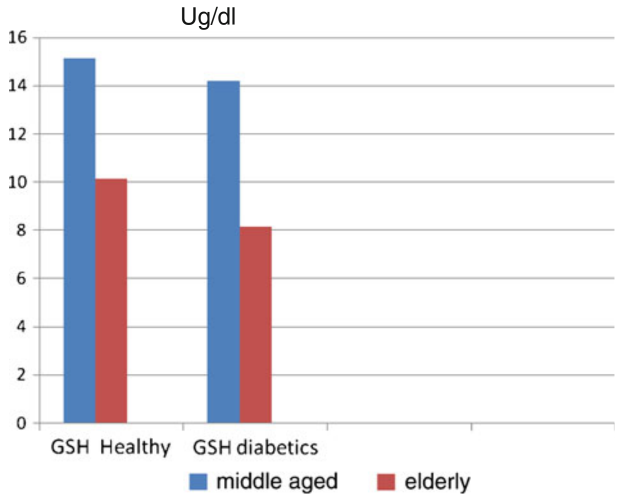


Fig. 6 GSH level in various groups

elevation increased with age [20]. Studies with an increase in the copper ion levels in patients with NIDDM might be attributed to hyperglycemia that may stimulate glycation and release of copper ions from copper-containing enzymes [11]; further studies are needed to verify this notion.

Our results revealed that hexavalent chromium was increased significantly in the diabetic groups in comparison to the healthy subjects. This is in agreement with Kruse-Jarres [21], who reported a significant increase in hexavalent chromium in patients with NIDDM, but in contrast to Kazi et al. [15] and Ruvz et al. [19], who reported that the chromium level was significantly reduced in the blood of diabetic patients (the trivalent type); the urinary levels of this element was shown to be higher in diabetic patients. Because hexavalent chromium is toxic, causing islets cell destruction, it is possible that pollution may precipitate diabetes through toxic destruction of the pancreas. The same possibility applies to another toxic element (cadmium). Cadmium is a widespread environmental pollutant that accumulates in the pancreas and exerts diabetogenic effects in animals, as well as humans [22–24]. Our results revealed a significant increase in the cadmium level in diabetics compared to healthy subjects in both age groups. This is consistent with the increasing rates of NIDDM worldwide, which suggests that diabetes may be caused by environmental toxins.

Our results showed no difference between diabetic and healthy subjects in both groups for lead, which may indicate that lead has no role in the development of diabetes in Egyptians. No data are available regarding the relationship between lead and diabetes.

Regarding lipid peroxidation and oxidative stress, our results revealed that there is a significant increase in TBARS and a decrease in GSH in the healthy and diabetic elderly groups compared to the middle-aged groups. There were insignificant changes in GST activity in all groups. In the middle-aged groups, the only difference detected involved the decreased GSH in the diabetic group.

The oral intake of a high glucose load increases TBARS. In humans, oxidative stress (OST) occurs in postprandial periods in healthy individuals, but diabetic patients are unable to compensate for the increased ROS. Increased OST also occurs in the basal state in both types of diabetes. Type 2 diabetics exhibit increases in TBARS and reduction in catalase activity. Plasma GSH levels are decreased and oxidized purines increase, illustrating DNA damage [25].

Hyperglycemia has a marked oxidant impact, as evidenced by the significant increase in lipid peroxidation products, as well as a significant decrease in antioxidants, including GSH content [11].

There was a significant decrease in the level of DHEAS in the elderly in comparison to middle-aged healthy and diabetic subjects.

Our results are partial consistent with a Japanese study which revealed that serum DHEAS levels were significantly lower in patients with NIDDM than patients with normal glucose tolerance. However, this difference was not significant when adjusted for age [26].

The DHEAS level was negatively correlated with age in all groups, except the elderly diabetic group. This is similar to what was reported, that starting at 50 years of age a significant age-related decrease in DHEAS is observed [27, 28].

Our results revealed a positive correlation between the DHEAS level and copper in the healthy elderly group.

Copper deficiency decreases the DHEA level in serum by approximately 50% in rats. The synthesis of DHEA and other steroid hormones from cholesterol requires oxidative transformation, and all the enzymes that require copper for activity are oxidative [6].

There was no correlation between DHEAS and oxidative or antioxidative parameters, with the exception of the elderly diabetic group, which had a positive correlation between the TBARS and DHEAS levels. These results are similar to the report by Brignardello et al. [29] because DHEA is not a scavenger compound and acts in a stoichiometric manner and exerts its antioxidant effects in a complex and incompletely defined way [29].

Our results regarding copper and lipid peroxidation parameters are not consistent with what the report of Heinecke et al. [11] who stated that increased copper levels, a transition metal that is redox-active and catalyzes lipid peroxidation, may enhance oxidation of LDL causing an increased level of TBARS in diabetic patients. This finding suggests other factors increase oxidative stress in diabetics rather than the copper level.

Conclusion

In conclusion, the DHEAS level correlates with age, copper, and TBARS. There were differences between the diabetic and healthy groups with respect to zinc, copper, chromium, and cadmium levels. The latter may point to the effect of pollution on the pathogenesis of diabetes in Egyptians.

The copper level was lower in diabetic patients than the healthy group. Oxidative stress seems to be affected.

Finally, we conclude that there is a lot of variation within a population in trace element status according to different environmental and nutritional factors, as well as economic status with a secondary impact on hormonal status.

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Conflict of Interest No conflicts of interest.

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