CHANGES IN THE CONCENTRATION OF FLUORIDE AND BIOGENIC ELEMENTS IN THE SERUM AND BONES OF FEMALE RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

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SUMMARY: The aim of this study was to determine if streptozotocin-induced diabetes in rats as a model for Type-1 human diabetes causes changes in the levels of fluoride (F) and biogenic elements in the bones and serum in the initial stage of the disease. Twenty-two female Wistar rats were given streptozotocin to induce diabetes; after 10 days the femoral bones and blood were collected for determination of F by potentiometric analysis, Ca and Mg by atomic absorption spectrometry, and estradiol by electroluminescence. At various levels of significance, F, Ca, and Mg increased in the bones. In the serum, F decreased, but Ca, Mg, and estradiol increased. The results indicate that diabetes in the early stages affects uptake of F, Ca, and Mg intake into the bones, which may result in defective crystal formation and an increase of amorphous mineral structure in the bone.

Keywords: Bone calcium; Bone magnesium; Bone fluoride; Crystal abnormality; Diabetic rats; Estradiol; Fluoride intake; Streptozotocin-induced diabetes.

INTRODUCTION

Type 1 diabetes is a metabolic disease characterized by hyperglycemia, resulting from an absolute insulin secretion deficit.1 Chronic hyperglycemia, both in experimentally induced diabetes in animals and in clinically visible Type 1 diabetes in people, leads to damage of tissues subjected to the activity of glucose.2,3 The group of chronic complications of diabetes in animals also involves osteopenia and osteoporosis.4 The etiology of osteopenia and osteoporosis in diabetes is still not fully understood. Chronic deficiency of insulin, occurring in Type 1 diabetes, can decrease the formation of the osseous matrix and tissue mineralization.5 Ketosis is an additional factor that also intensifies the resorption of the osseous tissue through the activation of osteoclasts.6 On the other hand, diabetic microangiopathy may contribute to the development of osteopenia and osteoporosis by impairing blood flow and nutrition of the osseous tissue,7,8 which may lead to changes in the concentration of biogenic elements in this tissue such as fluorine,9 calcium,10 and magnesium.11 To the best of our knowledge, few studies have been conducted to determine the manifestation of diabetes mellitus in the concentration of these elements in the bones and serum during the initial stage of the disease.

In this study, we aimed to examine what effect streptozotocin-induced diabetes has on the concentration of fluoride, calcium, and magnesium in the bones and

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serum of female rats in the acute early stages of the disease with an absolute deficit of insulin.

MATERIALS AND METHODS

The research was conducted on 29 clinically healthy, female Wistar rats. During the experiment the rats lived in standard conditions at the Pomeranian Medical University laboratory. The animals had free access to water and granulated fodder. After a one-week period of adaptation to their new environment, the rats were divided into two groups: a control group (n=7), which, on the first day of the experiment had 1 mL 0.01 M of citrate buffer with pH 4.5 injected into the caudal vein; and the experimental diabetes group (n=22). On the first day of the experiment all the animals in the experimental diabetes group were injected once with streptozocin STZ (Sigma), 55 mg/kg body weight in 1 mL 0.01 M of the citrate buffer with pH=4.5 directly into the caudal vein. Diabetes was recognized on the basis of a single measurement of glucose concentration in whole blood > 11.0 mms (200 mg/dL) on the tenth day of the experiment. Fourteen animals in the experimental group became diabetic, and these rats were selected for further analyses.

After euthanization of the animals on the tenth day, cardial blood was taken for coagulation and the thigh bones were collected. The blood was centrifuged, and the obtained serum was subjected to analysis for F, Ca, Mg, and estradiol. The bones were dried at 60°C, pulverized, weighed, and prepared for analysis. Samples (10 mg) were dissolved in 65% nitric acid for determination of Ca and Mg. For F determination, samples were dissolved in 0.1 M perchloric acid and the concentrations measured by the potentiometric method with an Orion ion-selective electrode. Ca and Mg were assayed by atomic spectrometry analysis (ASA). In the blood serum the concentration of estradiol (E2) was determined by the “ECLIA” (Electrochemiluminescence Immunoassay) method.

STATISTICAL ANALYSIS

For statistical analysis, each investigated parameter was characterized using an arithmetical mean and standard deviation (SD). Distributions of values for each parameter were analyzed using the Shapiro-Wilk test. Because most of the distributions deviated from the normal (Gauss) distribution, further analysis involved non-parametric tests. Correlations between the values of each parameter were examined using the Spearman's rank correlation test. In order to find statistically significant differences in the concentration levels of the examined elements in bones and blood serum we carried out Mann-Whitney tests. We assumed the level of significance to be p ≤0.05.

RESULTS

Figure 1 presents the mean concentration of F in the blood serum and bones of diabetic and healthy female rats. We observed a statistically significant increase in the concentration of F in the thigh bones of the diabetic rats when compared with the control group (p=0.031). On the other hand, the concentration of F in the blood
serum of the diabetic rats proved to be significantly lower than in the control group (p=0.016).

Figure 2 presents the mean Ca concentration (A) and Mg (B) in the blood serum and bones of diabetic and healthy female rats. In the bones, the concentration of Ca did not show significant differences between the two groups, but the serum Ca concentration was significantly higher in the diabetic group (p=0.000017). We also observed a statistically significant increase in the concentration of Mg in the bones of the diabetic rats compared with the control group (p=0.012). Moreover, the concentration of this element in blood serum of the diabetic rats proved to be significantly lower than in the control group (p=0.046).
As seen in Figure 3, the concentration of estradiol in the serum of the diabetic rats on the tenth day of the experiment was significantly higher compared to the control group (p=0.001). The Spearman's correlation test showed dependence between the concentration of estradiol in the serum and the levels of the examined elements in the blood serum and bones. The serum concentration of estradiol correlated significantly with the levels of F in the bones (p=0.0065; R=0.688) and with the levels of Ca (p=0.002; R=0.748) and glucose in the blood serum (p=0.000048; R=0.872). The remaining statistically significant correlations are presented in Table 1.
DISCUSSION

Experimental diabetes induced by STZ (streptozotocin) provides an appropriate animal model for Type 1 diabetes and is one of the most often used models in studying insulin-dependent diabetes. The research conducted here with rats on the tenth day following administration of STZ allowed study of the acute course of the disease with the absolute deficit of insulin.

Chronic Type 1 diabetes is associated with several disorders of the skeletal system, including decreased bone mineral density, an increased risk of developing osteoporosis, and fragility fracture. In the overwhelming majority of previous work, research was carried out on male individuals (humans or animals) and after a long duration of the disease or of an experiment. Low dynamic bone...
formation and osteoporosis are characterized in the STZ rat model with long-term diabetes.\textsuperscript{21} Our results concerning female rats, which were not given insulin, suggest the accumulation of elements (F, Ca, and Mg) in the osseous tissue during the initial stages of acute STZ-induced diabetes mellitus after 10 days of the experiment.

In our research we observed a statistically significant increase in the concentration of F in the bones of the diabetic rats, with a simultaneous decrease in the concentration of this element in serum. It is probable that the state of the absolute deficit of insulin observed in STZ-induced diabetes leads to the accumulation of ketonic compounds and hence the development of heavy metabolic oxidosis. A decrease in extracellular pH can drive the movement of F into tissue cells.\textsuperscript{22} As other studies have shown, F intake into the bone can interrupt and interfere with the normal apatite crystal nucleation process. Crystallization would occur continuously toward c-axis at the peripheral area, whereas the central area would remain amorphous, resulting in crystal defects with a fuzzy structure appearing in the bone.\textsuperscript{23}

The analysis of the estradiol concentration in the blood of both groups of rats showed that diabetic rats were characterized by statistically significantly higher concentration levels of this hormone compared with the diabetic group (Figure 3). Our results also support findings of a short-term (8 days) study by Le May et al. on mice.\textsuperscript{24} The correct morphology and function of the skeletal system depend on estrogens, which affect the mineralization of the osseous tissue, since a suitable level of estrogens helps prevent loss of calcium from bones. A high level of estrogens stimulates the formation of blood-vessels that penetrate epiphysial gristles, delivering inorganic substances including F, Ca, and Mg. Estradiol promotes mineralization of the chondral matrix\textsuperscript{25} and consequently an increase in the concentration in osseous tissue of the elements analyzed in this study, which may not be really very beneficial in view of the fact that a high level of F and Mg may result in an increase of amorphous mineralization in bone,\textsuperscript{23} facilitating future resorption of the osseous tissue.\textsuperscript{6}

A high concentration of estrogens resulting from STZ-induced diabetes, probably occurring through inhibition of alimentary tract motorics (hypoperistalsis), can increase the intestinal absorption of Ca and Mg,\textsuperscript{26} and hence their increased concentration levels being found in the blood serum observed in this research. Similar conclusions were reached by Hough et al.\textsuperscript{27} in their research on rats. Moreover, water consumption in diabetes is higher, and intake of large quantities of drinking water directly increases fluoride intake.\textsuperscript{28} The high concentration levels of Mg\textsuperscript{29} and estrogens\textsuperscript{26} in serum probably additionally cause a decrease in the resorption of F, Ca, and Mg from bone via the inhibition of PTH secretion.\textsuperscript{30} Moreover, an elevated level of estrogens in blood serum can result in the disrupted distribution of these elements (Ca and Mg) in the body through the increase in the sensitivity of estrogen receptors if in target cells it facilitates the intercept of these elements by cytoplasm of estrogen-sensitive cells, thus decreasing their availability to other tissues.\textsuperscript{30} This explains a higher
concentration of Ca and Mg in the osseous tissue of female diabetic rats in this study (Figure 4).

In conclusion, reports in the literature show that chronic insulin-dependent diabetes mellitus may lead to the occurrence of osteopenia and osteoporosis. However, in the first days of the disease when it is yet to be diagnosed, there may occur reactions leading to the deposition of F, Ca, and Mg in the osseous tissue as the result of complex biochemical conversions in which estrogens appear to play a crucial part. This may not be very beneficial because the resulting high level of F and Mg may promote amorphous bone mineralization.

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REFERENCES