The Relationship between Experimental Alimentary Obesity and Hard Tooth Tissues Mineralization

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Abstract

Objectives: Obesity has become a pan-European epidemic. More knowledge about the molecular and cellular mechanisms governing adipose tissue accumulation is needed to develop more effective preventative and therapeutic approaches to obesity. We aimed to evaluate the influence of dental micro- and macroelement contents on structural changes of hard tooth tissues in rats with diet-induced obesity.

Materials and Method: Experimental obesity was modelled by including sodium glutamate to the feed mixture of male, non-liner, white rats of around 3 months of age, in a ratio of 0.6:100.0 and using a high-calorie diet. The atomic absorption spectrophotometer with flame and graphite furnace was used to quantify micro- and macroelement content. To evaluate structural changes of hard tooth tissues we analysed histological specimens prepared from central incisors of upper and lower jaws.

Results: Overall, during the experiment period, the concentrations of minerals in hard tooth tissues decreased as follows: calcium, by 1.5 times; magnesium, by 11.8 times; zinc, by 3.6 times; and copper to practically negligible levels ($p<0.05$). Histological investigations showed significantly enlarged enamel areas with disrupted structure due to the destruction of enamel prisms. Dentine injury was characterized by dentine channels destruction. Their amount significantly decreased in the cement-enamel junction area and near pulpa area as well, and that results in trophic disorders and hard tooth tissues destruction.

Conclusion: These data provide evidence that mineralization process of hard tooth tissues was negatively affected in rats with diet-induced obesity.

Keywords: Obesity, Tooth, Mineralization.

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Introduction

The obesity epidemic receives insufficient attention from the general public and policy-makers alike. More than 1 billion adults in the world are overweight, at least 300 million of them - clinically obese. This may have been influenced by the earlier misrepresentation of health information, which led to the misguided notion of 'healthy' or 'benign' obesity[1-4].

According to the International Obesity Taskforce, obesity has become a pan-European epidemic and at least 135 million EU citizens are affected. Hence, it is not surprising that obesity has long been at the centre of health policy debate and the focus of academic research. Even in the developing world, obesity is escalating wildly, inflicting the paradoxical double burden of obesity and malnutrition on poorer nations[5].

According to WHO, in Ukraine 50.5% of men are overweight, 16 % of them are obese and 56% of women are overweight, 26 % of them are obese. In general, 45% of people of working age in Ukraine are obese.

The increasing prevalence of overweight and obesity is higher in Turkey than in the countries of Europe, where the prevalence of obesity in men ranges from 4.0% to 28.3% and in women from 6.2% to 36.5%. However, the prevalence of obesity in Turkish adults is similar to that of United States of America, where 35.6% of population aged 20 and over were obese in 2010[6,7,8].

Today, excess weight and obesity are being diagnosed as early as in childhood. This trend is expected to grow, and in 2020 childhood excess weight and obesity prevalence will reach 9.1% worldwide+. Unfortunately, approximately 90% of children with severe obesity will become obese adults with a BMI of 35 or higher[10-12]. As obesity is a major independent risk factor for hypertension, type 2 diabetes, and dyslipidemia, it is a big issue for health system[6,7].

More knowledge about the molecular and cellular mechanisms governing adipose tissue accumulation is needed to develop more effective preventative and therapeutic approaches to obesity. In this study, we aimed to evaluate the influence of dental micro- and macroelements contents on structural changes of hard tooth tissues in rats with diet-induced obesity.

Materials and Methods

Animals and experimental model: Experimental studies were conducted on male, non-liner, white rats of around 3 months of age, that were housed at 25±3°C and humidity of 55±2%, under a constant 12 h light and dark cycle. Water was available ad libitum. Experimental obesity was modelled by including sodium glutamate to the feed mixture in a ratio of 0.6:100.0 and using a high-calorie diet that consists of the standard meal (47%), sweetened concentrated milk (44%), corn oil (8%) and vegetable starch (1%) (diet # C 11024, Research Diets, New Brunswick, NJ, USA)[13].

When selecting for the optimal model of alimentary obesity, we stressed on the fact that sodium glutamate affects ventrolateral nuclei of the hypothalamus, where the hunger centre is located, and thus it stimulates appetite. To monitor the alimentary obesity model, animals were weighted, their nasal-anal length measured, and body mass index (body weight

in kg divided by the squared length in meters) calculated. The experimental animals were divided into the following groups: in group 1, the rats that were on the experimental diet for 14 days (EG 1), and in group 2, for 28 days (EG 2). The control groups (CG) consisted of animals maintained on a standard diet for 14 days (CG 1) and 28 days (CG 2), respectively. Animal euthanasia was carried out on the 14^{th} and 28^{th} days of the experiment by cardiac puncture under deep anaesthesia, in accordance with the requirements of the Animal Care Committee[14].

Mineral Contents Quantification: Atomic absorption spectrophotometer with flame and graphite furnace was used to quantify micro- and macroelement contents. For simultaneous analysis, it consists of eight turret lamps with a wavelength range of 190-900 nm. The spectroscopic conditions were the following: bandwidth 0.4 nm with a 1.0 filter factor for zinc and magnesium, bandwidth 0.1 nm with a 1.0 filter factor for calcium and copper. The integration time was 3.0 s set at 5.0 mA lamp current for calcium and zinc, at 3.0 mA lamp current for magnesium and 2.0 mA lamp current for copper. The elements were detected at the following wavelength: calcium – 422.7 nm, magnesium – 285.2 nm, zinc – 213.9 nm, and copper – 324.7 nm.

Tissue Preparation and Staining: To prepare tooth histological specimens from the rats' upper and lower central incisors, study samples were fixed in 15% formaldehyde solution for 4 weeks. Decalcification of central incisors was performed by placing them in the 10% nitric acid solution for 6 days, followed by neutralization with 5% potassium alum for 1 day. Specimens were dehydrated using increasing ethanol series, cleared with chloroform, soaked in liquid paraffin and embedded in solid paraffin for 6 hours. The paraffin embedded tissues were serially sectioned in a semiautomatic Microm–HM 340 E rotary microtome at a thickness of 7 μm. The sections were stained with haematoxylin and eosin and observed under a light microscope.

Statistical analysis: The results were analysed using Statistica 7.0 software and presented as mean with standard error of mean. The differences between all groups were determined using one-way ANOVA, followed by post hoc Least Significant Difference test. A p-value of <0.05 was considered statistically significant.

Results

On the 14^{th} day of experiment, the evaluation of hard tooth tissues mineral composition showed a decline in content of micro- and macroelements. In particular, the content of calcium decreased by 6.8%; magnesium, 58.1%; zinc, 40.5%; and copper, 62.6% compared to the control group ($p<0.05$). On the 28^{th} day, the deficiency of micro- and macroelements (calcium, magnesium, zinc and copper) in hard tooth tissues of animals in EG 2 became more prominent, even when compared with the EG1 ($p<0.05$). Overall, during the experiment period, the contents of minerals in hard tooth tissues have decreased as follows: calcium, by 1.5 times; magnesium, by 11.8 times; zinc, by 3.6 times; and copper to practically negligible levels ($p<0.05$) (Table 1).

Table 1. Contents of micro- and macroelements in hard tooth tissues of rats with diet-induced obesity (M±m)

Index	Control group 1 (n=12)	Experimental group 1 (n=12)	Control group 2 (n=12)	Experimental group 2 (n=12)
Calcium, mg/g	270.35±3.80	252.07±2.61	262.46±6.48	180.65±1.63
		$p_1<0.05$		$p_1<0.05$; $p_2<0.05$
Magnesium, mg/g	8.28±0.41	3.47±0.33	8.12±0.54	0.70±0.07
		$p_1<0.05$		$p_1<0.05$; $p_2<0.05$
Zinc, µg/g	0.33±0.03	0.20±0.01	0.36±0.05	0.09±0.03
		$p_1<0.05$		$p_1<0.05$; $p_2<0.05$
Copper, mg/g	0.60±0.08	0.23±0.04	0.52±0.09	0.01±0.01
		$p_1<0.05$		$p_1<0.05$; $p_2<0.05$

Notes: p_1 – significant difference compared to the control group; p_2 – significant difference compared to the other experimental group.

To evaluate structural changes of hard tooth tissues we analysed histological specimens prepared from central incisors of upper and lower jaws EG 2 animals. We observed enlarged enamel areas with disrupted structure due to the destruction of enamel prisms (Figure 1). Areas without prisms were located along the cement-enamel junction, as it is typical for the normal tooth organisation, and in these areas the enamel had almost the same thickness as in the control group. Areas without prisms also were located superficially, and formed solid homogenous layers in some places. Enamel lamellae were shortened, unravelled and reached only middle enamel.

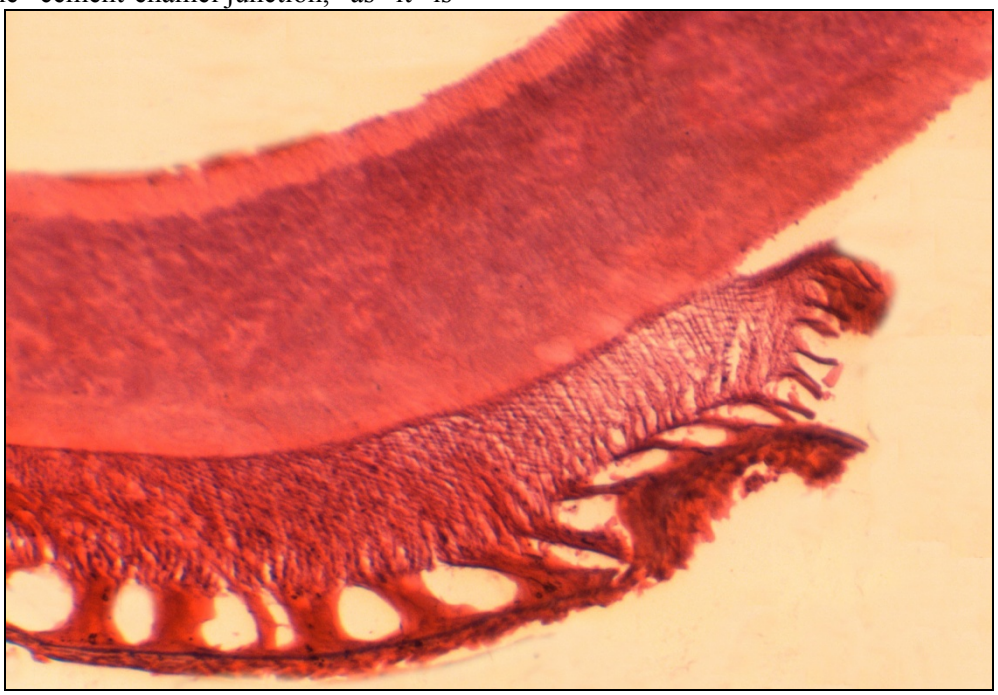

Figure 1: Destruction of hard tooth tissues in white rat of EG 2. Cross section, stained with hematoxylin and eosin. X 160

While preparing a longitudinal section, some enamel prisms strands were dissected lengthwise and others transversally. In histologic specimens, this was indicated by the presence of Hunter-Schreger bands. Another type of bands that look like curved asymmetrical arcs between enamel surface and cement-enamel junction was identified as so called striae of Retzius. These bands were found in control as well experimental groups, but in animals of the experimental group, the Hunter-Schreger bands and the striae of Retzius were more distinct and contoured (Figure 2). These characteristics indicate disorientation of enamel prisms and enamel matrix.

In the experimental group, dentine channels were located radially, and the same placement was found in the control group. However, in the experimental group, the channel outlines in dentine tissue near the enamel layer became less defined and unstructured areas were observed, suggesting dentine damage. At the same time, areas with elevated density of channels were identified, and this can be considered a manifestation of a compensatory event.

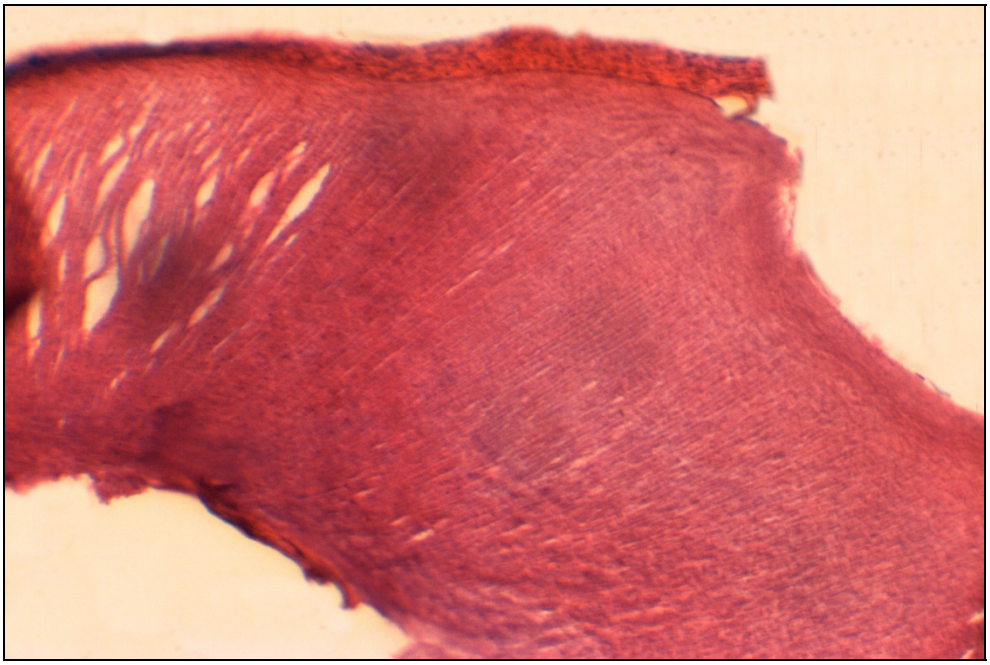

Figure 2: Destruction of hard tooth tissues in white rat of EG 2. Longitudinal section, stained with hematoxylin and eosin. X 160

DISCUSSION

We know that deficit of copper in bone tissue disrupts the process of collagen formation while increasing its soluble fractions, which decreases mineral bone density[15]. At the same time, with alimentary obesity, the facial skeleton loses its mineral bone density and develops progressing loss of periodontal tissues. The osteoporotic changes that affect bone tissues of dento-maxillary system increase the frequency of periodontal disorders[16]. We argue, that the results of this study point to copper deficit as one of the main causes of disorders of bone structure of the teeth.

Another microelement involved in many

hormone mediated reactions and in mineralization process of the body is magnesium. In our study, the levels of this particular element experienced significant decline. The exact role of magnesium in bone mineralization is still being debated in the literature. Some authors argue that the growth of apatite crystals, an important part of the mineralization process, intensifies with increased concentration of magnesium[17]. However, others link the increased concentration of magnesium in bone tissues to the development of osteoporotic processes[18]. Recent studies suggest that magnesium is involved in maintaining normal calcium concentration in bone tissues, and its prolonged deficit causes decreased activity of osteoblasts and increases bone fragility with subsequent clinical complications characteristic of osteoporosis[19]. Some researchers connect the action of magnesium on mineral metabolism in bone tissues with its influence on hormonal pathways as well as direct effects on the bone tissue itself[20]. There is a direct correlation between the level of magnesium in the bones and in the hard tissue of teeth[21]. Thus, our results suggest that the entire organism of the experimental animal has likely suffered reduced bone tissue mineralization.

Though copper and magnesium undoubtedly play an important role in mineralization, calcium that forms the mineral scaffolding of a tooth, ensuring its mechanical and support characteristics, is the clue element. Our study demonstrates decrease of calcium contents in hard tooth tissues. However, these changes were less pronounced compared to magnesium. Related investigations suggest that obesity can slow down bone formation while increasing adipogenesis, because both adipocytes and osteoblasts are derived from the same type of stem cell[22]. Sen et al. showed that certain factors suppressing lipogenesis at the same time stimulate osteoblast differentiation[23]. Our previous research indicates that experimental diet-induced obesity triggers development of oxidative stress[24]. This process, coupled with activation of anti-inflammatory cytokines, can activate osteoclasts, decreasing calcium content in hard tooth tissues, increasing reabsorption, and subsequently causing the destruction of bone tissue[25]. Another osteotropic element, zinc, modulates the intensity of metabolic processes in bone tissue. Zinc is involved in the process of calcification, accelerates collagen synthesis, and also is a structural element in many metalloproteinases (26). Obtained data indicate that zinc contents in hard tooth tissues decreased more than calcium, but less than magnesium. Research on experimental models showed that subcutaneous adipose tissue synthesizes metallothioneins, and concentration of these molecules also increases in liver under stress conditions[21,27,28]. Metallothioneins bind zinc in adipose tissue and liver, hindering its allocation throughout the body tissues. Thus, our results suggest that under experimentally-induced obesity, the significantly lower concentration of zinc in the hard tooth tissues is the consequence of low zink concentration in blood.

Histological investigations showed significantly enlarged enamel areas with disrupted structure. The presense of Hunter-Schreger bands and the striae of Retzius indicate disorientation of enamel prisms and enamel matrix[29]. Dentine injury was characterized by dentine channels destruction. Their amount significantly decreased in the cement-enamel junction area and near pulpa

area as well. Since metabolism of organic and inorganic compounds in dentine is due to the circulation of fluid in the channels then their destruction results in trophic disorders and hard tooth tissues destruction[30].

In summary, based on our results we conclude that diet-induced obesity in rats shows the negative impact on hard tooth tissues mineralization due to decreased micro- and macroelement content in hard tooth tissues. Thus, magnesium and copper can be regarded as systemic factors of reduced bone mineralization in experimental obesity. The damage to the organic matrix of the enamel and dentine channel destruction results in trophic disorders and hard tooth tissues destruction.

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العلاقة ما بين السمنة الغذائية التجريبية ومعدنة أنسجة الأسنان الصلبة

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الملخص

الأهداف: أصبحت السمنة مرضاً وبائياً في جميع أوربا. نحتاج لمعلومات أكثر حول الميكانيكية الجزئية والخلوية حول تراكم الأنسجة الدهنية لتطوير طرق فعالة أكثر لوقاية وعلاج السمنة. سعينا إلى تقييم تأثير محتوى العناصر الصغيرة والكبيرة في الأسنان على التغييرات الهيكلية في الأسنان الصلبة على الفئران تحت تأثير نظام غذائي يسبب السمنة.

الأدوات والخطة: حثينا على السمنة التجريبية بالإضافة غلوتامات الصوديوم إلى خليط غذاء جرذان بيضاء غير مخططة أعمارهن يقارب الثلاثة أشهر، على نسبة 0.6.100.0 بالإضافة إلى نظام غذائي ذو سعرات حرارية عالية. استخدمنا جهاز طيف الامتصاص الذري وفرن الجرافيت لتحديد كمية الجزيئات الصغيرة والكبيرة. ولتقييم التغييرات الهيكلية لأنسجة الأسنان الصلبة، حللنا عينات انسجة معدة من القواطع الوسطى التابعة للفك العلوي والسفلي.

النتائج: اثناء الفترة التجريبية، لقد تناقصت تركيز المعادن على النحو الآتي؛ كالسيوم ب 1.5 مرة، مغنيسيوم ب 11.8 مرة، زنك بـ3.6 مرة؛ والنحاس بكميات ضئيلة $p > 0.05$. أظهرت التحليلات النسيجية كبر في مناطق المينا مع اختلال في الهيكل بسبب تدمير موشور المينا. وضرر العاج بسبب تدمير القنوات العاجية. ونسبتهم بشكل كبير في منطقة وصل الملاط والمينا وكذلك في منطقة الجذر. وتَسبَبَ ذلك في خلل ضامر وتدمير أنسجة الأسنان الصلبة.

الخلاصة: تشير هذه المعطيات إلى ان معدنة أنسجة الاسنان الصلبة قد تأثرت بشكل سلبي في الجرذان التي خضعت لنظام غذائي يسبب السمنة..

الكلمات الدالة: السمنة، الأسنان، التمعدن.