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# THE EXAMINATION OF REMODELING PROCESSES OF HARD PERIODONTAL TISSUES IN RATS WITH THE DISORDERS OF PANCREATIC INCRETORY FUNCTION

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***Abstract.*** **Topicality.** Pancreatic hormones play an important role in the process of remodeling of hard periodontal tissues. Violations of carbohydrate metabolism that occur under conditions of isolated insulin resistance (IR) and in combination with iodine deficiency (ID) can be the cause of dysmetabolic disorders of mineralization/demineralization physiological system of tooth-maxillary complex.

**The aim**. To study the changes of remodeling processes of hard periodontal tissues in rats with isolated IR and impaired glucose tolerance against the background of ID.

**Materials and methods.** The examination was carried on 90 male rats, which were divided into three groups: control (intact animals), group of rats with IR under conditions of adequate iodine supply, and with IR against the background of ID. The system of carbohydrate metabolism was studied by the level of insulin in blood serum, glucose and glycosylated hemoglobin (HbA1c) of blood with the following calculation of HOMA-IR index. The processes of mineralization/demineralization were detected by the content of calcium, magnesium, zinc, manganese, copper in cementum of tooth root and alveolar process, and by the activity of acid and alkaline phosphatases in blood serum.

**Results.** Keeping of animals on a high-fructose diet led to the development of carbohydrate metabolism disorders (increase blood glucose and HbA1c levels, blood serum insulin, HOMA-IR index) and changes of remodeling processes in hard periodontal tissues (decrease the level of calcium, magnesium and manganese against the background of increase the zinc content in cementum of tooth root; decrease the content of macroelements in alveolar process; activation of acid phosphatase against the background of inhibition of alkaline phosphatase activity). The development of combined endocrinopathy was accompanied by more pronounced changes of studied parameters.

**Conclusions.** The violation of glucose tolerance against the background of ID slows down the mineralization processes of hard periodontal tissues mainly due to the intensification of osteoresorptive processes.

***Keywords: acid and alkaline phosphatases; calcium homeostasis; bioelement panel of tooth-maxillary system; insulin resistance; iodine deficiency.***

**Statement of the problem and analysis of the latest research**

The state of human health significantly depends on the characteristics of the lifestyle in modern conditions and the factors of external environment. However, under any conditions, the regulatory influence of humoral factors on the course of key links of metabolic processes and maintaining the physiological parameters of the body remains unchanged. An important role in such regulation play the pancreatic hormones, and disorders of their secretion or biological activity cause violations of metabolic and energy metabolism at the level of entire organism [1, 2].

According to the data of the World Health Organization, the diseases of pancreas take the first place in the structure of endocrinopathies. Thus, more than 366 million of people suffer from diabetes mellitus, moreover the number of new cases with a confirmed diagnosis tends to increase. This problem is no less relevant for Ukraine, where the number of patients is more than two million people [3].

It is considered that a predictor of diabetes mellitus development is insulin resistance (IR), the prevalence of which is global, and clinical manifestations can have the systemic character [4]. Taking into account the multicomponent pathogenic mechanisms of metabolic disorders development in case of IR, pathological changes under such conditions can also affect the tooth-alveolar complex [5]. It is known that hyperglycemia leads to a decrease of periodontal resistance, which is associated with the activation of pathogenic microflora, osteopenic changes in bone tissue, suppression of the immune response, and the development of metabolic acidosis [6]. An important aspect is the development of microangiopathies, the occurrence of which is associated with impaired glycosaminoglycan metabolism, non-enzymatic glycosylation of proteins, and direct glucose toxicity. It should be noted, that hyperglycemia also has a direct and indirect damaging effect on bone tissue cells, which is manifested by a decrease of bone density by reducing the absorption of calcium ions in the intestine and their reabsorption in the nephron tubules [7]. Along with this, glycation processes increase the manifestations of osteoblasts apoptosis, inhibit osteocytes differentiation, and the synthesis of osteonectin, osteocalcin, and type I collagen. Thus, the bone remodeling processes that provide bone microarchitecture and maintain the strength of alveolar processes undergo the changes, which can lead to partial or complete teeth loss [8].

**The purpose.** To study the changes of remodeling processes of hard periodontal tissues in rats with isolated IR and impaired glucose tolerance against the background of iodine deficiency (ID).

**Materials and methods**

The research was carried on 90 non-linear male rats weighing 150-180 g, which were divided into three groups for 30 animals in each: control (1st group), rats with IR under conditions of adequate iodine supply (2nd group), animals with IR on the background of ID (3rd group). The control group was represented by intact animals.

IR was simulated by replacing the drinking water of rats with a 10 % fructose solution for 8 weeks [9]. For the reproduction of iodine deficiency state an experimental model was used, according to which the animals were on an iodine-deficient diet during the experiment [10]. The rats of control group were kept on a standard food diet and drinking regime of the vivarium.

The system of carbohydrate metabolism was studied by the level of insulin in the blood serum, glycosylated hemoglobin of the blood (HbA1c) and the concentration of glucose in the blood. The degree of IR was determined by the HOMA-IR (Homeostasis Model Assessment Insulin Resistance) index [11]. The content of mineral substances (calcium, magnesium, zinc, manganese, copper) was examined in the cementum of the tooth root and the alveolar process by the method of atomic absorption spectrophotometry on the S-115 PK device (Selmi, Sumy). The tissue samples were previously mineralized by the method of dry ashing. After that, the acid extraction was performed. The acid (APs) and alkaline (AP) phosphatase activity was determined with a set of reagents from the "Filisit-diahnostyka" company (Dnipro, Ukraine).

Statistical processing of the research results was performed using the mathematical software package StatisticSoft 7.0. Student`s t test was used to assess the significance of differences between groups. The difference at $p<0.05$ was considered as statistically significant.

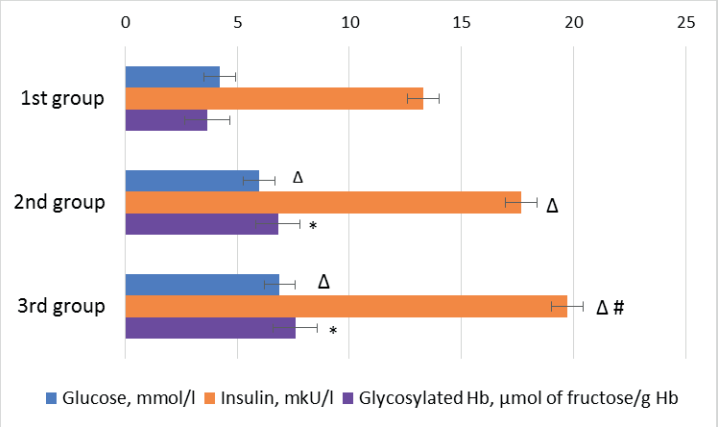


**Fig. 1. Markers of carbohydrate metabolism of intact and insulin-resistant rats under conditions of adequate iodine supply and iodine deficiency (M±m, n=30)**

Note: here and in the following figures *$p<0.05$, Δ$p<0.001$ – relative to the data in intact animals; #$p<0.001$ – in relation to the data in animals with insulin resistance

**Results and discussion**

The violations of carbohydrate metabolism in rats with isolated IR were indicated by the changes of the main markers of carbohydrate metabolism (fig. 1, 2).

Thus, maintaining animals on a high-fructose diet led to an increase of blood glucose levels by 41.7 % ($p<0.001$) compared to control values. A decrease of the peripheral sensitivity of cells to insulin under such conditions was confirmed by an increase the level of insulin in the blood serum by 32.7 % ($p<0.001$) compared to the initial indexes.

The changes of HbA1c content, which in rats of the 2nd group became higher by 86.6 % ($p<0.05$) in comparison with the similar values of intact animals, were co-directed. The development of IR is confirmed by the elevation of HOMA-IR index, which under such experimental conditions increased by 68.1 % ($p<0.001$) compared to the control parameters.

It should be noted that dysmetabolic manifestations of impaired carbohydrate homeostasis were more pronounced under the conditions of combined endocrinopathy (see fig. 1, 2). In particular, in animals of the 3rd group, the blood glucose level increased by 63.3 % ($p<0.001$) compared to the control values. At the same time, the insulin content in blood serum increased by 48.2 % ($p<0.001$) in comparison with the initial data. According to the excessive accumulation of glucose in the blood under such experimental conditions, an increase of HbA1c level by 2.1 times ($p<0.05$) compared to the corresponding indexes of intact rats was noted. A reliable criterion for the development of IR in animals with combined endocrinopathy was an increase the HOMA-IR index by 2.1 times ($p<0.001$) in comparison with similar values in rats of the control group.

During the comparative analysis of the carbohydrate metabolism markers in animals of the 2nd and 3rd groups, only

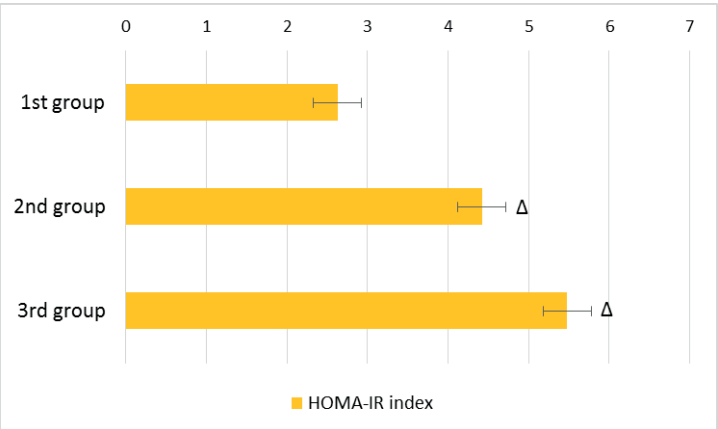


**Fig. 2. HOMA-IR index of intact and insulin-resistant rats under conditions of adequate iodine supply and iodine deficiency (M±m, n=30)**

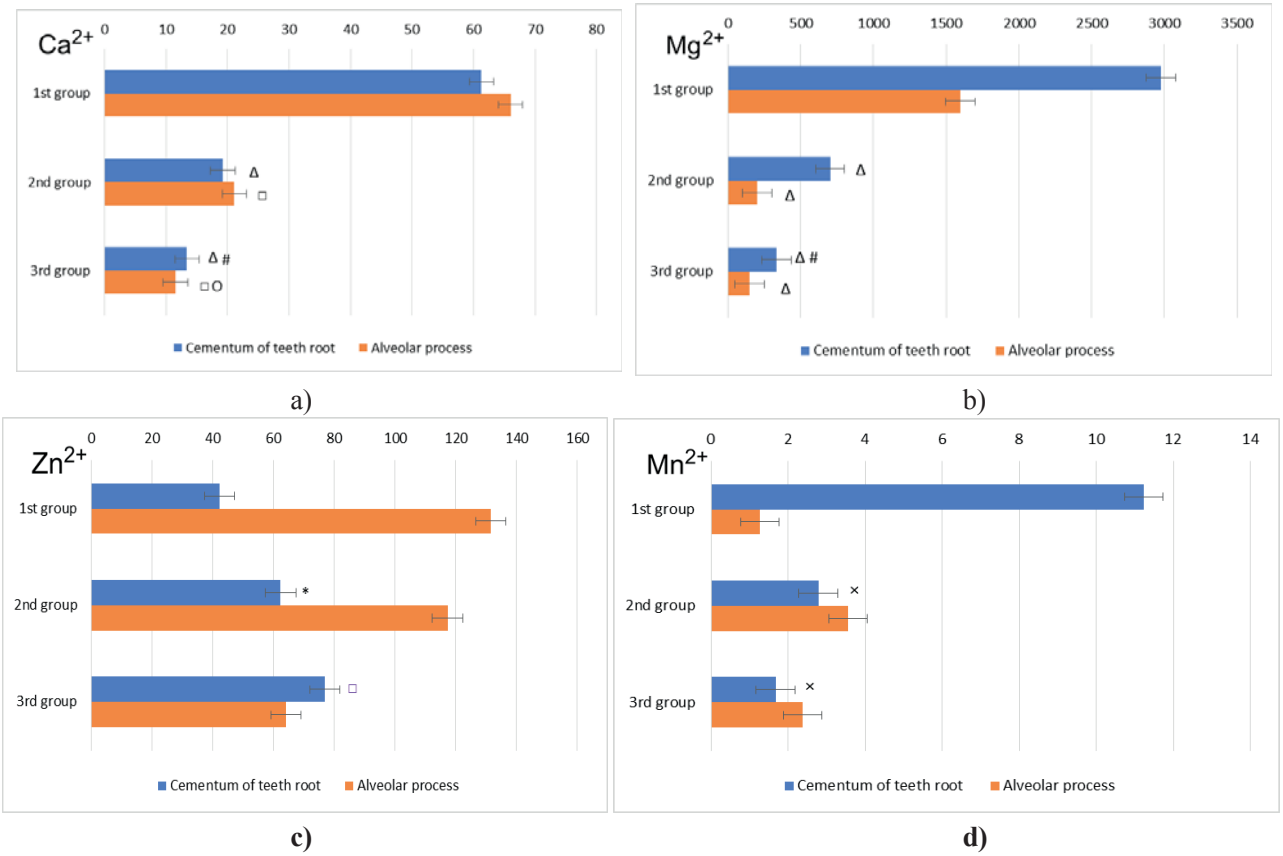


**Fig. 3. The content of macro- and microelements (calcium (mg/kg) – a, magnesium (μg/g) – b, zinc (μg/g) – c, manganese (μg/g) – d in the cementum of the tooth root and the alveolar process of intact and insulin-resistant rats under conditions of adequate iodine supply and iodine deficiency (M±m, n =30)**

Note: here and in the following figures □ $p<0.02$ – relative to the data in intact animals; × $p<0.01$ – relative to the data in intact animals; O $p<0.01$ - relative to the data in animals with insulin resistance

the level of insulin experienced the reliable changes, which in rats with IR on the background of ID increased by 11.7 % ($p_{2-3}<0.001$) compared to similar indexes under the condition of isolated fructose loading. The obtained results can be explained by the regulatory effect of thyroid hormones on the sensitivity of cells cytoreceptors to insulin and the development of hyperinsulinemia in response to ID.

During the analysis of the mineral composition of teeth-alveolar complex in rats that were on a normal diet, the highest content of calcium and zinc was noted in the alveolar process, and magnesium, manganese and copper – in the cementum of the tooth root.

In animals with endocrinopathies, an imbalance of mineralization/demineralization physiological system of hard periodontal tissues was observed. In particular, under IR conditions the redistribution of minerals in the examined tissues took place (fig. 3a, b, c, d). Thus, in the cementum of the tooth root the content of calcium became lower by 68.7 % ($p<0.001$), magnesium – by 76.4 % ($p<0.001$), manganese – by 75.2 % ($p<0.01$) against the background of increase the zinc content by 47.5 % ($p<0.05$) compared to the similar indexes of intact animals. At the same time, the mineral composition of the alveolar process under these experimental conditions significantly changed only due to the calcium and magnesium, the content of which decreased by 68.0 % ($p<0.02$) and by 87.4 % ($p<0.001$) respectively compared to control data. It is known that calcium participates in the formation of the core of crystallization in hard tissues, therefore, a decrease in its content in the bone tissue of the jaws reduces the mineralization of the organic matrix of the bone, which can disrupt the structure of the tooth-alveolar complex in general [12].

It should be noted that the development of combined endocrine pathology was accompanied by the more pronounced changes of the studied parameters of the mineral spectrum (see fig. 3a, b, c, d). In particular, a decrease of calcium content by 78.1 % ($p<0.001$), magnesium – by 88.8 % ($p<0.001$), manganese – by 85.1 % ($p<0.01$) was observed in the cementum of the tooth root with the simultaneous increase of zinc concentration by 82.1 % ($p<0.02$) compared to the similar data in control rats. Taking into account the fact that zinc is a component of cell division during the process of dentinogenesis, an increase of its content in tooth tissues can be a compensatory replacement of other inorganic substances as a result of demineralization against the background of hyperglycemia [13].

At the same time, in the alveolar process only the content of macroelements underwent the significant changes, where the level of calcium and magnesium became lower by 82.5 % ($p<0.02$) and 90.7 % ($p<0.001$), respec-

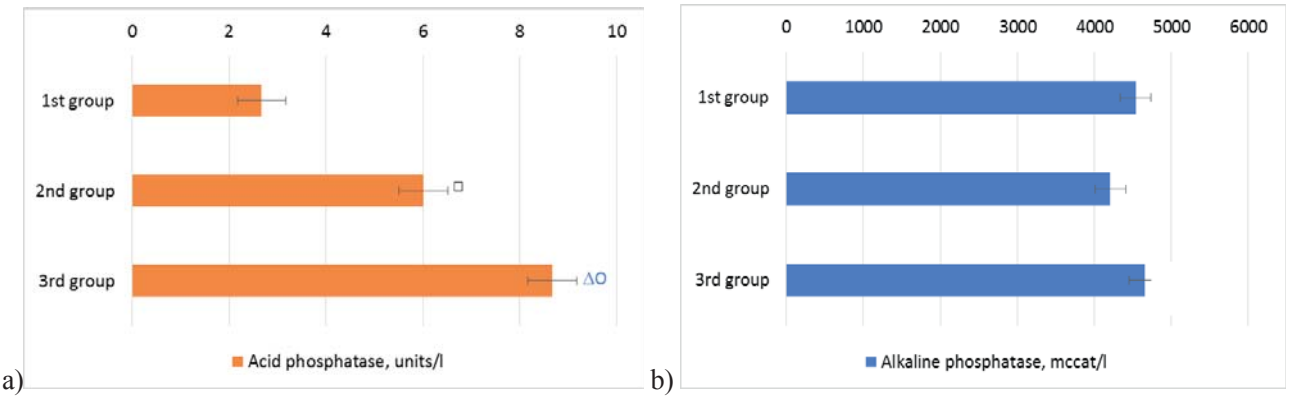


**Fig. 4. Acid (a) and alkaline (b) phosphatases activity of blood serum of intact and insulin-resistant rats under conditions of adequate iodine supply and iodine deficiency (M±m, n=30)**

tively, compared to initial indexes. The obtained results of the mineral spectrum may be one of the pathogenetic mechanisms of inhibition of bone tissue remodeling processes of the alveolar process due to the accumulation of glycation end products in them.

During the study of features of mineral substances accumulation in the tissues of rats with isolated IR and IR on the background of ID, a predominant decrease of their content under the conditions of combined endocrine pathology was established, mainly due to macroelements (see fig. 3a, b). In particular, in the cementum of the tooth root of rats of the 3rd group, the level of calcium decreased by 30.0 % ($p_{2-3}$<0.01) and magnesium – by 52.3 % ($p_{2-3}$<0.001) compared to the corresponding values in animals with impaired glucose tolerance. It should be noted that only the content of calcium in the alveolar process underwent the significant changes, which in rats with IR on the background of ID was lower by 45.3 % ($p_{2-3}$<0.01) compared to the similar indexes of animals with isolated IR.

The important biochemical markers of physiological mineralization of hard tissues of the body are determination of serum phosphatase activity (APs and AP) (fig. 4a, b).

The analysis of activity of these enzymes in animals with endocrinopathies indicates the reliable changes of APs activity. Thus, the keeping of animals on a high-fructose diet led to increase of APs activity by 2.3 times (p<0.02) compared to the data in intact animals (see fig. 4a). It should be noted that the activity of AP under such conditions had the tendency to decrease (see fig. 4b).

Under the conditions of combined endocrinopathy, the processes of demineralization of the examined tissues were more pronounced, what is confirmed by an increase of blood serum APs activity by 3.3 times (p<0.001) compared to the initial values (see fig. 4a). During the comparative analysis of the activity of serum phosphatases in rats with isolated IR and combined endocrinopathy, a significant increase of APs activity in the blood serum of animals of the 3rd experimental group by 44.3 % ($p_{2-3}$<0.05) was found in comparison with the corresponding indexes of rats with impaired glucose tolerance. Considering the fact, that APs is secreted by osteoclasts, which are activated during the demineralization of hard tissues, and AP characterizes the activity of osteoblasts in the process of osteosynthesis [4], the obtained results may indicate a violation of the processes of remodeling of hard tissues in animals with IR against the background of ID precisely due to the increase of bone resorption intensity.

**Prospects for further research**

A comprehensive study of all links of the remodeling process of hard periodontal tissues under the conditions of isolated carbohydrate metabolism disorder and in combination with ID will allow to systematize the manifestations of metabolic imbalance and the mineral spectrum changes of the tooth-alveolar complex under the conditions of endocrinopathies. At the same time, the study of the mineralization/demineralization processes of hard tissues of the body under the conditions of the development of carbohydrate metabolism disorders will contribute to the expansion of existing knowledge regarding the possibilities of treatment and prevention of complications from the dento-maxillary system in patients with comorbid pathology, in particular, impaired glucose tolerance against the background of ID.

**Conclusions**

The keeping of animals on a high-fructose diet led to the development of carbohydrate metabolism disorders, which is confirmed by an increase of blood glucose and HbA1c levels, blood serum insulin level and the HOMA-IR index. The development of combined endocrinopathy was accompanied by more pronounced changes of carbohydrate metabolism markers. The specified manifestations prove the potentiating effect of ID on the incretory function of the pancreas.

The violation of carbohydrate homeostasis was manifested by the changes of the remodeling processes of hard periodontal tissues due to the redistribution of minerals in the cementum of tooth root (decrease the level of calcium, magnesium and manganese against the background of an increase the zinc content) and a decrease the content of macroelements in the alveolar process. Co-directed to the changes of the inorganic spectrum

of the examined tissues under such conditions was the increase of APs activity against the background of the decrease of AP activity. The obtained results indicate the intensification of osteoresorptive processes of the tooth-alveolar complex in animals with IR. At the same time, the combined effect of glucose excess and ID led to more pronounced changes in the hard periodontal tissues, which may be the reason of suppression of the physiological capacity of the tooth-maxillary system and the development of structural disorders of bone tissue and teeth in patients with endocrine disorders.

**Ethics Policy.** All experiments were carried out according to the legislation of Ukraine (Law of Ukraine № 3447-IV "On protection of animals from cruel treatment", 2006), the rules of European Convention for the protection of vertebrate animals used in experimental research and for other scientific purposes (Strasbourg, 1986) and approved by the Local Ethics Committee.

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**Conflict of Interests.** The author declare that no conflict of interests exist.

**References**

1. Griffith A Bell, Tuija Männistö, Aiyi Liu, Kurunthachalam Kannan, Edwina H Yeung, Un-Jung Kim, et al. The joint role of thyroid function and iodine concentration on gestational diabetes risk in a population-based study. Acta Obstet. Gynecol. Scand. 2019;98(4):500-506. doi: 10.1111/aogs.13523

2. Wang D, Wan S, Liu P, Meng F, Zhang X, Ren B,et al. Relationship between excess iodine, thyroid function, blood pressure, and blood glucose level in adults, pregnant women, and lactating women: A cross-sectional study. Ecotoxicol. Environ. Saf. 2021;208:111706. https://doi.org/10.1016/j.ecoenv.2020.111706

3. Ponyrko AA. Microscopic structure and morphometric parametres of long tubular bones of senile rats with induced hyperglycemia. Actual problems of modern medicine: Bulletin of Ukrainian Medical Stomatological Academy. 2019;19(3(63)):151-6. DOI:10.31718/2077-1096.19.3.151

4. Dubey P, Thakur V, Chattopadhyay M. Role of Minerals and Trace Elements in Diabetes and Insulin Resistance. Nutrients 2020;12:1864. DOI: 10.3390/nu12061864

5. Pankiv VI. State of mineral density of bone tissue of women in postmenopausal period with primary hypothyroidism. Clin. & Exper. Pathol. 2016;15(1):104-10.

6. Kimak HB, Melnychuk HM, Ersteniuk AM. Peculiarities of Carbohydrate Exchange in Patients with Generalized Periodontitis of Young Age Persons. Galician Medical Journal. 2018;25(2):19-22. http://nbuv.gov.ua/UJRN/glv_2018_25_2_7

7. Indurkar MS, Maurya AS, Indurkar S. Oral manifestations of diabetes. Clin. Diabetes. 2016;34:54-7. doi: 10.2337/diaclin.34.1.54

8. Laclaustra M, Moreno-Franco B, LouBonafonte JM. Impaired sensitivity to thyroid hormones is associated with diabetes and metabolic syndrome. Diabetes Care. 2019;42(2):303-10. doi: 10.2337/dc18-1410

9. Stetseviat VB, Voronych-Semchenko NM. Influence of insulin resistance on the thyroid homeostasis of rats in conditions of congenital and acquired iodine deficiency. Art of medicine. 2019;4(12):119-23. DOI: 10.21802/artm.2019.4.12.119

10. Martijn JL Verhulst, Bruno G Loos, Victor EA Gerdes, Wijnand J Teeuw. Evaluating All Potential Oral Complications of Diabetes Mellitus. Front. Endocrinol. (Lausanne). 2019;10:56. doi: 10.3389/fendo.2019.00056

11. Vatseba TS, Skrypnyk NV, Didushko OM. The value of cytokine imbalance in the formation of insulin resistance in patients with primary hypothyroidism. Art of medicine. 2017;3(3):48-53.

12. Ihnatiev OM, Yermolenko TO, Turchyn MI. The markers of bone tissue metabolism. Journal of Marine Medicine. 2020;2:127-48. DOI http://dx.doi.org/10.5281/zenodo.3976574

13. Monzio CG, et al. Mitochondrial dysfunction in fibroblasts of multiple system atrophy. Biochim Biophys Acta Mol Basis Dis. 2018;1864(12):3588-97. doi: 10.1016/j.bbadis.2018.09.018

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