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CIRCADIAN RHYTHM OF METABOLISM INDICATORS IN HEALTHY PEOPLE ACCORDING TO SALIVA STUDY FINDINGS

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Abstract

In recent years, the concept of functional-metabolic continuum (FMC) has been formulated, which indicates the relatively constant nature of metabolism and its parameters in the intercellular environment. Thus, in its turn, is closely and adequately connected to the functional activity of organs and cells, thus forming a single system – the continuum. In addition, the functional activity of key low molecular weight antioxidants in the tissues and biological fluids of the body may, to a large extent, indicate the adaptive abilities and reactions at the level of the entire organism. One of the possible approaches to FMC study, depending on the physiological activity of the body, is to determine the parameters of metabolism and regulatory molecules relative to the daily rhythm, i.e., their comparison in the morning and in the evening, when the changes in the body over the day are observed, in other words, when there is an overall load. The aim of this research was to examine the circadian rhythm of FMC in healthy people according to saliva study findings.

Thus, the obtained results, firstly, represent the existence of daily changes in FMC, and secondly, constitute a possible information channel that can be used to study these changes, to determine and compare indicators in the morning and evening about the functional activity of the body involving the regulatory systems.

Key words: functional-metabolic continuum, circadian rhythm, metabolism

In recent years, the concept of functional-metabolic continuum (FMC) has been formulated, which indicates the relatively constant nature of metabolism and its parameters in the intercellular environment. Thus, in its turn, is closely and adequately connected to the functional activity of organs and cells, thus forming a single system – the continuum [1].

In addition, the functional activity of key low molecular weight antioxidants in the tissues and biological fluids of the body may, to a large extent, indicate the adaptive abilities and reactions at the level of the entire organism [2]. There are numerous researches to confirm the non-digestive overall body function of the salivary glands, which is to support the homeostasis of the blood – the major internal environment of the body [3]. The presence of features in the FMC functioning in different types of pathology makes it possible to consider the changes in biological composition of saliva as possible informative markers for the diagnosis of certain pathological conditions and their severity.

Hence, it is known that for the purpose of studying the FMC indicators, in order to diagnose the functional states and pathologies in the body, the possibility of its dynamic monitoring is essential. In this regard, defining the regulatory and metabolic parameters in saliva should be considered as one of the promising methods.

It is also known that the nature of the circadian curve of concentration, for example, of hormones in saliva, can be an indicator for the degree of functioning of a particular endocrine gland, and the study of rhythmology in electrolytes excretion with saliva helps, for instance, in assessing the functional state of the sympathoadrenal system. Most researchers who studied the hormonal composition of saliva believe that defining their concentrations in saliva can be of a diagnostic value, since there is a close correlation between the content of a number of hormones in blood and saliva not only in healthy people, but also in people with health problems [4].

It should be noted that the use of saliva can be not only an additional method in clinical trials, but it also has many advantages over the analysis of blood and urine: the collection of saliva is simple and convenient in case of non-clinical settings; it is painless; the risk of infecting medical staff is much lower than when working with blood; the content of many molecules in saliva reflects their concentration in blood [5, 6]. Despite numerous anatomical and physiological data on salivary glands and their secretion, the mechanism that manages the formation of saliva biochemical composition is still an unresolved issue [6, 7, 8, 9, 10, 11, 12, 13].

One of the possible approaches to FMC study, depending on the physiological activity of the body, is to determine the parameters of metabolism and regulatory molecules relative to

the daily rhythm, i.e., their comparison in the morning (minimum functional activity) and in the evening, when the changes in the body over the day are observed, in other words, when there is an overall load.

The aim of the research

In saliva, most metabolites and regulatory molecules can be detected. There is a certain dependence between their content in plasma and saliva, which makes it possible to use saliva as an information channel for evaluation of metabolism in the human body. However, there is no complete correspondence, which indicates a more complex nature of saliva formation. Consequently, certain metabolites and regulators in saliva play an important role in the physiological and pathophysiological reactions of the body.

The aim of this research was to examine the circadian rhythm of FMC in healthy people (the group included 21 subjects) according to saliva study findings.

Research methods

The object of the research embraced unstimulated oral fluid (mixed saliva), which was collected in the morning, on an empty stomach, after careful hygienic measures in the oral cavity, by spitting in a sterile test tube. Subsequently, the oral fluid in test tubes was centrifuged for 10 minutes at 2000 rpm, and the sediment portion of the fluid was used for biochemical studies.

ADMA concentration was determined using liquid chromatograph LC 5000 (INGOS, Czech Republic), wavelength 340 nm, in isocratic mode. For solid-phase extraction (purification and concentration), Absolut Nexus (Varian) cartridges were used [14].

The activity of lipid peroxidation processes was evaluated by the concentration of TBC-active products. [15]

SOD activity was determined by spectrophotometric method of V.A. Kostiuk, A.N. Potapov and Zh.V. Kovalev, which is based on quercetin oxidation [16].

Catalase activity was determined by spectrophotometric method (according to S. Chevvari et al.) [17].

The content of potassium, sodium, calcium, magnesium, total lipids, triglycerides and cholesterol was determined by spectrophotometric methods using a set of reagents of “Filisit – Diagnostika” (Ukraine, Dnipro).

The content of zinc and copper was determined using a set of reagents of DAC-Spectro Med S.R.L (Moldova).

The content of mucin in saliva was determined by spectrophotometric method (according to E.I. Ilyinykh) [18].

The content of lysozyme in saliva was determined using the *Micrococcus Lysodeicticus* culture test, strain N2665 (by the degree of emulsion lightening) [19].

Results and discussion

Table 1

The content of ionic composition and ADMA in saliva of healthy people in the morning and evening

Parameter	Magnesium	Sodium	Copper	Calcium	ADMA
Time of day	mmol / l	mmol / l	μmol / l	mmol / l	μmol / l
8-00 n= 21	0.34 ± 0.01	7.45 ± 0.52	3.55 ± 0.23	1.85 ± 0.12	0.052±0.003
20-00 n= 21	0.26 ± 0.02 p < 0.05	6.94 ± 0.43 p>0.05	3.71 ± 0.28 p>0.05	1.63 ± 0.09 p>0.05	0.087±0.003 p< 0.02

Note: p - reliable differences in the parameter's value in the morning and evening.

The data in the table show that conditionally all parameters of saliva can be divided into two main groups according to their changes in the morning / evening.

The first group includes the homeostatic parameters that are most accurately controlled in the body: sodium, calcium and copper (Table 1). These ions did not virtually change, thus indicating the high stability of ionic homeostasis, which is known to belong to the most accurately controlled constants of the internal environment. Interesting is the fact that only Mg⁺⁺ concentration decreased in the evening from 0.34 ± 0.01 to 0.26 ± 0.02 (p <0.05).

We consider that this may indicate that the extracellular concentration of this ion does not refer to strictly controlled constants of ionic homeostasis. Probably, it is Mg⁺⁺ that enters the intracellular environment with high muscle activity during the day, as against the state of night rest, and takes part, for example, in muscular contraction reactions, etc.

The second group of metabolic parameters is represented by energy substrates (lipids and glucose) (Table 2). In this regard, we recorded their increase toward the evening. The obtained results, at first glance, are not entirely logical, because it would seem that the increase in functional activity during the day could lead to their use and reduced concentration of energy substrates in plasma and, accordingly, saliva. Therefore, their increase can be explained only by mobilization from the depot (adipose tissue and liver). This, on the one hand, indicates the possibility of regulatory activation, and, on the other hand, their higher

concentration, in case of increased functional load, is precisely that “metabolic tail” and reflects changes in FMC.

Table 2

Metabolic parameters in saliva of healthy people in the morning and evening

Parameter	Proteins	Lipids	Choleste- rol	Glucose	Urea	Uric acid	Lactate
Time of day	g / l	g / l	mmol / l	mmol / l	mmol / l	mmol / l	mmol / l
8-00 n = 21	2.11 ± 0.15	0.45 ± 0.03	0.12 ± 0.01	0.064 ± 0.003	0.95 ± 0.06	0.053 ± 0.002	0.37 ± 0.02
21-00 n= 21	2.29 ± 0.13 p>0.05	0.51 ± 0.02 p>0.05	0.15 ± 0.01 p > 0.05	0.072 ± 0.004 p > 0.05	1.22 ± 0.08 p <0.05	0.082 ± 0.005 p <0.02	0.46 ± 0.03 p<0.05

Note: p - reliable differences in the parameter's value in the morning and evening.

One can conclude that this is due to the need for increased energy supply of the body, where the level of lipids and cholesterol can be reliably increased by mobilizing from the fat depot, while glucose levels only tended to increase, due to much fewer possibilities of the carbohydrate depot and neoglycogenesis.

As our results showed, the concentration of glucose in saliva changed least of all: in the evening it tended to increase from 0.064 to 0.077 (by 0.008).

The second group of metabolic parameters includes the data on the increased concentration of uric acid from 0.053 ± 0.002 to 0.082 ± 0.005 (p <0.02), urea from 0.95 ± 0.06 to 1.22 ± 0.08 (p <0.05) and lactic acid from 0.37 ± 0.02 to 0.46 ± 0.03 (p <0.05). We consider this as an indication that catabolism of proteins (amino acids) and purine bases has increased over the course of the day. However, in the study group, judging by the data on the unreliable increase of lactic acid, anaerobic glycolysis did not significantly increase, as there was no significant energy deficiency – all of the subjects lived a usual life without significant physical exertion. At the same time, significant features of the free radical oxidation processes were detected in FMC.

There is a physiologically normal level of activity of free radical processes, necessary for regulation of lipid composition and permeability of membranes, as well as a number of biosynthetic processes, which is also determined by the functioning of a complex tissue-specific system of free radical oxidation inhibitors [2]. In oxidative stress, antioxidant

protection from oxygen radicals is disrupted, which causes the damage to molecules and disturbance of physiological processes. Antioxidant activity (AOA) of human saliva has a pronounced circadian rhythm, possibly implemented through the effect of melatonin, due to its stimulating effect on a number of enzymic systems [2].

The nature of changes in the state of the main parts of AOP is studied in various tissues and organs, intra- and extracellular fluids (bronchoalveolar, cerebrospinal fluid, blood plasma), parietal mucous layer of the upper parts of the digestive tract, and also in saliva [20,21]. Asymmetric dimethylarginine (ADMA) is one of the leading parameters, indicating the level of oxidative stress.

Table 3

Activity of enzymes of antioxidant system, contents of lysozyme, mucin and some hormones in saliva of healthy people at different times of day

Parameter Time of day	Catalase mmol sec · g protein	SOD U/g protein	Lysozyme µmol / l	Mucin g / l	Cortisol nmol / l	Adrena- line nmol / l
8-00 n= 21	10.45 ± 1.03	0.95± 0.06	0.194 ±0.011	1.35 ± 0.07	94.57 ± 4.11	1.88 ± 0.12
21-00 n = 21	9.57 ± 0.48 p< 0.05	0.89 ± 0.04 p<0.05	0.145 ± 0.015 p< 0.02	1.08 ± 0.08 p<0.05	98.69 ± 5.03 p< 0.05	1.46 ± 0.08 p< 0.05

Note: p - reliable differences in the parameter's value in the morning and evening.

We have shown (Table 1) that the level of ADMA increased slightly, but reliably, in the evening from 0.052 to 0.087 (by 0.035) (p <0.02). The level of SOD, on the contrary, decreased (p <0.05) (Table 3). The level of catalase also reliably decreased (p <0.05).

Daily fluctuations can be explained by the fact that the AOA parameter also reflects the content of low molecular weight substances in biological fluids that have antioxidant properties (vitamins, sulfur-containing amino acids, glutathione, melatonin, etc.) [20], although it is oxidative stress that stimulates the AOA activity.

The AOA acrophase in the daytime may also be associated with increased activity of catalase, at night – of SOD [22].

The obtained parameters of oxidative stress indicate that during the daytime, the LPO activity changes in the body and, consequently, an increase in the oxidation products (in particular, arginine) occurs with formation of ADMA. This, on the one hand, indicates the activity of free radical oxidation processes, and on the other hand it can affect the NO-dependent functions, in particular, vasodilation processes.

Thus, the obtained results, firstly, represent the existence of daily changes in FMC, and secondly, constitute a possible information channel that can be used to study these changes, to determine and compare indicators in the morning and evening about the functional activity of the body involving the regulatory systems.

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