ACTA ABP BIOCHIMICA POLONICA

Epub: No 6408
Paper in Press
https://doi.org/10.18388/abp.2020_6408

Regular paper

Influence of age, gender characteristics, chronotype on the expression of core clock genes *Per1*, *Clock*, *Bmal1* and *Cry1* in buccal epithelium

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The purpose of the study is to determine the expression of the core clock genes in buccal epithelial cells of healthy people with different chronotypes. Materials and methods. Fourteen healthy volunteers with a healthy periodontium and oral mucosa (7 women and 7 men) were selected for participation in the trial. The buccal epithelium sampling was performed at 07:00 am and 07:00 pm in one day by cytological brush. The surveyed patients were examined chronotypically using the Horn-Ostberg test. The determination of the mRNA expression of the *Per1, Clock, Bmal1, Cry1* genes was performed by quantitative real-time PCR. Statistical analysis was performed using two-way analysis of variance followed by Bonferroni post hoc tests. Results. *Per1* expression was higher in the morning, regardless of chronotype, age, and gender. The expression of the *Clock* demonstrated the prevalence of the evening in both chronotypes, in both men and women. *Bmal1* was better expressed in the evening, regardless of age, gender, and chronotype. The expression of *Cry1* did not show statistically significant differences between the indicators. Conclusions. The evening expression of *Clock* was higher in people with the evening chronotype than in people with the morning chronotype. The chronotype did not show any effect on the expression of *Per1, Bmal1, and Cry1*. Age and sex did not show any effect on the expression of the core clock genes.

Keywords: buccal epithelium, chronotype, Per1, Clock, Bmal1, Cry1

Received: 29 June, 2022; revised: 21 September, 2022; accepted: 23 September, 2022; available on-line: 17 October, 2022

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Abbreviations: ANOVA, analysis of variance; Bmal1, Brain Muscle Arnt-Like Protein-1; CCA1, Circadian Clock-Associated1; CCGs, clock-controlled genes; cDNA, complementary deoxyribonucleic acid; Clock, Circle Output Kaput; Cry, Cryptochrome; Dbp, D-Box binding PAR BZIP transcription factor; DBT, Doubletime; DNA, deoxyribonucleic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GI, Gigantea; HPA, hypothalamus-pituitary-adrenal; LHY, Late Elongated Hypocotyl; mRNA, messenger ribonucleic acid; Nfil3, nuclear factor, interleukin 3 regulated; PCR, polymerase chain reaction; Per, Period; PTMs, post-transcriptional modifications; RNA, ribonucleic acid; Rorα, RAR-related orphan receptor alpha; SCN, suprachiasmatic nucleus; Tim, Timeless

INTRODUCTION

Daily endogenous rhythms are triggered by mechanisms called circadian clock. A circadian clock, or circadian oscillator, is a biochemical oscillator that cycles with a stable waves and is synchronized with 24 hours (the earth's current day). In most living things, internally synchronized circadian clocks allow the organism to anticipate daily environmental changes that correspond to the day–night cycle and adjust its biology and behavior accordingly.

Robust circadian rhythms in physiological functions and behaviors are conserved across all organisms, from cyanobacteria to humans. Cyanobacteria were the first prokaryotes reported to have the circadian clock regulated by a cluster of three genes: *kaiA*, *kaiB*, and *kaiC*, wich are responsible for fundamental physiological processes such as the regulation of nitrogen fixation, cell division, and photosynthesis. More than 20 circadian clock-related genes have been identified in Arabidopsis, with homologs of these present in other plants, including crops. Such circadian clock genes as *CCA1* (Circadian Clock-Associated1), *LHY* (Late Elongated Hypocotyl), *GI* (Gigantea), and others are involved in internal metabolic and hormonal signals, ranging from the control of metabolism, photosynthesis, growth, and development. The discovery of genes such as *Per* (Period), *Tim* (Timeless), and *DBT* (Doubletine) in Drosophila and later in mice contributed to the functioning of the circadian clock in humans (Dodd *et al.*, 2015; Panter *et al.*, 2019).

The human biological clock plays a fundamental role in regulating the rhythmic course of all physiological processes occurring in the body, including organismal, organ, and cellular ones. Body temperature, pulse rate, respiration, blood pressure, brain activity, hormone production, cell regeneration, and other processes are subordinated to circadian rhythm (Jagannath *et al.*, 2017; Douma *et al.*, 2018; Nirvani *et al.*, 2018; Farshadi *et al.*, 2020).

The dynamics of daily biorhythms parameters is called chronotype. It referrs to behavioral patterns or manifestations based on biological processes controlled by circadian rhythms. Based on the intrinsic circadian rhythm of the individual, individuals differ in his preferred time of sleep and activity. The tradition of dividing people by the type of their peak of activity on owls (evening peak) and larks (morning peak) arose in 1939 (Potter *et al.*, 2016; Chi-Castañeda *et al.*, 2018; Montaruli *et al.*, 2021).

Clock genes, as key regulators of physiological function and circadian clock dysfunction have been linked to various diseases and multiple morbidities such as diabetes mellitus, obesity, thrombosis, neurodegenerative diseases, cardiovascular disease, cancer, psychiatric disorders, autoimmune and inflammatory diseases, and sleep disorders. Perturbation of the internal clock system has been found to be comorbid with major oral, head and neck pathologies, such as oral cancer and Sjögren syndrome (Papagerakis *et al.*, 2014; Adeola *et al.*, 2019).

Circadian regulation of normal physiological and metabolic processes comes from fluctuations in the expression of core genes of the clock and the proteins they encode and differ in each individual organ or tissue. Key genes include *Per* (Per), *Cry* (Cryptochrome), *Bmal1* (Brain Muscle Arnt-Like Protein-1) and C*lock* (Circle Output Kaput) (Andreani *et al*., 2015; Hurley et al., 2016; Panda, 2016; Anna *et al*., 2021).

Analysis of scientific data has revealed that the circadian clock is the main determinant of cellular homeostasis of the maxillofacial region, which regulates the proliferation and differentiation of salivary glands, odontoblastst cells and oral cavity epithelium; influences on the differentiation of ameloblasts and mineralization of dental tissue, influences on pulp sensibility. Some publications also suggest a correlation between circadian periodicity, cross-striations, and incremental lines in histological tooth sections. The circadian rhythm of gene expression was detected in basal cells of the oral epithelium, including the palatal and connective epithelium, remnants of the Malassez epithelium; in ameloblasts and odontoblast cells, tooth pulp cells, periodontal dental ligament cells, osteoblasts, and alveolar bone osteoblasts (Lech *et al*., 2016; Panda, 2016; Adeola *et al*., 2019; Janjic *et al*., 2019).

For example, *Bmal1*, *Cry2*, and *Per2* influence bone mass and bone volume trough regulation of osteoclast parameters and differentiation, playing an important role during enamel formation (Xu *et al*., 2016). However, the specific functions of the respective peripheral clocks in oral tissues and the mechanisms that are implied on the way to the fulfillment of such a function or behavior are still widely unknown.

Recently, there are limited data on the correlation between chronotype and mRNA expression of the core genes of the clock *Per1*, *Clock, Bmal* and *Cry1* in human buccal epithelial cells at different times of the day.

The purpose of the study is to determine the expression of core clock genes in buccal epithelial cells of healthy people with different chronotypes.

MATERIALS AND METHODS

Fourteen healthy volunteers with a healthy periodontium and oral mucosa (7 women and 7 men) were selected for participation in the trial. All participants were fully informed about the nature, potential risks and benefits of their participation in the study and signed an inform consent form. The study protocol was reviewed and approved by the ethical committee of Poltava State Medical University (№ 188, 25.11.2020). Research was carried out in full accordance with the Helsinki Declaration of 1975, as revised in 2013.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows:

1) the age 36-45 years (middle adult age group) (Tsyigankov *et al*., 2009);

2) good general health;

3) healthy periodontium and oral mucosa;

4) written informed consent form.

Exclusion criteria were as follows:

1) antibiotics or anti-inflammatory medications were in the preceding 3 months;

2) periodontal therapy within the previous 6 months;

3) pregnancy and breast feeding;

4) the presence of sever uncontrolled (decompensated) internal organ disease, or neuropsychiatric disorders;

5) the presence of other conditions that determined the participant's inability to understand the nature and possible consequences of the study.

The chronotype

The chronotype was identified by the Horn-Ostberg test that assessed belonging to a certain type of biorhythm by the sum of scored points. It is a self-assessment questionnaire, whose main purpose is to measure whether a person's circadian rhythm produces peak alertness in the morning, in the evening, or between. The Horn-Ostberg assessment consists of 23 multiple-choice questions, each having four or five response options. The responses to the questions are combined to form a composite score that indicates the degree to which the respondent favors morning versus evening (Reiter *et al*., 2021). The study included only people of the morning chronotype and the evening chronotype.

Sampling of the buccal epithelium

The buccal epithelium sampling was performed at 07:00 am and 07:00 pm in one day in the autumn-winter period. Sampling was performed early in the morning and late in the evening, i.e., at the two extreme points of the 24-hour cycle, in order to record the peak expression of morning and evening genes and to obtain the statistical significance of the differences between the expression levels. The study was carried out by the minimally invasive method using a cytological brush with a narrow plastic bristle and a blunt end. For a tighter contact of the cytological brush with the buccal mucosa, rotational movements were performed in place for 10 s in one direction with pressure on the buccal mucosa. The brush was then removed and immediately immersed in RNA stabilizing solution (RNAlater™ Stabilization Solution Invitrogen™ (ThermoFisher, USA) at room temperature and frozen at –80°C for further usage. Before RNA isolation, the samples were kept at –20°C overnight (Gu *et al*., 2021).

Quantification of the expression of the *Per1*, *Clock*, *Bmal1*, *Cry1* genes in the buccal epithelium

General RNA was isolated from biological samples using a set of reagents for isolation and purification of RNA with a magnetic sorbent (UkrGenTech, Kyiv, Ukraine). A set of reagents for the reverse transcription reaction (UkrGenTech, Kyiv, Ukraine) was used to obtain cDNA. For each reaction, we used: 5×PCR mixture containing 5 mm deoxynucleotide triphosphate, 2.5 mm MgCl2 in appropriate buffer solution, random hexameric primer at a final concentration of 20pM, reverse transcriptase ML-RT at a final concentration of 100U, deionized water free of RNases. Reverse transcription was

Table 1. Primers for determining gene expression

Gene	The sequence of primers
Clock	F: 5′-AAA ATA CTC TCT ACT CAT CTG CTG G-3′ R: 5′-ATG GCT CCT TTG GGT CTA TTG-3′
Bmal1	F: 5′-CTG GCT AGA GTG TAT ACG TTT GG-3′ R: 5′-GGT CAC CTC AAA GCG ATT TTC-3′
Per1	F: 5′-ATT CCG CCT AAC CCC GTA TGT GAC C-3′ R: 5′-GTG TGC CGC GTA GTG AAA ATC CTC TTG T-3′
Cry1	F: 5′-TTA CAC TAT GCT CAT GGC GAC-3′ R: 5′-GTG CTC TGT CTC TGG ACT TTA G -3′
β-actin	F: 5′-TCC ACC TTC CAG CAG ATG TG-3′ R: 5′-GCA TTT GCG GTG GAC GAT -3′

Table 2. Expression of core clock genes depending on chronotype ($2^{-\Delta CT}$)

Clock gene	Morning (n=8)		Evening (n=6)	
	07:00 am	07:00 pm	07:00 am	07:00 pm
Per1	1.43±0,07	1.09±0.08 P_1<0.05	1.57±0.12	1.23±0.11 P_1<0.05
Clock	0.000051±0.000014	0.000147±0.000017 P_1<0.05	0.000122±0.000067	0.000592±0.000215 P_1<0.05 P_2<0.05
Bmal1	0.0017±0.0004	0.0068±0.0019 P_1<0.001	0.0019±0.0001	0.0100±0.0023 P_1<0.001
Cry1	2.11±0.06	2.38±0.17	2.20±0.22	2.30±0.14

P, value calculated by two-way ANOVA followed by Bonferroni post-hoc tests: P_1, compared with 07:00 am; P_2, compared with 07:00 pm morning chronotype

performed using the «T100 thermal cycler» (BIO-RAD, Hercules, USA) at 50°C for 45 minutes (Biassoni *et al.*, 2014; Fraga *et al.*, 2014).

The etermination of the mRNA expression of the *Per1*, *Clock*, *Bmal1*, *Cry1* genes was performed by the CFX96TM Real Time PCR Detection System (BIO-RAD, Hercules, USA) in the reaction mixture: 10×Buf for amplification with dye SYBR Green I; 25 mm magnesium chloride; 2.5 mM deoxynucleotide triphosphate; 10 µmol/µl of primers (Table 1); SynTag DNA polymerase, 5 IU/µl; 20-50 ng cDNA.

PCR was carried out under the following conditions: the first cycle – 95°C – 300 s and the next 45 cycles: 55–60°C – 40 sec; 95°C – 15 sec. The GAPDH gene was used as a reference gene. The relative $2^{-\Delta CT}$ method was used for data analysis (Biassoni *et al.*, 2014; Fraga *et al.*, 2014).

Statistical analysis

Statistical analysis «SPSS for Windows» 13.0 was used by means of two-way ANOVA followed by Bonferroni post-hoc tests. *P* values of <0.05 were considered statistically significant in all analyses (MacFarland, 2012).

The null hypothesis tested was that chronotype, age, and gender had no influence on expression of core genes of the clock in buccal epithelium cells of healthy volunteers.

RESULTS

The study population consisted of 14 healthy volunteers. These participants were divided into two chronotypes – morning (n=8) and evening (n=6) due to the results of the Horn-Ostberg test.

The results of the expression level determination of these genes depending on the chronotype are represented in Table 2.

Per1 was determined to be better expressed early in the morning than late in the evening, regardless of chronotype (P_1<0.05). The difference between the indicators of morning and evening expression of *Per1* in the two chronotype groups was not statistically significant. The level *Clock* expression of was higher in the evening in both "larks" and "owls" (P_1<0.05). The difference between the indicators of the morning expression of *Clock* in the two groups was not statistically significant. The difference between the indicators of the *Clock* expression at 07:00 pm in the two chronotype groups was statistically significant (P_2<0.05). The expression of *Bmal1* in the two groups was found to be higher in the evening than in the morning (P_1<0.001). There was no statistically significant difference between indicators of morning and evening expression of *Bmal1* in the two groups. *Cry1* demonstrated the same night prevalence in both chronotypes, but was not statistically significant. Additionally, there were no statistically significant differences between indicators of morning and evening *Cry1* expression in two chronotype groups.

Thus, the results obtained indicated the statistically significant morning prevalence of *Per1* expression and the evening prevalence of *Clock* and *Bmal1* expressions in both chronotype groups of the study population. In addition, the difference between the indicators of evening expression of *Clock* in the two chronotype groups was statistically significant. The expression of *Per1*, *Bmal1,* and *Cry1* was not demonstrated statistically significant difference in the indicators depending on chronotype.

The results of the determination the gene expression level according toage are presented in Table 3.

The expression of *Per1* was found to be higher in the morning than in the evening in both age groups (P_1<0.001). There was no statistically significant difference between indicators of morning and evening *Per1* expression in the two groups. The level of *Clock* expression was higher at 07:00 pm among participants of

Table 3. Age dependency of core clock genes expression ($2^{-\Delta CT}$)

Clock gene	36–40 years (n=9)		41–45 years (n=5)	
	07:00 am	07:00 pm	07:00 am	07:00 pm
Per1	1.53±0.09	1.24±0.08 P_1<0.001	1.43±0.10	0.99±0.08 P_1<0.001
Clock	0.000098±0.000046	0.000439±0.000158	0.000052±0.000015	0.000156±0.000023
Bmal1	0.0019±0.0003	0.0093±0.0019 P_1<0.001	0.0016±0.0004	0.0062±0.0021 P_1<0.001
Cry1	2.19±0.14	2.28±0.13	2.07±0.09	2.46±0.21

Table 4. Gender dependencies of core clock genes ($2^{-\Delta CT}$)

Clock gene	Women (n=7)		Men (n=7)	
	07:00 am	07:00 pm	07:00 am	07:00 pm
Per1	1.47±0.07	1.12±0.07 P_1<0.05	1.52±0.12	1.18±0.11 P_1<0.05
Clock	0.000057 ±0.000017	0.000191±0.000024 P_1<0.05	0.000106±0.000058	0.000484±0.000204 P_1<0.05
Bmal1	0.0016±0.0002	0.0069±0.0016 P_1<0.05	0.0020±0.0004	0.0095±0.0025 P_1<0.05
Cry1	2.03±0.05	2.43±0.12	2.27±0.18	2.26±0.19

36–40 years and 41–45 years, but it was not statistically significant. Furthermore, there was no statistically significant difference between indicators of morning and evening expression in two age groups. *Bmal1* was better expressed in the evening in the first and second age groups (P_1<0.001). The difference between the indicators of morning and evening expression of *Bmal1* in the two groups was not statistically significant. *Cry1* demonstrated the prevalence of expression in the evening among both age groups og the participants, but was not statistically significant. There was no statistically significant difference between the indicators of morning and evening expression of *Cry1* in two age groups.

Thus, the results obtained indicated the statistically significant morning prevalence of *Per1* expression and the evening prevalence of *Bmal1* expression in both age groups of the study population. The expression of *Per1*, *Clock, Bmal1* and *Cry1* did not show statistically significant differences in the indicators depending on age.

The gender dependencies of the expression level of these genes are illustrated in Table 4.

The level of *Per1* expression was higher early in the morning in both women and men (P_1<0.05). There was no statistically significant difference between the indicators of morning and evening expression of *Per1* in the two gender groups. The *Clock* was expressed better in the evening than in the morning in both men and women (P_1<0.05). There was no statistically significant difference between the indicators of morning and evening expression of the *Clock* in the two gender groups. *Bmal1* demonstrated the prevalence of expression in the evening, regardless of gender (P_1<0.05). The difference between the indicators of morning and evening expression of *Bmal1* in two gender groups was not statistically significant. The level of *Cry1* expression was higher in the evening in women and almost equal at 07:00 am and 07:00 pm in men, but was not statistically significant. There was no statistically significant difference between the indicators of morning and evening expression of *Cry1* in two age groups.

Thus, the results obtained indicated the statistically significant morning prevalence of *Per1* expression and the evening prevalence of *Clock* and *Bmal1* expressions in both gender groups of the study population. The expression of *Per1*, *Clock*, *Bmal1,* and *Cry1* did not demonstrated statistically significant differences between the indicators depending on gender.

DISCUSSION

Several authors have studied the role, patterns, and potential of the circadian clock in human oral cavity (Bjarnason *et al*., 2001; Zheng *et al*., 2012; Papagerakis *et al*., 2014; Nirvani *et al*., 2018; Adeola *et al*., 2019; Janjic *et al*., 2019; Gu *et al*., 2021). They determined the expression level of core clock genes at different times of the day in fibroblasts of the gingiva and periodontal ligament (Janjic *et al*., 2017; Fleissing *et al*., 2018; Hilbert *et al*., 2019), in developing teeth (Zheng *et al*., 2011; Zheng *et al*., 2013), salivary glands (Zheng *et al*., 2012), and oral mucosa (Bjarnason *et al*., 2001; Bjarnason *et al*., 2007; Zieker *et al*., 2010; Gu *et al*., 2021).

Furthermore, the analysis of scientific literature showed the presence of a large number of articles that have studied the expression of core clocl genes in human oral squamous cell carcinoma cells (Li *et al*., 2016; Zhao *et al*., 2016; Qin *et al*., 2017; Yang *et al*., 2020; Gong *et al*., 2021). In contrast, there are a relatively few scientific papers that have studied the expression of core clock genes in the healthy oral mucosa depending on chronotype. We revealed the expression of the *Per1*, *Clock*, *Bmal1* and *Cry1* genes in the healthy oral mucosa in patients without any somatic or dental disease and traced the level of expression of each individual gene depending on chronotype, age, and gender.

As in other similar scientific works, we had a small sample size, which limited the assessment of the correlation between gene expression and population characteristics (Bjarnason *et al*., 2001; Bjarnason *et al*., 2007; Zieker *et al*., 2010; Kurbatova *et al*., 2014; Cho *et al*., 2016; Gu *et al*., 2021; Sato *et al*., 2021). Bjarnason G.A. and others (Bjarnason *et al*., 2001; Bjarnason *et al*., 2007), Zieker and others (Zieker *et al*., 2010), Cho and others (Cho *et al*., 2016) and Gu and others (Gu *et al*., 2021), who investigated the expression of core genes of the clock in the healthy oral mucosa, the sampling were carried out every 4 hours within 24 hours. In our study, we performed the sampling at 07:00 am and 07:00 pm, in order to record the peak expression of morning and evening genes and to obtain the statistical significance of the differences between the expression level.

Analysis of the study results revealed statistically significant time differences in the expression of the core clock genes *Per1*, *Clock, Bmal1* and *Cry1*.

Previously published data demonstrated that *Per1*expression in the oral mucosa and in human skin is maximal in the morning and gradually decreases during the day (Bjarnason *et al*., 2001; Gu *et al*., 2021). Gu and others (Gu *et al*., 2021) in their study determined that eight participants had peak times of *Per1* expression between 10:30 am and 02:10 pm and three participants had peak times at 6:30 am, 7:00 am, and 9:00 am. The average peak times of of *Per1'* RNA expression were approximately 10:45 am. It was also found that a later *Per1* peak time was found in the elderly, but the small number of samples analyzed did not reach statistical significance. Regarding the chronotype, they did not find correlations

between gene expression peak times and chronotype (Gu *et al*., 2021).

In our study, it was found that *Per1* exhibits the same rhythmic expression that peaks early in the morning (at 07:00 am) and decreases in the evening, regardless of the chronotype, age, and gender. The chronotype, age, and gender did not have influence on *Per1* expression, which correlates with previous published data.

According to the study of Bjarnason and others the *Clock* expression was not rhythmic (Bjarnason *et al*., 2001). On the contrary, Zhanfeng and others in their study determined that the expression peak of *Clock* was at 12:00 pm (Zhanfeng *et al*., 2019). But in our study *Clock* expression demonstrated the evening prevalence in both morning and evening chronotypes, in both men and women. The evening expression of the *Clock* was higher among the population with evening chronotype than with morning chronotype. Sex and age did not influence *Clock* expression.

In a previously published study of Bjarnason and others it was found that *Bmal1* shows a rhythmic expression, peaking at night (late activity) (Bjarnason *et al*., 2001). On the contrary, Zieker and others in their study indicated that maximum expression of *Bmal1* was observed at 06:00 am (Zieker *et al*., 2010). But according to the study Zhanfeng and others, the expression peak of *Bmal1* was at 12:00 pm (Zhanfeng *et al*., 2019).

In our study, *Bmal1* expression showed a maximal level in the evening (at 07:00 pm) and gradual decrease in early in the morning (at 07:00 am), regardless of chronotype, gender, and age. Chronological, gender, and age did not influence *Bmal1* expression.

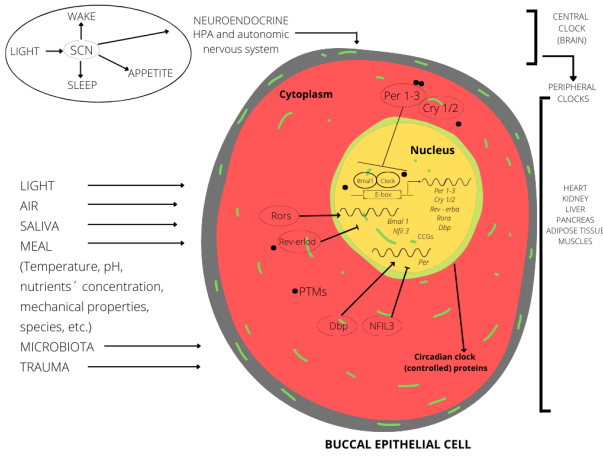

Figure 1. Schematic illustration of the main mechanisms of action in both the central nervous system and peripheral clocks
The central pacemaker of the circadian system is located in the hypothalamic suprachiasmatic nucleus (SCN). Oscillating neurons and astrocytes are integrated as a single circadian unit and produce a coordinated circadian signal. The SCN forwards the rhythmic signal trough the hypothalamus-pituitary-adrenal (HPA) axis and the autonomic nervous system (Wang *et al*., 2021). The mammalian circadian clock network at the molecular level consists of the transcription of genes that contain E-box elements in their promoters, such as *Per1-3*, *Cry1/2*, *Rora*, *Rev-erba*, and *Dbp*. The Per and Cry proteins translocate back into the nucleus and repress the activity of the Bmal1/Clock complex, which subsequently inhibits their own expression. Rorα and Rev-erbα fine-tune the expression of *Bmal1* and *Nfil3*, trough activation and repression, respectively. The stability and nuclear translocation of circadian clock proteins are modulated by post-transcriptional modifications (PTMs). Cricadian clocks also control the rhythmic expression of numerous clock-contolled genes (CCGs) and biological processes (Wang *et al*., 2021). Due to the complex interaction between external Zeitgeber and internal circadian rhythms, the greatest beneficial effects of entrainment on circadian function are seen when food intake coincides with the activity phase (Salgado-Delgado *et al*., 2013; Schilperoort *et al*., 2019), while an inhibitory effect occurs when food is taken during the rest phase (Yasumoto *et al*., 2016).

Previous analyses of *Cry1* detected peak gene expression in oral mucosa during the afternoon (Bjarnason *et al*., 2001; Zieker *et al*., 2010). In the study of Bjarnason and others, it was found that *Cry1* demonstrates peak of expression at late afternoon (Bjarnason *et al*., 2001). In the study of Zieker and others, it was observed that *Cry1* shows peak time of expression at 06:00 pm (Zieker *et al*., 2010).

In our study, the results obtained about *Cry1* expression in different chronotype groups, different age groups, and among men and women were not statistically significant. Chronotype, sex, and age did not influence *Cry1* expression.

We can hypothesize that the buccal epithelium might be regulated not only by the dark-light cycle but by the direct influence of meal and fluid on the oral mucosa (Fig. 1).

Our study has several limitations. The small sample size of this study limited the statistical power to find genes with circadian rhythmic expression and to assess correlations between genes expression and population characteristics. Future studies with a larger sample size are needed to confirm our findings. The sampling was carried out exclusively at 07:00 am and 07:00 pm on one day in the autumn-winter period. Therefore, we were limited in time and were unable to determine the expression level of core genes of the clock at other times of the day or in the other season of the year. Additionally, we did not take into account the influence of such factors as lifestyle, diet, intensity of physical activity, and sleep quality.

The prognosis for further investigation is to study the correlation between the expression of core clock genes and mealtime, as well as with the concentrations of rhythmically expressed hormones such as melatonin.

The results of the study on the expression of *Per1*, *Cry1*, *Bmal1*, *Clock* in the healthy oral mucosa can also be used in patients with periodontitis. In the future, we plan to study the expression of core genes of the clock *Per1, Cry1, Clock* and *Bmal1* in buccal epithelial cells of patients with periodontitis.

CONCLUSIONS

The data obtained indicate that the chronotype has its influence on the evening expression of *Clock*, which was higher in people with the evening chronotype than in people with the morning chronotype.The addition to the morning or evening chronotype did not show any effect on the expression of the core clock genes *Per1, Bmall* and *Cry1*. The chronotype does not have significant effect on the circadian regulation of physiological and metabolic processes in the buccal epithelium. Age and sex did not show any effect on the expression of core clock genes.

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