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CHANGES OF DIURNAL ORGANIZATION OF FREE-RADICAL MODIFICATION OF MACROMOLECULES, ANTIOXIDANT DEFENSE IN HEALTHY PEOPLE EXPOSED TO PROLONGED PHOTOPERIOD

Key words: *healthy volunteer, diurnal rhythms, antioxidants.*

Abstract. *Prolonged artificial light period as a consequence of civilisation, as proven, may disturb some physiological circadian patterns. This may be a background for illnesses development. The aim of investigation was to compare daily profiles of free radical oxidation products and antioxidant patterns in people with usual and disturbed sleeping regimen. 41 healthy volunteers were examined. 6 blood samples were taken during 24 h. 24 people had stable sleep regimen from 11.00 p.m. to 7.00 a.m. (group 1); rest went to bed systematically around 2.00-3.00 a.m. at least 3-4 days per week (group 2). Free radical oxidation products (malonyc dialdehyde, products of free radical protein modification) and antioxidants (restored glutathione content, activity of catalase, gluthathioneperoxidase, glutathione transferase) were measured by standard biochemical methods. All results were approved statistically. Maximal values of free radical oxidation metabolytes were inverse to antioxidant activity profiles in both groups. But, we established that daily profiles of all investigated parameters were violated in people with late falling asleep. They had lower daily average levels of antioxidants and higher - of products of macromolecules' free radical modification. In addition, shift of acrophase of free radical oxidation*

Introduction

Periodic changes of light and darkness cycles of environment maintained establishment of circadian rhythms of majority of organism's physiological functions - from development up to aging, violations appearance and diseases development. Significant role of circadian system in regulation of vital processes on a cellular level is a proven fact, and rhythmic fluctuations of indices reflecting biological processes are external manifestation of the mentioned regulation.

All life forms have developed endogenous molecular circadian clocks to synchronize their behavioural, biological, and metabolic rhythms to environmental cues, with the aim to perform at their best over a 24-h span. Hence, the coordinated circadian regulation of sleep/wake, rest/activity, fasting/feeding, and catabolic/anabolic cycles is crucial for optimal health.

Sharp reorganization of natural environment of a human being is a consequence of civilisation and recent technical progress. Artificial illumination causes prolonged activity and in some people - inversion of regimens of sleep and activity. Chronic sleep limitation is associated with significant increases in a number of consequent problems: sleepiness-related accidents, social disruption, and psychiatric disturbances. Chronic exposure to shift work has now been shown to be an independent risk factor for the development of numerous chronic diseases. But, people mostly suggest they have to spend years in shifted regimen before organism will change something on biochemical level. So, question, whether systematically prolonged activity at dark time may influence any biochemical parameters and induce changes remains actual.

Aim: to compare daily profiles of free radical oxidation products, antioxidant patterns in people with usual and several months' disturbed sleeping regimen

Material and Methods

41 healthy volunteers who had no chronic pathology of respiratory organs or gastro-intestinal tract, cardiovascular system as well as no acute illnesses during last 3 months were examined. All patients were synchronized by meals intake time. 6 blood samples (8 ml) were taken during 24 h. Female:male ratio was 1:1, their average age - 33,7 ± 5,8 years. 24 people had stable sleep regimen from 11.00 p.m. to 7.00 a.m. (group 1); 17 went to bed systematically around 2.00-3.00 a.m. 3-4 days per week for at least 3 months (group 2).

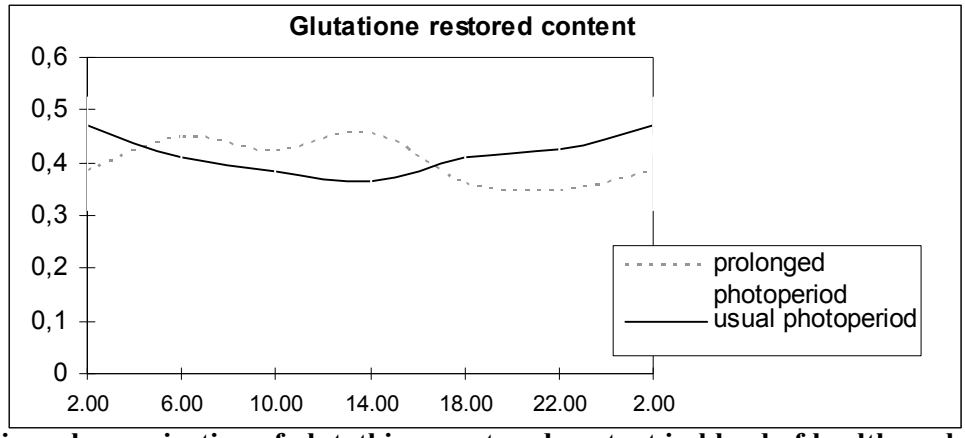
Free radical oxidation products (malonyc dialdehyde (MD), products of free radical modification of proteins (PFPM)) and antioxidants (restored glutathione (RG) content, activity of catalase, glutathione

peroxidase, glutathione transferase) were measured by standard biochemical methods. All trial procedures were in agreement with standards of Helsinki Statement (1975) and its amendment (1983).

Mathematical processing of the obtained data was performed employing variation statistical analysis on IBM PC Pentium III. Significance of variations changes was determined using paired Student's test in case of normal distribution in data sets, non-parametric paired Wilcoxon's T-test was used in other cases. Results were considered as reliable when significance quotient was less or equal to 0,05. Rhythm parameters evaluation was done using individual or group Cosinor-analysis by means of Chronos-Fit software (Heidelberg, Germany, 2002).

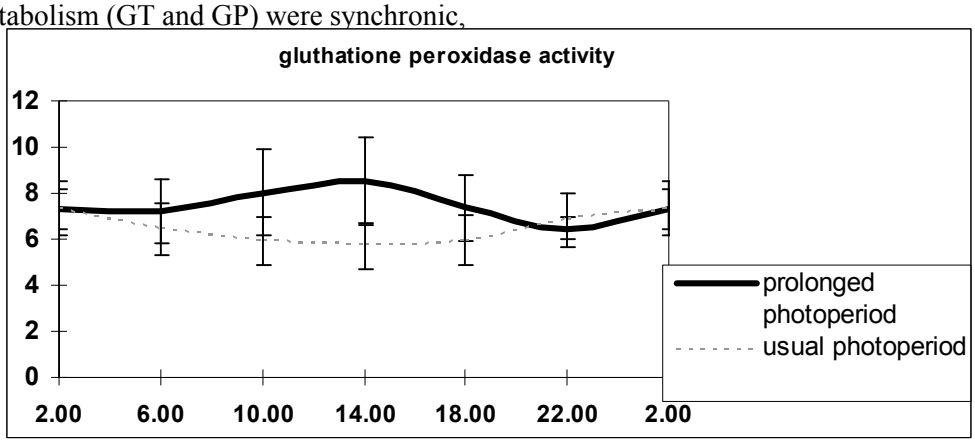
Obtained results and discussion.

Changes of diurnal rhythm of GR in healthy people systematically staying under prolonged photoperiod conditions (group 2 vs 1) were detected; they manifested with trend to mesor decrease (15,2%, $p<0,05$), rhythm amplitude (18,1%, $p<0,05$) and acrophase shift to day time (around 14.00). Individual cosinor-analysis revealed abolishment of circadian character of GR content variations in 1/3 of group 2 subjects, despite the fact that circadian properties in group data were preserved (pic.1).

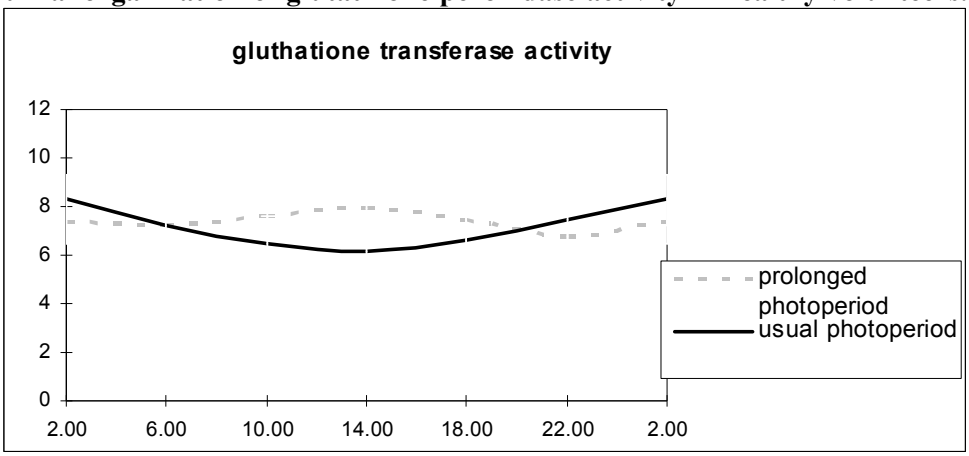


Pic.1. Diurnal organization of glutathione restored content in blood of healthy volunteers.

Profiles of activity of enzymes responsible for glutathione metabolism (GT and GP) were synchronic, in phase with GR in both groups (pic. 2-3).

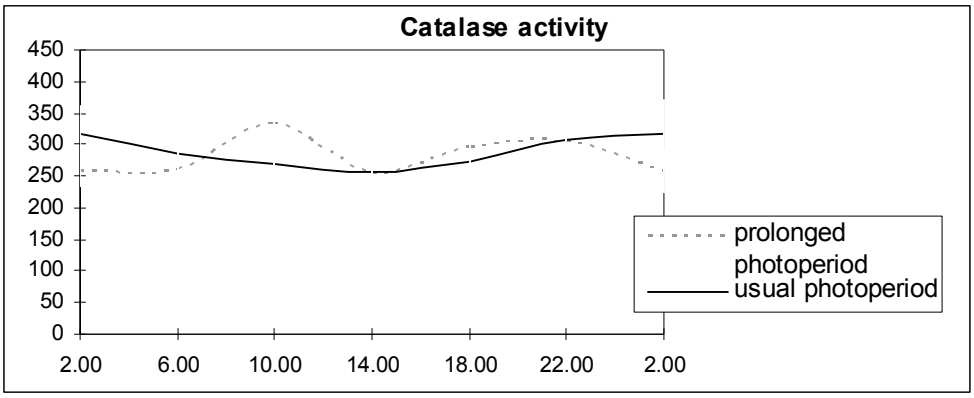


Pic.2. Diurnal organization of glutathione peroxidase activity in healthy volunteers.



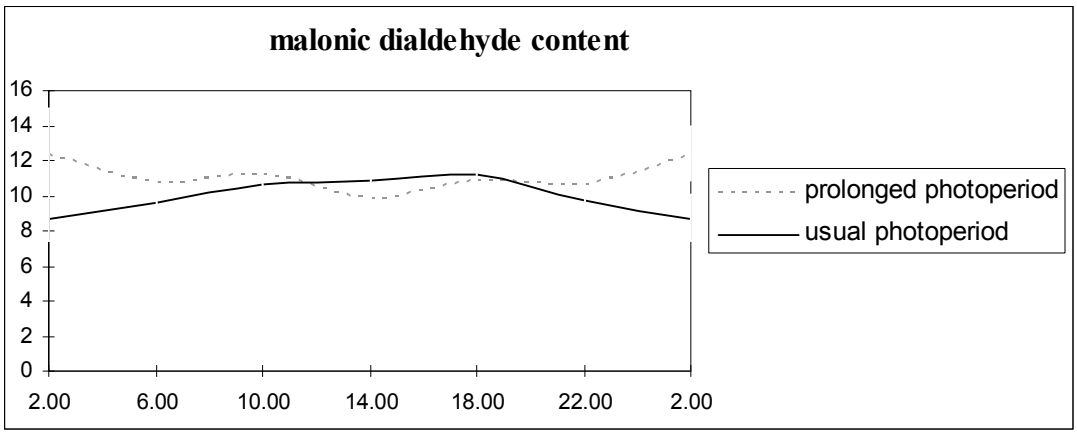
Pic. 3. Diurnal organization of glutathione transferase activity in healthy volunteers.

Investigation of catalase activity variations throughout diurnal interval demonstrated similar results: circadian periodicity, acrophase at evening hours (around 00.00) in group 1 subjects (pic.4). Prolonged exposure to artificial illumination lead to periodicity and circadian character disappearing in majority investigated people of second group. Maximal values shift to earlier hours was discovered (pic.4).

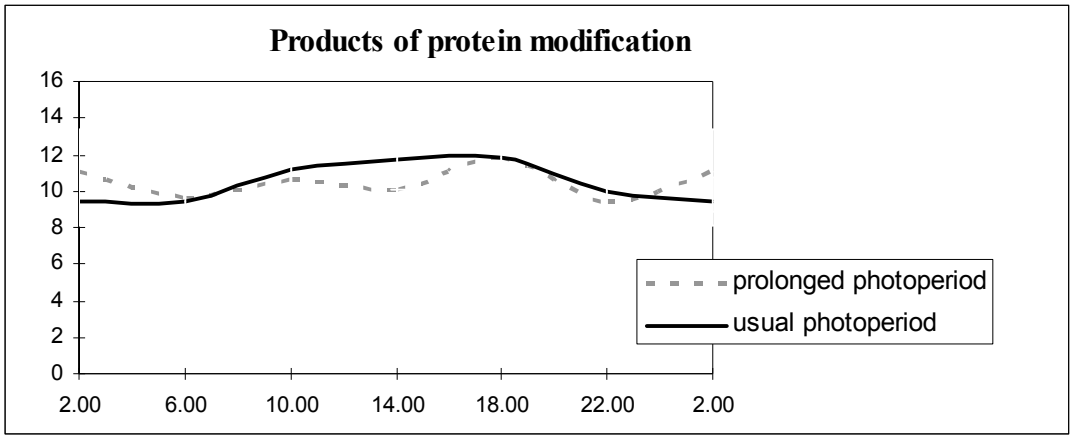


Pic. 4. Diurnal organization of catalase activity in healthy volunteers.

Systematic exposure of healthy volunteers to prolonged photoperiod lead to abolishment of circadian character of biological rhythms of MD and PFPM in one-third of the group 2 subjects (rhythmicity value remained within 72,0-80,0% in rest, $p<0,05$); group chronogram preserved circadian signs. Despite no significant difference was discovered when comparing absolute values in different time points, as well as average daily levels of mentioned indices, cosinor-analysis revealed acrophase shift to night hours in group 2 (2.22 vs 16.00) (pic.5,6).



Pic. 5. Diurnal organization of malonic dialdehyde content in healthy volunteers.



Pic. 6. Diurnal organization of products of free radical modification of proteins in healthy volunteers

Obtained results and discussion.

As demonstrated, activity growth at physiological rest time produces marked masking effects over internal time-related organization of biological parameters. These effects increase depending on exposure duration. That may explain detected

changes and differences from normal physiological profiles in healthy subjects systematically exposed to prolonged illumination conditions.

We observed acrophase shift of antioxidant enzymes to day time, and products of free radical modification of molecules - to later hours in target population. Several reasons may cause that: from excess of physical activity up to absence or lower efficacy of antioxidants induction by melatonin: its synthesis and secretion are blocked by light directly and immediately. Such reaction belongs to immediate response type ones, is adaptive by origin and must not cause marked changes in organism in case of shorter duration of unusual conditions (we observed shift phase only with preserving circadian character of biological rhythms of investigated values). Despite, change of values of free-radical processes as response for quite short photostimuli may indicate that even short-lasting temporary disbalance of physiological functions and environment may be accompanied by activity of adaptive mechanisms and manifests with tension of regulatory system and lowering functional reserve sources.

Biological rhythms, being maintained genetically, are well-synchronized and entrained by external factors - light, food availability, temperature regimen etc. Extreme external values cause their misbalance that may lead to physiological or psychological stress, decreased resistance to infections. That is maintained by changes of cortisol and katecholamines expression rhythms and may be initial factor in a chain of further biochemical interactions. Modern life aspects altering biological rhythms in key periods of development - in kids and teenagers - are suggested to be extremely unfavorable and harmful.

It has been proved recently that melatonin secretion alterations are strongly associated with sleep disturbances in elder age population. Elder patients suffer from insomnia more frequently comparing younger ones, and that may be the reason why they have higher average daily levels of pro-inflammatory cytokines: tumor-necrotising factor-α, interleukin-6, interleukin-1β with higher blood content at day time. Initial desynchronosis is characterised by disappearing of night peak of interleukin-1β with parallel increase of β-endorfine; that may induce euphoria-like states with under-estimation of situation and inclination of physical and psychological overloading. Desynchronization of cytokines and cortisol synthesis may cause disintegration of internal metabolic processes and increase exposure of the organism (cardiovascular and locomotor systems, in partial) to pro-inflammatory stimuli.

Conclusion.

Violation of regimen of illumination with prolongation of activity at night time in healthy volunteers leads to desorganization of biological rhythms of antioxidant defence values, free-radical processes. Disbalance in the investigated system may be early bird of desynchronosis and initiator (risk factor) for different pathology.

Perspectives of further investigations.

No exact data are described relating duration of exposure to prolonged illumination, intensity, regularity which may induce changes. Considering chronobiological regularities of the described biochemical processes may serve as a background to further understanding of shift work risks.

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ИЗМЕНЕНИЯ СУТОЧНОЙ ОРГАНИЗАЦИИ ПРОЦЕССОВ СВОБОДНОРАДИКАЛЬНОЙ МОДИФИКАЦИИ МАКРОМОЛЕКУЛ, АНТИОКСИДАНТНОЙ ЗАЩИТЫ ЗДОРОВЫХ ЛЮДЕЙ ПРИ ПРОЛОНГАЦИИ ФОТОПЕРИОДА

Бойчук Т.Н., Илащук Т.А., Микитюк О.П.

Резюме. Показано, что пролонгация периода искусственного освещения как следствие цивилизации может вызывать нарушение суточной организации некоторых физиологических показателей. В свою очередь, это может стать основой развития ряда заболеваний.

Целью исследования стало сравнение суточных профилей продуктов свободнорадикального окисления и антиоксидантов у людей, пребывающих в условиях обычного и нарушенного режима ночного сна. С этой целью обследовано 41 здорового добровольца. У каждого их них получено 6 образцов крови в течение суток. 24 обследованных придерживались стабильного режима ночного сна - с 23.00 до 7.00 (группа 1); остальные систематически ложились спать около 2.00-3.00 - не реже, чем 3-4 раза в неделю (группа 2).

Изучение показателей свободнорадикальной моди-

фикации макромолекул (малонового альдегида и продуктов свободнорадикальной модификации белков), состояния антиоксидантной защиты (содержание восстановленного глутатиона, активности каталазы, глутатионпероксидазы и глутатионтрансферазы) проведено с использованием стандартных биохимических методик. Все результаты оценены статистически.

Максимальные уровни метаболитов свободнорадикальных процессов были инверсными в обеих группах. Выявлены изменения суточных профилей всех исследуемых показателей у обследованных второй группы - с поздним уходом ко сну. У них наблюдали снижение среднесуточного содержания антиоксидантов и повышение уровня продуктов свободнорадикальной модификации макромолекул. Параллельно, у исследуемых второй группы отмечали сдвиг акрофаз с сопутствующим снижением концентрации антиоксидантов на позднее вечернее время. Поскольку ночь принято считать периодом восстановления для организма, описанные нарушения могут быть проявлением десинхроноза в условиях продления времени активности и предвестником серьезных факторов риска развития патологии.

Ключевые слова: здоровые добровольцы, суточные ритмы, антиоксиданты.

ЗМІНИ ДОБОВОЇ ОРГАНІЗАЦІЇ ПРОЦЕСІВ ВІЛЬНОРАДИКАЛЬНОЇ МОДИФІКАЦІЇ МАКРОМОЛЕКУЛ, АНТИОКСИДАНТНОГО ЗАХИСТУ ЗДОРОВИХ ЛЮДЕЙ ЗА ПРОЛОНГАЦІЇ ФОТОПЕРІОДУ

Бойчук Т.М., Ілащук Т.О., Микитюк О.П.

Резюме. Показано, шо пролонгація періоду штучного освітлення як наслідок цивілізації може спричиняти порушення добової організації декотрих фізіологічних показників. У свою чергу, це може стати основою розвитку ряду захворювань.

Метою дослідження стало порівняння добових профілів продуктів вільнорадикального окислення і антиоксидантів у людей, що перебувають за умов звичайного і порушеного режиму нічного сну. З цією метою обстежено 41 здорового добровольця. У кожного з них забрано 6 взірців крові впродовж доби. 24 обстежених пдотримувались стабільного режиму нічного сну - з 23.00 до 7.00 (група 1); решта систематично лягали спати біля 2.00-3.00 - не рідше, ніж 3-4 рази на тиждень (група 2).

Вивчення показників вільнорадикальної модифікації макромолекул (малонового альдегіду і продуктів вільнорадикальної модифікації білків), стану антиоксидантного захисту (вміст відновленого глутатіону, активності каталази, глутатіонпероксидази і глутатіонтрансферази) проведено з використанням стандартних біохімічних методик. Всі результати оцінено статистично.

Максимальні рівні метаболітів вільнорадикальних процесів та антиоксидантів були инверсними в обох групах. Виявлено зміни добових профілів всіх досліджуваних показників у другій групі - з пізнім відходом до сну. У обстежених спостерігали зниження середньодобового вмісту антиоксидантів і підвищення рівня продуктів вільнорадикальної модифікації макромолекул. Паралельно, у досліджуваних другої групи відмічали зсув акрофаз із супутним зниженням концентрації антиоксидантів на пізній вечірній час. Оскільки ніч прийнято вважати періодом відновлення для організму - описані порушення можуть бути проявом десинхронозу за умов подовження часу активності і передвісником серйозних факторів ризику розвитку патології.

Ключевые слова: здорові добровольці, добові ритми, антиоксиданти.

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