SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSPHY (PhD)

Link between the irisin/BDNF axis and circadian regulation, and its implications in clinical diseases

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Moto: "Open your arms to change, but don't let go of your values"

Dalai Lama

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1. Background and aims of the thesis

1.1. Diseasome of physical inactivity)

The term “diseasome of physical inactivity” was coined by Pedersen to explain clustering of chronic diseases linked to physical inactivity. This diseasome includes type 2 diabetes, cardiovascular diseases, colon cancer, postmenopausal breast cancer, dementia, and depression. Accordingly, physical inactivity per se promotes the accumulation of metabolically active visceral fat, which, by generating low-grade systemic inflammation, contributes to clustering of chronic, non-communicable diseases. Additionally, parallel decrease of total fat-free mass and body mass index, impaired glucose tolerance, and blunted postprandial lipid metabolism were witnessed. These findings indicate that absence of contractile activity of muscle directly contributes to systemic derangements in several organs possibly by releasing soluble mediators. Accordingly, skeletal muscle was coined as an endocrine organ, after myokines, -muscle derived polypeptides with auto-, para- and endocrine function—were discovered and thus offered a mechanism that may explain these elaborate effects.

1.2. The irisin/BDNF axis

Recently, a novel contraction-regulated myokine, irisin, has been identified, and was shown to have peripheral and central effects (4). Irisin is formed by proteolytic cleavage from the fibronectin type III domain-containing protein 5 (FNDC5). FNDC5 is a transmembrane protein that is expressed under the regulation of the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α).

In the periphery, the mostt significant action of irisin is adipocyte browning by inducing translation of mitochondrial uncoupling protein 1 (UCP1) and consequent non-shivering thermogenesis. Additionally irisin was shown to have central effects related to the induction of brain-derived neurotrophic factor (BDNF) in the brain. BDNF is a potent neurotrophin that is synthetized in a pre-pro form and released into the circulation either as pro-BDNF (which is then converted to BDNF by plasma tissue plasminogen activator) or as mature BDNF (the active form, referred to as BDNF further on). It is

able to cross the blood-brain barrier and exerts central and peripheral effects. BDNF is known for enhancing the dopamine content in the ventral tegmental area (VTA) and for mediating neuronal plasticity, learning, and memory in limbic structures. Nevertheless, the mechanisms underlying physical inactivity and its link to the diverse array of diseases included in the diseasome of physical inactivity remain elusive, giving way to the notion that potential derangement of the irisin/BDNF axis may be involved.

1.3. Circadian regulation

The circadian system comprises of a network of molecular clocks ubiquitously present in virtually every cell. The omnipresence of the circadian system in light-sensitive organisms posits that adaptation to circadian periodicity (due to the Earth's rotation around its axis) carries a significant evolutionary advantage, possibly by enabling the organism to anticipate regular environmental changes. In humans circadian rhythm is driven by a cell autonomous core clock machinery. The master clock is located bilaterally in the suprachiasmatic nucleus (SCN) of the ventrolateral hypothalamus. It orchestrates top-down synchronization of tissue specific subsidiary clocks. The SCN transmits the environmental rhythm to other areas of the brain and the peripheral organs via its neuronal connections, by altering sympathetic outflow, endocrine signals (e.g., melatonin), body temperature rhythm, and other indirect cues. The free-running period of the molecular clocks generally differs from the 24-h day (is usually longer), hence molecular clocks must be entrained by environmental cues (Zeitgebers). Inappropriate entrainment of the master clock leads to the evolution of varying diseases and disorders. Entrainment e.g., adjusting the phase of the molecular clock to be aligned with the timing of an environmental cue, may occur in response to photic (light) and non-photic (food, temperature, exercise, stress, or arousal) environmental stimuli. The master clock is innervated by a specialized light-response pathway, the retinohypothalamic tract, emanating from the retina (26). Light sensitivity of these retino-recipient pacemaker cells shows pronounced circadian fluctuation and are entrained by light.n machinery

The limited capability of the central clock to be entrained is well reflected by plethora of health disturbances caused by the disorganization of the timing system, which leads to circadian misalignment. Chronic jet lag, social jet lag, or shift work causes the phase shift of the individual's internal central clock due to objective (e.g.,

intercontinental travel) or subjective (conflicting social schedule) causes. In addition, marked lag in internal circadian timing is also defined as delayed sleep-wake phase disorder, by the 5th edition of the Diagnostic and statistical manual of mental disorder In this disorder the biological rhythms governed by the SCN e.g., the sleep-wake cycle, oscillations in core body temperature and endogenous melatonin secretion are considerably delayed with respect to the normal 24 h physical and social environment of a person. Preclinical and clinical studies have shown the causative role of circadian misalignment in the evolution of metabolic syndrome, obesity and diabetes, cardiovascular disease, depression, prostate and breast cancer, and dementia.

The SCN is most readily entrained by light, however resent reports posit that exercise and restricted feeding may also be of some, albeit considerably less influence. BDNF has emerged as a possible mediator of cognition-enhancing effects of exercise and intermittent fasting (95), a finding underscored by simultaneous increase of BDNF expression in multiple brain regions and improved cognition in response to voluntary aerob exercise and/or intermittent fasting (96–98). The finding that β-hydroxybutyrate, a ketone produced during fasting and exercise, induces BDNF expression in hippocampal cell culture (5, 99) also corroborates this link.

The ability of light to reset the intrinsic SCN pacemaker is only present during subjective night, as light is not able to reset SCN during subjective day, making it possible for the SCN to align circadian behavior with the day and night cycle. Photic entrainment is mediated by complex signalization, BDNF, that is postulated to play a critical role by gating the circadian system to light. BDNF expression within the SCN is rhythmic, showing basal and elevated BDNF levels during subjective day and night, respectively. On one hand, BDNF signaling during subjective day is insufficient to permit excitatory neurotransmitter release, inhibiting the transmission of the light signal through the retinohypothalamic tract-SCN synapse. On the other hand, elevated BDNF levels during subjective night via activating the TrkB receptor is sufficient to induce functional and structural changes that lead to potentiation of the light-induced retinohypothalamic activation in the SCN. These findings are in line with the observations that photic input is unable to entrain the master clock during subjective day, rendering the SCN insensitive to perturbation by light, while the SCN responds to light by prominent phase shifts during subjective night.

1.4. Obstructive sleep apnea hypopnea syndrome (OSAHS)

OSAHS is a sleep-related breathing disorder characterized by excessive daytime sleepiness paralleled by intermittent collapse of upper airway leading to impaired gas exchange and consequent hypoxemia, events often terminated by arousal [3, 4]. Airway pathology triggers sympathetic hyperactivation, inflammation and oxidative stress paving the way along the continuum of cardio-metabolic diseases, by contributing to endothelial dysfunction, insulin resistance, and subsequently results in arterial hypertension, diabetes mellitus, stroke, myocardial infarction, heart failure and sudden death.

Sleep regulation is well conceptualized by the two-process model of sleep, that posits the continuous interaction of oscillating homeostatic processes (growing and declining sleep debt experienced during wakefulness, and sleep, respectively) and the circadian pacemaker process, governed by the master clock. As a result, the phase, amplitude and periodicity of oscillations are entrained in a way that sleep is properly aligned with the 24-h day-night cycle. Optimal timing of sleep with regards to the circadian phase is an important regulatory signal aligning the central circadian and peripheral tissue specific rhythms. Homeostatic and circadian pacemaker processes interact albeit are regulated separately. Circadian misalignment e.g. altered sleep phase, with respect to the circadian environment, and/or altered amplitude of underlying circadian processes leads to diminished sleep quality and excessive sleepiness, and impaired cognitive performance.

1.5. Aims

1. The aim of the current study was to reconceptualize the diseasome of physical inactivity by meticulously reviewing its links with the circadian rhythm. Accordingly, a model describing the link between the regulation of circadian rhythm at the level of the suprachiasmatic nucleus (e.g. the master clock) and peripheral biomarkers. Therefore the possible interaction between BDNF, present at the site of photic entrainment in the suprachiasmatic nucleus and irisin, an exercise borne myokine known to induce BDNF levels in the CNS was elaborated. This hypothesis offers a mechanistic relationship between certain chronic non-communicable diseases (in which the irisin/BDNF axis is altered) and impaired regulation of the circadian rhythm.

2. The goal of the clinical study involving OSASH patients was to elucidate the potential link between the shift of circadian rhythm (characterized by the score obtained on the Epworth Sleepiness Scale) and the serum level of of BDNF, a neurotrophin involved in photic entrainment.

3. Our further goal was to assess if it is possible to identify the role of irisin, as an upstream mediator of BDNF in the regulation of circadian rhythm, in this patient population.

2. Materials and methods

2.1. Conceptualization

The diseasome of physical inactivity was conceptualized by elucidation of its relationship with circadian rhythm regulation. Accordingly an interdisciplinary, comprehelsive assessment was undertaken. it builds mainly on the conceptual and the experimental findings of others, cited throughout the text. Exploratory searches were conducted on PubMed, in English for the following topics: diseasome of physical inactivity, molecular clocks, master clock, peripheral clocks, entrainment, synchronization, oxidoreductive cycle, metabolism, muscle, BDNF, hypothalamus, irisin. Articles of high relevance were identified subjectively and used as starting points for identifying further articles (by reviewing both the articles cited by these and those that have cited them latter) of high impact.

2.2. Study design and protocol

2.2.1. Patients and protocol

The present study was designed in line with the STROBE statement for cross-sectional studies [38] and was approved by the Ethical Committee of the University of Debrecen (DEOEC RKEB/IKEB 3715–2012). Informed consent was obtained from each participant. The investigation conforms to the principles outlined in the Declaration of Helsinki.

The current study is based on the analysis of a cohort of patients who attended the accredited Sleep Medicine Center of the Department of Neurology (University of Debrecen) between October 1, 2012 and April 30, 2013 and was diagnosed to have OSAHS. Accreditation was made by the Hungarian Society for Sleep Medicine in compliance with European guidelines for the accreditation of Sleep Medicine Centres. Every patient meeting the diagnostic criteria for OSAHS according to the relevant Hungarian [40] and concordant international guidelines were invited to participate, given they met the inclusion criteria and failed to meet any of the exclusion criteria.

Inclusion criteria were age between 18 and 80 years and diagnosis of OSAHS at the time of inclusion. The clinical diagnosis of OSAHS was made if the number of obstructive events (apneas, hypopneas + respiratory event related arousals) on polysomnography was greater than 5 events/hour, and the patient reported excessive daytime sleepiness and/or at least two of the following: repeated nighttime awakening, unrefreshing sleep, decreased concentration and impaired memory, fatigue, repeated gasping or choking while asleep. Sleep related events were scored according to standard operational procedures and the manual of the American Academy of Sleep Medicine. Exclusion criteria included inability to provide informed consent, pregnancy, kidney disease, bronchial asthma or chronic obstructive pulmonary disease (COPD), inflammatory diseases of the face or oral cavity, any systemic autoimmune disease.

Overall, 70 patients were recruited, data from one patient was excluded from the analysis due to a highly significant outlier (serum irisin > 15 ng/mL). Patients were diagnosed with OSAHS at the time of their recruitment, thus received no therapy at inclusion for this syndrome.

2.2.2. Poliszomnography

In-laboratory full-night polysomnography was performed according to the European standard operational procedures (Fisher 2011) (Philips Alice IV and V, Respiromed, Hungary. Patients were directed to take their medication as usual on the night of their sleep study. Patients were mounted with 6 EEG electrodes (F3-M2, C3-M2, C4-M1, O2-M1), 2 EOG electrodes, 1 submental EMG, ECG (one channel), pulsoxymeter, body position sensor, nasal pressure/thermal flow sensor, snore sensor. Proper, artefact-free functioning of the recording devices, trouble-shooting was provided by the continuous oversight of nurses certified in clinical electrophysiology. Every sleep study was reviewed and interpreted by a qualified sleep physician..

2.2.3. Blood samples

After an overnight fast blood, samples were drawn in the morning of the examination. Routine laboratory investigations were performed according to the standard clinical practice of the Department of Laboratory Medicine (University of

Debrecen), making use of the locally used reference values. Serum or plasma samples were used for determining measures descriptive of carbohydrate homeostasis (glucose, insulin, hemoglobin A1c (HgA1c)), lipid homeostasis (total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol, Lp(a), apoA1, apoB), kidney function (urea, creatinine), liver function (GOT, GPT, γGT), status of skeletal muscles (CK, LDH) and systemic inflammation (C-reactive protein (CRP)). CRP was dichotomized as high vs. normal with the cutoff being 4.6 mg/L and 5.2 mg/L for female and male patients, respectively. Serum samples used to determine irisin and BDNF were frozen within 60 min and stored at − 80 °C until further analysis

2.2.4. Determination of serum irisin and BDNF

Serum BDNF and irisin levels were measured in accordance with the manufacturer's protocol (Sigma-Aldrich, MO, USA – BDNF; Phoenix Pharmaceuticals, Burlingame, CA, USA – az irisin).

2.2.5. Questionnaires

Several questionnaires were completed by the study participants in a supervised administration setting, including the Epworth Sleepiness Scale (ESS), the Pittsburgh sleep quality index (PSQI) and A kutatás résztvevői felügyelt környezetben a következő kérdőíveket töltötték ki: Epworth Álmosság Skála (ESS), Pittsburgh Alvásminőség Index (PSQI), Beck Depression Inventory Beck Depresszió Kérdőív. A kérdőívek magyar verzióit a Magyar Alvástársaság biztosította.

2.2.6. Data analysis

Normality of continuous variables was checked by the Shapiro-Wilk test. In case of Gaussian distribution, Student's t-test was used for the comparison of two data sets, if not, Mann-Whitney U test was carried out. Frequencies were compared with Pearson's $\chi 2$ test

Demographic, anthropometric, anamnestic, laboratory and polysomnography data were compared based on the severity of the OSAHS, using AHI, with cutoff levels

of < 30/h vs ≥ 30/h for mild to moderate severity vs severe OSAHS, respectively).

For linear regression, parameters showing Gaussian distribution were used in their raw forms, whereas those not normally distributed were appropriately transformed to obtain normal distribution. Accordingly, the parameters were transformed as follows: (log) systolic blood pressure, (log) weight, 1/body mass index (1/BMI), (log) abdominal circumference, (log) systolic blood pressure, log (triglyceride), (log) PSQI, (sqrt) arousal index, (sqrt) central apnea index, (sqrt) obstructive apnea index,, (sqrt) hypopnea index, (sqrt) Beck depression score, (log) triglyceride, (log) HDL-cholesterol.

To identify determinants of Epworth Sleepiness Scale Score, serum irisin and BDNF levels, simple linear regression was carried out including traditional confounding factors (age, gender), anthropometric parameters (height, (log) weight, 1/BMI, neck and (log) abdominal circumference), blood pressure ((log) systolic blood pressure, diastolic blood pressure), intima media thickness, polysomnographic parameters, laboratory parameters characteristic of carbohydrate and lipid homeostasis, (log) PSQI and (sqrt) Beck score. Furthermore, information regarding the number of people living in a household, smoking, benzodiazepine use, presence of diabetes mellitus, arterial hypertension, coronary artery disease, cerebrovascular disease, metabolic syndrome (the latter six parameters dichotomized as yes vs. no) were also assessed. Polysomnography parameters yielding significant regressors were combined into a single parameter using principal component analysis. Missing data were omitted. Then, in order to eliminate effects of potential confounders, multiple linear regression modeling was performed including both irisin and BDNF levels, and all significant regressors determined with the simple linear regression as well as age and gender (as a priori variables). Variables were introduced into the initial multiple model simultaneously, then factors not contributing significantly to the model were deleted. The final model contained all variables identified a priori, and (log) PSQI. In addition, the final model was assessed for the interaction of irisin and BDNF. Heteroskedasticity and goodness of fit for the model was assessed by Cook-Weisberg and Ramsey test.

Statistical analysis was performed with Stata 18.0 software (Stata Corporation). Values are given as mean ± SD or median (with the interquartile range: IQR), regression coefficients are presented with their 95% confidence interval (CI).

3. Results

3.1. Elméleti eredmények

risin in the brain is able to induce BDNF expression at varying sites e.g., in the hippocampus, and the VTA. Elevation of the circulating level of irisin was shown to induce BDNF expression in the hippocampus, as was forced hippocampal expression of FNDC5. Moreover, decrease of cortical BDNF expression was evidenced, following mRNA expression-mediated knockdown of FNDC5 expression. Another stream of experiments further articulated the possibility of irisin serving as a cross-organ messenger linking skeletal muscle and brain to enable the organism to react adaptively to its environment. Accordingly, irisin's direct locomotor activity was proposed, when central administration of irisin into the third ventricle of rats, lead to an increase of physical activity (characterized by increase of total travel distance, ambulatory count and time, vertical counts in treated rats vs. controls receiving IgG Fc peptide). Summarizing the above findings, we put forward the speculative notion that the exercise-related irisin mediates brain-muscle crosstalk by possibly linking sedentary lifestyle and circadian rhythms.

This hypothesis is underscored by recent findings that seem to contradict earlier notions regarding the inability of the SCN to be entrained by non-photic Zeitgebers. Nevertheless, in order to generate a coherent rhythm, the SCN must integrate environmental Zeitgebers (mainly light) as well as information from the peripheral tissues. The presence of sufficient signalization for entraining the central clock by peripheral signals, e.g., exercise and feeding/fasting, is indicated by the ability of exercise and feeding to induce shifts in the SCN under constant darkness. Albeit light is the main entraining stimuli for the SCN, restricted feeding schedules, hypocaloric feeding, and scheduled exercise can also entrain the SCN to a limited extent, under dark-light conditions. Human studies have shown that the combination of light and exercise is needed for entraining the SCN. This is indicated by finding that the sleep-wake cycle may be entrained by the sleep schedule alone, while phase advancement of the circadian rhythm of melatonin was dependent on both the sleep-wake cycle and exercise

3.2. Clinical results

3.2.1. Patients

Data from the 69 patients were analyzed. The average age was 53.81 ± 10.72 years, and 18 patients were female. Of the 69 patients, 16 patients did not take any medications, 16 patients took statins, 41 patients took antihypertensives (any of the following: ACE inhibitor, ARB, beta receptor blocker, Ca channel antagonist, diuretic, or other), 11 patients took oral antidiabetics, 19 patients received aspirin, 8 patients took benzodiazepines and 10 patients used proton pump inhibitors regularly.

3.2.2. Comparison of patients regarding OSAHS severity

The two strata of our OSAHS cohort, dichotomized by AHI (cutoff at < 30/h for low to moderate severity) was homogenous with respect to basic demographic data (age and gender distribution) and most parameters (Table 2). However, patients suffering from severe OSAHS had higher prevalence of arterial hypertension, were more obese indicated by significantly higher weight, BMI, larger neck and abdominal circumference. These patients had a less favorable cardiometabolic risk profile indicated by significantly higher IMT, serum glucose and HbA1c, triglyceride Lp(a) and Apo-A1 levels, and lower HDL-cholesterol level. Higher CRP levels and arterial hypertension were also more prevalent in this subset of patients. Naturally polysomnography measures were significantly worse in the subset of patients with severe OSAHS. Furthermore, higher proportion of patients suffered from daytime sleepiness as indicated by the ESS in this patient subset (Table 2). Sleep quality and depression characterized by the PSQI and Beck Depression Inventory showed no significant difference.

3.2.3. Associations between Epworth sleepiness score, serum irisin and BDNF levels

Upon assessing the linear relationship between ESS and serum irisin levels, we found that their association is on the verge of statistical significance ($p = 0.051$), and no significant association was seen between ESS and serum BDNF levels ($\beta = 0.008$

(− 0.0023; 0.017, p = 0.129). If the influence of the interaction between serum irisin and BDNF were assessed, the results were yet again close to statistical significance (p = 0.055). Significant regressors of irisin included (log) weight, and (log) triglyceride, while serum BDNF showed a significant association with the number of people living in the patient's household. ESS showed significant relationships with gender, number of people living in the same household, several polysomnography parameters, measures reflective of obesity, (sqrt) Beck depression score and (log) PSQ)

This relationship between ESS and the two independent variables of interest, irisin and BDNF showed strong significant association in the final multiple regression model (βirisin: 1.53; CI: 3.55, 2.70; p = 0.012; βBDNF: 0.014; CI: 0.005, 0.023; p = 0.002) that contained a priori determinants and (log) PSQI. Furthermore, significant interaction between serum irisin and BDNF levels were identified by the final model. The models not containing and containing the interaction between the two explanatory variables of interest were significant ($p < 0.001$ for both models). The Cook-Weisberg test showed no heteroskedasticity for either model (p = 0.08 and p = 0.08, respectively). The Ramsey test showed good fit for both models (p = 0.42; p = 0.46, respectively). Furthermore, good fit was also reflected by the locally weighted scatterplot smoothing (model devoid of the interaction term) and also by visual comparison of the three dimensional plots (axis x, y and z presenting serum irisin, BDNF levels and ESS, respectively) for the original data set and that fitted to the data yielded by our model (containing the interaction term) (Fig. 2). When plotting the final model addressing the interaction between irisin and BDNF, it is interesting to note that significant changes in the ESS are experienced if serum irisin level changed by 1 ng/mL, given the serum level of BDNF is within the range of ~ 280 to 470 ng/mL. Vice versa, ESS shows a significant increase in response to 1 ng/mL increase of BDNF if serum irisin levels are within the range of ~ 6.1 to 8.1 ng/mL. The final multiple linear regression model (built for the ESS) contained only (log) PSQI additional to the a priori identified parameters. The model showed that sleep quality is significantly associated with daytime drowsiness experienced by OSAHS patients (β: 7.01; CI: 0.85, 13.35; p = 0.026) (this relationship was significant whether or not the interaction term was included in the model).

4. Discussion

4.1. FNDC5/irisin, a novel link between photic and non-photic entrainment signals

Summarizing, we propose that the diseasome of physical inactivity evolves due to the derangement of complex interactions between the circadian system, the redox homeostasis, inflammation and the PGC-1α/irisin/BDNF axis. Furthermore, based on previous findings, that sufficiently high BDNF levels are mandatory for photic entrainment, rendering BDNF as the gatekeeper regulating light's ability to shift the phase of the master clock, we propose that irisin, either by crossing the blood-brain-barrier, or by its direct expression in the hypothalamus is able to increase BDNF levels in the retinohypothalamic synapse, and hence may modulate BDNF's effect on photic entrainment. This is supported by the fact that peripheral effects per se are unable to shift the master clock, however simultaneous presence of light and peripheral factors (exercise, and fasting, both known to induce PGC-1α and consequently irisin expression) may do so. Nevertheless, the need for further investigation into the possible role of the irisin/BDNF axis in linking sedentary lifestyle and circadian misalignment are need. In the current work we suggest, that muscle-brain crosstalk enables non-photic entrainment signals to fine-tune the master clock, by inducing the release of the irisin into the systemic circulation. We hypothesize that irisin after crossing the blood-brain barrier in turn increases the expression and release of BDNF in the pre- and postsynaptic terminals of the retinohypothalamic tract, facilitating photic entrainment in the SCN in response to the peripheral signals of fasting, and exercise

4.2. Interpretation of the clinical results

The main finding of our study is that circadian misalignment indicated by excessive daytime sleepiness (characterized by the ESS) has a strong positive linear relationship with serum irisin and BDNF levels in a multiple linear regression model that corrects for age, gender and the only other significant regressor, (log) PSQI. Furthermore, we identified an interaction between serum irisin and BDNF levels with respect to their effect on ESS, suggestive of a concentration-response relationship. Our findings suggest that the effect of irisin on BDNF and subsequently on ESS follows the

kinetics of the Hill equation [58]. Accordingly, in our OSAHS patient sample, increasing serum irisin level by 1 ng/mL fails to cause a significant increase of the ESS score if serum BDNF levels are in the lower range (e.g. below 280 ng/mL), indicating that low irisin levels have a small, nonsignificant effect on BDNF levels and consequently on ESS score. Conversely, changing serum irisin level by 1 ng/mL also fails to exert a significant increase in the outcome measure of interest at high serum BDNF levels (e.g. above 470 ng/mL), implicating that a theoretical maximal effect (Emax) has been reached.

Similarly, increasing serum BDNF level by 1 ng/mL only leads to a significant increase in the ESS score if serum irisin levels are midrange e.g. fall between 6.1 ng/mL and 8.1 ng/mL.

Worthy of notice is the finding that (log) PSQI was also found to be a significant predictor in the final multiple model especially if one considers the concept of two process model of sleep [22]. Our results suggest that poorer sleep quality (indicated by higher PSQI scores) results in more pronounced subjective daytime sleepiness. Allowing a more speculative interpretation, this model may intuitively posit that in the context of OSAHS alteration of sleep-related processes should be interpreted in terms of disrupted sleep homeostasis evolving due to intermittent airway obstruction, consequent hypoxia, and arousals and also via the alteration of the intertwining circadian regulatory processes reflected by the complex interaction between excessive daytime sleepiness and the irisin/BDNF axis. This interpretation provides a sound explanation for the phenomena of excessive daytime sleepiness reported in CPAP-treated OSAHS patients, in whom objective polysomnography-derived indices of OSAHS were shown to be normalized, while daytime sleepiness was preserved along with increased risk for morbidities and mortality.

To the best of our knowledge there are no reports concerning the serum level of irisin in OSHAS patients. In one study evaluating the effects of CPAP therapy in OSHAS patients, irisin levels were determined, however no absolute serum levels were reported, only changes from baseline values, mean difference between groups and correlation coefficients were published. Previously our group has reported serum irisin levels in the range of 7.22 ng/mL (IQR: 6.63–8.10 ng/mL) in COPD and 7.87 ng/mL (IQR: 7.15–8.82 ng/mL) in asthma patients. Others have reported irisin levels of 26.3 (IQR: 22.6–32.4) ng/ml, 53.7 (IQR: 46.7–62.8) ng/ml, 58.5 (42.8–78.9) ng/ml in

smokers with and without COPD, and in non-smoking individuals, respectively.

The serum BDNF levels reported in the present investigation are considerably higher, but fall within the range reported by others. Studies including healthy individuals and other patient populations reported serum BDNF levels spanning over four orders of magnitude, ranging from 0.005 ng/ml to 280 ng/ml, [71,72,73,74] using different ELISA kits. Our group has formerly reported serum BDNF levels of 345.6 ng/mL (IQR 294.20–387.90 ng/mL) and 314.46 ± 118.68 ng/mL in cohorts of chronic obstructive pulmonary disease patients [75] and bronchial asthma [76], respectively. To the best of our knowledge there are no reports concerning the serum level of irisin in OSHAS patients. In one study evaluating the effects of CPAP therapy in OSHAS patients, irisin levels were determined, however no absolute serum levels were reported, only changes from baseline values, mean difference between groups and correlation coefficients were published [77]. Previously our group has reported serum irisin levels in the range of 7.22 ng/mL (IQR: 6.63–8.10 ng/mL) in COPD and 7.87 ng/mL (IQR: 7.15–8.82 ng/mL) in asthma patients. Others have reported irisin levels of 26.3 (IQR: 22.6–32.4) ng/ml, 53.7 (IQR: 46.7–62.8) ng/ml, 58.5 (42.8–78.9) ng/ml in smokers with and without COPD, and in non-smoking individuals, respectively

Choosing only the Epworth Sleepiness Scale to characterize the possible alteration of circadian rhythm could be regarded as a limitation of the current study. The ESS is considered as a standard questionnaire appropriate in the clinical setting to provide a subjective measure of daytime sleepiness [41]. Furthermore, that objective measure of excessive daytime sleepiness was not obtained should also be included among the limitations. It is acknowledged that daytime sleepiness may be caused by several diseases and conditions (e.g. poor sleep hygiene, primary hypersomnias, depression, obesity etc). These possible confounders were handled by recruiting only OSAHS patients (note that one of the diagnostic criteria for the clinical diagnosis of OSAHS is excessive daytime sleepiness that is not due to any other known causes e.g. primary hypersomnia), by excluding patients with certain co-morbidities (malignancies, kidney disease, COPD and asthma) and by controlling for parameters known to reflect conditions in which excessive daytime sleepiness may be present (e.g. BMI, neck circumference, Beck depression inventory score, included in the initial model).

In OSAHS, excessive daytime sleepiness is generally considered to be the consequence of intermittent nocturnal hypoxemia that leads to sleep fragmentation,

hence poorer sleep quality. To attest for the possible influence of reversible upper airway obstruction on daytime hypersomnolence, significant polysomnography derived indices (AHI, oxygen desaturation index and obstructive apnea index) were also included in the model as well as (log) PSQI gauging perceived nocturnal sleep quality.

Previous studies have shown a poor association between ESS and PSQI as well as between PSQI, ESS and polysomnography measures to the extent that it was suggested that these measures reflect distinct aspects of sleep. After controlling for causes other than altered circadian rhythm, we feel that ESS may be considered as an indicative parameter reflective of altered circadian rhythm in our explorative study. Nevertheless, characterization of the circadian rhythm by obtaining serial measurements of either salivary or serum melatonin or core body temperature could have added value at the cost of extra inconvenience for the patients and possibly have interfered with other measures e.g. polysomnography. However, alteration of the circadian rhythm in OSAHS has been established previously, allowing the speculative notion that altered irisin/BDNF axis may be causative for circadian misalignment in OSAHS, and thus, it may be suggested that excessive daytime sleepiness is a result of the deterioration of circadian pacemaker process. This alternative mechanism could account for the phenomenon of residual EDS as well as the excess risk of cardiovascular and all-cause morbidity and mortality in CPAP treated OSAHS patients. Measurements from a single timepoint may be considered as further limitation of the study, as serum BDNF levels are themselves circadian. Nevertheless, the current findings suggest a putative relationship between the subjective measure of excessive daytime sleepiness and the alteration of the irisin/BDNF axis as reflected by the single measurement. Furthermore, this finding may contribute to teasing apart two parallel mechanisms underlying excessive daytime sleepiness e.g. one stemming from the mechanical obstruction of the airways and the other from the alteration of the circadian regulation.

5. New results of the thesis

1. Our theoretical findings posit that the pathomechanism of chronic non-communicable diseases emerging in context of sedentary lifestyle (physical inactivity) includes the disturbance of the circadian regulation. We hypothesize that decreased activity of the peripheral striated muscle yields lower serum irisin levels that after crossing the blood-brain barrier leads to limited production of BDNF in the superchiasmatic nucleus (SCN). It should be noted that BDNF has a permissive effect regarding entrainment of the master clock by peripheral mediators. Due to the decreased BDNF concentration in the SCN entrainment by light and caloric restriction are insufficient, rendering the circadian rhythm and consequential harmonization of organ-level metabolic processes insufficient.

2. Our main findings in untreated patients newly diagnosed with obstructive sleep apnoe/hypopnoe syndrome (OSAHS) showed a strong linear relationship between the Epworth Sleepiness Scale (ESS) score, a metric quantifying excessive daytime sleepiness (an indicator of circadian shift) and serum irisin and BDNF levels in a multiple linear regression model.

3. ESS was positively correlated with one other metric, the logarithm of the Pittsburgh Sleep Quality Index (PSQI), an in index that characterizes sleep quality (the global index ranges between 0 and 21 points, and 21 indicates severe difficulties in distinct domains of sleep). Reliability of the multiple linear regression model is further reflected by the fact that the ESS score (e.g. the extent of excessive daytime sleepiness) is significantly related to the (logarithm) of PSQI reflecting sleep quality.

4. The relationship between serum irisin and BDNF concentration with ESS proved to be concentration dependent and saturable. Upon assessing the final model addressing the interaction between irisin and BDNF, it should be noted that significant changes in the ESS are experienced if serum irisin level changed by 1 ng/mL, given the serum level of BDNF is within the range of ~ 280 to 470 ng/mL. Vice versa, ESS shows a significant increase in response to 1 ng/mL increase of BDNF if serum irisin levels are within the range of ~ 6.1 to 8.1 ng/mL. The final multiple linear regression model (built for the ESS) contained only (log) PSQI additional to the a priori identified parameters.

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7. Publications

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Jelölt: Móré Elek Csaba
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A PhD értekezés alapjául szolgáló közlemények

1. **Móré, E. C.**, Papp, C., Kolozsváriné Harsányi, S., Gesztelyi, R., Mikáczó, A., Tajti, G., Kardos, L., Seres, I., Lőrincz, H., Csapó, K., Zsuga, J.: Altered irisin/BDNF axis parallels excessive daytime sleepiness in obstructive sleep apnea patients.
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2. Zsuga, J., **Móré, E. C.**, Erdei, T. D., Papp, C., Kolozsváriné Harsányi, S., Gesztelyi, R.: Blind spot for sedentarism: redefining the diseasome of physical inactivity in view of circadian system and the irisin/BDNF axis.
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További közlemények

3. Szőke-Kovács, Z., **Móré, E. C.**, Szőke-Kovács, R., Máthé, E., Frecska, E.: Selective Inhibition of the Serotonin Transporter in the Treatment of Depression: Sertraline, Fluoxetine and Citalopram.
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5. Frecska, E., **Móré, E. C.**, Vargha, A., Luna, L. E.: Enhancement of creative expression and entoptic phenomena as after-effects of repeated ayahuasca ceremonies.
J. Psychoact. Drugs. 44 (3), 191-199, 2012.
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