Sleep and circadian rhythm alterations in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Post-COVID-19 syndrome and its association with cardiovascular risk factors: a prospective cohort study

María Fernanda Zerón-Rugerio[a,b], Maria Cleofé Zaragozá[c], Joan Carles Domingo[d], Ramon Sanmartín-Sentañes[e,f], Jose Alegre-Martín[e,f], Jesús Castro-Marrero[f,¶,*], and Trinitat Cambras[b,g,¶,*]

[a]Department of Clinical and Fundamental Nursing, Faculty of Nursing, University of Barcelona, Spain; [b]Nutrition and Food Safety Research Institute (INSA-UB), University of Barcelona, Barcelona, Spain; [c]Clinical Research Department, Laboratorios Viñas, Barcelona, Spain; [d]Department of Biochemistry and Molecular Biomedicine, Faculty of Biology, University of Barcelona, Barcelona, Spain; [e]Division of Rheumatology, ME/CFS Clinical Unit, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain; [f]Division of Rheumatology, Research Unit in ME/CFS and Long COVID, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain; [g]Department of Biochemistry and Physiology, School of Pharmacy and Food Sciences, University of Barcelona, Barcelona, Spain

[¶] These authors contributed equally to this work and share co-senior first authorship.

[*] **Corresponding authors:**

Prof. Trinitat Cambras, Ph.D.

Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Sciences, Universitat de Barcelona, Avenida Joan XXIII 27-31, 08028 – Barcelona, Spain

E-mail: cambras@ub.edu

Dr. Jesús Castro-Marrero, Ph.D.

Division of Rheumatology, Research Unit in ME/CFS and Long COVID, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Passeig de Vall d'Hebron 119-129, 08035 – Barcelona, Spain

E-mail: jesus.castro@vhir.org

Abstract

This study aimed to investigate circadian rhythm manifestations in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) patients (including a subpopulation of long-COVID patients) and matched healthy controls, while also exploring their association with cardiovascular health variables. Thirthy-one ME/CFS patients (75% females), 23 individuals diagnosed with post-COVID ME/CFS (56% females) and 31 matched healthy controls (68% females) were enrolled in this study. Demographic and clinical characteristics were assessed using validated self-reported outcome measures. Actigraphy data, collected over one week, were used to analyze the 24-hour profiles of wrist temperature, motor activity, and sleep circadian variables in the study participants. Associations between lipid profile with endothelial dysfunction biomarkers (such as endothelin-1, ICAM-1 and VCAM-1) and with sleep and circadian variables were also studied. No differences were found in these variables between the two group of patients. Patients showed lower activity and worse sleep quality than matched healthy controls, together with a worse lipid profile than controls, that was associated with disturbances in the circadian temperature rhythm. ICAM-1 levels were associated with plasma lipids in healthy controls, but not in patients, who showed higher levels of endothelin-1 and VCAM-1. These findings suggest that lipid profiles in ME/CFS are linked to disrupted circadian rhythms and sleep patterns, likely due to endothelial dysfunction. Furthermore, they highlight the intricate relationship between sleep, circadian rhythms, and cardiovascular health in this condition.

Keywords: chronic fatigue syndrome; circadian temperature rhythm; Actigraphy; endothelium inflammation; plasma lipids.

Introduction

Myalgic encephalomyelitis, commonly referred to as chronic fatigue syndrome (ME/CFS), is a complex and debilitating disorder that affects a significant part of the global population. The precise etiology of ME/CFS remains elusive, but it is believed to arise from a combination of immunogenetic, metabolic, and environmental factors (Castro-Marrero et al. 2017). ME/CFS is characterized by distressing symptoms, including post-exertional malaise, cognitive impairment, autonomic dysfunction, and disrupted sleep patterns, potentially related to disturbances in circadian rhythms (McCarthy 2022). ME/CFS symptomatology significantly hamper daily activities, leading to a considerable decline in overall functioning (Cambras et al. 2018; Castro-Marrero et al. 2018). Unfortunately, to date there are no definitive case criteria, no accurate diagnostic markers, and no FDA-approved medication exist for modifying the course of the disease (Rivera et al. 2019).

Previous research indicated that individuals with ME/CFS experience imbalances in the autonomic nervous system (Tanaka et al. 2015), which may contribute to the prevalent dysautonomia symptoms observed in ME/CFS (Carruthers et al. 2011) and to alterations in thermoregulation and circadian rhythms. At present, some of the patients that suffer from Long-COVID-19 also show similar clinical profile that that described above (Carfi et al. 2020), overlapping with ME/CFS in immunology, neurological and mitochondrial dysfunctions (Marshall-Gradisnik and Eaton-Finch, 2022, and Sukocheva et al., 2022).

The circadian system, with its central clock located in the hypothalamic suprachiasmatic nuclei, governs circadian rhythm manifestations, influencing, through peripheral clocks, hormonal release, temperature regulation, the balance between sympathetic (daytime) and parasympathetic (nighttime) activity, as well as sleep (Buijs et al. 2021; Buijs, Escobar and Swaab 2013; Riganello et al. 2019). Actigraphy is a wide commonly employed method for studying circadian rhythms under ambulatory conditions, although its relationship with gold standard circadian measures like dim light melatonin onset (DLMO) is weak (Reiter et al 2020). Note that actigraphy allows continuous monitoring of motor activity and, in some cases, skin (wrist) temperature. Peripheral temperature measured at wrist has been found to be correlated with DLMO (Bonmatí-Carrion et al., 2014) and with sleep quality (Tai et al., 2023). Unlike core temperature rhythm, peripheral temperature increases at night and decreases during the day.

Circadian disturbances in peripheral temperature reflect abnormalities in vasoconstriction and vasodilation processes, which can lead to difficulties in falling asleep (Kräuchi and Wirz-Justice 2001). Nevertheless, vasoconstriction is influenced not only by sympathetic nervous system activity but also by the regulation of local blood vessels via endothelial function, which has been reported to be impaired in ME/CFS (Haffke et al. 2022; Kennedy et al. 2004; Paschos and FitzGerald 2010).

Recent studies have highlighted the role of endothelin-1 (ET-1), a potent vasoconstrictor produced by endothelial cells, in the regulation of circadian rhythms (Douma, Barral, and Gumz 2020). As such, elevated levels of circulating ET-1 have been observed in individuals with ME/CFS (Haffke et al. 2022), along with elevated levels of vascular cell adhesion protein-1 (VCAM-1), suggesting endothelial dysfunction in this disease. Endothelial dysfunction is characterized by overexpression of adhesion molecules, including vascular cellular adhesion molecule (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) (Habas and Shang 2018), which can also be considered as biomarkers for cardiovascular disease (Kaur et al. 2022).

Since plasma lipids have been linked to endothelium dysfunction (Goldberg and Bornfeldt 2013) and to serotonin levels (Watanabe et al. 2010) all of which, in turn, influencing well-being (de Vries, van de Weijer, and Bartels 2022), our hypothesis suggests the possibility that lipid profiles could be associated with endothelial dysfunction, contributing to the sleep and circadian rhythm alterations.

Thus, this study aimed to investigate sleep and circadian rhythm manifestations in ME/CFS patients (including a subgroup of Post-COVID-19 patients) compared with matched healthy controls, while also exploring their association with circulating biomarkers associated to endothelial dysfunction (ET-1, VCAM-1, and ICAM-1) and cardiovascular health (blood pressure, glucose levels and lipid profiles). As secondary aim, we explored sex-dependent differences in sleep and the circadian manifestation among the study participants.

Materials and methods

Participants

A single-center, prospective, cross-sectional, case-control study was conducted involving 31 ME/CFS patients (75% females), 23 individuals with post-COVID ME/CFS (Long-COVID subgroup, 56% females) and 31 matched healthy controls (68% females) consecutively recruited

by a specialist clinic from a single outpatient tertiary-referral center (ME/CFS Unit, Vall d'Hebron University Hospital, Barcelona, Spain) from October 2020 to March 2022. Patient selection involved consecutive enrollment and exclusion criteria were those as previously described by our group (Cambras et al. 2018). All the patients were potentially eligible if they were aged ≥ 18 years with a confirmed diagnosis by a specialist of ME/CFS according to the 2011 International Consensus Criteria (ICC), being the recommended case criteria for ME/CFS from an updated EUROMENE report for research purposes (Carruthers et al. 2011). In the case of patients with Post-COVID fatigue syndrome, they were potentially eligible if they were adults with self-reported exercise intolerance following post-exertional malaise that developed during or after an acute COVID-19 infection (confirmed or probable by SARS-CoV-2 RT-PCR testing) which was not explained by an alternative diagnosis.

Healthy control volunteers were eligible if they aged ≥ 18 years and had absence of self-reported autonomic symptoms, neurological, cardiac, endocrine or neuroimmune disorders, alcohol or drug dependence or use of daily medications, as previously reported (Cambras et al. 2018). All healthy volunteers were recruited through word-of-mouth from the same local community, did not meet the case criteria for ME/CFS nor long COVID at the time of enrollment. All study participants were Caucasians, from the same geographical area.

Ethics statement and consent to participate

After receiving verbal and written information on the study protocol, all participants gave signed informed consent prior to enrolment in the research protocol, which was approved by the local Vall d'Hebron University Hospital Institutional Ethical Committee on Human Research (reference number: PR/AG 201/2016). The study was carried out in accordance with the 2013 Declaration of Helsinki and with all the International Conference on Harmonization and Good Clinical Practice Guidelines. After providing consent, all participants underwent a clinical examination and experimental research protocols. A summary of the study participants' demographic and clinical features and biochemical variables is displayed in **Table 1** according to the group and gender.

Experimental procedures

Participants underwent a comprehensive clinical assessment at the hospital on Wednesday morning, between 8 a.m. and 11 a.m., between September 2020 and December 2022, following the suggestion made in a previous study not to record data during the summer (Cambras et al.

2018). Demographic information and self-reported outcome measures were collected, as previously outlined in our referenced study (Cambras et al. 2023). Specifically, participants completed validated general health-status questionnaires to evaluate fatigue perception, sleep problems, anxiety/depression symptoms, dysautonomia and health-related quality of life. Blood pressure (BP) was measured in supine position with an automated BP cuff with a monitor (Beurer BM-26, Beurer GmbH & Co., Ulm, Germany) on the left arm, recording the systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in each study participant. Additionally, a blood sample was taken for routine biochemical analysis. Concurrently, participants were equipped with an ambulatory actigraphy device (ActTrust®, Condor Instruments, Sao Paulo, Brazil) which, captured data on motor activity (accelerometer), skin temperature (ºC) at one-minute intervals continuously for seven days. Exceptions were made for showering or swimming pool activities. The device recorded and stored data in its memory for subsequent analysis. After one week, participants went back to the hospital to return the actigraphy device and to submit the filled health status questionnaires.

Data collection and self-reported outcome measures

Participants were asked to provide complete validated self-report questionnaires on their current health status one week after the first clinical assessment under the supervision of two trained investigators, who ensured compliance. Changes in fatigue perception were assessed using fatigue severity questionnaire (FIS-40) (Fisk et al. 1994); sleep quality, using the Pittsburgh Sleep Quality Index (PSQI) (Royuela and Macías 1997); anxiety/depression, using the Hospital Anxiety and Depression Scale (HADS) (Herrero et al. 2003); autonomic symptoms, using the Composite Autonomic Symptom Score-31 (COMPASS-31) (Sletten et al. 2012) and health-related quality of life, using the 36-Item Short Form Survey (SF-36) (Alonso, Prieto, and Antó 1995). A summary of the questionnaires used has been provided elsewhere (Cambras et al. 2018; Cambras et al. 2023).

Actigraphy analysis

Skin temperature records (WT, wrist temperature) and activity data (detected by accelerometer) were obtained with the actigraphy device and analyzed with “El-temps, version 314”, an integrated package for chronobiological analysis (A. Díez-Noguera, University of Barcelona, Spain; http://www.el-temps.com). The rhythmic variables of WT and activity were determined by

adjusting the data to a 24-hour sinusoidal curve. Subsequently, we calculated the following variables: the mean 24-hour value (mean), the phase of the rhythm (acrophase) and the amplitude of the adjusted rhythm (Acos). Moreover, we calculated the relative amplitude (RA), which refers to the amplitude related to the daily mean value, and is an indicator of the robustness of the rhythm. Non-parametric circadian analysis was also performed as previously described (van Someren, Mirmiran, and Swaab 1993). In this case, since activity increases during the day and WT during the night, M10 (or M5 in the case of WT) and L10 (or L5 in the case of WT) denoted the mean values in the 10 (or 5) consecutive hours with highest values (night temperature or activity during the day) and the 10 (or 5) hours with lowest values (day temperature or night activity values). In addition, we estimated the intra-daily variability (IV, which depicts the frequency and extent of transitions between the rest and the activity, or higher and lower values of temperature, within the day), the stability of the acrophases (Rayleigh's test, Rt, that reflects the grouping of the acrophases), the inter-daily stability (IS, that indicates the day to day consistency of the rest-activity, or temperature, rhythm) were also calculated. For a better comprehension of the results, in this paper, variables started with "T_" referred to values calculated for temperature data, and with "A_" for activity data.

Sleep assessment

First, using the actigraphy software (ActStudio Alpha v1.0.21.1, Condor Instruments, Sao Paulo, Brazil), we identified the main sleep period of each day based on recordings and the observation of a sharp decrease in activity with subsequent increase in WT. Additionally, we analyzed activity and WT rhythms during daytime to identify any period of daytime sleep or so-called secondary sleep time. Then, we estimated the time in bed (min), total sleep time (TST, min), onset sleep latency (OSL, min), sleep efficiency (%), wake after sleep onset (WASO, min), and the number of awakenings for each main sleep period. OSL is defined as a length of time from bedtime to sleep onset time, and sleep efficiency as the percentage of total sleep time/total time in bed. Furthermore, we calculated the mean number of secondary sleep periods per day (number of secondary sleeps during the week/7) and the total time of secondary sleep in 24h (TST_2). All sleep variables were calculated for each study day using the Cole-Kripke algorithm (Cole et al. 1992) and then averaged for further analyses.

Blood sampling and processing

Twenty milliliters of fasting blood samples from each participant were collected directly into K_2EDTA anticoagulant tubes (Vacutainer, BD Biosciences, Madrid, Spain) by venipuncture. Plasma samples were obtained by centrifugation at 2000 x g for 15 min at 4ºC within one hour after the blood collection. Samples were collected immediately and frozen in aliquots at -80ºC until further analysis. Levels of glucose, cortisol, serotonin, and lipids were measured at the hospital's chemistry laboratory using routine methods. Each of the biochemical variables (glucose, cortisol, serotonin, triglycerides (TG), Total cholesterol (TChol), HDL cholesterol (cHDL), LDL cholesterol (cLDL), the difference between TChol – cHDL (non-cHDL) as well as clinical indices: ratio of TChol/cHDL, ratio of cLDL/cHDL, and ratio of TG/cHDL were evaluated. Circulating levels of ET-1 and cellular adhesion molecules (such as ICAM-1 and VCAM-1) were quantified using ELISA kits according to the manufactures' instruction manual (Quantikine R&D Systems, Minneapolis, MN, USA) as indicators of assessment of vascular endothelium function as previously described (Cambras et al. 2023).

Statistical analysis

Continuous variables are shown as mean ± standard error of the mean (SEM) and categorical ones as numbers and percentages. Data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. Due to a limited sample size, statistical comparisons were performed using non-parametric methods. The Kruskal-Wallis test and post-hoc tests were used to compare variables among the three independent groups. The associations between the different variables were evaluated with partial correlation analyses controlling for sex, age, and BMI. For each studied variable, paired data with missing values were excluded from the analysis. Circadian rhythm variables were tested for collinearity. Variables with tolerance values greater than 0.1 and variance inflation factor (VIF) values less than 10 were considered acceptable for inclusion in the regression analysis. Moreover, to test if associations among variables differed among the groups, a General Linear Model (GLM) for multiple dependent variables was carried out. In several cases, stepwise linear regression models with backward elimination were performed to select significant predictors for circadian variables. Regression analysis was performed for patients and controls, and the results of the ANOVA (significance of the global model), unstandardized beta coefficients, and their significance levels were obtained.

Data were analyzed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). A two-tailed $p < 0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics of the studied population

Table 1 summarizes the demographic characteristics, clinical profiles, and blood analyses of the participant groups. The groups did not differ in terms of sex proportion, age, or BMI. Results showed that patients reported higher scores for fatigue status, anxiety/depression, sleep quality, autonomic symptoms and lower health-related quality of life ($p < 0.001$) than controls. In addition, the plasma lipid profile was different between the groups, with patients having worse lipid profile than controls. No significant differences among groups were found for cortisol, glucose or ICAM-1 levels. However, ET-1 and VCAM-1 were higher in both groups of patients than in HC ($p<0.005$ in all cases). Moreover, serotonin levels could only be determined on 18 ME/CFS, 12 Long-COVID and 24 healthy control participants and no differences among groups are shown. No significant differences among ME/CFS and Long-COVID group were found in none of the studied variables.

Circadian wrist temperature and activity rhythms

The analysis of the variables obtained from the circadian rhythms (**Table 2**) indicates that the differences between the groups were manifested with the activity rhythm profile and not in temperature rhythm. In the case of the activity rhythm variables the mean daily activity (A_m), maximum activity (A_M5) and IS were higher in HCs then in the patient groups. Since no differences were detected between the two patient groups, we show the mean circadian rhythm profiles of both groups together, distinguishing them from that of healthy controls (**Figure 1**). Although not shown in tables, it should be mentioned that the light intensity detected by the actigraphy revealed higher values of light intensity in healthy controls (HC) than in patients during the day ($p < 0.002$) but not at night, similar to previously described findings (Cambras et al, 2018).

Sleep variables

Analysis of sleep variables (**Table 2**) indicates that groups differed in the time in bed and TST, with a mean of 77 and 64 minutes respectively longer in patients than in controls ($p=0.001$ and $p= 0.004$ respectively). The number of days with secondary sleep was 1 day as the mean for HC, while patients had a mean of 4.6 days in a week ($p=0.001$). In addition, the time of the secondary

sleep, this is, the mean diurnal sleep time, was on average, 5.8 hours longer in patients than in controls (p=0.001).

Association analysis

Because no significant differences were found between the two groups of patients in neither lipid or circadian variables, the data were pooled for the association analysis, while maintaining the healthy control (HC) group as a separate entity. Associations between biochemical variables and circadian and sleep related variables were calculated, separately for controls and patients.

Association between lipid profiles with endothelial and circadian rhythm variables

For the sake of simplicity and interpretability, we first focused our study on the association between lipid ratios (TChol/cHDL, cLDL/cHDL and TG/cHDL) and the endothelial and circadian variables. The individual paired associations between lipid ratios and endothelial, variables are shown in **Table S1**, demonstrating that circadian variables are more associated with lipid ratios in patients than in HC. Despite this, to examine the relationship between lipid profile parameters and circadian variables, we used a linear regression approach. First, we conducted a general linear model (GLM) separately for patients and controls to analyze the global relationships between the lipid ratios and variables related to the temperature circadian rhythm that remain after tested for collinearity (T_RA, T_IV, T_IS, and T_M5). The significance of the General Linear Model (GLM) is assessed through the p-values associated with the F-statistics for each dependent variable and group combination. Results indicate that the overall model significantly explains the variation of circadian variables in the patients group, being, the model significant for the following dependent variables: T_RA: (p=0.043), T_IS (p=0.020) and T_M5 (p=0.033). However, the model is not significant for the control group. Additionally, we tested the same model with activity circadian rhythm variables and found no significant results for none of the two groups. To further investigate the relationship between wrist temperature circadian variables with the studied biomarkers, we carried out several regression models with each circadian variable as the dependent variable and variables related to endothelial dysfunction and lipid profiles (ET-1, VCAM-1, ICAM-1, and the three lipid ratios TChol/cHDL, cLDL/cHDL, and TG/cHDL) as predictors. Predictor selection was performed through regression analysis with backward selection. We examined the significance of the overall model using ANOVA and reported the unstandardized coefficients (B) and their significance levels for the predictors in both the initial

and final steps of the stepwise regression. **Table 3** shows the model selection process with the final coefficients and their statistical significance. The final models were always statistically significant (in all cases, $p < 0.05$) and showed that lipid ratios were predictors of the temperature rhythm variables in patients, while this was not observed in the control group.

Associations between circadian variables

Table 4 shows the associations between the circadian temperature rhythm variables with those of the activity rhythm, which should be mostly related in physiological conditions. Only in the case of HC, as expected, several associations between two rhythms were found, but none of them was significant in the case of patients.

Sex-related differences in sleep and circadian variables

As a secondary objective we tested specific differences between males and females within the ME/CFS, Long-COVID and HC groups separately (table not included). In the groups of patients, no significant differences were found in any of the variables, except for ME/CFS group, where sex differences were detected in ET-1 and serotonin levels, with females having higher values than males (p= 0.016 and p=0.01, respectively). In the case of controls, HC males showed higher values of SBP [females: 110.8 (2.3), males: 124 (3.6), p=0.023] and females showed more stability (Rt value) in the circadian activity rhythm [females: 0.89 (0.02), males: 0.81 (0.06), p=0.001]. Also, sex differences in HC were found for several variables related with sleep: time in bed (females: 8.4h (9.72 min), males: 7.1 h (16 min), p= 0.014); sleep efficiency, (females: 86% (1.04), males: 92.3% (1.6), p=0.022)); WASO (females: 65(4.5), males: 30(8.2), p=0.007).

Discussion

To the best of our knowledge, our study stands as the first to establish a relationship between the manifestation of circadian rhythms and cardiovascular biomarkers in individuals diagnosed with ME/CFS, encompassing both genders and including a subpopulation of Long COVID patients. Note that although the Long COVID group exhibited a higher degree of homogeneity in terms of illness origin and duration compared to the broader ME/CFS cohort, where the antecedent infection or causal factors remain uncertain, within the context of, lipid

profile, sleep and circadian variables, we were unable to differentiate between the two subgroups of patients diagnosed with ME/CFS. Actually, sleep disturbances and unrefreshing sleep are major complaints from both, ME/CFS (Jackson and Bruck 2012) and Long COVID patients (Tedjasukmana et al. 2023), which have been previously linked to autonomic nervous system dysfunction (Mohamed et al. 2023), but also to proinflammatory cytokine levels in women with ME/CFS (Milrad et al. 2017).

The primary findings of this study underscore a noteworthy distinction between patients and controls regarding the association of plasma lipid concentrations with sleep and circadian variables. As such, our study indicates that patients show a less healthy lipid profile than controls, characterized by higher TG and elevated ratios of TG/cHDL and Chol/cHDL. Considering the pivotal role of cHDL in normal endothelial function and the prevention of cardiovascular diseases (Riwanto and Landmesser 2013), and noting that low cHDL levels are associated with an increased burden of cardiovascular disease (Kontush 2014), it is plausible to regard plasma lipid profiles as potential additional risk factors for blood vessel functioning. This includes processes such as vasodilation and vasoconstriction, which can interfere with blood distribution and thus, with sleep and temperature rhythm alterations. This fits with other authors that demonstrated similar impairment in vascular function among Long COVID and ME/CFS patients (McLaughlin et al. 2023), thereby suggesting an increased cardiovascular risk in both populations.

The use of actigraphy provides an accurate objective approach to identify changes in sleep patterns and circadian rhythms. As reported by other studies, actigraphy measures have demonstrated lower values of total activity in post-infectious ME/CFS patients compared to controls (Walitt et al 2024). However, conducting a sleep analysis through actigraphy poses considerable challenges due to extended periods of rest frequently observed among patients. Consequently, in this study, we have made a clear demarcation between the main sleep period, indicative of patients spending more time in bed, and thus yielding longer total sleep time and secondary sleep episodes. It is worth noting that secondary sleep is a somewhat intricate variable, largely attributable to the fact that many patients engage in daytime resting. Nonetheless, it represents an objective metric that underscores a distinct behavioral pattern exhibited by patients, warranting quantitative assessment. Along these lines, our results revealed that secondary sleep time was significantly related with higher wrist temperature values during daytime, implying

greater sleepiness during this time of the day. Hypersomnolence, characterized by difficulties in maintaining wakefulness during daytime hours, has been reported as a noteworthy phenomenon associated with both COVID-19 (Sarkanen et al. 2023) and ME/CFS (Cambras et al. 2023; Maness et al. 2019). It is plausible that this hypersomnolence may serve as a manifestation of underlying alterations in nocturnal sleep patterns. This would be in line with previous research by our group has shown that greater diurnal values of wrist temperature are associated with poor sleep quality among a sample of young women (Zeron-Rugerio et al. 2020). It is interesting to note that in the case of controls, as expected, activity and temperature rhythms are highly associated. However, in ME/CFS, the number of variables associated is lower, leading us to surmise that a certain degree of chronodisruption may be contributing to the altered dynamics observed in patients, exacerbating symptoms experienced by these patients, such as fatigue, daytime sleepiness, cognitive issues and sleep disturbances.

However, we would also like to highlight that the significant differences between patients and controls mostly refer to mean activity and day-to-day variability, but do not include amplitude or acrophase. This may suggest an overall decrease in activity, but the involvement of the circadian system in this process remains to be elucidated. Moreover, the lower light intensity that patients received throughout the day, compared with healthy controls (Cambras et al., 2018), may potentiate the low activity levels in patients and consequently lead to sleep alterations.

Interestingly, we show herewith that in patients, significant associations emerged between temperature rhythms variables and plasma lipids concentrations, in such a way that the poorer lipid profile, the more circadian rhythm disturbances. The circadian temperature rhythm not only depends on the rest/waking cycle, but also it is sensitive to the endothelium state, as has been demonstrated in women with ME/CFS when temperature rhythm variables were related with soluble ET-1 levels (Cambras et al. 2023). Interestingly, when testing inflammation, ICAM-1 and VCAM-1 levels were associated with TG/cHDL in controls and with glucose in patients, suggesting a potential risk of endothelium inflammation. TG/cHDL has been associated with a marker for metabolic syndrome and cardiovascular disease, and is also a marker for insulin resistance in nondiabetic adults (Kosmas et al. 2023). ICAM-1 and VCAM-1 are cell adhesion molecules that regulate leukocyte adherence to endothelial cells in acute or chronic inflammatory state and have a pathophysiological role in various inflammatory or cardiovascular diseases (Singh et al. 2023).

Both are expressed by endothelial cells, but VCAM-1, which is notably higher in patients, is expressed after the stimulation of cytokines, molecules that correlate with fatigue symptoms in ME/CFS (Montoya et al. 2017). This suggests that the high levels of VCAM-1 could mask the association between lipids profile and the adhesion molecules observed in controls. Thus, the endothelial dysfunction documented in this disease (Blauensteiner et al. 2021; Sandvik et al. 2023) may make the temperature rhythm more dependent on the lipid profile. It is also of interest that only in patients, glucose levels are associated positively with ICAM-1 and VCAM-1 and negatively with nocturnal wrist temperature. This observation fits with the fact that among patients with Long COVID, diabetes mellitus has been independently associated with sleep disturbances (Islam et al. 2022). Therefore, multiple mechanisms contribute to sleep propensity and among them there is the increase of peripheral nocturnal temperature due to blood vessels dilation, which may be altered by endothelium dysfunction as suggested by our research group (Cambras et al. 2023) and might be influenced by blood glucose levels (Rajendran et al. 2021). Contrarily to what other researchers reported in ME/CFS (Chang et al. 2021), or in long COVID (Klein et al, 2023) cortisol levels in patients did not differ from controls and neither they were associated with circadian rhythms (Morgan et al. 2017).

In our study, differences in sleep variables between sexes, were only detectable within the HC group, with females displaying higher values of variables associated with sleep disturbances than males. This suggests that ME/CFS may mask the mechanisms that generate sex differences in both circadian and sleep variables, perhaps due to highest endothelium inflammation. It could be of interest to test ET-1 inhibitors to evaluate improvement in patients affected of ME/CFS and to improve endothelium function.

Taking together, these findings suggest that in ME/CFS sleep alterations are associated with cardiometabolic risk factors in a distinct manner compared to controls. Therefore, understanding the relationship between lipid concentrations and circadian rhythms could help identify potential interventions in this group of patients. Interestingly, in adolescents (Feliciano et al. 2018) longer sleep duration and higher sleep efficiency were associated with a more favorable cardiovascular profile, suggesting the need to amend the role of sleep quantity and quality for improving cardiovascular profiles. These results suggest that lipid profiles may have a more pronounced impact on the vasoconstriction/vasodilation process in patients compared to controls,

highlighting the potential importance of circadian regulation in managing lipid-related health issues in this population. Since sleep alterations are a characteristic feature of ME/CFS and physical exercise is often challenging for these patients, nutritional interventions may offer an avenue to improve metabolic profile and contribute to improved sleep and circadian patterns.

Limitations

This study has some limitations and strengths that should be mentioned. First, the single-center, cross-sectional nature of the design prevents us from identifying causation. In addition, the relatively small sample size, especially in the case of males, the use of self-reported data, and the inclusion only of patients with mild/moderate disease severity may mean that the results are not representative of the entire population. However, as strength, we maintained the proportion of both sexes, similar to that of the sex prevalence in the disease, and that all analyses were meticulously corrected by considering age and BMI as covariates. Further limitations included several potential confounders, such as the lack of data on comorbid health conditions, level of physical activity, and other sedentary lifestyles. Finally, the analyses derived from associations are exploratory and may not have been appropriately powered.

Conclusions

This research contributes to a better understanding of the intricate connections between circadian dysregulation, lipid metabolism and the complex symptomatology of ME/CFS. The main findings indicate that patients with ME/CFS diagnosis exhibited a poorer lipid profile, characterized by higher levels of TG, non-cHDL and higher values of the atherogenic index TG/cHDL compared to the controls, which is linked to alterations in sleep and circadian rhythms. All this suggests a possible role of some lipid pattern in promoting inflammation and maybe endothelial dysfunction. Moreover, sex differences in sleep disturbances were observed in healthy controls, but not in ME/CFS patients, suggesting that the disease may mask sex-related sleep differences. Since sleep is essential for patients' recovery and improving the quality of life, the use of actigraphy may be useful to record circadian disturbances in patients, in order to improve their regular sleep patterns and thus mental health. Our results highlight the potential differences in lipid metabolism or its effects between patients and healthy controls, warranting further investigation into lipid-related biomarkers in ME/CFS. Studying plasma lipids in a group of ME/CFS in the context of

circadian rhythms offers unique insights into the disease pathophysiology and it has the potential to aid in the development of personalized treatments.

Disclosure of interest

The authors report no conflict of interest.

Funding

This study has been funded by the National Institute of Health Carlos III (Madrid, Spain) within the framework of the Strategic Action Plan on Health (Grant number: PI19/00629 and PI19/00731) and co-funded by the European Union (European Regional Development Fund – ERDF 2014-2020): "a way to build Europe". Vall d'Hebron Research Institute (VHIR) is member of the CERCA programme (Generalitat de Catalunya, Barcelona, Spain).

Authors' contributions

Conceptualization and design: JC-M and TC; Methodology: MFZ-R, MCZ, JCD, RS-S, JA-M, JC-M, TC. Formal analysis and investigation: MFZ-R, MCZ, JA-M, JC-M, and TC. Writing original draft preparation: MFZ-R, JC-M and TC. Writing, reviewing and editing final manuscript: MFZ-R, MCZ, JCD, JA-M, JC-M, TC. Funding acquisition: JA-M, JC-M, and TC. All authors read and approved the final version of the manuscript.

Acknowledgments

The authors would like to thank the ME/CFS patients and healthy volunteers for their participation in the study and also the nursing team at the local clinical research support unit (Carla Aguilar and Gisela Gili from USIC, Vall d'Hebron University Hospital, Barcelona, Spain).

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FIGURES

Figure 1: Sex-dependent circadian profiles. Mean 24-hour profile of activity (upper graphs) and wrist temperature (lower graphs) with data obtained every minute for healthy controls (grey) and patients with ME/CFS and Long COVID (black). Left graphs correspond to females and right graphs to males. To facilitate visualization, standard error is not added into the graph. The horizontal line represents the mean daily value of the group and asterisk indicate statistically significant differences ($p < 0.05$).

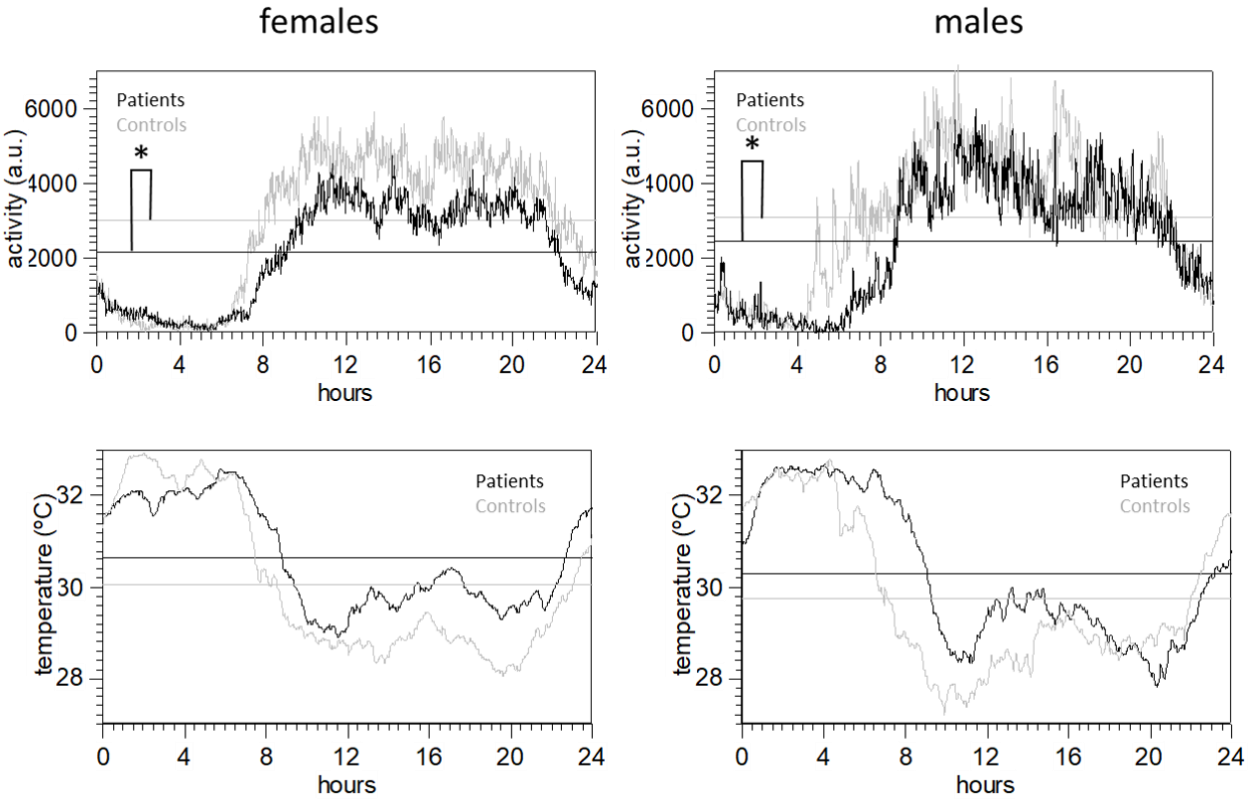

Table 1. Demographic and clinical characteristics of the study population

	Healthy controls	ME/CFS	Long-COVID	p-values	
	n= 31	n= 31	n= 23	p (1)	p(2)
Age, years	43.06 (1.99)	48.39 (2.16)	49.61 (2.09)	n.s.	n.s.
BMI, kg/m^2	24.98 (0.7)	26.57 (1.07)	25.75 (0.93)	n.s.	n.s.
SBP, mmHg	115.56 (1.99)	128.11 (2.97)	124.63 (2.89)	**0.002**	**0.02**
DBP, mmHg	73.71 (1.62)	81.77 (1.55)	77.85 (1.28)	**0.001**	n.s.
Heart rate, bpm	65.55 (1.71)	73.61 (1.83)	67.24 (2.18)	**0.003**	n.s.
Questionnaires (global score)					
FIS-40 (0-160)	17.03 (3.55)	128.58 (4.14)	127.52 (3.65)	**<0.001**	**<0.001**
HADS (0-42)	6.33 (0.82)	24.16 (1.39)	22.48 (1.66)	**<0.001**	**<0.001**
COMPASS-31 (0-100)	11.23 (1.75)	54 (2.27)	40.87 (3.72)	**<0.001**	**<0.001**
SF-36 (0-100)	89.23 (1.4)	28.54 (2.69)	29.1 (3.04)	**<0.001**	**<0.001**
PSQI (0-21)	5 (0.47)	14.23 (0.82)	13.96 (0.87)	**<0.001**	**<0.001**
Cardiometabolic variables					
TChol, mg/dl	200.84 (7.36)	223.3 (8.15)	205.41 (6.91)	n.s.	n.s.
cHDL, mg/dl	67.77 (3.45)	61.8 (2.47)	60.36 (2.73)	n.s.	n.s.
cLDL, mg/dl	120.47 (6.24)	140.47 (6.79)	123.36 (6.33)	n.s.	n.s.
TG, mg/dl	71.19 (5.32)	104.7 (10.35)	103.36 (11.23)	**0.011**	**0.012**
Non-cHDL, mg/dl	134.47 (6.64)	161.5 (7.82)	145.05 (6.18)	**0.011**	n.s.
Glucose, mg/dl	87.03 (1.51)	92.4 (2.27)	99.04 (5.09)	n.s.	n.s.
Lipid ratios					
Tchol/cHDL	3.14 (0.15)	3.73 (0.18)	3.5 (0.16)	**0.012**	n.s.
LDL/cHDL	1.9 (0.13)	2.36 (0.15)	2.11 (0.13)	n.s.	n.s.
TG/cHDL	1.21 (0.14)	1.82 (0.22)	1.88 (0.28)	**0.015**	**0.024**
Hormones					
Cortisol, ng/ml	11.16 (0.75)	14.72 (1.31)	14.21 (1.1)	n.s.	n.s.
Serotonin, ng/ml	116.58 (9.02)	85.72 (13.58)	165.51 (39.8)	n.s.	n.s.
Endothelial variables					
ET-1, pg/mL	1.17 (0.12)	1.5 (0.14)	1.09 (0.07)	**0.001**	**0.004**
VCAM-1, ng/mL	502.16 (18.49)	702.38 (30.55)	671.48 (40.89)	**0.001**	**0.001**
ICAM-1, ng/mL	282.84 (17.8)	298.91 (14.88)	269.82 (12.45)	n.s.	n.s.

BMI, body mass index; DBP, diastolic blood pressure; FIS-40, 40-item Fatigue Index Scale; HADS, Hospital Anxiety and Depression Scale; HDL, high density lipoproteins; LDL, low density lipoproteins; ME/CFS, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure; SF-36, Medical Outcome Study 36-item Short Form Health Survey; TChol, Total cholesterol; cHLD, HLD cholesterol; cLDL, LDL cholesterol; TG, Triglycerides; non-HDL, TChol-HDL. Data are shown as mean (SEM) for each item. Differences between groups were tested using Krukal-Wallis test and post-hoc comparisons among the groups, thus, p values denote statistical significance at $p < 0.05$ between HC and ME/CFS, p(1) and between HC and Long-COVID, p(2).

Table 2. Circadian and sleep variables for each group of participants

	Healthy controls	ME/CFS	Long-COVID	p-values	
	n= 31	n= 31	n= 23	**p (1)**	**p(2)**
Circadian variables (temperature rhythm)					
T_m, ºC	29.93 (0.2)	30.49 (0.15)	30.23 (0.23)	n.s.	n.s.
T_Acos, ºC	2.09 (0.16)	1.7 (0.16)	1.86 (0.25)	n.s.	n.s.
T_RA, ºC	0.07 (0.01)	0.05 (0)	0.06 (0.01)	n.s.	n.s.
T_IV, a.u.	0.01 (0)	0.01 (0)	0.01 (0)	n.s.	n.s.
T_R, a.u.	0.89 (0.02)	0.79 (0.04)	0.78 (0.06)	n.s.	n.s.
T_IS, %	41.85 (2.49)	38.38 (3.14)	41.43 (4.31)	n.s.	n.s.
T_M5, ºC	32.52 (0.17)	32.49 (0.22)	32.52 (0.26)	n.s.	n.s.
T_L10, ºC	28.41 (0.29)	29.19 (0.17)	28.83 (0.37)	n.s.	n.s.
Circadian variables (activity rhythm)					
A_m, a.u.	2935.46 (195.94)	2113.18 (132.31)	2334.84 (178.75)	**0.001**	**0.019**
A_Acos, a.u.	2373.94 (205.14)	1854.46 (146.57)	1935.94 (150.59)	n.s.	n.s.
A_RA, a.u,	0.92 (0.01)	0.89 (0.02)	0.92 (0.01)	n.s.	n.s.
A_IV, a.u.	0.38 (0.02)	0.42 (0.01)	0.44 (0.01)	n.s.	n.s.
A_R, a.u.	0.91 (0.02)	0.92 (0.02)	0.96 (0.01)	n.s.	n.s.
A_IS, %	30.2 (1.16)	32.32 (1.54)	33.69 (1.34)	**0.024**	**0.039**
A_M10, a.u.	4716.07 (363.61)	3518.11 (237.68)	3817.58 (283.09)	**0.003**	**0.027**
A_L5, a.u.	209.08 (40.08)	167.76 (24.53)	158.68 (15.42)	n.s.	n.s.
Sleep variables					
Time in bed, min	481.17 (9.33)	561.67 (20.82)	521.86 (16.45)	**0.034**	**0.001**
TST, min	408.6 (14.33)	492.47 (18.23)	447 (15.69)	**0.001**	n.s.
Onset latency, min	2.17 (0.41)	2.13 (0.63)	1.19 (0.09)	n.s.	n.s.
Sleep efficiency, %	87.7 (0.94)	85.16 (2.94)	85.72 (1.53)	n.s.	n.s.
WASO, min	55.37 (4.78)	63.57 (6.07)	71.86 (6.87)	n.s.	n.s.
Awakenings, n	14.93 (1.09)	16.2 (1.11)	17.57 (1.55)	n.s.	n.s.
TST_2, n days	0.97 (0.29)	4.47 (0.33)	4.62 (0.38)	**0.001**	**0.001**
TST_2 min	66.33 (23.89)	383.03 (62.82)	499.48 (122.17)	**0.001**	**0.001**

A.U., arbitary units; IV, intraday variability; L5 (or L10), minimum value of the variable (see text for further explanation); ME/CFS, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; M10 (or M5) maximum value of the variable; IS, interdaily stability; R, Rayleigh test; TST, total sleep time. Variables started with "T_" refer to values calculated for temperature data, and with "A_" for activity data; WASO, Wake after sleep onset. Data are shown as mean (SEM) for each item. Differences between groups were tested using Krukal Wallis test and post-hoc comparisons among the groups, p values denote statistical significance at $p < 0.05$, between HC and ME/CFS (p(1) and between HC and Long-COVID (p(2).

Table 3: Regression analysis with backward selection of predictors (biomarkers and lipid ratios) for the various wrist temperature circadian variables

Wrist temperature circadian rhythm variables

	control				**Patients**			
	T_RA ºC	T_ IV, a.u.	T_IS, %	T_M5, ºC	T_RA ºC	T_ IV, a.u.	T_IS, %	T_M5, ºC
predictors	B[a]	B[a]	B[a]	B[a]	B[a]	B[a]	B[a]	B[a]
Initial model, ANOVA p value.	*0.929*	*0.679*	*0.527*	*0.869*	*0.173*	*0.434*	*0.418*	*0.202*
ET-1 pg/mL	-0.004	0.003	-9.303	0	-0.008	0.001	-0.838	0.225
VCAM-1 ng/mL	0.0001	0.0001	-0.004	0.002	-0.0001	0.001	-0.01	-0.001
ICAM-1 ng/mL	0.0001	0.0001	0.093	0.002	-0.0001	0.001	0.005	-0.0001
Tchol/HDL	-0.059	0.026	-66.380	-3.558	-0.054	-0.003	-13.89	-1.878
LDL/HDL	0.071	-0.027	71.871	4.008	0.052	0.001	12.138	1.967
TG/HDL	-0.006	-0.004	-4.087	-0.01	0.001	0.001	-3.097	-0.014
Final model, ANOVA p value.	*n.s.*	***0.04***	*n.s.*	*n.s.*	***0.012***	***0.038***	***0.003***	***0.005***
ET-1 pg/m		**0.003**						
VCAM-1 ng/mL						**0.0001**		
Tchol/HDL					**-0.048**	**-0.001**		**-2.488**
LDL/HDL					**0.042**			**2.887**
TG/HDL							**-8.133**	

[a] Values are unstandardized beta coefficients derived from stepwise regression models. Table shows Step 1 (initial model, which was conducted with all predictors entered together) and the predictors obtained in the final step after backwards selection (final model). Significance at *p < 0.05 and **p < 0.01 are shown in bold. When the final model was not obtained since there was no one significant predictor n.s is indicated.

ET-1 endothelin-1, VCAM-1 vascular cell adhesion molecule-1, ICAM-1 intercellular adhesion molecule-1, Patients include ME/CFS and Long-Covid groups, IV intraday variability, IS, interdaily stability; M10 (or M5) maximum value of the variable, L5 (or L10) minimum value of the variable (see text for further explanation). Variables starting with T_ or A_ were obtained respectively from the temperature or activity data of the actigraphic.

Table 4. Association between circadian variables (correlation coefficient (p values)) for healthy controls and patients.

Heathy controls	**T_m**	**T_Acos**	**T_RA**	**T_IV**	**T_PV**	**T_M5**	**T_L10**
A_m, a.u.	**-0.468 (0.014)**	0.338 (0.084)	**0.42 (0.029)**	-0.03 (0.884)	0.112 (0.58)	-0.16 (0.425)	**-0.46 (0.016)**
A_Acos, a.u.	**-0.418 (0.03)**	**0.461 (0.015)**	**0.516 (0.006)**	0.047 (0.816)	0.236 (0.236)	0.016 (0.936)	**-0.469 (0.014)**
A_RA, a.u,	-0.015 (0.942)	0.296 (0.134)	0.273 (0.168)	0.03 (0.883)	**0.411 (0.033)**	0.292 (0.139)	-0.124 (0.539)
A_IV, a.u.	**0.381 (0.05)**	-0.16 (0.426)	-0.204 (0.307)	-0.189 (0.346)	0.139 (0.49)	0.313 (0.112)	0.339 (0.084)
A_IS, %	0.043 (0.832)	0.313 (0.112)	0.291 (0.141)	-0.127 (0.528)	**0.474 (0.012)**	**0.384 (0.048)**	-0.079 (0.694)
A_M10, a.u.	**-0.459 (0.016)**	**0.407 (0.035)**	**0.477 (0.012)**	0.016 (0.939)	0.168 (0.401)	-0.084 (0.677)	**-0.478 (0.012)**
A_L5, a.u.	-0.028 (0.892)	-0.196 (0.328)	-0.16 (0.426)	-0.049 (0.81)	-0.294 (0.137)	-0.21 (0.293)	0.06 (0.765)

Patients	**T_m**	**T_Acos**	**T_RA**	**T_IV**	**T_PV**	**T_M5**	**T_L10**
A_m, a.u.	-0.199 (0.19)	0.292 (0.052)	0.238 (0.116)	0.011 (0.943)	0.162 (0.289)	0.083 (0.586)	-0.274 (0.068)
A_Acos, a.u.	-0.092 (0.549)	0.227 (0.133)	0.171 (0.261)	0.011 (0.942)	0.114 (0.455)	0.088 (0.566)	-0.176 (0.249)
A_RA, a.u,	-0.073 (0.633)	0.131 (0.39)	0.11 (0.474)	0.144 (0.345)	0.102 (0.506)	0.048 (0.756)	-0.131 (0.389)
A_IV, a.u.	0.064 (0.675)	-0.164 (0.283)	-0.169 (0.266)	-0.172 (0.259)	-0.003 (0.987)	-0.09 (0.559)	0.163 (0.285)
A_IS, %	-0.134 (0.381)	0.277 (0.066)	0.27 (0.073)	-0.177 (0.245)	0.291 (0.052)	0.134 (0.381)	-0.273 (0.07)
A_M10, a.u.	-0.145 (0.341)	0.27 (0.073)	0.212 (0.163)	0.003 (0.982)	0.149 (0.329)	0.093 (0.546)	-0.229 (0.13)
A_L5, a.u.	0.055 (0.718)	0.077 (0.614)	0.041 (0.79)	-0.149 (0.329)	0.032 (0.837)	0.071 (0.642)	0.025 (0.87)

A.U., arbitary units; IV, intraday variability; L5 (or L10), minimum value of the variable (see text for further explanation); M10 (or M5) maximum value of the variable; IS, interdaily stability; R, Rayleigh test. Variables started with "T_" refer to values calculated for temperature data, and with "A_" for activity data. Partial correlations (controlled for age, body mass index, and sex) were condusted to test the associations between variables. Significant associations ($p < 0.05$) are marked in bold.

Supplementary table. Correlation between lipid ratios and with endothelial, sleep and circadian variables (person correlation coefficient (p values)) for healthy controls and patients.

	Healthy Controls			Patients		
	Tchol/cHDL	**cLDL/cHDL**	**TG/cHDL**	**Tchol/cHDL**	**cLDL/cHDL**	**TG/cHDL**
Endothelial variables						
ET-1	0.047 (0.814)	0.087 (0.659)	-0.117(0.554)	0.061 (0,673)	0.113 (0.434)	-0.155(0.283)
VCAM-1 ng/mL	0.19 (0.354)	0.128 (0.532)	**0.423 (0.031)**	-0.066 (0.666)	-0.117 (0.444)	0.143 (0.349)
ICAM-1 ng/mL	0.334 (0.095)	0.269 (0.184)	**0.64 (0.001)**	0.05 (0.742)	-0.015 (0.92)	0.227 (0.134)
Circadian variables (wrist temperature rhythm)						
T_m, ºC	-0.03 (0.884)	-0.042 (0.84)	0.036 (0.86)	0.242 (0.11)	0.244 (0.106)	0.083 (0.587)
T_Acos, ºC	0.018 (0.932)	0.043 (0.835)	-0.105 (0.609)	**-0.367 (0.013)**	**-0.295 (0.049)**	**-0.416 (0.004)**
T_RA, ºC	0.018 (0.931)	0.04 (0.845)	-0.108 (0.6)	**-0.338 (0.023)**	-0.254 (0.092)	**-0.437 (0.003)**
T_IV, a.u.	-0.015 (0.944)	-0.005 (0.981)	-0.087 (0.671)	-0.204 (0.179)	-0.249 (0.1)	0.049 (0.748)
T_Rt, a.u.	-0.078 (0.704)	-0.044 (0.831)	-0.188 (0.357)	-0.238 (0.116)	-0.164 (0.283)	**-0.376 (0.011)**
T_IS, %	-0.202 (0.323)	-0.176 (0.39)	-0.252 (0.214)	-0.28 (0.063)	-0.209 (0.168)	**-0.382 (0.01)**
T_M5, ºC	0.026 (0.899)	0.04 (0.844)	-0.066 (0.749)	-0.117 (0.445)	-0.038 (0.806)	**-0.33 (0.027)**
T_L10, ºC	-0.015 (0.944)	-0.031 (0.882)	0.071 (0.73)	**0.398 (0.007)**	0.35 (0.019)	**0.334 (0.025)**
Circadian variables (activity rhythm)						
A_m, a.u.	-0.213 (0.297)	-0.245 (0.228)	0.011 (0.959)	**-0.323 (0.031)**	**-0.294 (0.05)**	-0.269 (0.073)
A_Acos, a.u.	-0.235 (0.249)	-0.252 (0.214)	-0.099 (0.631)	**-0.383 (0.009)**	**-0.358 (0.016)**	-0.284 (0.059)
A_RA, a.u,	-0.275 (0.174)	-0.257 (0.205)	-0.294 (0.146)	**-0.301 (0.044)**	**-0.302 (0.044)**	-0.179 (0.239)
A_IV, a.u.	0.325 (0.105)	0.365 (0.067)	0.028 (0.891)	0.167 (0.274)	0.112 (0.463)	0.274 (0.069)
A_Rt, a.u.	**-0.597 (0.001)**	**-0.566 (0.003)**	**-0.59 (0.001)**	-0.259 (0.086)	-0.29 (0.053)	-0.018 (0.909)
A_IS, %	-0.298 (0.139)	-0.29 (0.15)	-0.277 (0.171)	-0.272 (0.07)	-0.269 (0.074)	-0.176 (0.247)
A_M10, a.u.	-0.227 (0.265)	-0.254 (0.211)	-0.042 (0.837)	**-0.36 (0.015)**	**-0.334 (0.025)**	-0.278 (0.064)
A_L5, a.u.	0.141 (0.493)	0.109 (0.597)	0.267 (0.188)	0.063 (0.681)	0.065 (0.673)	0.053 (0.731)

HDL, high density lipoproteins; IV, intraday variability; LDL, low density lipoproteins; L5 (or L10), minimum value of the variable (see text for further explanation);; M10 (or M5) maximum value of the variable; IS, interdaily stability); R, Rayleigh test; TChol, Total cholesterol; cHLD, HLD cholesterol; cLDL, LDL cholesterol; TG, Triglycerides; nonHDL, TChol-HDL; TST, Total sleep time.. Partial correlations (controlled for age, body mass index, and sex) were conducted to test the associations between variables. Significant associations ($p < 0.05$) are marked in bold.