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Relationship of morningness-eveningness questionnaire score to ferritin, gamma glutamyl-transpeptidase and alanine amino-transferase concentrations in a large cohort

Abstract

Background: The circadian rhythms of sleep propensity and melatonin secretion are driven by the suprachiasmatic nuclei (SCN) of the hypothalamus. The liver is also considered as an extra-SCN pacemaker and its biological parameters have proved to follow a circadian pattern of secretion. Only one study in the literature was found to investigate the relation between liver biomarkers and Morningness-Eveningness traits, revealing higher levels of ALAT in evening type patients among diabetic subjects.¹ There is currently no study about whether subjective Morningness-Eveningness correlates with hepatic biomarkers biological rhythm in a large population.

Objective and design: This retrospective cohort aimed to evaluate the relation between preferred timing of sleep behaviour and selected hepatocellular biological parameters. A large cohort of 6846 subjects from authors' patients' clinical records, among which 564 completed both the reduced Morningness-Eveningness questionnaire (MEQ) and blood samples, was used. A multivariate analysis was performed to evaluate the association between chronotypes and these hepatocellular parameters.

Results: The results found a significant association between low ferritin ($p=0.0401$), gamma-glutamyl transpeptidase (GGT) ($p=0.0173$) and alanine aminotransferase (ALAT) ($p=0.007$) levels and the evening chronotypes. Contrary to the previous cited study, ALAT levels were found to be lower for the evening type. Also, the parallel decrease of ALAT, GGT, and ferritin levels from morning to evening chronotypes support the hypothesis of a chronobiological pattern for liver biological parameters. These results would indicate that chronotypes have an important role in the circadian rhythms of hepatocellular function. Furthermore, this study found a significant correlation between the morning chronotypes and the elderly population, well known by now, and between the evening chronotypes and higher fatigue scores.

Keywords: circadian rhythms, iron, ferritin, haemoglobin, somnolence, depression, fatigue, hepatocellular biomarkers

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Didier Cugy,^{1,4} Liliana Cruz Lopez,² Imad Ghorayeb^{1,2,3}

¹Département de Neurophysiologie Clinique, CHU de Bordeaux, France

²Université de Bordeaux, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, France

³CNRS, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, France

⁴Hypnologie Clinique, France

Correspondence: Didier Cugy, Hypnologie Clinique, 47 rue Gaston Lespault 33000 Bordeaux, France, Tel +33 5562 4228 6, Email didier@cugy.fr

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Abbreviations: MEQ, morningness-eveningness questionnaire; GGT, gamma-glutamyl transpeptidase; ALAT, alanine aminotransferase; SR, sedimentation rate; SCN, suprachiasmatic nuclei

Introduction

Daily rhythms in circulating levels of various biochemical, endocrine factors and behavioural processes such as sleep/wake are controlled by the master central clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus.²⁻⁵ The SCN distributes its rhythmic signal to all tissues of the body that in turn will act as peripheral circadian oscillators although it is unclear how these multiple rhythms are coupled together to form a coherent system.^{6,7} Circadian preference or chronotypes, is another individual trait that modulates the preferred timing of sleep behaviour.⁸ Definite objective physiological differences between chronotypes have been reported demonstrating that individuals differ in their biological rhythms according to their subjective preferences for daytime. Diurnal morning and evening types varied in their circadian rhythms of urinary catecholamine excretion^{9,10} and a number of biological parameters are associated

with the Morningness-Eveningness chronotypes as defined by the Morningness-Eveningness questionnaire (MEQ),⁸ including body temperature, cortisol rhythm, melatonin secretion, sleep patterns, and sleep architecture.¹¹⁻¹⁷

Ferritin is the most widely used haematological parameter to assess iron stores¹⁸ and, although still a matter of debate, it has been shown that its concentration shows day to day and circadian variation both in animals and in humans.¹⁹⁻²⁴ These results, linking ferritin levels and circadian variations, may also be correlated to chronotypes, as circadian rhythms have already been proved to be modified by the subjective evaluation of sleep preference for other biological parameters as mentioned before. To our knowledge, there is only one study in the literature that correlates biological liver parameters and Morningness-Eveningness preferences, and it was among a group of diabetic subjects.¹ The results of this study showed higher levels of ALAT concentrations in evening-type subjects. The present study includes a larger population to assess the relation between these liver parameters and MEQ categories.

As ferritin is one of the major component of iron storage in the liver, an extra-SCN pacemaker²⁵⁻²⁷ we sought to determine whether

ferritin, together with biomarkers of hepatocellular function such as gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALAT) levels, vary in relation to the categories of the MEQ in a large cohort. A secondary aim was to evaluate the relationship of MEQ score and ferritin levels with self-reports of excessive daytime sleepiness, depression and/or fatigue.

Methods

Samples

We retrospectively investigated patient's medical records extracted from the authors' database from January 1, 2005 to December 31, 2014. Patient's demographic and biological information were collected at their initial visit. Patients were informed in conformity with the ARTICLES 26, 34 ET 40 DE LA LOI N° 78-17 DU 6 JANVIER 1978 RELATIVE À L'INFORMATIQUE, AUX FICHIERS ET AUX LIBERTÉS and have the right to demand removal of their record. The present study respected the declaration of Helsinki.

The individuals were outpatient clinic of the Cabinet d'Hypnologie Clinique, Bordeaux, France seeking diagnosis and treatment for complaint of all kind of sleep disorders. Subjects' descriptive medical records included age, sex, body mass index (BMI), personal and family medical history and full clinical examination. Subjects were evaluated for their depressive symptoms, fatigue, daytime sleepiness, and Morningness-Eveningness chronotypes.

Symptoms of depression were measured using the Q2DA questionnaire including 13 questions.³⁵ Scores ranged from 0 to 13 points, and subjects with a score greater than 6 were considered as having depressive symptoms. Subjective fatigue was assessed with the ADA Pichot fatigue scale consisting of 8 questions scored progressively from 0 "not at all" to 4 "extremely".^{28,29} An excessive fatigue was considered if the score was higher than 22. The Epworth sleepiness scale (ESS) is an auto-questionnaire that rates the likelihood of dozing in 8 different situations.³⁰ Scoring of the answers is 0 to 3, with 0 being "would never doze" and 3 being "high chance of dozing". A sum of 10 or more is widely considered to be indicative of excessive daytime sleepiness.³⁰

Finally, chronotypes were classified into five groups, "definitely morning type", "morning type", "neither type" or "intermediate type", "definitely evening type" and "evening type", based on results of shortened version of the MEQ, validated in a previous study³¹ and used in the daily bases of a sleep consult by the author. The ADA, QD2A and ESS questionnaires are also used by the author in the daily bases of a sleep consult, as well as blood samples, within adult subjects.

Biochemical tests were assumed to be performed in the morning as it is the common rule in French laboratories and included routine haematology parameters. Although, the day of the blood sample was left to the patient's decision. For the purpose of this study, only sedimentation rate (SR), haemoglobin, iron, ferritin, gamma-glutamyl transpeptidase (GGT) and alanine aminotransferase (ALAT) levels were considered for final analysis.

The database included 6846 patients (2854 women). Of these, 6263 had unavailable lab results for ferritin. Among the 583 final subjects, 564 (332 women) fully completed the MEQ. Thus 583 were retained for the main analysis. The 564 patients who performed both the blood sample and the MEQ were retained for gender analysis, as shown in Figure 1.

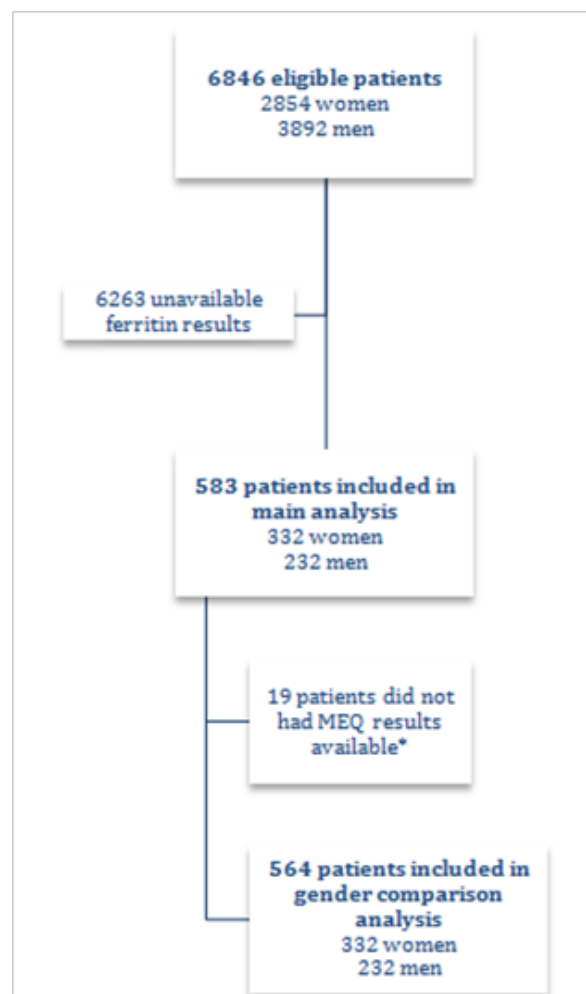


Figure 1 Patient's flowchart.

Statistical analysis

Statistical analyses were performed with Stat view software and with R software release 3.2.1 GUI 1.66 OSX Mavericks build (6956). A first descriptive analysis on all quantitative and qualitative variables was performed. A multivariate analysis after normalisation of variables was used to assess associations between the presence of a given chronotype, biological hepatocellular parameters, sleepiness, depression and fatigue scores. All reported *p* values are two-tailed, with statistical significance below.

Participants' characteristics were summarized as means \pm standard deviation (SD) for continuous variables and counts and percentages for categorical variables. Sex, age and chronotype were considered as independent variables. Normalization of data was performed by log transformation for iron, ferritin, GGT, ALAT and by square root transformation for ESS, ADA and Q2DA scores. Comparisons were performed using the chi-square test for categorical variables and the T-Student test for normally distributed continuous variables. Multiple analysis of variance (MANOVA) was used to analyse the effect of gender, age and chronotype factors on biological parameters and auto-evaluation scores. The alpha risk was set at 0.05.

Results

Relevant demographic, clinical characteristics and biological parameters values of the considered population according to the chronotype are shown in Table 1. The total evening group (definitely and moderately evening group) accounted for only 8.16% of the total cohort whereas the total morning group (definitely and moderately morning group) and the intermediate group accounted for 36.17% and 55.67%, respectively. Statistically significant associations were found between chronotypes and mean age ($p < 0.0001$), ferritin levels ($p = 0.0401$), GGT levels ($p = 0.0173$), ALAT levels ($p = 0.007$) and ADA score ($p = 0.005$).

As shown in (Figure 2), ferritin, GGT and ALAT levels showed similar shape of variation in relation to MEQ categories with significantly increased blood concentrations in relation with the morning chronotype. Multiple analysis of variance showed ferritin levels to be significantly correlated with the ESS scores, no matter the chronotype ($F(3,134) = 2.927$, $p = 0.0361$). Increased ferritin levels with age was not related to that of SR ($F(41, 10) = 0.754$, $p = 0.751$). Higher BMI and higher ESS scores were significantly associated with lower fatigue and depression scores ($F(190, 130) = 1.531$, $p = 0.00482$) and ($F(41, 130) = 1.634$, $p = 0.02005$), respectively.

Table 1 Demographic, clinical and biological characteristics of the study cohort

	Total	Definitely morning type (DMT)	Moderately morning type (MMT)	Intermediate type (IT)	Moderate evening type (MET)	Definitely evening type (DET)	P
MEQ n= (%)	564	34 (6.03)	170 (30.14)	314 (55.67)	42 (7.45)	4 (0.71)	
Age (±SD)	537	54.429 (12.381)	51.243 (13.726)	44.438 (14.858)	38.55 (17.832)	24.5 (9.327)	< 0.0001
Gender (men/women)	242/341	16/18	80/90	117/197	17/25	2-2	
Ferritin (±SD) ng/mL	583	185.938 (181.509)	141.187 (150.298)	127.28 (120.523)	109.919 (86.112)	54.6 (35.5)	0.0401
GGT (±SD) UI/L	303	65.619 (113.521)	35.61 (30.008)	31.55 (39.435)	24.391 (12.116)	17 (2)	0.0173
ALAT (±SD) UI/L	331	33.762 (26.625)	28.894 (17.965)	25.594 (14.055)	20.087 (7.728)	14.25 (3.403)	0.007
Pichot fatigue (±SD) ADA	564	15.212 (7.193)	15.186 (8.087)	16.467 (7.878)	20.095 (7.615)	21.25 (8.221)	0.00549
Pichot depression Q2DA (±SD)	517	5.179 (3.973)	5.604 (3.643)	6.058 (3.631)	7.049 (3.911)	8.75 (4.425)	NS
Epworth sleepiness scales (±SD)	567	12.088 (5.739)	11.061 (5.677)	10.371 (5.762)	10.2 (6.525)	10 (11.532)	NS
Iron (±SD) µmol/L	221	28.945 (34.237)	26.386 (32.047)	20.118 (13.816)	27.937 (43.47)	36.05 (n=1)	NS
Hb (±SD) g/dL	429	14.072 (1.23)	14.229 (1.248)	14.248 (2.841)	14.368 (1.677)	14.725 (1.636)	NS
SR (±SD) mm/s	299	15.65 (15.08)	9.349 (7.981)	10.411 (11.744)	8.885 (8.071)	3.75 (2.63)	NS
BMI (±SD) kg/m ²	576	27.531 (4.780)	25.382 (4.648)	25.121 (6.169)	24.806 (6.136)	20.407 (2.43)	NS

Values are given as mean±SD (p calculated after normalization); Non-significant (NS).

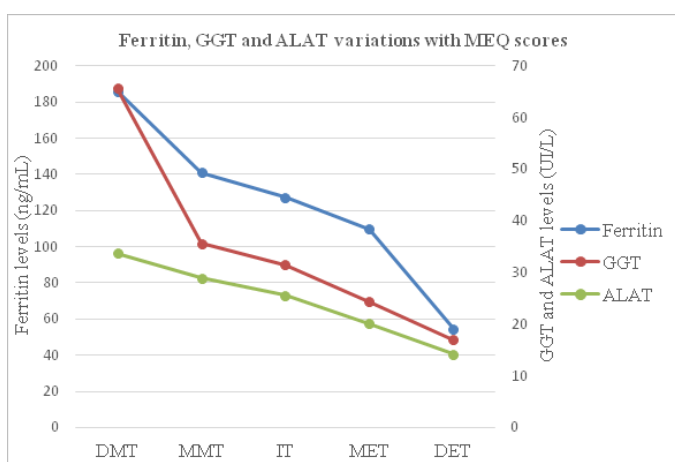


Figure 2 Ferritin, GGT and ALAT levels variation with MEQ categories.

An analysis of variance was performed independently of the chronotype to compare hepatocellular biomarkers, clinical features and auto-questionnaires scores between men and women. Compared to men, and as shown in (Table 2), ferritin, GGT and ALAT levels were significantly lower in women ($F(1,571) = 180.366$, $p < 2.10 \cdot 10^{-16}$), $p < 5.22 \cdot 10^{-8}$, $p < 2.76 \cdot 10^{-14}$, respectively. Men showed significant higher BMI ($p < 1.44 \cdot 10^{-5}$) but women showed significantly higher fatigue scores ($p = 0.000141$).

Finally, a regression analysis was made to evaluate the association of the chronotype with GGT ($F = 7.317$, $p = 0.007$), ALAT ($F = 4.53$, $p = 0.0336$), ferritin levels ($F = 4.844$, $p = 0.0281$) and the ADA score ($F = 65.525$, $p < 0.0001$). All results found significant associations.

Discussion

The main findings of the present study are that serum levels of ferritin, GGT and ALAT significantly varied in relation to chronotype, with the morning chronotypes showing higher levels of ferritin,

GGT and ALAT than the evening types. Only one previous study investigated the relationship between liver function and morningness and eveningness trait in patients with type 2 diabetes mellitus and showed subjects of the evening type to have higher levels of ALAT.¹ The results in the present study contradict these ones, and the reason for this discrepancy is presently unknown but the exclusive diabetic

cohort of the former survey may in part account for this result. In our study, the parallel variation of ferritin, GGT and ALAT, checked in a fixed time slot, in relation to chronotype favours a chronobiological pattern of hepatic metabolic activity that may be driven by central pacemaker.

Table 2 Gender comparison of biological and clinical parameters

	Female	Male	p
Number of subjects	332 (58.9%)	232 (41.1%)	
Age	45.4±14.82 (25)	47.8±15.99 (16)	NS
Ferritin (ng/mL)	83.9±84.11	203.8±161.99	<2.10-16
GGT (UI/L)	26.6±31.09 (181)	43.1±55.08 (94)	5.22 10-8
ALAT (UI/L)	21.0±10.11 (161)	32.7 ±18.89 (81)	2.76 10-14
ADA	17.3±7.78 (11)	14.9±8.07 (7)	0.000141
ESS	10.8±5.83 (9)	10.4±5.81 (6)	NS
Q2DA	6.0±5.83 (28)	5.9±3.71 (35)	NS
BMI (kg/m ²)	24.5±5.99 (3)	26.5±5.03 (1)	1.44 10-5

Results are given as mean±SD (missing values); Non-significant (NS).

A body of evidence support the circadian variation for iron blood levels in the human body. Indeed, these studies found levels of iron to vary within the day, and are found to be higher in the morning, decreasing during the day.³²⁻³⁷ However the studies are inconclusive for ferritin circadian variations, as literature supports ferritin levels have not been found to follow a circadian rhythm.^{21,33-35,38-40} In these studies, the relationship between iron biomarkers and subjective preferred timing of activity was never investigated. Our study found however a contradictory result for ferritin levels, as the significant differences according to chronotype suggests the hypothesis of a circadian variation for this parameter.

Our results point to a positive correlation between age and morningness, which is consistent with previous findings.^{19,41} It could be argued that high prevalence of elevated iron stores in the elderly could be related to higher prevalence of disease in aged individuals. This is likely not to be the case in our study as in our cohort SR values did not correlate with those of haemoglobin or with blood iron and ferritin concentrations. High prevalence of elevated iron stores with insignificant effects of chronic disease on these iron status estimates was previously reported.⁴² Ferritin is the mirror of iron stores and, as also confirmed by our results, is commonly lower in women compared to men in the general population.⁴³⁻⁴⁵ Although most women in reproductive age appear with negative iron balance due to poor diet and menstrual blood loss, the underlying mechanisms of gender differences in ferritin levels are not entirely attributed to the said negative balance and presumably other unknown mechanisms remain.⁴⁶ Elevated ferritin concentrations in men have been significantly related to higher risk of metabolic diseases (for instance, hyperlipidemia, obesity, and diabetes) with consequent increase of cardiovascular risk.⁴⁷ Accordingly, here we also show that serum levels of GGT, ALAT levels and BMI are significantly higher in male population.

Iron deficiency may promote fatigue but not depression nor ESS, although surveys assessing these correlations are scarce.⁴⁸⁻⁵¹ A

great body of research indicates that eveningness is associated with negative psychological outcomes, including depressive mood.⁵²⁻⁵⁵ Our data extend previous results by demonstrating evening type to have significantly higher Pichot fatigue ADA scores indicating a higher degree of fatigue complaint. Significance was not reached however for Pichot depression scores. Daytime sleepiness was also reported in evening-type individuals in comparison with morning-types but objective measures failed to show overall differences in daytime sleepiness across chronotypes.^{16,55} Here, we failed to demonstrate correlations with MEQ categories and differential levels of sleepiness, and although ferritin levels dropped together with increased fatigue and depression scores, significant association was not found between ferritin levels and auto-questionnaires scores. However, women had significantly higher ADA scores than men, indicating a higher level of fatigue. Whether this is related to decreased ferritin levels remains to be investigated.

ESS scores were rather negatively correlated to higher fatigue and depression scores. This result, as surprising as it might seem, may be due to the population of the study daytime.

Interestingly, increased ferritin concentration significantly correlated with higher Epworth scores no matter the chronotype. Only one study reported low serum ferritin levels not to predict Epworth scores in patients with obstructive sleep apnea⁴⁸ Further studies are mandatory to further investigate iron metabolism interaction with reports of complex subjective complaints such as sleepiness, tiredness and depression. Biochemical screenings are biased by diurnal variations, which must be considered when blood concentrations of these parameters are interpreted in the clinical setting.³⁵ In our study, the influence of sampling time may have interfered with the results. This however is unlikely as most of the French medical laboratories recommend blood withdrawal early in the morning.

It is well known that iron-related biological parameters show day to day and within day individual variations.^{20,21,33-35} However, diurnal

variations in serum ferritin concentration were not demonstrated in most of the studies performed in humans.^{38-40,21,34,35} Not only do circulating levels of various endocrine factors oscillate over the 24h period, but so too does responsiveness of target tissues, including the liver, to these signals or stimuli.^{3,5,56} The circadian clock system in the liver plays important roles in regulating metabolism and energy homeostasis. Circadian-controlled hepatic metabolism is partially achieved by controlling the expression and/or activity of key metabolic enzymes, transcription factors, signaling molecules, and transporters. Reciprocally, intracellular metabolites modulate the molecular clock activity in response to the energy status.

Limitations

This study has certain limitations. First, the cross-sectional design does not allow inference of causal relationship between a given chronotypes and metabolic control. Second, the very little size of the definitely evening type weakens the statistical power of our results. Third, other lifestyle factors that could have influenced the results, including sleep quality, energy intake, physical activity, alcohol consumption and smoking were not taken into consideration. Fourth, blood samples were not necessarily performed the same day as the questionnaires were filled, therefore sleeping patterns might have been modified between the two dates.

Conclusion

Irrespective of the causal relationship between chronotype and hepatocellular metabolic function, MEQ types were significantly associated to hepatocellular parameter's levels, especially ferritin levels, the most widely used haematological parameter to assess iron stores, were found to be lower in the evening groups. This could be considered when blood concentrations of this parameter are interpreted in the clinical setting. However, more studies should be performed to corroborate or contradict the results found in the present study.

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Conflict of interest

The authors declared that there are no conflicts of interest.

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