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Chronodisruption enhances inflammatory cytokine release from visceral adipose tissue in obesity

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Abstract

Background Chronodisruption, marked by circadian rhythm misalignment, is linked to inflammatory diseases like obesity. Chronotypes, reflecting individual circadian behavior, include morning, intermediate, and evening types, with evening chronotypes showing worse body composition and higher metabolic risk. This study evaluated the inflammatory profile of visceral adipose tissue (VAT) across chronotypes in individuals with obesity and examined clock gene expression.

Methods Twenty-five participants with obesity (11/14 F/M, BMI 41.59 ± 7.69 kg/m^2, age 41.13 ± 11.08 years) candidates for bariatric surgery were classified using the Morningness-Eveningness Questionnaire (MEQ): morning (36%), intermediate (28%), or evening (36%) chronotypes. VAT biopsies were analyzed for cytokines, chemokines, and growth factors via multiplex ELISA, and clock genes (PER1, CLOCK, BMAL1) were assessed using qPCR.

Results Body composition and biochemical parameters were similar across groups, but evening chronotypes had higher triglyceride levels ($p = 0.012$) and lower phase angle ($p = 0.035$). VAT inflammatory markers, including IL-1β ($p = 0.04$), IL-8 ($p = 0.03$), bFGF ($p = 0.01$), MCP-1 ($p = 0.01$), and MIP-1β ($p = 0.05$), were highest in evening and lowest in morning chronotypes. Evening chronotypes had significantly elevated bFGF levels compared to other groups ($p = 0.04$). PER1 mRNA expression was also higher in evening chronotypes ($p = 0.02$) and correlated with VAT-released bFGF ($p = 0.03$) and IL-1β ($p = 0.03$). MEQ scores negatively correlated with VAT bFGF ($p = 0.02$), MCP-1 ($p = 0.02$), and PER1 expressions.

Conclusion Despite similar metabolic profiles, evening chronotypes exhibit heightened VAT inflammation and altered clock gene expression, potentially worsening their metabolic risk.

Keywords Chronotype, Obesity: inflammation, Cytokines, Clock genes, Visceral adipose tissue

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Introduction

Daily metabolic and behavioral cycles are synchronized with the external day-night cycle by molecular circadian clocks [1]. These clocks, which prime energy metabolism for recurrent alterations on a 24-hour basis, are closely related to health and metabolic homeostasis [1]. Metabolic alterations, ranging from the promotion of obesity and type 2 diabetes to the onset of metabolic syndrome, frequently accompany changes in the circadian rhythm, such as those occurring during shift work or sleep disturbances [2]. In addition, circadian misalignment—often referred to as chronodisruption—encompasses disruptions in the endogenous circadian system due to behavioral or environmental factors, such as irregular sleep-wake cycles or mistimed feeding. While closely related to sleep disruption, chronodisruption more broadly refers to disturbances in circadian rhythmicity that extend beyond sleep patterns [1]. This misalignment promotes systemic low-grade inflammation, which first manifests in adipose tissue and appears to be a significant predictor of the negative consequences of obesity [1], a widespread epidemic disease [3].

Adipose tissue regulates metabolic and inflammatory responses through cytokine secretion [4]. In obesity, immune cells infiltrate fat, amplifying systemic low-grade inflammation—a hallmark of metabolic syndrome [5, 6]. Neutrophils are the first responders, releasing elastases that impair insulin sensitivity [7, 8]. Macrophages further sustain this inflammation through rhythmic secretion of TNF-α and IL-6, driven by their internal clock [9]. In fact, like most tissues, adipose tissue harbor its own circadian clock, and local metabolic processes, like carbohydrate and lipid metabolism, adipogenesis, and cytokine release, are rhythmically regulated [10]. Inflammation affects the molecular clock machinery. The circadian clock, in turn, controls multiple inflammatory pathways, including cytokine release [11, 12].

In shift workers, who are at higher risk for metabolic syndrome, leukocyte counts increase, indicating elevated inflammation [13]. Studies in rats show that chronic, advanced light-dark shifts increase inflammation [14], while dim light at night enhances TNF-α expression in adipose tissue under a high-fat diet [15]. This inflammation disrupts triglyceride and glucose homeostasis [16]. Treatments targeting circadian rhythms, such as glucocorticoids, reduce adipose inflammation by altering immune pathways and limiting immune cell recruitment [17]. Melatonin, with dose-dependent effects, improves inflammatory markers in obese animals, counteracts endocrine rhythm disruptions from high-fat diets, and supports metabolic health by normalizing biochemical parameters [18, 19]. Additionally, the gut microbiome, influenced by circadian genes, modulates adipose tissue inflammation, with disruptions linked to obesity and diabetes [20, 21]. In germ-free mice, loss of microbiome rhythms promotes low-grade adipose inflammation [20, 21]. In humans, however, clear scientific evidence on the direct impact of circadian rhythm disruptions on adipose inflammation remains limited.

In this context, chronotype plays an important role in the circadian typology of an individual [22]. Chronotype is a construct designed to identify the preferred or actual timing of daily activities, such as eating and sleeping. Using tools such as the Morningness-Eveningness Questionnaire (MEQ) [23], it is often grouped into morning, intermediate, and evening chronotypes. Morning chronotypes prefer earlier clock times and tend to wake up and go to bed early, whereas evening chronotypes have later wake-up and bedtimes and peak performance later in the day. Morning or evening preference has proven to be a stable human behavioral trait with measurable and predictable impacts on various physiological systems and psychological parameters, such as body temperature, blood pressure, catecholamine secretion, sleep patterns, subjective activation and arousal, and lifestyle regularity [22]. Evidence indicates that evening chronotype is associated with a greater degree of misalignment between internal timing and external conditions, potentially leading to disruptions in central and peripheral rhythms [24]. Furthermore, evening chronotypes are at higher risk for developing poor health outcomes, including obesity and metabolic diseases [24–27].

Building on this background, the current study aimed to evaluate the inflammatory secretory profile of adipose tissue while also investigating the expression levels of master clock genes in individuals with obesity across different chronotype categories, potentially clarifying how chronotype and circadian misalignment impact inflammation in obesity.

Methods

Study design

For this cross-sectional observational study, we enrolled 25 patients who were candidates for bariatric surgery at the University Hospital Federico II in Naples, Italy, from September 2023 to December 2023. The study was conducted in accordance with the ethical guidelines of the 1961 Declaration of Helsinki and current legislation on clinical research in humans. The Ethics Committee approved the protocol, and written informed consent was obtained from all participants.

Inclusion criteria

- Age ≥ 18 years;
- Body mass index (BMI) ≥ 35 kg/m^2;
- First-time undergoing bariatric surgery.

Exclusion criteria

- Night shift workers;
- History of previous bariatric surgery;
- Chronic use of medications known to disrupt circadian rhythms (e.g., corticosteroids, melatonin);
- Glucose metabolism dearrangements.

Human visceral adipose tissue (VAT) biopsies were obtained at the time of bariatric surgery and stored at − 80 °C. A flow chart of study participants is shown in Fig. 1.

Anthropometrical and body composition assessment

Before bariatric surgery, anthropometric evaluations were carried out by a nutritionist in the morning hours, specifically between 8:00 and 10:00 a.m., following a period of overnight fasting. Participants dressed in lightweight clothing and remained barefoot during the measurements. Body weight was determined using a calibrated beam balance scale (Seca 711; Seca, Hamburg, Germany), while height was measured with a wall-mounted stadiometer (Seca 711; Seca, Hamburg, Germany). BMI was then computed by dividing weight in kilograms by the square of height in meters (kg/m^2), and participants were categorized based on the classification system established by the World Health Organization (WHO).

Waist circumference (WC) was measured in line with the protocols provided by the National Center for Health Statistics [28]. A non-elastic measuring tape was positioned at the natural waistline, or, in cases where no natural indentation was apparent, at the midpoint between the lower margin of the ribcage and the iliac crest. Measurements were rounded to the nearest 0.1 cm. Hip circumference was assessed by placing the same non-elastic tape around the widest section of the buttocks, ensuring the tape remained level and parallel to the ground, with results also recorded to the nearest 0.1 cm. Finally, the waist-to-hip ratio (WHR) was determined by dividing the waist circumference by the hip circumference.

Chronotype assessment

At baseline, participants' chronotypes were evaluated using the Morningness-Eveningness Questionnaire (MEQ), a tool composed of 19 multiple-choice items. Each question offered four or five response options aimed at exploring sleep patterns and daily performance [22]. For instance, the questionnaire assessed preferences regarding the time of day when individuals feel most energetic, when they experience the greatest fatigue, and when they would ideally wake up to perform at their best.

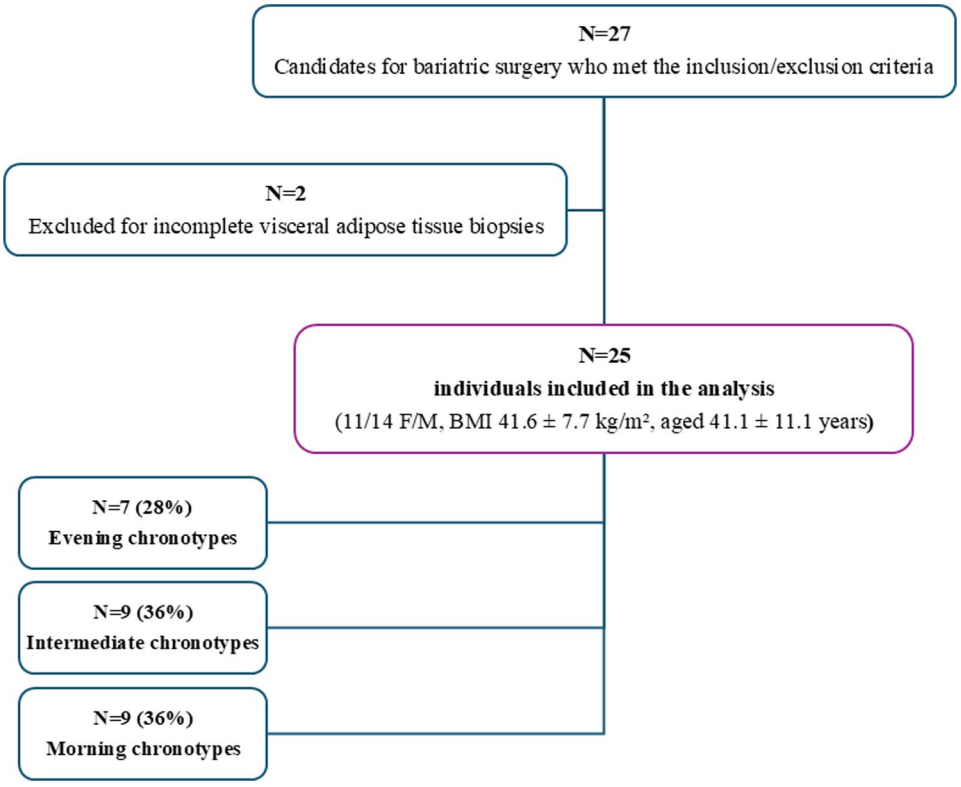

Fig. 1 Flow chart of study participants. BMI, body mass index

The MEQ provided a total score ranging from 16 to 86, which was used to classify participants into one of three chronotype categories: morning types (scores between 59 and 86), intermediate types (scores between 42 and 58), or evening types (scores between 16 and 41) [23].

Conditioned medium

Conditioned medium was prepared from VAT biopsies by incubating the tissue samples in Dulbecco's Modified Eagle Medium (DMEM) containing 0.25% BSA (100 mg:250 µl) at 37 °C for 24 h [29]. Following incubation, the supernatant was collected and stored at -80 °C.

Cytokines, chemokines, and growth factors screening.

The concentration of cytokines, chemokines, and growth factors in VAT conditioned medium samples was determined as previously described [30]. Specifically, the analysis included IL-6, IL-1ra, IL-7, IL-1β, IL-10, IL-8, IL-2, IL-4, IL-9, IL-13, IL-5, IL-12(p70), IL-15, IL-17, basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), eotaxin, interferon-γ (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1)β, MIP-1α, interferon-γ inducible protein 10 (IP-10), C–C motif chemokine ligand 5 (CCL5)/RANTES, tumor necrosis factor-α (TNF-α), platelet-derived growth factor (PDGF-BB), and vascular endothelial growth factor (VEGF). The Bioplex multiplex human cytokine, chemokine, and growth factor kit (Bio-Rad, Hercules, CA, USA; cat. # M500KCAF0Y) was employed for this purpose. Measurements were conducted using the Bio-Plex 200 System (Bio-Rad, Hercules, CA, USA), a platform based on magnetic bead technology. All results fell within the detection ranges specified by the manufacturer (bio-rad.com/Bio-Plex/AnalyteGuide) [30].

RNA extraction and quantitative Real-Time PCR (Q-RT-PCR)

Total RNA was isolated from VAT biopsies using the QIAZOL (Qiagen, Hilden, Germany) solution according to the manufacturer's instructions (39501160). 500 ng of RNA were reverted out of each sample with the SuperScript III Reverse Transcriptase with oligo dT primers (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR was performed with iTaq Universal SYBR Green Supermix (Biorad, Hercules, CA, USA). All reactions were performed in duplicate, and Ribosomal Protein L13 (*RPL13*) was used as the internal reference. Primer sequences for Clock Circadian Regulator (*CLOCK*), Period 1 (*PER1*), basic helix-loop-helix ARNT like 1 (*BMAL1)*, and *RPL13* genes were the following: *CLOCK* forward TGGGTTGAATTTTGGTTCCGT and reverse AGATGTTTGCTGACTGTGCC; *PER1* forward GATG TGGATGAAAGGGCTGC and reverse GATAGCCAG CATGAGGGGTC; *BMAL1* forward CCCTGGGCCAT CTCGATTAT and reverse TCATCCAGCCCCATCTTT GT; and *RPL13* forward CTTTCCGCTCGGCTGTTTT C and reverse GCCTTACGTCTGCGGATCTT. Through the 2 – ΔCt formula, fold changes were calculated.

Biochemical assessment

Blood samples were collected via venipuncture by the nursing staff between 8:00 and 10:00 a.m., after an overnight fast. The samples were then transported to a nearby laboratory and analyzed according to standardized local protocols. Laboratory assessments included glucose and lipid profiles. Furthermore, the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated for each participant using the formula: [fasting glucose (mmol/L) × fasting insulin (mU/mL) / 22.5] [31].

Statistical analysis

Statistical analyses were conducted using GraphPad® Prism version 8.4.2 (GraphPad Software Inc., La Jolla, CA, USA) and the R programming language version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) via the RStudio® Integrated Development Environment (Posit PBC, Boston, MA, USA). Categorical variables were summarized as relative frequency and percentages and compared using Fisher's exact test. Continuous variables were presented as mean ± standard deviation (SD) or median with interquartile range (IQR, 25th–75th percentile), depending on data distribution.

The D'Agostino-Pearson normality test was employed to assess the normality of continuous data. For comparisons among more than two groups, ANOVA with Tukey's correction was applied for parametric data, while the Kruskal-Wallis test with Dunn's post hoc correction was used for non-parametric data. Additionally, the Jonckheere-Terpstra test was utilized to evaluate trends involving ordinal independent variables.

Correlations between two continuous variables were analyzed using either Pearson's or Spearman's rank correlation (ρ), with corresponding 95% confidence intervals (CIs) reported. A p-value of < 0.05 was considered indicative of statistical significance.

Results

Table 1 displays sex, age, anthropometric, body composition, and biochemical parameters for the entire population and stratified by chronotype categories. Twenty-five individuals with obesity (BMI 41.59 ± 7.69 kg/m², age 41.13 ± 11.08 years), comprising 11 females (44.0%) and 14 males (56.0%), were included in the analysis. Among these, 7 (28.0%) were evening chronotypes, 9 (36.0%) intermediate chronotypes, and 9 (36.0%) morning chronotypes. No significant differences were observed in sex, age, anthropometric, body composition, or biochemical parameters across chronotype categories, except

Table 1 Sex, age, anthropometrical, body composition and biochemical parameters in the entire population and according to chronotype categories

Parameters	Total (*n* = 25)	Evening chronotypes (*n* = 7, 28.0%)	Intermediate chronotypes (*n* = 9, 36.0%)	Morning chronotypes (*n* = 9, 36.0%)	*p*-value
Sex					
Males (n, %)	11, 44.0%	3 (42.86%)	5 (55.56%)	3 (33.34%)	0.64
Females (n, %)	14, 56.0%	4 (57.14%)	4 (44.44%)	6 (66.66%)	
Age (years)	41.13 ± 11.08	43.22 ± 11.99	39.54 ± 10.09	41.1 ± 12.33	0.82
Anthropometrical parameters					
BMI (kg/m^2)	41.59 [39.04; 48.21]	40.31 [39.35; 44.87]	45.43 [40.93; 54.22]	39.89 [36.91; 48.48]	0.35
WC (cm)	141 [121; 148]	131 [120; 148]	147 [124.5; 157]	132 [120; 150]	0.59
WHR	1.04 [0.96; 1.12]	1.01 [0.92; 1.12]	1.03 [0.97; 1.09]	1.05 [0.96; 1.16]	0.83
Body composition					
PhA (°)	5.7 [5.4; 6.5]	5 [4.8; 5.4][a, b]	5.9 [5.6; 6.3]	6.6 [5.7; 6.85]	0.04
FM (Kg)	55.1 [47; 67.9]	50.7 [47; 60.6]	55.5 [47.1; 63.3]	53.1 [45.65; 69.6]	0.80
FM (%)	45.2 [40.3; 47.3]	45.2 [41.1; 46.1]	45.7 [40.3; 48.45]	44.4 [39.6; 48.45]	0.99
FFM (Kg)	74.4 [60.5; 88.9]	73.4 [54.3; 97]	76.9 [66.8; 89.9]	74.4 [64.2; 78.9]	0.80
FFM (%)	54.8 [52.7; 59.7]	54.8 [53.9; 58.9]	54.3 [52.7; 59.7]	55.6 [51.55; 60.4]	0.99
MM (Kg)	43.3 [34.8; 47.1]	41.3 [27.9; 43.4]	43.3 [42.3; 48.6]	45.2 [34.25; 48.5]	0.21
MM (%)	32.5 [28.4; 35.4]	28.4 [26.5; 30.2]	33 [31.3; 35.1]	35.9 [26.7; 44.15]	0.22
Biochemical parameters					
Glycaemia (mg/dl)	85.5 [78.25; 96.5]	85 [79.25; 91.5]	84 [76.5; 123]	91 [72.5; 114]	0.87
Insulin (μU/ml)	26.1 [21.85; 32.25]	24.4 [13.5; 47.1]	27.6 [23.58; 32.55]	24.7 [22.3; 31.1]	0.91
HOMA-IR	5.4 [4.03; 6.82]	3.86 [3.03; 10.58]	5.4 [5.01; 6.7]	5.99 [4.68; 6.72]	0.84
Tot-COL (mg/dl)	179.5 [167.2; 209.7]	189 [179; 216]	179 [167.5; 189.5]	177 [154; 215]	0.62
LDL (mg/dl)	132 [117; 147]	128 [114; 137]	137 [120.5; 148.5]	132 [116.8; 163.8]	0.72
HDL (mg/dl)	43 [38; 49]	42 [39; 43]	45 [39; 47]	45.5 [39; 49]	0.68
Triglicerydes (mg/dl)	129 [92.75; 213]	213 [122; 422][a, b]	135.5 [89.25; 184.5]	118 [78; 141]	**0.01**

BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; PhA, phase angle; FM, fat mass; FFM, fat-free mass; MM, muscle mass; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein

Data are expressed as numbers and percentage, median and IQR (25th percentile: 75 h percentile) or mean ± SD

[a] versus intermediate chronotype

[b] versus morning chronotype

for phase angle and triglycerides. Evening chronotypes exhibited higher phase angle values ($p = 0.04$), and elevated triglyceride levels ($p = 0.01$) than morning and intermediate chronotypes.

Table 2 displays cytokine, chemokine, and growth factor levels released from VAT across the entire population and stratified by chronotype categories. A significant decreasing trend was observed in IL-1β ($p = 0.04$), IL-8 ($p = 0.03$), bFGF ($p = 0.01$), MCP-1 ($p = 0.01$), and MIP-1β ($p = 0.05$) across the three groups (evening chronotypes ≥ intermediate chronotypes ≥ morning chronotypes). Additionally, evening chronotypes demonstrated significantly higher levels of bFGF compared to morning and intermediate chronotypes ($p = 0.04$).

Figure 2 represents master clock gene (PER1, CLOCK, and BMAL1) mRNA levels in VAT biopsies. Evening chronotypes exhibited significantly higher mRNA levels of PER1 compared to morning types ($p = 0.017$). Although not significant, a slight increase in CLOCK was also observed in evening chronotypes compared to morning chronotypes.

Table 3 displays the correlation analysis between MEQ scores and VAT markers. Notably, MEQ scores showed a negative correlation with VAT-released bFGF ($p = 0.02$) and MCP-1 ($p = 0.02$) and with *PER1* mRNA levels ($p = 0.01$).

Table 4 shows the correlations among master clock genes expression in VAT with the levels of cytokines, chemokines, and growth factors released from VAT. PER1 exhibited significant positive correlations with IL-1β ($p = 0.03$) and bFGF ($p = 0.03$). Similarly, CLOCK showed significant positive correlations with bFGF ($p = 0.03$) and MCP-1 ($p = 0.02$). In contrast, BMAL1 mRNA levels demonstrated a negative correlation with MIP-1β ($p = 0.005$) and a negative trend for IL-8 ($p = 0.07$).

Discussion

This cross-sectional observational study examined the associations between chronotypes and inflammatory markers secreted by VAT in 25 individuals with obesity, also investigating the expression levels of clock master genes. As main findings, inflammatory markers in

Table 2 Cytokine, chemokine, and growth factor levels released from VAT in the entire population and according to chronotype categories

VAT markers	Total (*n* = 25)	Evening chronotypes (*n* = 7, 28.0%)	Intermediate chronotypes (*n* = 9, 36.0%)	Morning chronotypes (*n* = 9, 36.0%)	*p*-value*	*p*-value**
IL-1β	0.48 [0.34; 1.19]	1.19 [0.61; 1.29]	0.42 [0.36; 1.03]	0.38 [0.25; 0.52]	0.15	0.04
IL-1ra	747 [392; 858]	1333 [747; 2367]	715 [360; 837]	456 [389; 760]	0.06	0.07
IL-2	1.34 [0.42; 2.43]	1.84 [1.24; 2.14]	0.58 [0.42; 2.6]	1.44 [0.42; 2.43]	0.90	0.63
IL-4	0.81 [0.58; 1.5]	1.46 [1.11; 1.58]	0.8 [0.63; 1.63]	0.63 [0.42; 0.87]	0.17	0.07
IL-5	10.42 [3.32; 15.01]	12.33 [3.32; 13.59]	14.85 [9.09; 18.55]	7.88 [5.92; 10.26]	0.66	0.94
IL-6	10.89 [6.3; 17.36]	16.86 [13.85; 57.69]	5.14 [2.05; 13.8]	8.34 [7.68; 9.99]	0.08	0.13
IL-7	3.59 [1.79; 4.55]	4.24 [2.18; 4.86]	1.4 [1.4; 3.59]	3.59 [2.91; 4.24]	0.39	0.87
IL-8	7.09 [1.81; 17.35]	17.35 [15.57; 22.85]	4.62 [1.84; 7.38]	2.04 [1.76; 2.94]	0.08	0.03
IL-9	3.64 [2.83; 4.91]	5.54 [3.64; 14.1]	3.72 [3.64; 4.39]	3.07 [1.98; 3.56]	0.14	0.05
IL-10	0.38 [0.19; 1.04]	0.76 [0.57; 1.33]	0.19 [0.05; 0.62]	0.38 [0.31; 0.8]	0.20	0.31
IL-12	2.03 [1.02; 3.53]	2.03 [2.03; 2.03]	2.03 [1.02; 4.03]	1.02 [0; 3.03]	0.81	0.56
IL-13	0.2 [0; 0.38]	0.19 [0; 0.38]	0.1 [0; 0.2]	0.2 [0; 0.54]	0.69	0.67
IL-17	4.74 [3.47; 8.88]	8.88 [5.54; 9.79]	4.16 [3.5; 9.74]	3.18 [2.12; 4.86]	0.15	0.06
EOTAXIN	0.47 [0.29; 0.86]	0.86 [0.45; 0.9]	0.38 [0.25; 0.85]	0.42 [0.26; 0.58]	0.39	0.17
bFGF	473 [413; 898]	934 [805; 1036][a, b]	473 [452; 792]	342 [152; 462]	0.04	0.01
gCSF	37.2 [26.39; 44.78]	43.57 [30.82; 45.19]	43.84 [31.02; 44.52]	25.09 [22.76; 30.26]	0.32	0.15
gmCSF	1.72 [1.15; 2.16]	2.1 [1.45; 2.16]	1.51 [1.07; 2.18]	1.56 [1.03; 2.07]	0.63	0.33
IFN-γ	2.83 [2.24; 4.1]	4.93 [2.19; 9.11]	2.69 [2.41; 3.08]	2.69 [2.26; 3.54]	0.75	0.47
IP-10	4 [3.37; 5.09]	5.09 [4; 5.34]	3.83 [3.37; 4.89]	3.68 [2.82; 4.43]	0.39	0.19
MCP-1	14.79 [6.18; 20.05]	18.88 [17.69; 32.61]	17.42 [8.54; 28.73]	6.18 [4.61; 10.12]	0.06	0.01
MIP-1α	0.5 [0.34; 0.58]	0.53 [0.46; 0.53]	0.5 [0.47; 0.73]	0.32 [0.07; 0.52]	0.36	0.17
MIP-1β	1 [0.58; 1.41]	3.81 [1.49; 9.03]	1 [0.72; 1.27]	0.65 [0.44; 0.86]	0.15	0.04
PDGF	9.21 [1.56; 18.66]	15.2 [8.2; 30.84]	14.21 [7.19; 20.13]	2.08 [1.04; 9.21]	0.34	0.13
RANTES	29.41 [14.95; 62.69]	62.69 [17.34; 175]	33.05 [21.48; 60]	15.18 [7.27; 27.74]	0.18	0.09
TNF-α	6.75 [3.77; 11.38]	11.38 [7.07; 17.73]	5.12 [4.11; 9.81]	6.43 [2.72; 8.33]	0.23	0.09

VAT, visceral adipose tissue; IL, interleukin; bFGF, basic fibroblast growth factor; gCSF, granulocyte colony-stimulating factor; gmCSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1 alpha; MIP-1β, macrophage inflammatory protein-1 beta; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; TNF-α, tumor necrosis factor alpha

Data are expressed as numbers and percentage, median and IQR (25th percentile: 75 h percentile) or mean ± SD

*overall

** for trend

[a] versus intermediate chronotype

[b] versus morning chronotype

VAT, including IL-1β, IL-8, bFGF, MCP-1, and MIP-1β, showed a decreasing trend across chronotype groups, with the highest levels detected in evening chronotypes and the lowest in morning chronotypes, despite similar anthropometric, body composition, and biochemical parameters across the groups. Notably, bFGF levels were significantly elevated in evening chronotypes compared to the others. Gene expression analyses revealed chronotype-dependent differences: evening chronotypes exhibited higher expression of the clock gene PER1 compared to morning chronotypes, with PER1 expression positively correlating with IL-1β and bFGF. In contrast, BMAL1 expression showed an inverse correlation with IL-8 and MIP-1β. Furthermore, MEQ scores were negatively correlated with bFGF, MCP-1, and PER1 expressions.

It is important to highlight that the homogeneity of participants in terms of anthropometric, body composition, and biochemical characteristics enabled us to specifically isolate the effects of chronotype on VAT inflammation. This suggests that the observed differences reflect an early stage of metabolic dysfunction, where chronotype independently influences inflammation, regardless of other metabolic factors. Furthermore, the ease with which chronotype can be assessed through a simple, widely accessible tool, such as the MEQ questionnaire, underscores its potential clinical value for early identification of at-risk individuals and the development of targeted interventions.

The pro-inflammatory environment in VAT observed in evening chronotypes, highlighted by elevated levels of IL-1β, IL-8, bFGF, MCP-1, and MIP-1β, aligns with previous evidence linking evening chronotypes to systemic inflammation [32, 33]. The increase in bFGF is particularly noteworthy, as this growth factor is implicated in

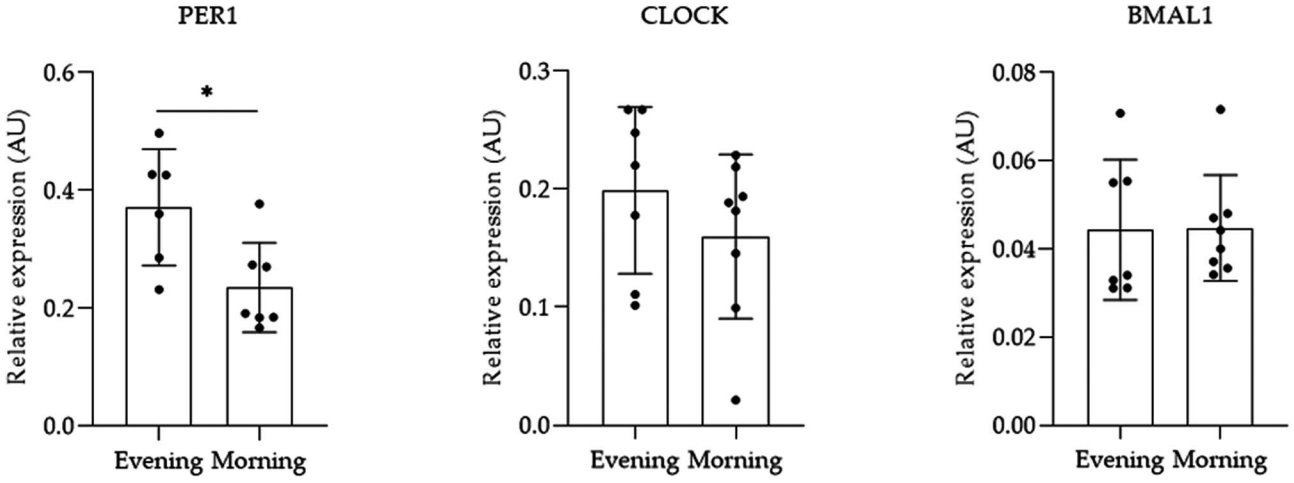

Fig. 2 VAT mRNA levels of *PER1*, *CLOCK*, and *BMAL1* in evening or morning chronotypes. PER1, period circadian 1; CLOCK, circadian locomotor output cycles kaput; BMAL1, brain and muscle ARNT-like 1. mRNA levels of *PER1*, *CLOCK*, and *BMAL1* were determined in VAT biopsies of individuals with evening or morning chronotype by qPCR. Data were normalized on the Ribosomal Protein L13 (*RPL13*) gene as an internal standard. Individual values are expressed in absolute units (AU) and are represented by bar graphs. * Denotes a statistically significant p-value ($p < 0.05$)

adipose tissue fibrosis, expansion [34], and the promotion of inflammatory responses through activation of the NLRP3 inflammasome [35]. Moreover, bFGF has a harmful effect on the development of type 2 diabetes through metabolism reprogramming and attenuation of the insulin signal [36]. The positive correlation between bFGF and PER1 further supports the role of circadian misalignment in driving VAT inflammation.

Altered expression of clock genes in VAT provides additional insights into the mechanisms underlying the observed inflammatory responses. Of note, we have shown, for the first time, that in individuals with obesity, chronotype could impacts VAT, modifying the release of inflammatory cytokines and the expression of PER1, which belongs to the master clock gene family [37]. Cytokines govern the low-grade inflammation in adipose tissue in dysmetabolic states [38]. For instance, in obesity, adipose tissue-derived cytokines are early markers of impaired glucose tolerance [39]. The elevated levels of IL-1β, IL-8, bFGF, MCP-1, and MIP-1β in VAT of evening chronotypes, particularly IL-1β and IL-8, underscore the pro-inflammatory state potentially experienced by this group. Elevated levels of these markers suggest a greater inflammatory burden [38], which is consistent with prior studies linking unhealthy dietary patterns and chronodisruption with inflammation [40]—a characteristic trait of evening chronotypes [41]. The misalignment of PER1 and, to the least extent, of CLOCK in evening chronotypes is also suggestive of adipose tissue impairment. PER1 and CLOCK are the main regulators of the thousand clock-controlled genes that, in adipose tissue, are involved in adipokine secretion, redox state, lipid metabolism, and angiogenesis [42]. In vivo and in vitro studies have clearly demonstrated that obesity and inflammation impact on this machinery [42]. However, studies in humans are very poor [10, 11]. Here, we have found that in VAT of individuals with obesity, PER1 and CLOCK are increased in evening chronotypes and that their expression correlated positively with the release of the proinflammatory molecules IL-1β and MCP1, highlighting the connection between chronotype disruption-gene misalignment- inflammation. Accordingly, the time-restricted feeding resulted in healthy adipose tissue remodeling with reduced expression of PER1 [43]. In addition, we have provided evidence that in adipose tissue BMAL1 expression is inversely correlated with IL-8 and MIP-1β. This finding is consistent with literature data showing that inflammation, with sustained activation of NF-kB pathway (that is, upstream IL-8 transcription), results in marked inhibition of BMAL1 [44].

Finally, evening chronotypes exhibited significantly higher triglyceride levels and lower phase angle values compared to morning and intermediate chronotypes. The elevated triglyceride levels in evening chronotypes may reflect maladaptive dietary patterns [45], sedentary behavior [46], and disrupted sleep [47], all of which are commonly associated with this chronotype [46, 48]. Triglycerides are known precursors of insulin resistance and contribute to the development of metabolic disorders [49]. Similarly, the lower phase angle observed in evening chronotypes suggests compromised metabolic health, as phase angle is a well-recognized marker of cell membrane integrity and function [50, 51].

One of the key strengths of this study is the homogeneity of the study population in terms of anthropometric and metabolic characteristics. In fact, while behavioral and lifestyle factors such as dietary habits, sleep duration, and physical activity could influence VAT inflammation

Table 3 Correlation analysis for MEQ scores with VAT markers

VAT markers	ρ (95% CI)	*p*-value
IL-1β	-0.48 (-0.78–0.02)	0.06
IL-1ra	-0.41 (-0.75–0.10)	0.10
IL-2	-0.19 (-0.65–0.37)	0.50
IL-4	-0.42 (-0.76–0.12)	0.11
IL-5	-0.24 (-0.72–0.41)	0.46
IL-6	-0.43 (-0.77–0.10)	0.10
IL-7	0.15 (-0.40–0.63)	0.60
IL-8	-0.47 (-0.78–0.03)	0.06
IL-9	-0.45 (-0.77–0.06)	0.08
IL-10	-0.31 (-0.74–0.31)	0.31
IL-12	0.00 (-0.56–0.56)	0.99
IL-13	0.29 (-0.47–0.80)	0.46
IL-17	-0.44 (-0.77–0.06)	0.08
Eotaxin	-0.34 (-0.72–0.21)	0.20
bFGF	-0.58 (-0.84– -0.10)	0.02
gCSF	-0.33 (-0.72–0.21)	0.21
gmCSF	-0.19 (-0.64–0.35)	0.48
IFN-γ	-0.21 (-0.65–0.33)	0.43
IP-10	-0.32 (-0.70–0.20)	0.21
MCP-1	-0.57 (-0.84– -0.09)	0.02
MIP-1α	-0.02 (-0.54–0.51)	0.96
MIP-1β	-0.35 (-0.78–0.30)	0.27
PDGF	-0.34 (-0.76–0.28)	0.26
RANTES	-0.33 (-0.72–0.21)	0.21
TNF-α	-0.47 (-0.79–0.05)	0.07
PER1	-0.64 (-0.88-0.14)	0.01
CLOCK	-0.25 (-0.67-0.31)	0.37
BMAL1	0.16 (-0.40-0.63)	0.56

VAT, visceral adipose tissue; IL, interleukin; EOTAXIN, eotaxin; bFGF, basic fibroblast growth factor; gCSF, granulocyte colony-stimulating factor; gmCSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1 alpha; MIP-1β, macrophage inflammatory protein-1 beta; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T cell expressed and secreted; TNF-α, tumor necrosis factor alpha

For every correlation, rho (ρ) correlation coefficient is reported, as well as its relative p-value

independently of chronodisruption, participants across the three chronotype categories did not exhibit differences in anthropometric parameters, body composition, or insulin resistance, which are key factors involved in obesity-related inflammation. This uniformity allowed us to isolate and directly evaluate the effects of chronotype on inflammatory markers in VAT without interference from other metabolic or compositional variables. However, a limitation of this study is the relatively small sample size, which may restrict the generalizability of our findings. Additionally, chronotype classification was based on self-reported MEQ scores, which may introduce recall bias. Future studies could strengthen these findings by incorporating objective measures, such as actigraphy, to provide a more precise assessment of sleep-wake patterns and circadian rhythms. Nonetheless, as an initial investigation, this study provides important insights into the relationship between chronotype, clock gene expression, and VAT inflammation. It lays the groundwork for future research with larger and more diverse populations to confirm and expand upon these results.

Conclusion

In conclusion, this study demonstrates that evening chronotypes are associated with an altered inflammatory profile in VAT, mediated by dysfunctional clock gene expression. These findings provide novel evidence of the role of circadian misalignment in obesity-related inflammation and highlight the importance of interventions aimed at restoring circadian alignment to improve metabolic outcomes. Moreover, since chronotype can be easily assessed using simple questionnaires, it represents a practical and accessible tool in clinical settings to identify individuals at risk of VAT-related inflammation, even before overt metabolic alterations occur. This underscores its potential value in early risk stratification and targeted preventive strategies.

Abbreviations

BMI	Body Mass Index
WC	Waist Circumference
WHR	Waist-to-Hip Ratio
PhA	Phase Angle
FM	Fat Mass
FFM	Fat-Free Mass
MM	Muscle Mass
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
LDL	Low-Density Lipoprotein
HDL	High-Density Lipoprotein

Table 4 Correlation among VAT markers and master clock genes

VAT markers	PER1		CLOCK		BMAL1	
	ρ (95% CI)	*p*-value	ρ (95% CI)	*p*-value	ρ (95% CI)	*p*-value
IL-1β	0.77 (0.14–0.96)	**0.03**	0.44 (-0.27–0.84)	0.21	-0.39 (-0.83–0.34)	0.26
IL-8	0.49 (-0.33–0.89)	0.22	0.42 (-0.28–0.83)	0.22	-0.61 (-0.91–0.10)	*0.07*
bFGF	0.75 (0.09–0.95)	0.03	0.69 (0.12–0.92)	0.03	-0.36 (-0.81–0.37)	0.31
MCP-1	0.70 (-0.22–0.87)	0.11	0.79 (0.04–0.93)	0.02	-0.35 (-0.79–0.42)	0.36
MIP-1β	0.67 (-0.38–0.94)	0.16	0.71 (-0.36–0.94)	0.14	-0.99 (-0.99– -0.61)	0.05

VAT, visceral adipose tissue; PER1, period circadian 1; CLOCK, circadian locomotor output cycles kaput; BMAL1, brain and muscle ARNT-like 1; IL-1β, interleukin 1 beta; IL-8, interleukin 8; bFGF, basic fibroblast growth factor; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1 beta

For every correlation, rho (ρ) correlation coefficient is reported, as well as its relative p-value

VAT	Visceral Adipose Tissue
MEQ	Morningness-Eveningness Questionnaire
qPCR	Quantitative Polymerase Chain Reaction
IL	Interleukin
bFGF	Basic Fibroblast Growth Factor
G-CSF	Granulocyte Colony-Stimulating Factor
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
IFN-γ	Interferon Gamma
IP-10	Interferon Gamma-Inducible Protein 10
MCP-1	Monocyte Chemoattractant Protein-1
MIP-1α	Macrophage Inflammatory Protein-1 Alpha
MIP-1β	Macrophage Inflammatory Protein-1 Beta
PDGF	Platelet-Derived Growth Factor
RANTES	Regulated on Activation, Normal T Cell Expressed and Secreted
TNF-α	Tumor Necrosis Factor Alpha
PER1	Period Circadian 1
CLOCK	Circadian Locomotor Output Cycles Kaput
BMAL1	Brain and Muscle ARNT-Like 1
RPL13	Ribosomal Protein L13
SD	Standard Deviation
IQR	Interquartile Range
CI	Confidence Interval
AU	Absolute Units

Author contributions
G.M. conceived the research. L.V., M.F.D.T., A.P. and F.D.A. performed the experiments. M.F.D.T. and L.Z. analyzed the data. L.Z., L.Z. and M.F.D.T. wrote the manuscript. L.B., A.C., V.D.E, P.F. and G.M. revised the final manuscript. All authors have approved the submission of the final draft.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study has been approved by the Local Ethical Committee and carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments that involved humans. The aim of the study was clearly explained to all the study participants and a written informed consent was obtained.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Kolbe I, Oster H, Chronodisruption. Metabolic homeostasis, and the regulation of inflammation in adipose tissues. Yale J Biol Med. 2019;92(2):317–25.
2. Hernandez-Garcia J, Navas-Carrillo D, Orenes-Pinero E. Alterations of circadian rhythms and their impact on obesity, metabolic syndrome and cardiovascular diseases. Crit Rev Food Sci Nutr. 2020;60(6):1038–47.
3. Verde L, Barrea L, Bowman-Busato J, Yumuk VD, Colao A, Muscogiuri G. Obesogenic environments as major determinants of a disease: It is time to re-shape our cities. Diabetes Metab Res Rev. 2024;40(1):e3748.
4. Vazquez-Vela ME, Torres N, Tovar AR. White adipose tissue as endocrine organ and its role in obesity. Arch Med Res. 2008;39(8):715–28.
5. [Japanese literature on antibiotics 86]. J Antibiot B. 1966;19(6):472–82.
6. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112(12):1821–30.
7. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol. 2005;6(12):1191–7.
8. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med. 2012;18(9):1407–12.
9. Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, et al. A circadian clock in macrophages controls inflammatory immune responses. Proc Natl Acad Sci U S A. 2009;106(50):21407–12.
10. Shostak A, Meyer-Kovac J, Oster H. Circadian regulation of lipid mobilization in white adipose tissues. Diabetes. 2013;62(7):2195–203.
11. Hepler C, Bass J. Circadian mechanisms in adipose tissue bioenergetics and plasticity. Genes Dev. 2023;37(11–12):454–73.
12. Vieira E, Mirizio GG, Barin GR, de Andrade RV, Nimer NFS, La Sala L. Clock genes, inflammation and the Immune System-implications for diabetes, obesity and neurodegenerative diseases. Int J Mol Sci. 2020;21(24).
13. Sookoian S, Gemma C, Fernandez Gianotti T, Burgueno A, Alvarez A, Gonzalez CD, et al. Effects of rotating shift work on biomarkers of metabolic syndrome and inflammation. J Intern Med. 2007;261(3):285–92.
14. Herrero L, Valcarcel L, da Silva CA, Albert N, Diez-Noguera A, Cambras T, et al. Altered circadian rhythm and metabolic gene profile in rats subjected to advanced light phase shifts. PLoS ONE. 2015;10(4):e0122570.
15. Fonken LK, Lieberman RA, Weil ZM, Nelson RJ. Dim light at night exaggerates weight gain and inflammation associated with a high-fat diet in male mice. Endocrinology. 2013;154(10):3817–25.
16. Fuster JJ, Zuriaga MA, Ngo DT, Farb MG, Aprahamian T, Yamaguchi TP, et al. Noncanonical wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction independent of adipose tissue expansion. Diabetes. 2015;64(4):1235–48.
17. Lee MJ, Gong DW, Burkey BF, Fried SK. Pathways regulated by glucocorticoids in omental and subcutaneous human adipose tissues: a microarray study. Am J Physiol Endocrinol Metab. 2011;300(3):E571–80.
18. Agil A, Reiter RJ, Jimenez-Aranda A, Iban-Arias R, Navarro-Alarcon M, Marchal JA, et al. Melatonin ameliorates low-grade inflammation and oxidative stress in young Zucker diabetic fatty rats. J Pineal Res. 2013;54(4):381–8.
19. Rios-Lugo MJ, Cano P, Jimenez-Ortega V, Fernandez-Mateos MP, Scacchi PA, Cardinali DP, et al. Melatonin effect on plasma adiponectin, leptin, insulin, glucose, triglycerides and cholesterol in normal and high fat-fed rats. J Pineal Res. 2010;49(4):342–8.
20. Weger BD, Gobet C, Yeung J, Martin E, Jimenez S, Betrisey B, et al. The mouse microbiome is required for sex-specific diurnal rhythms of Gene expression and metabolism. Cell Metab. 2019;29(2):362–82. e8.
21. Caesar R, Tremaroli V, Kovatcheva-Datchary P, Cani PD, Backhed F. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR Signaling. Cell Metab. 2015;22(4):658–68.

22. Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, et al. Epidemiology of the human circadian clock. Sleep Med Rev. 2007;11(6):429–38.
23. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976;4(2):97–110.
24. Ekiz Erim S, Sert H. The relationship between chronotype and obesity: a systematic review. Chronobiol Int. 2023;40(4):529–41.
25. Barrea L, Verde L, Vetrani C, Docimo A, de Alteriis G, Savastano S, et al. Evening chronotype is associated with hormonal and metabolic disorders in polycystic ovary syndrome. J Pineal Res. 2023;74(2):e12844.
26. Barrea L, Vetrani C, Altieri B, Verde L, Savastano S, Colao A et al. The Importance of Being a 'Lark' in Post-Menopausal Women with Obesity: A Ploy to Prevent Type 2. Diabetes Mellitus? Nutrients. 2021;13(11).
27. Docimo A, Verde L, Barrea L, Vetrani C, Memoli P, Accardo G et al. Type 2 diabetes: also a clock matter? Nutrients. 2023;15(6).
28. STATISTICS NCFH. Waist Circumference Measurement Methodology Study: National Health and Nutrition Examination Survey, 2016 2016 [Available from: https://www.cdc.gov/nchs/data/series/sr_02/sr02_182-508.pdf
29. Cabaro S, Conte M, Moschetta D, Petraglia L, Valerio V, Romano S, et al. Epicardial adipose tissue-derived IL-1beta triggers postoperative Atrial Fibrillation. Front Cell Dev Biol. 2022;10:893729.
30. D'Esposito V, Di Tolla MF, Lecce M, Cavalli F, Libutti M, Misso S, et al. Lifestyle and Dietary habits affect plasma levels of specific cytokines in healthy subjects. Front Nutr. 2022;9:913176.
31. Shashaj B, Luciano R, Contoli B, Morino GS, Spreghini MR, Rustico C, et al. Reference ranges of HOMA-IR in normal-weight and obese young caucasians. Acta Diabetol. 2016;53(2):251–60.
32. Swanson GR, Kochman N, Amin J, Chouhan V, Yim W, Engen PA, et al. Disrupted Circadian Rest-Activity cycles in inflammatory bowel Disease are Associated with Aggressive Disease phenotype, subclinical inflammation, and Dysbiosis. Front Med (Lausanne). 2021;8:770491.
33. Zhai S, Tao S, Wu X, Zou L, Yang Y, Xie Y, et al. Associations of Sleep Insufficiency and Chronotype with Inflammatory cytokines in College Students. Nat Sci Sleep. 2021;13:1675–85.
34. Yamashita H, Sato Y, Kizaki T, Oh S, Nagasawa J, Ohno H. Basic fibroblast growth factor (bFGF) contributes to the enlargement of brown adipose tissue during cold acclimation. Pflugers Arch. 1994;428(3–4):352–6.
35. ZhuGe DL, Javaid HMA, Sahar NE, Zhao YZ, Huh JY. Fibroblast growth factor 2 exacerbates inflammation in adipocytes through NLRP3 inflammasome activation. Arch Pharm Res. 2020;43(12):1311–24.
36. Kihira Y, Yamano N, Izawa-Ishizawa Y, Ishizawa K, Ikeda Y, Tsuchiya K, et al. Basic fibroblast growth factor regulates glucose metabolism through glucose transporter 1 induced by hypoxia-inducible factor-1alpha in adipocytes. Int J Biochem Cell Biol. 2011;43(11):1602–11.
37. Patke A, Young MW, Axelrod S. Molecular mechanisms and physiological importance of circadian rhythms. Nat Rev Mol Cell Biol. 2020;21(2):67–84.
38. Kawai T, Autieri MV, Scalia R. Adipose tissue inflammation and metabolic dysfunction in obesity. Am J Physiol Cell Physiol. 2021;320(3):C375–91.
39. D'Esposito V, Ambrosio MR, Liguoro D, Perruolo G, Lecce M, Cabaro S, et al. In severe obesity, subcutaneous adipose tissue cell-derived cytokines are early markers of impaired glucose tolerance and are modulated by quercetin. Int J Obes (Lond). 2021;45(8):1811–20.
40. Khorasaniha R, Siassi F, Khajehnasiri F, Qorbani M, Sotoudeh G. Dietary patterns in relation to inflammation in shift workers. BMJ Mil Health. 2020;166(4):221–6.
41. Teixeira GP, Guimaraes KC, Soares A, Marqueze EC, Moreno CRC, Mota MC, et al. Role of chronotype in dietary intake, meal timing, and obesity: a systematic review. Nutr Rev. 2022;81(1):75–90.
42. Vatier C, Christin-Maitre S. Epigenetic/circadian clocks and PCOS. Hum Reprod. 2024;39(6):1167–75.
43. Zhao L, Hutchison AT, Liu B, Wittert GA, Thompson CH, Nguyen L, et al. Time-restricted eating alters the 24-hour profile of adipose tissue transcriptome in men with obesity. Obes (Silver Spring). 2023;31(Suppl 1Suppl 1):63–74.
44. Hong HK, Maury E, Ramsey KM, Perelis M, Marcheva B, Omura C, et al. Requirement for NF-kappaB in maintenance of molecular and behavioral circadian rhythms in mice. Genes Dev. 2018;32(21–22):1367–79.
45. Muscogiuri G, Barrea L, Aprano S, Framondi L, Di Matteo R, Laudisio D et al. Chronotype and Adherence to the Mediterranean Diet in obesity: results from the Opera Prevention Project. Nutrients. 2020;12(5).
46. Sempere-Rubio N, Aguas M, Faubel R. Association between Chronotype, physical activity and sedentary behaviour: a systematic review. Int J Environ Res Public Health. 2022;19(15).
47. Verde L, Barrea L, Vetrani C, Frias-Toral E, Chapela SP, Jayawardena R, et al. Chronotype and sleep quality in obesity: how do they change after menopause? Curr Obes Rep. 2022;11(4):254–62.
48. van der Merwe C, Munch M, Kruger R. Chronotype differences in body composition, Dietary Intake and Eating Behavior outcomes: a scoping systematic review. Adv Nutr. 2022;13(6):2357–405.
49. Kraegen EW, Cooney GJ, Ye J, Thompson AL. Triglycerides, fatty acids and insulin resistance–hyperinsulinemia. Exp Clin Endocrinol Diabetes. 2001;109(4):S516–26.
50. Annunziata G, Paoli A, Frias-Toral E, Marra S, Campa F, Verde L, et al. Use of phase angle as an indicator of overtraining in sport and physical training. J Transl Med. 2024;22(1):1084.
51. da Silva BR, Orsso CE, Gonzalez MC, Sicchieri JMF, Mialich MS, Jordao AA, et al. Phase angle and cellular health: inflammation and oxidative damage. Rev Endocr Metab Disord. 2023;24(3):543–62.

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