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Article

Dietary Intake and Body Composition of Fixed-Shift Workers During the Climacteric: An Intervention Study with Exogenous Melatonin

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Abstract: Poor sleep quality is associated with unhealthy dietary choices and worse body composition among night-shift workers and women during the climacteric period. This study aimed to evaluate the effect of exogenous melatonin administration on the dietary intake and body composition in healthcare workers exposed to fixed-shift work during the climacteric period. A phase II randomized, double-blind, placebo-controlled clinical trial of climacteric women working fixed morning, afternoon, or night shifts was conducted. Baseline data collection included sociodemographic information, sleep parameters, dietary intake, and body composition using bioelectrical impedance. Participants received either 0.3 mg of melatonin or a placebo for three months. Baseline total caloric and carbohydrate intake was higher among night-shift workers than afternoon-shift workers, though afternoon-shift workers a had higher body weight and fat percentage. Post-intervention, caloric intake differences dissipated due to reduced intake among night-shift workers and increased intake among afternoon-shift workers, independent of melatonin or placebo administration. However, differences in body composition persisted. Postmenopausal participants showed reduced fat mass, while premenopausal participants experienced an increase, regardless of intervention. The results suggest that physiological nighttime melatonin doses, administered on non-consecutive nights for three months, were ineffective for changing dietary intake or body composition.

Keywords: climacteric; night shift; melatonin; food intake; body composition

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1. Introduction

There is strong evidence supporting the association between shift work and adverse health outcomes, such as metabolic syndrome [1] and cardiovascular diseases [2]. These consequences are linked to the misalignment between light–dark cycles and workers' activity–rest rhythms [3]. However, a greater understanding of the physiological and behavioral mechanisms underlying these processes is needed [3]. Regarding the diets of night-shift workers, studies have shown that they tend to adopt less healthy eating patterns compared to day-shift workers, including a higher intake of saturated fats [4]. This behavior may be explained by sleep restriction and fatigue, commonly experienced by shift workers, which can influence food choices and promote unhealthy dietary habits. Conversely, better sleep quality and longer sleep duration are associated with healthier eating behaviors, including higher consumption of vegetables, fruit, fish, water, and dietary fiber [4–6]. Consequently, shift workers are more prone to weight gain, central obesity, and impaired lipid metabolism compared to day workers [7,8].

Furthermore, exposure to light at night, as occurs during night shifts, leads to a reduction in melatonin levels. Melatonin is a hormone produced by the pineal gland in humans, whose synthesis is regulated by the light–dark cycle through the circadian timing system, ensuring its production occurs during the night and in the absence of light. Hence, melatonin is secreted at night and in the dark, playing a crucial role in regulating various functions, including the sleep–wake cycle and energy metabolism [9,10]. Melatonin is also recognized as a hormonal factor that improves sleep quality in humans [11]. Low melatonin concentrations can disrupt circadian rhythms, leading to poorer sleep quality among night-shift workers [12,13].

A number of studies have demonstrated that melatonin administration can help prevent metabolic, cardiac, and immune disorders, improve sleep quality, and promote general well-being by restoring physiological homeostasis [13,14]. Additionally, some evidence suggests that melatonin therapy can contribute to a reduction in body mass index (BMI) [15,16].

Sleep disturbances are frequently reported during the climacteric period—a natural transition in women's lives from a reproductive to a non-reproductive phase [17]. For some women, these disturbances are severe and impact their quality of life, with potential long-term consequences for mental and physical health [18]. Regarding diet, poor sleep quality during the climacteric has been linked to the consumption of diets rich in processed foods, saturated fats, refined grains, fried foods, sweets, and sugary beverages [19,20]. Studies have suggested that exogenous melatonin may improve sleep quality in menopausal women and, given the numerous health benefits of the hormone, its use during the climacteric can be considered [15,21].

As in other stages of life, body composition during the climacteric is influenced by dietary choices. Adherence to the Mediterranean diet—a dietary pattern considered healthy and anti-inflammatory—is associated with better body composition, characterized by lower fat mass percentage, coupled with higher percentages of muscle mass and fat-free mass [22].

In this context, the aim of the present study was to evaluate the effect of exogenous melatonin administration on dietary intake and body composition in healthcare workers exposed to fixed-shift work during the climacteric. The study hypothesis holds that exogenous melatonin improves food choices, positively affecting both dietary intake and body composition. Theoretically, this indirect effect of melatonin on food choices could occur through improvements in sleep quality promoted by melatonin administration, as previously documented in the literature. Moreover, the effect is expected to be greater among night-shift workers, who presumably experience poorer sleep quality, make less healthy dietary choices, and have a worse body composition.

2. Materials and Methods

2.1. Study Design

A phase II randomized, double-blind, placebo-controlled clinical trial was conducted at a large hospital in São Paulo, Brazil. The facility has a registered capacity of 806 beds, comprising 583 serving the private sector and 223 serving the public sector. Data collection occurred between September 2022 and September 2023.

2.2. Population

The study population consisted of female nurses or nursing technicians working three different fixed shifts as follows: morning (7:00 a.m. to 1:00 p.m.), afternoon (1:00 p.m. to 7:00 p.m.), and night (7:00 p.m. to 7:00 a.m.). Shift schedules were fixed, with six consecutive workdays followed by one day off for morning and afternoon shift workers. Night-shift workers had a schedule of 12 h on alternating with 36 h off, working one night and resting the next. Regarding duties, nursing technicians performed practical and routine patient care tasks under the supervision of nurses, such as administering prescribed medications, assisting with patient hygiene, and collecting samples. Nurses had greater autonomy and responsibility in clinical and administrative decision making, including performing detailed assessments, devising care plans, and supervising and training nursing technicians.

2.3. Study Sample

Only nurses and nursing technicians aged 38 years or older were invited to participate in the study. The first step involved an interview to evaluate inclusion and exclusion criteria. A total of 160 workers were initially eligible based on the inclusion criteria. Of these, 80 participants were excluded based on the exclusion criteria, and 24 refused to participate. The final sample included 56 women, of which 46 completed the study. Of the total 10 dropouts, 1 was due to a job change and 9 to withdrawal (Figure 1).

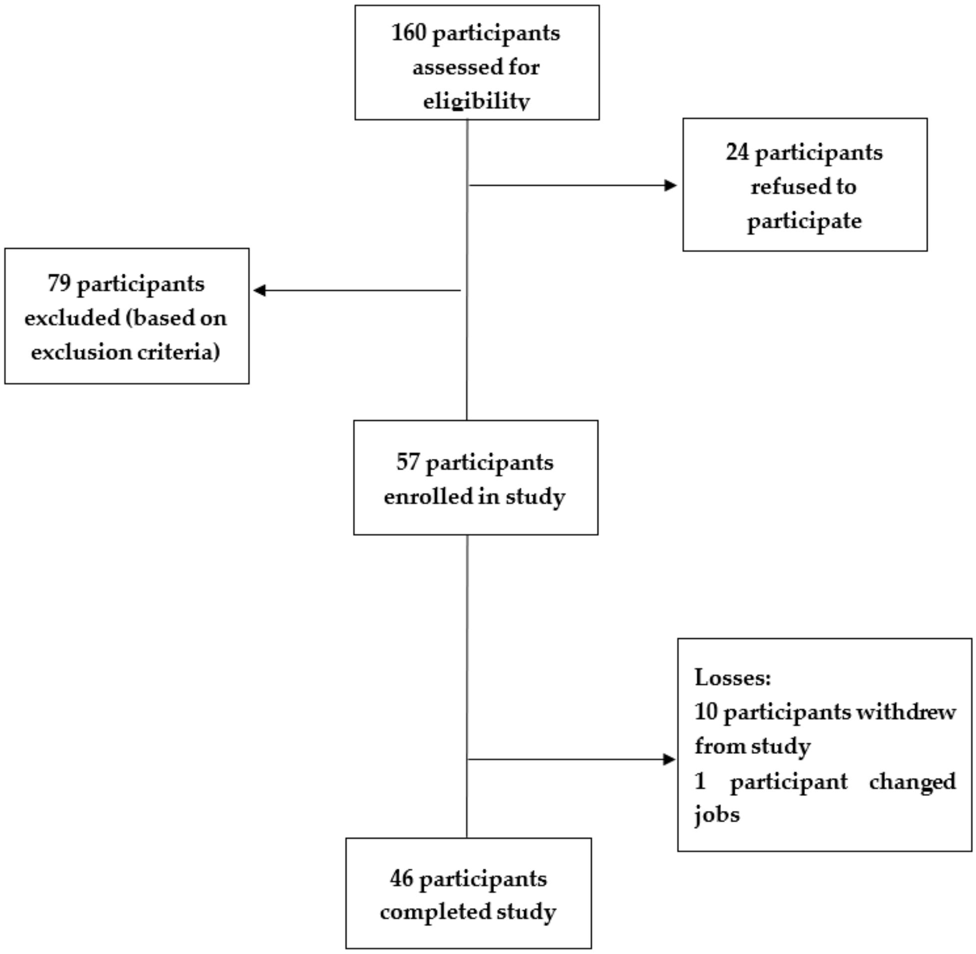

Figure 1. Steps in inclusion process of study participants.

2.4. Sample Calculation

The sample size calculation was performed *a priori*, adopting an effect size of 0.30, alpha error of 5%, and a β power of 95%. The statistical test employed was repeated measures ANOVA (within–between interaction) with two groups (work shift and intervention: melatonin vs. placebo) and two time points (pre- and post-intervention). Based on these parameters, the calculated sample size was 38 participants. Allowing for a 20% dropout rate, the study sought to recruit 46 participants. However, 56 women were initially eligible and, after commencement of the protocol there were 10 dropouts, as depicted in Figure 1, resulting in a final sample of 46 participants and a β power of 98%. Following melatonin intervention in the present study, a 30% improvement in dietary intake and body composition was expected. This rate was selected based on the 2021 goal set by the World Health Organization (WHO) and the Pan American Health Organization (PAHO) to reduce sodium intake by 30% in the population by 2025 [23,24].

2.5. Inclusion and Exclusion Criteria

The inclusion criteria for the study subjects were as follows: female, nursing professionals from a hospital in São Paulo, aged 38 years or older (menopausal transition), and those who reported symptoms of menopause. The menopause symptoms considered for inclusion were the following: hot flashes, night sweats, and insomnia.

The exclusion criteria constituted workers who were using or planning to use hormone replacement therapy or who had taken exogenous melatonin in the last three months, as well as individuals with conditions affecting sleep (such as fibromyalgia and anemia), or those undergoing treatment with medications that influenced sleep or alertness. Additionally, night-shift workers that held a second job were also excluded. Women who were on a diet or planned to go on a diet within the next three months, as well as those using medications affecting appetite, were also excluded from the study.

2.6. Intervention Group (Melatonin) and Control Group (Placebo)

Participants took a capsule containing 0.3 mg of melatonin (Intervention Group) or a placebo (Control Group) one hour before their usual bedtime for a period of three months. More specifically, night-shift workers took the capsule on nights off, while daytime workers (morning and afternoon shifts) took the capsule on alternate days to match the night-shift workers. All capsules were taken at night. Thus, melatonin was administered to both groups for 45 nights throughout the three-month period. Given the study was double-blind, a simple random draw was conducted to determine allocation to the intervention or placebo groups. The draw was performed by a team member who did not participate in the data collection.

2.7. Urine Collection and Analysis

To investigate the melatonin production profile and subsequently adjust the correct individual melatonin dose to avoid daytime escape, two urine collections were conducted. Participants collected urine in a sterile container to prevent contamination. Upon receipt of the samples, researchers separated aliquots into Falcon and Eppendorf tubes for analysis. Physical characteristics (volume, color, turbidity, and odor) were recorded and specialized analysis was subsequently performed. All collected urine samples (Urine 0 and Urine 1) were independently analyzed by a single researcher to assess the concentration of the melatonin metabolite (6-sulfatoxymelatonin), blinded to group allocation (intervention or control).

The first collection, referred to as the baseline collection, was used to determine the participants' basal 6-sulfatoxymelatonin concentration before the intervention. Subsequently, after the first night in which participants took either a melatonin or placebo capsule, a second urine collection was performed to evaluate potential 6-sulfatoxymelatonin escape in the second urine of the day.

The urine collection adhered to the following protocol: participants were instructed to use the restroom around 18:30 to eliminate any urine in the bladder. Subsequently, all nocturnal urine was collected, including the first morning void upon waking. The second urine was collected in a smaller container after participants were fully awake for later analysis to check the presence of the melatonin metabolite (6-sulfatoxymelatonin).

In the event of any morning "spillover" of 6-sulfatoxymelatonin, the melatonin dose was to be reduced, although no adjustments proved necessary.

2.8. Data Collection

Data collection spanned from September 2022 to September 2023 and took place one work shift at a time. For each shift type, baseline sociodemographic data; lifestyle habits, including sleep patterns, sedentary behavior (time spent sitting on rest days), and 24 h food diaries were initially recorded, along with bioimpedance measurements. Following baseline collection, participants took melatonin or placebo for three months, after which, post-intervention data collection was conducted. A detailed description of the data and instruments used are presented below (Figure 2).

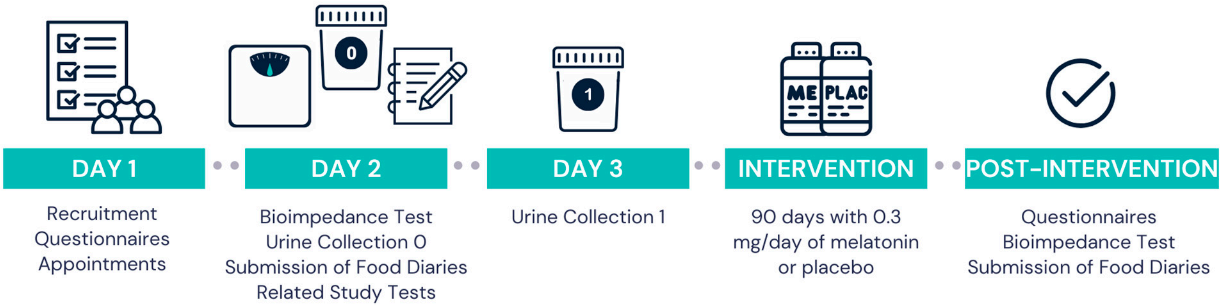

Figure 2. Study illustration. Recruitment was conducted on Day 1, when participants also filled out questionnaires and scheduled the bioimpedance test, Urine 0 collection, 24 h food diaries, and the additional related study tests (arterial and blood tests), for the next day. On Day 2, the bioimpedance test was performed, the food diaries and Urine 0 were collected, and the intervention capsules (melatonin or placebo) were provided for 90 alternating days. The related study tests were also conducted. On Day 3, after the first night of using the melatonin or placebo capsule, Urine 1 was collected. The 90-day intervention then began with administration of 0.3 mg/day of melatonin or placebo. After this period, questionnaires were filled out, the bioimpedance test repeated, and food diaries submitted again.

2.9. Dietary Intake Data Collection

Two 24 h food diaries were kept by participants as follows: one on their workday and another on their day off. These diaries were recorded at two different time points as follows: one at baseline and another immediately after the intervention, for a total of four food diaries per participant (two at baseline and two post-intervention).

The food diary is a prospective method of assessing dietary intake, meaning it collects current information. This type of survey does not rely on memory, provides greater accuracy and precision for foods consumed, and allows for the identification of foods and preparations, as well as mealtimes [25].

For each food diary completed, a preliminary check of the information quality was performed to identify potential inconsistencies. The multiple-pass method (MPM), developed by the U.S. Department of Agriculture (USDA) was used [26]. This method entails the following:

(1) Listing all foods and beverages consumed on the previous day without interrupting the participant or asking detailed questions.

(2) The interviewer quickly reviews the list and asks detailed questions about each item, including description, quantity, brand, and preparation method.
(3) The interviewer reviews the times and occasions of meals and snacks, helping the participants recall any items not initially reported.
(4) Checking for commonly omitted items such as juices, snacks, and alcoholic beverages.
(5) Final review by the interviewer, rechecking times and quantities to ensure all information is accurately recorded.

The food diaries were sent to the researchers electronically. The participants either took photos of their food diaries or recorded the information on their phones and sent it to the researchers with all the necessary details (time, quantity, brand, etc.). The MPM was followed through text messages exchanged between the researchers and participants [26,27].

Participants were instructed to provide detailed information about the foods and beverages consumed, including commercial brands, ingredients used in homemade preparations, and mealtimes. Portions were estimated using household measures, such as spoons and cups, and later converted into units of mass (grams) and volume (milliliters). To facilitate the characterization of food items by participants and avoid researcher bias, a photographic manual for food quantification was provided, including pictures of various portion sizes [28]. The data obtained from the food diaries were individually checked by a trained nutritionist to monitor the quality of the information collected. The quantitative assessment of dietary intake was performed with the aid of the Virtual Nutri Plus® and the Brazilian Food Composition Table (TACO) [27].

2.10. Body Composition Data Collection

Initially, participants were weighed using a calibrated analog scale with a capacity of 150 kg and precision to the nearest 100 g. Participants were asked to remove footwear and wear light clothing, with the same requirements for the bioimpedance test performed after the weighing procedure.

Bioimpedance (BIA), tetrapolar BIA1010 Sanny® (São Paulo, Brazil, was used to estimate the lean mass and fat mass percentages. BIA is a non-invasive, easy-to-apply assessment used for evaluating body composition and estimating the structural components of the body, including muscles, bones, organs, and mass fat. To perform the inter-individual analyses, the fat mass and fat-free mass were adjusted for the participants' height, using the Fat Mass Index (FMI) and Fat-Free Mass Index (FFMI). The evaluation was performed by the researcher, using a tetrapolar BIA1010 Sanny® bioimpedance device with Ag/AgCl (silver/silver chloride) surface electrodes, according to the following protocol: participants had to be outside their menstrual period, wear light clothing, and wear no metal accessories. At the time of the test, participants must have fasted (food and liquids) for at least four hours before the exam. Additionally, participants were recommended to avoid coffee or alcohol in the 24 h leading up to the test and were instructed not to engage in physical activities during the six hours leading up to the test.

2.11. Sleep Quality

The Pittsburgh Sleep Quality Index (PSQI) was used to assess participant sleep quality. This instrument has been validated for evaluating sleep quality in Brazilians [29]. The results are grouped into seven components as follows: subjective sleep quality; sleep latency; sleep duration; habitual sleep efficiency and sleep disturbances; use of sleep medications; and daytime dysfunction. The total score ranges from 0 to 21 points and the results are classified as follows: a score of 0 to 4 indicates good sleep quality; a score of 5 to 10 indicates poor sleep quality; and a score above 10 indicates sleep disturbances.

2.12. Ethical Procedures

This study was submitted to and approved by the Ethics Committees of the participating institutions, namely, the University of São Paulo (permit no. 5.468.644 and 5.650.216); Catholic University of Santos (permit no. 5.650.216 and 5.650.216); and the Hospital (permit no. 5.658.804 and 5.650.216). All participants were volunteers, and data collection ensued only after the subjects had read and signed the two Informed Consent Forms (ICF), in accordance with all ethical guidelines related to human research stipulated in the Helsinki Declaration.

2.13. Statistical Analysis

The Shapiro–Wilk test was applied to test the normality of quantitative variables. Parametric quantitative variables were expressed as the means and standard deviations (SD).

Descriptive statistical analyses were performed using the chi-square or Fisher Exact tests to compare qualitative variables (race, role, marital status, education, presence of children, smoking, and income) by work shift. Comparisons of quantitative variables were conducted using ANOVA or ANCOVA tests for parametric variables (age, years working at hospital, time working night shifts, calorie intake, protein, carbohydrate, lipids, weight, BMI, fat mass percentage, lean mass, and skeletal muscle mass).

The average food intake during days off and on workdays was calculated, and all baseline food intake analyses were performed applying the adjustment variables time working in hospital (years), role (nurse or nursing technician), and presence of children. The adjustment variable used for the baseline body composition analyses was time working night shifts (years).

To compare baseline data against post-intervention data, the percentage change in dietary intake and body composition was calculated to determine the increase or decrease in percentage terms, facilitating the comparison of changes between the study time points. The formula for calculating the percentage change was as follows:

$$\text{PercentageChange} = \frac{FINAL\ VALUE - INITIAL\ VALUE}{INITIAL\ VALUE} \times 100\%$$

A positive percentage indicates an increase from the initial value, a negative percentage indicates a decrease from the initial value, while a value of zero indicates no change.

The generalized linear model (GLM), adjusted for education and sedentary behavior (time spent sitting), was used to assess the effects of melatonin administration on dietary intake and body composition, considering the following three factors: group (melatonin and placebo), work-shift type (morning, afternoon, or night), and menopausal status (menopausal or non-menopausal), with the application of the post hoc Bonferroni test. On all tests, a p-value < 0.05 was considered significant. The data were analyzed using Jamovi software Version 2.3.28. The graphs were plotted using GraphPad Prism® software, while the figures and visual elements were produced using Canva®.

3. Results

3.1. Sociodemographic Data

The mean age of participants was 46 ± 5.5 years. No statistically significant group differences were observed for work shift in relation to sociodemographic variables. Regarding comparisons between the intervention and placebo groups within shifts, participants in the placebo group on the night shift were older and spent more time sitting on their days off compared to those in the intervention group. No other differences were found (Table 1).

Table 1. Sociodemographic characteristics of participants by work shift (n = 46).

Shift	MORNING (*n* = 16)		*	AFTERNOON (*n* = 15)		*	NIGHT (*n* = 15)		*	**
Variable	*n* (%) or Mean ± SD			*n* (%) or Mean ± SD			*n* (%) or Mean ± SD			
	Intervention (*n* = 7)	Placebo (*n* = 9)		Intervention (*n* = 8)	Placebo (*n* = 7)		Intervention (*n* = 7)	Placebo (*n* = 8)		
Age (years)	49.9 ± 6.6	45.1 ± 4.3	n.s	47.1 ± 5.7	48.5 ± 5.87	n.s	43.0 ± 3.5	50.0 ± 4.9	**0.01**	n.s
Years working at hospital	14.4 ± 8.4	11.3 ± 3.7	n.s	6.4 ± 3.6	10.4 ± 9.32	n.s	9.9 ± 2.5	9.8 ± 4.3	n.s	n.s
Years working night shifts	4.6 ± 3.5	5.2 ± 4.5	n.s	9.2 ± 9.2	5.2 ± 4.4	n.s	7.3 ± 7.0	7.7 ± 3.8	n.s	n.s
Minutes sitting on days off	244 ± 82	251 ± 150	n.s	300 ± 137	326 ± 129	n.s	197 ± 96.2	251 ± 142	**0.04**	n.s
Menopausal status			n.s			n.s			n.s	n.s
Menopausal	3 (18.8)	3 (18.8)		1 (6.7)	3 (20.0)		3 (20.0)	3 (20.0)		
Non-menopausal	4 (25.0)	6 (37.5)		7 (46.7)	4 (26.7)		4 (26.7)	5 (33.3)		
Race/Ethnicity			n.s			n.s			n.s	n.s
White	2 (12.5)	6 (37.5)		4 (26.7)	4 (26.7)		2 (13.3)	4 (26.7)		
Non-White (Brown, Black, and Yellow)	5 (31.3)	3 (18.8)		4 (26.7)	3 (20.0)		5 (33.3)	4 (26.7)		
Occupation			n.s			n.s			n.s	n.s
Nurse	2 (12.5)	2 (12.5)		0 (0)	3 (20.0)		2 (13.3)	3 (20.0)		
Nursing Technician	5 (31.3)	7 (48.8)		8 (53.3)	4 (26.7)		5 (33.3)	5 (33.3)		
Marital status			n.s			n.s			n.s	n.s
No partner (single, widowed, or separated/divorced)	1 (6.3)	2 (12.5)		5 (33.3)	3 (20.0)		2 (13.3)	3 (20.0)		
With partner (married or living with partner)	6 (37.5)	7 (43.8)		3 (20.0)	44 (26.7)		5 (33.3)	5 (33.3)		
Education level			n.s			n.s				n.s
High school graduate	0 (0)	0 (0)		0 (0)	1 (6.7)		0 (0)	0 (0)		
Completed high school and technical education	3 (18.8)	7 (43.8)		5 (33.3)	3 (20.0)		3 (20.0)	1 (6.7)		
Higher education in progress or incomplete	1 (6.3)	0 (0)		0 (0)	0 (0)		1 (6.7)	1 (6.7)		
Completed higher education	0 (0)	1 (6.3)		1 (6.7)	0 (0)		1 (6.7)	0 (0)		
Postgraduate studies in progress or incomplete	3 (18.8)	1 (6.3)		2 (13.3)	3 (20.0)		2 (13.3)	3 (20.0)		
Has children			n.s			n.s			n.s	n.s
Yes	6 (37.5)	8 (50.0)		6 (40.0)	6 (40.0)		7 (46.7)	6 (40.0)		
No	1 (6.3)	1 (6.3)		2 (13.3)	1 (6.7)		0 (0)	2 (13.3)		
Smoking status			n.s			n.s			n.s	n.s
Never smoked	7 (43.8)	8 (50.0)		8 (53.3)	6 (40.0)		6 (40.0)	6 (40.0)		
Not currently smoking but past smoker	0 (0)	1 (6.3)		0 (0)	1 (6.7)		1 (6.7)	2 (13.3)		
Yes	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)		
Income			n.s			n.s			n.s	n.s
≤5400.00	6 (37.5)	9 (56.3)		8 (53.3)	4 (26.7)		6 (40.0)	6 (40.0)		
5401.00–9001.00	1 (6.3)	0 (0)		0 (0)	3 (20.0)		1 (6.7)	2 (13.3)		
Does not know/does not want to respond	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)		

* *p*-value for difference in work shifts between intervention and placebo groups (*t*-test for quantitative variables and Fisher's exact test for qualitative variables). ** *p*-value for difference between work shifts (two-way ANOVA—shift and group for quantitative variables and Fisher's exact test for qualitative variables). n.s: not significant.

3.2. Food Consumption and Body Composition

At baseline, significant differences were evident between work shifts in terms of average total calorie and carbohydrate intake (Table 2). The average calorie intake was higher among night-shift workers than afternoon-shift workers (post hoc Bonferroni $p = 0.02$), and the mean carbohydrate intake was also significantly greater in night-shift workers than evening-shift workers (post hoc Bonferroni $p < 0.01$).

Table 2. Dietary intake and body composition of participants at baseline by work shift ($n = 46$).

Shift	MORNING ($n = 16$)		*	AFTERNOON ($n = 15$)		*	NIGHT ($n = 15$)		*	**
Variable	n (%) or Mean ± SD Intervention ($n = 7$)	Placebo ($n = 9$)		n (%) or Mean ± SD Intervention ($n = 8$)	Placebo ($n = 7$)		n (%) or Mean ± SD Intervention ($n = 7$)	Placebo ($n = 8$)		
Dietary intake										
Calories (kcal)	1.522 ± 437	1.927 ± 442	n.s	1.609 ± 269	1.524 ± 254	n.s	2.010 ± 408	1.921 ± 402	n.s	**0.03**
Proteins (g)	66.3 ± 21.1	69.4 ± 15.1	n.s	65.2 ± 10.6	76.7 ± 23.3	n.s	95.9 ± 31.6	78.0 ± 19.8	n.s	0.12
Carbohydrates (g)	191 ± 41.4	248 ± 71.6	n.s	207 ± 33.4	185 ± 26.5	n.s	234 ± 34.9	261 ± 58.5	n.s	**0.02**
Lipids (g)	56.4 ± 30.7	72.4 ± 13.7	n.s	59.3 ± 20.7	53.0 ± 21.8	n.s	78.4 ± 25.3	69.8 ± 20.1	n.s	0.11
Body composition										
Weight (kg)	66.1 ± 7.15	73.7 ± 12.4	n.s	77.8 ± 20.5	83.6 ± 13.2	n.s	70.3 ± 10.9	70.4 ± 13.2	n.s	**<0.01**
BMI (kg/m²)	26.0 ± 3.32	28.2 ± 3.89	n.s	28.3 ± 6.69	31.5 ± 4.74	n.s	27.8 ± 4.55	27.1 ± 5.51	n.s	**0.04**
Fat-free mass (%)	62.5 ± 6.76	56.0 ± 6.71	n.s	60.0 ± 10.5	52.5 ± 6.47	n.s	60.1 ± 8.29	62.7 ± 7.07	n.s	**0.02**
Fat-free index (FFMI)	16.1 ± 0.96	15.6 ± 1.18	n.s	16.5 ± 1.06	16.3 ± 0.99	n.s	16.5 ± 1.76	16.7 ± 1.69	n.s	0.58
Fat mass (%)	37.5 ± 6.76	44.0 ± 6.71	n.s	40.0 ± 10.5	47.5 ± 6.47	n.s	39.9 ± 8.29	37.3 ± 7.07	n.s	**0.02**
Fat mass index (FMI)	9.93 ± 2.81	12.6 ± 6.02	n.s	11.9 ± 3.79	15.2 ± 3.54	n.s	11.3 ± 4.14	10.4 ± 3.92	n.s	**0.02**
Skeletal muscle mass (%)	30.1 ± 3.71	27.2 ± 3.63	n.s	29.5 ± 5.67	25.8 ± 2.93	n.s	29.2 ± 5.03	30.6 ± 4.67	n.s	0.07

* p-value of the difference in work shifts between the intervention and placebo groups (t-test). ** p-value of the difference between the shift (morning, afternoon, and night) (ANCOVA). Analysis of covariance (ANCOVA) for dietary intake was adjusted for years of hospital employment, job role (nurse or nursing technician), and whether participants had children. ANCOVA for body composition was adjusted for the time working night shifts (years). n.s: not significant.

Body composition data, adjusted for time working night shifts, revealed that the body weight of evening-shift workers was significantly higher than that of workers on the morning or night shifts (post hoc Bonferroni $p < 0.01$ and $p < 0.01$). The mean fat-free mass percentage (total amount of non-fat tissue in body, including muscles, bones, water, organs, and other lean tissues), fat mass percentage, and fat mass index were significantly higher among evening-shift workers than night-shift workers (post hoc Bonferroni $p = 0.02$, $p = 0.02$ and $p = 0.02$).

As expected at the initial stage of the study, no statistically significant differences were found between the intervention and placebo groups at baseline (Table 2).

At baseline, no significant differences in sleep quality were observed between work shifts (morning, evening, and night) or between intervention and placebo groups, regardless of whether assessments were conducted on workdays or days off. All work shifts were associated with poor sleep quality, exhibiting a score of >5 on the Pittsburgh Sleep Quality Index (PSQI).

3.3. Results After Intervention

Urine analysis results indicated that the dose administered was not excessively high, as no morning escape of 6-sulfatoxymelatonin was detected in the samples analyzed. Therefore, the initially planned dose was maintained without any modifications throughout the intervention period.

Following the intervention period, the baseline differences in average calorie and carbohydrate intake between work shifts were no longer evident. However, differences in body composition persisted. Evening-shift workers continued to have significantly higher body weight compared to both morning and night-shift workers (post hoc Bonferroni

$p = 0.01$ and $p < 0.01$). Additionally, differences in the proportions of fat-free mass percentage, fat mass percentage, and fat mass index between evening and night-shift workers remained significant (post hoc Bonferroni $p = 0.05$, $p = 0.05$, and $p = 0.03$) (Table 3).

Table 3. Dietary intake and body composition of participants after melatonin intervention by work shift ($n = 46$).

Shift	MORNING ($n = 16$)			AFTERNOON ($n = 15$)			NIGHT ($n = 15$)			
Variable	n (%) or Mean ± SD Intervention ($n = 7$)	Placebo ($n = 9$)	*	n (%) or Mean ± SD Intervention ($n = 8$)	Placebo ($n = 7$)	*	n (%) or Mean ± SD Intervention ($n = 7$)	Placebo ($n = 8$)	*	**
Dietary intake										
Calories (kcal)	1.604 ± 447	1.732 ± 374	n.s	1.738 ± 315	1.517 ± 219	n.s	1.624 ± 400	1.649 ± 277	n.s	0.98
Proteins (g)	69.8 ± 21.0	71.3 ± 19.3	n.s	68.4 ± 10.9	71.5 ± 14.8	n.s	74.1 ± 12.7	67.3 ± 14.1	n.s	0.79
Carbohydrates (g)	197 ± 56.5	212 ± 61.5	n.s	222 ± 55.9	175 ± 32.3	n.s	199 ± 45.6	224 ± 48.0	n.s	0.62
Lipids (g)	60.1 ± 23.3	68.7 ± 20.5	n.s	67.4 ± 15.7	59.2 ± 30.7	n.s	61.7 ± 23.0	55.6 ± 14.2	n.s	0.84
Body composition										
Weight (kg)	65.2 ± 7.26	73.7 ± 12.9	n.s	78.7 ± 20.6	81.4 ± 14.3	n.s	71.8 ± 13.5	70.4 ± 13.6	n.s	**<0.01**
BMI (kg/m²)	25.6 ± 3.31	28.2 ± 4.00	n.s	28.7 ± 6.68	30.6 ± 5.21	n.s	28.2 ± 5.16	27.0 ± 5.51	n.s	**0.04**
Fat-free mass (%)	59.2 ± 8.81	56.0 ± 7.02	n.s	59.3 ± 9.17	53.8 ± 8.63	n.s	58.4 ± 5.87	62.7 ± 7.88	n.s	**0.05**
Fat-free index (FFMI)	15.6 ± 0.85	15.5 ± 0.83	n.s	16.6 ± 1.30	16.2 ± 1.27	n.s	16.4 ± 2.04	16.6 ± 1.70	n.s	0.24
Fat mass (%)	38.6 ± 7.58	44.0 ± 7.02	n.s	40.7 ± 9.17	46.2 ± 8.63	n.s	41.6 ± 5.87	37.3 ± 7.88	n.s	**0.05**
Fat mass index (FMI)	10.1 ± 3.12	12.6 ± 3.72	n.s	12.2 ± 5.58	14.5 ± 4.54	n.s	12.0 ± 3.84	10.4 ± 3.99	n.s	**0.03**
Skeletal muscle mass (%)	28.5 ± 3.00	26.3 ± 3.06	n.s	30.1 ± 4.68	26.8 ± 4.10	n.s	27.9 ± 3.47	30.1 ± 3.65	n.s	0.15

* p-value of the difference in works shift between the intervention and placebo groups (t-test). ** p-value of the difference between the shift (morning, afternoon, and night) (ANCOVA). Analysis of covariance (ANCOVA) for dietary intake was adjusted for years of hospital employment, job role (nurse or nursing technician), and whether participants had children. ANCOVA for body composition was adjusted for time working night shifts (years). n.s: not significant.

After the intervention, only morning-shift workers showed good sleep quality, while evening- and night-shift workers continued to exhibit poor sleep quality, as observed at baseline ($p < 0.001$).

3.4. Dietary Intake

Among climacteric workers, exogenous melatonin intervention improved sleep quality only for morning-shift workers. Despite this improvement, there were no significant changes in dietary intake ($p = 0.16$) or body composition ($p = 0.07$), as measured by the average calorie intake and fat mass percentage. Thus, the study hypothesis was not confirmed (Figure 3).

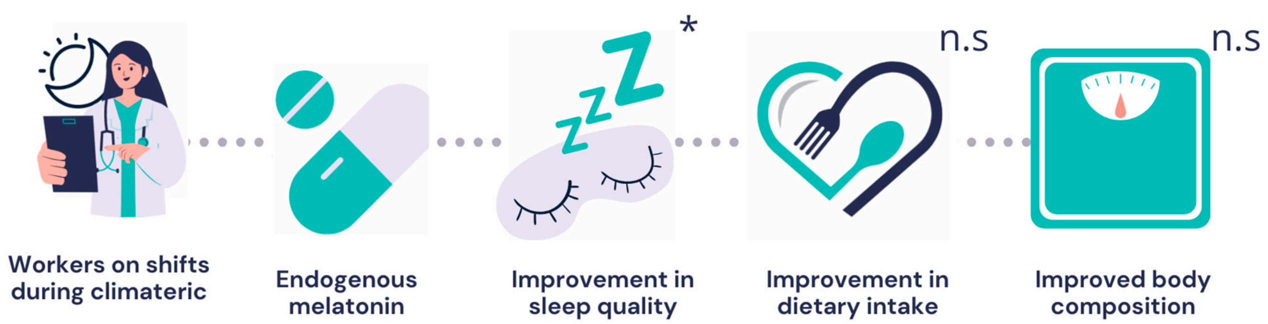

Figure 3. Flowchart of study hypothesis and outcomes ($n = 46$). Legend: * The p-value indicates a statistically significant difference; n.s: not significant.

The percentage change in calorie intake (pre- and post-intervention) showed no significant group difference between participants who received melatonin and those given placebo ($p = 0.16$). However, a significant difference was observed between work shifts ($p = 0.01$), particularly between evening- and night-shift workers (post hoc Bonferroni $p = 0.01$), regardless of intervention or placebo.

Evening-shift participants increased their calorie intake by an average of 3.1% after the intervention, whereas night-shift participants decreased their caloric intake by an average of 16.3% over the three-month intervention period (Figure 4). No differences were found in the average intake of carbohydrates, proteins, or lipids between shifts or groups (intervention or placebo). Additionally, no differences were found in the total calorie intake between postmenopausal and non-postmenopausal participants ($p = 0.97$).

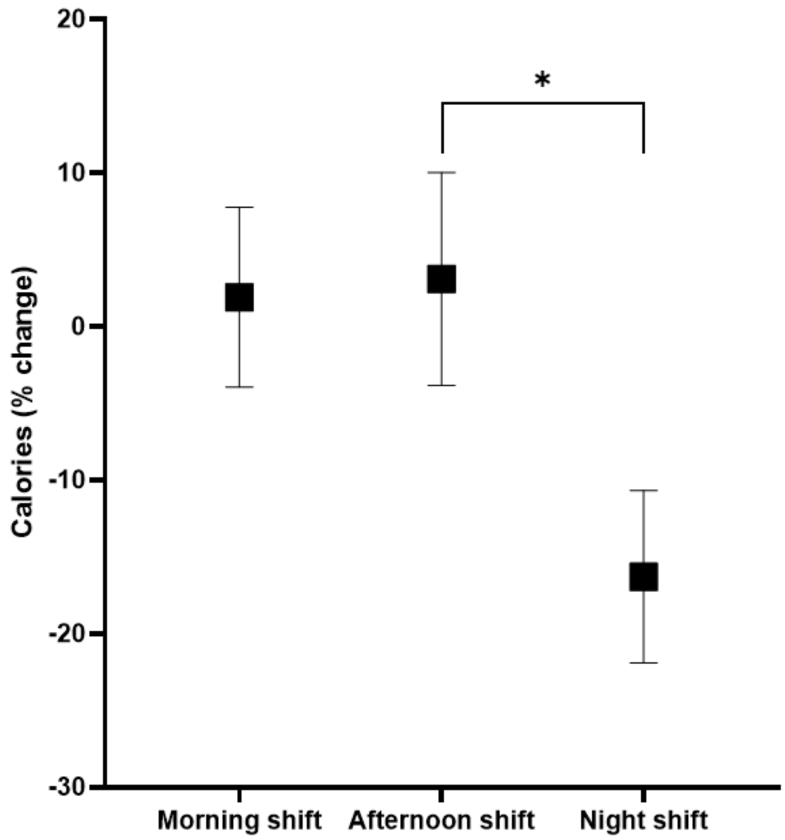

Figure 4. Mean and standard error of percentage change in calorie intake by work shift, irrespective of intervention or placebo ($n = 46$). Significance level: $p < 0.05$ = *. p-values adjusted for education level and sedentary behavior (sitting time). Analysis conducted using GLM test.

3.5. Body Composition

The percentage change in body composition did not differ significantly between the melatonin and placebo groups ($p = 0.07$). No differences in body composition were observed across work shifts.

However, a significant difference was found in the body composition between postmenopausal and non-postmenopausal participants before and after the intervention ($p = 0.04$). The percentage of women in the premenopausal group was 65%, while 35% were postmenopausal. Postmenopausal participants experienced a 2.74% reduction in fat mass percentage, while non-postmenopausal participants exhibited a 3.81% increase; significant differences were also observed in the fat mass index (Figure 5). Similar changes were noted in the average body weight and BMI ($p = 0.03$ and $p = 0.04$, respectively), with postmenopausal participants showing a 1.8% reduction in weight and a 1.67% reduction in BMI. No significant changes were found in the fat-free mass or skeletal muscle mass.

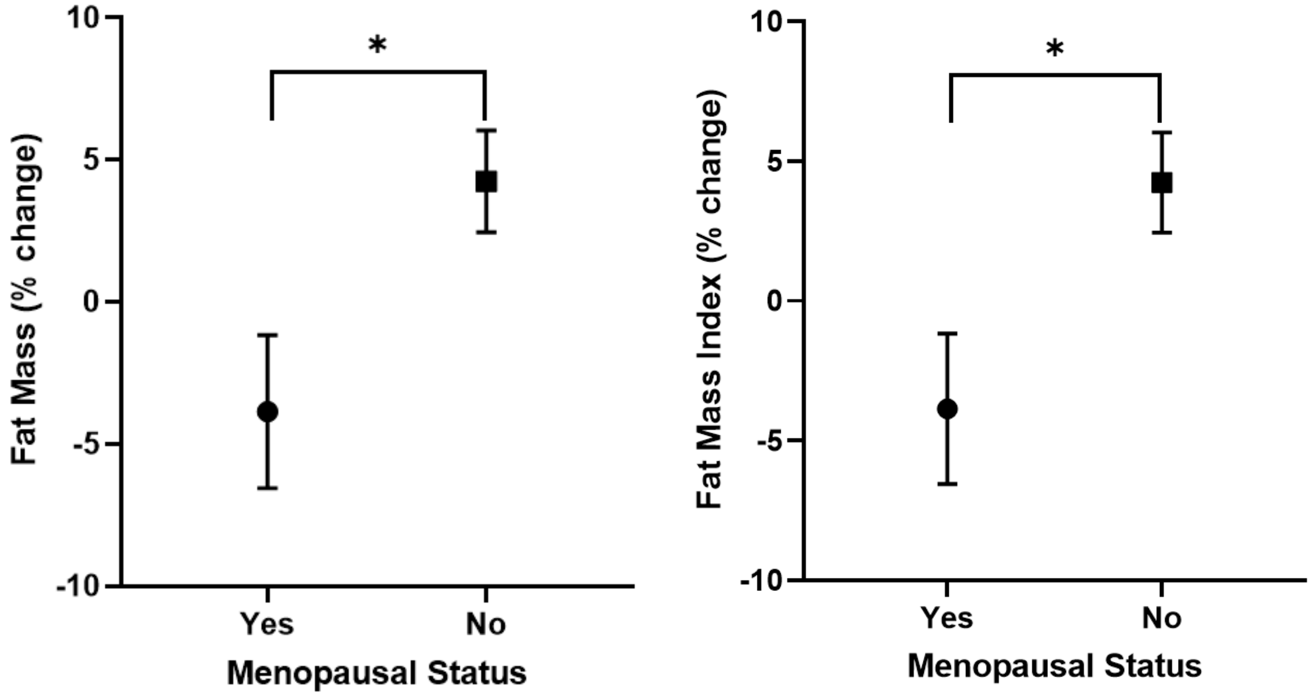

Figure 5. Mean and standard error of percentage change in fat mass by menopausal status (n = 46). Significance level: $p < 0.05$ = *. p-values adjusted for education level and sedentary behavior (sitting time). Analysis conducted using GLM test.

4. Discussion

The initial hypothesis posited that administering exogenous melatonin would enhance dietary choices, positively influencing food consumption and body composition among workers. However, post-intervention analyses revealed that changes in these parameters were more closely associated with work shifts than with melatonin or placebo administration. Notably, only the morning-shift participants exhibited significant improvements in sleep quality, underscoring the potential impact of work schedules on sleep patterns. In contrast, no such enhancements were observed in the afternoon and night shifts. It is important to consider that factors such as melatonin dosage, duration of the intervention, and the demographic characteristics of participants may have significantly influenced melatonin's efficacy in these outcomes [30].

A 2024 meta-analysis involving the general population, after an intervention with exogenous melatonin, revealed no changes in body weight or body mass index (BMI) [30]. In subgroup analyses, lower BMIs were found only in specific populations, such as larger samples ($\geq$50 participants) and younger individuals (<45 years) [30]. These characteristics differ from the population of the present study which comprised a sample of 46 participants with a mean age of 47.2 $\pm$ 5.5 years. Another study assessing overweight night-shift workers showed that dietary habits were unchanged after melatonin administration [31].

Studies involving melatonin have emphasized the importance of evaluating both the formulation and the administration method to maximize its benefits [32]. In this context, a melatonin supplement, combined with magnesium in the form of a coffee capsule, showed improvements in sleep quality in healthy individuals with sleep disorders. However, the scores from the Pittsburgh Sleep Quality Index (PSQI) questionnaire still indicated poor average sleep quality (PSQI > 5) [32]. This result, along with the data obtained in our study, underscores the need for further investigations to identify the specific conditions, doses, and formats in which melatonin exhibits the highest efficacy.

Several randomized controlled trials (RCTs) have investigated the effects of melatonin on body composition and blood pressure (BP) [30,33]. However, the findings from these studies remain controversial, reflecting the variability in outcomes across different populations and study designs [30]. A systematic review with a dose–response meta-analysis suggested that the impact of melatonin supplementation is significantly influenced by factors such as age, gender, dosage, and study duration. While some studies suggest modest benefits in reducing blood pressure and improving body composition, others fail to identify significant changes [30,33].

In the present sample, consisting of mostly overweight workers on fixed day and night shifts in the climacteric phase, the administration of 0.3 mg of melatonin for three months promoted no significant changes in food consumption or body composition. It is important to note that more recent studies have used equivalent physiological doses of melatonin to evaluate different bodily responses [34]. However, a previous study conducted by our group found that melatonin administration in individuals with high circadian misalignment (participants with morning chronotypes) significantly decreased body weight, BMI, waist circumference, and hip circumference, without a change in calorie intake or physical activity levels. The cited study was a double-blind, randomized, placebo-controlled, crossover trial using a 3 mg dose of melatonin administered for 12 weeks in nurses who were younger than those in the present study (mean age 37.1 $\pm$ 5.9 years), though all were overweight [16]. Also, in the present clinical trial, no changes in calorie intake after melatonin intervention were evident. However, unlike the study in Ref. [16], no changes in body composition associated with melatonin administration were found in the present sample.

On the other hand, there was a significant baseline difference between work shifts in terms of dietary consumption for the groups studied. Night-shift workers had a higher calorie and carbohydrate intake than evening-shift workers (the night group consumed 393 calories and 51 g of carbohydrates more than the evening group). Heath et al. [5] found similar results in nurses working evening (1:00–9:30 p.m.) and night shifts (9:00 p.m.–7:30 a.m.). Evening shifts were associated with a lower energy intake compared to night shifts, with a significant difference of 478.02 calories. The study also included a morning shift group working from 7:00 a.m. to 3:30 p.m., where no differences in dietary intake were found between morning, evening, or night shifts, corroborating the findings of the present study.

In qualitative interviews with day- and night-shift nurses, Saggi et al. [35] observed different eating habits between shifts. Participants reported that the availability of energy-dense foods, such as chocolate, chips, and cookies, during the night shift encouraged their consumption due to easy access. In addition to reporting increased consumption of energy-dense foods during the night shift, participants also agreed that their daily eating patterns were altered when working these shifts.

The results of the present study, akin to previous reports, show higher food consumption during night shifts; however, poor body composition was not observed among these workers, and was instead observed in evening-shift workers. Evening workers had higher body weight, fat mass percentage, and fat mass index and lower fat-free mass percentage compared to night-shift workers. Several factors may explain these results. The first relates to the number of years working on night shifts, where evening-shift workers reported working night shifts for an average of 7.25 years, a similar period to that of night-shift workers (7.57 years). By contrast, morning-shift workers had only 4.9 years of previous night-shift work experience. Furthermore, the evening workers reported significant weight gain during their night-shift years and a subsequent difficulty losing weight.

In the present study, postmenopausal women showed a reduction in BMI, body weight, fat mass percentage, and fat mass index after three months, regardless of whether they were in the melatonin or placebo group.

Amstrup et al. [36] reported a significant 7.2% reduction in total body fat percentage in postmenopausal women after one year of combined melatonin treatment (1 and 3 mg). Although the current study found a significant 2.7% reduction in body fat among post-menopausal women compared to those who had yet to reach menopause, this reduction occurred independently of the study group (intervention or placebo). It is possible that the physiological doses of melatonin are insufficient for achieving similar results, i.e., reduced body fat.

After the intervention period, the evening-shift group reported an increase in food consumption, while the night-shift group reported a decrease, irrespective of group allocation (intervention or placebo). This behavior might be attributed to factors such as variations in dietary habits associated with differences in attention levels and fatigue or other behavioral factors influencing data recording [5,37].

This study has some limitations, such as the frequency of melatonin administration. Depending on clinical application, a wide variety of melatonin dosages are used, such as 0.1 mg/day for central clock synchronization and 0.6–5.0 mg/day for treating sleep disorders [13]. The dose used in this study was 0.3 mg every other day. Alternate-day administration was necessary so that all workers could standardize with night-shift workers. However, this may have impaired daytime workers, who had the opportunity to sleep every night but took melatonin alternately, thereby failing to yield the expected results after 90 nights of melatonin administration. Nevertheless, it is important to note that the exogenous melatonin dose in this study was established so as to ascertain the effect of a dose close to the physiological concentration. Furthermore, dietary intake was analyzed using 24 h food diaries, a widely used method. Although direct observation with food weighing is the most accurate approach for assessing dietary intake, its application in field studies is impractical. For decades, food diaries have been employed to produce reliable and valid records of food intake, making them the method of choice for investigating human eating behaviors [38]. Additionally, the 24 h dietary diary offers the advantage of recording foods and beverages as they are consumed, thereby minimizing the omission of items due to memory failure [39], which further supports their utility in our analysis. Future research should explore alternative methodologies that enhance the accuracy of dietary intake assessment while maintaining feasibility. The incorporation of specific biomarkers and the refinement of self-reported dietary assessment tools may contribute to improving methodological rigor and the reliability of the results.

5. Conclusions

In the present study, calorie and carbohydrate intake among participants before the intervention period was higher for night-shift workers than evening-shift workers. After the intervention, calorie intake increased among evening-shift workers and decreased among night-shift workers, resulting in no significant differences in dietary intake between shift groups, regardless of melatonin or placebo administration. This outcome may suggest that the study participants modified their eating habits due to factors independent of the intervention.

Regarding body composition, a difference was evident between women who had already undergone menopause and those still in the climacteric phase, irrespective of the intervention. Postmenopausal participants showed a reduction in body weight, BMI, and fat mass compared to individuals in the climacteric phase. Despite changes in fat mass after the intervention, baseline differences persisted, with evening-shift workers continuing to have higher BMI, weight, and fat mass than night-shift workers.

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Institutional Review Board Statement: This study was submitted to and approved by the Ethics Committees of the participating institutions, namely, the University of São Paulo (permit no. 5.468.644 and 5.650.216); Catholic University of Santos (permit no. 5.650.216 and 5.650.216); and the Hospital (permit no. 5.658.804 and 5.650.216). All participants were volunteers, and data collection ensued only after the subjects had read and signed the two Informed Consent Forms (ICF), in accordance with all ethical guidelines related to human research stipulated in the Helsinki Declaration.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Correspondence and requests for materials should be addressed to crmoreno@usp.br.

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