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Melatonin reduces muscle damage, inflammation and oxidative stress induced by exhaustive exercise in people with overweight/obesity

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ABSTRACT

Background: Intense physical exercise leads to inflammation, oxidative stress and muscle damage, and these responses are of greater magnitude in people with obesity. Melatonin (MLT) is considered an endogenous antioxidant which may have beneficial effects against inflammation, oxidative stress and promote tissue repair after exercise. The aim of this study was to examine the effect of MLT on inflammatory parameters,

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oxidative stress and muscle damage in people with overweight/obesity after a high-intensity interval exercise (HIIE). *Methods:* A total of 23 subjects with obesity (9 men and 14 women) age: 33.26 ± 9.81 years, BMI: 37.75 ± 8.87 $kg.m^{-2}$ were randomized to participate in two experimental sessions: HIIE + Placebo and HIIE + MLT (3 mg). The HIIE protocol corresponds to 8 intervals of 1 min (90% of the maximal aerobic power (MAP)) alternating with 2 min recovery (45% of the MAP). Blood samples were drawn before and 5 min after each exercise session. *Results:* MLT ingestion attenuated the increase of inflammation (C-reactive protein, white blood cells ($P < 0.001$, $\eta p^2 = 0.45$; for both) and Neutrophils ($P < 0.01$, $\eta p^2 = 0.36$)) and hepatic and muscle damage (Aspartate aminotransferase ($P < 0.01$, $\eta p^2 = 0.25$), Alanine aminotransferase ($P < 0.01$, $\eta p^2 = 0.27$) and Creatine kinase ($P = 0.02$, $\eta p^2 = 0.23$). MLT also attenuated the exercise induced lipid and protein peroxidation (i.e., Malondialdehyde ($P = 0.03$, $\eta p^2 = 0.19$) and AOPP ($P < 0.001$, $\eta p^2 = 0.55$)). Concerning the antioxidant status, MLT intake increased Thiol ($P < 0.01$, $\eta p^2 = 0.26$) and Catalase ($P < 0.01$, $\eta p^2 = 0.32$) and decreased Uric acid ($P = 0.02$, $\eta p^2 = 0.2$) and Total bilirubin ($P < 0.01$, $\eta p^2 = 0.33$). *Conclusions:* MLT intake before HIIE reduced muscle damage by modulating oxidative stress and preventing overexpression of the pro-inflammatory mediators in people with obesity.

KEYWORDS

melatonin, obesity, strenuous exercise, inflammation, oxidative stress

INTRODUCTION

Obesity, the biggest health issue of the 21st century, is a chronic condition that affects human quality of life, physiologically, socially, regardless of cultural, financial or ethnic background [1]. Obesity is related with the development of wide variety of comorbidities, such as type 2 diabetes, dyslipidemia, cardiovascular complications, cancer, liver and kidney dysfunction, infertility and asthma [2]. Particularly, it is well established that abdominal fat is the major factor responsible for these pathologies [3]. Exercise interventions have been used to mitigate the harmful impacts of obesity by improving body composition, blood pressure, lipid profile and glycemic control [4]. In recent times, high intensity interval training has become a popular alternative to provide similar benefits as those obtained by moderate intensity continuous training including body fat reduction primarily because of its time efficiency [5–7] and its ability to increase lipid oxidation through the excessive consumption of oxygen [8] and decreased appetite after exercise [9] which reduces waist circumference and the risk of cardiovascular diseases [10]. Paradoxically, intense exercise have been considered as a strong stressor leading to a myriad of adverse effects such as fatigue, impaired immune function and muscle damage manifested by muscle pain, prolonged loss of muscle function and muscle protein leakage [11]. This damage is associated with a high production of free radicals (FR) and stimulation of inflammatory cells [12] such as neutrophils, macrophages, T lymphocytes and mast cells [13]. Increased oxygen uptake in muscle fibers decreases intracellular pressure during exercise [14], which may promote increased generation of reactive oxygen and nitrogen species in skeletal muscle [15]. Exercise-induced FR production lead to increase lipid peroxidation and protein denaturation, inactivation of enzymes, modification of DNA and RNA, and cells function alteration [16].

Melatonin (N-acetyl-5-methoxytryptamine) (MLT) is an endogenous pineal gland neurohormone whose secretion is controlled by the light/dark cycle [17]. This indole may be presented in high concentration in mitochondria, an organelle in which free radicals are produced

in abundance, presumably via its uptake through the oligopeptide transporters PEPT1 and PEPT2 and due to its intramitochondrial synthesis. Thus, MLT may have a key role to scavenge radicals and reduce the degree of oxidative damage [18, 19]. MLT receptors can be located throughout the body (e.g., skeletal muscles, mitochondria, pancreas, adipose tissue, retina, brain, platelets, and skin) [20]. Particularly, MLT plays an important role in the management of insomnia, obesity, diabetes, and metabolic syndrome [21]. Moreover, MLT can cross cell membrane and directly scavenge FR which reduces oxidative stress [22]. Recent findings support the beneficial effects of MLT against oxidative stress, inflammation and cellular apoptosis in humans living with obesity and animal models of obesity [23]. It has been shown that the level of MLT is inversely correlated with obesity and its reduction is strongly linked to insulin resistance [24]. Indeed, MLT ingestion can stimulate insulin secretion, which reduces plasma glucose levels and FR production [24]. In addition, MLT suppresses the translocation of nuclear factor kappa B (NF-κB) which inhibits the release of pro-inflammatory cytokines such as interleukin-6 (IL-6) and IL-1β [22]. Few studies have examined the combined effect of MLT and exercise. For example, in diabetic rats, it has been demonstrated that MLT administration inhibits the increase of FR production and inflammation and promotes antioxidant activity after acute swimming exercise [25]. Another study conducted in young footballers has shown that acute MLT (6 mg) ingestion before high intensity continuous exercise (heart rate (HR) = 135 beats/min) improved the immune defenses and lipid metabolism and reversed the oxidative damage generated after exercise by increasing total antioxidant activity which can lead to improve physical performance [26]. To the best of the authors' knowledge, no study has been conducted to investigate the acute effect of MLT intake on biochemical responses following a high-intensity interval exercise (HIIE) in people with obesity. Thus, the aim of the present study was to explore the effect of MLT ingestion on inflammatory parameters, muscle and liver damage, and biomarkers of oxidative stress in subjects living with overweight/obesity following a HIIE session. We hypothesized that MLT would exert a protective effect against inflammation, tissue damage and oxidative stress after the proposed exercise.

MATERIALS AND METHODS

Subjects

A total of 23 subjects with overweight/obesity (9 men and 14 women) participated voluntarily in this study. Participants' characteristics are listed in Table 1. Weight and body composition were measured with a bioelectrical impedance (Tanita BC-418, Tokyo, Japan). Participants were informed about the experimental protocol and aims of the research. Subjects were not involved in regular physical activity in the 6 months preceding the study and reported no history of injury or disease, these conditions were determined after answering the SF-36 questionnaire [27]. All participants signed an informed consent and they were free to withdraw their participation at any time during the study. The study complies with the declaration of Helsinki, approved by the regional ethics committee and registered with the Pan African Clinical Trials Registry (PACTR202006677216503).

Study procedure

Participants performed a maximal exercise test, a familiarization session and two experimental exercise trials, separated by a washout period of one week. The first visit was planned to

Table 1. Characteristics of the subjects

Characteristics	Mean ± (SD)
Age (years)	33.26 ± 9.81
Weight (kg)	107.38 ± 24.05
height (cm)	169 ± 7.86
Body mass index ($kg.m^{-2}$)	37.75 ± 8.87
waist circumference (cm)	111.59 ± 16.27
hip circumference (cm)	127.35 ± 18.27
waist-to-hip ratio	0.88 ± 0.09
% fat	41.23 ± 11.11
Fat mass (kg)	45.67 ± 20.68
Lean mass (kg)	61.43 ± 9.88
Hydric mass (kg)	44.65 ± 7.02
Basal metabolism (kcal)	1911.74 ± 285.86
Max aerobic power (W)	164.23 ± 41.73
VO_{2max} ($L.min^{-1}$)	2.92 ± 1.18
VO_{2max} ($mL.kg.min^{-1}$)	28.76 ± 2.13
Resting heart rate (bpm)	86.54 ± 9.12
Resting systolic blood pressure (mmHg)	121.38 ± 16.65
Resting diastolic blood pressure (mmHg)	75.69 ± 13.06

determine maximal oxygen consumption (VO_{2max}) and maximal aerobic power (MAP). At the second visit, participants were familiarized with experimental trials. The remaining experimental trials included a HIIE session with MLT ingestion prior to exercise and the other session was a HIIE with placebo (PLA) consumption. Participants were therefore asked to report all food and drink eaten and to refrain from any activity throughout the 48 h prior to their next visit. All participants have finished PLA and MLT sessions in a single blind randomized design. All procedures were carried in hospital and under the supervision of the physicians. The following sections describe the procedures in detail.

Graded exercise test

The participants performed an incremental test on an ergocycle (Ergoline, Ergoselect 100, France) to confirm the absence of a cardiorespiratory pathology and to determine VO_{2max}, the ventilation thresholds and MAP during pedaling. The cadence rate was maintained between 60 and 70 rpm. After 5 min of warm-up empty pedaling, the protocol started by an initial power of 35 W then the intensity increased gradually 15 W every minute until the participant could no longer maintain the power (the rate fell under 50 rpm) [8].

The test was considered maximal if it filled at least two of the following criteria: a plateau of VO_2, HR greater than 90% HR max, perceived exertion >9 and a respiratory exchange ratio ≥ 1.15 [28].

Experimental sessions

All subjects performed HIIE in a randomized order, after taking MLT or PLA. A rest period of at least one week was set between sessions. Subjects arrived at the laboratory between 7:00 a.m. and

9:00 am., after an overnight fasting for at least 8 h. They were also advised to follow a similar diet and abstain from vigorous physical activity for at least 48 h before taking blood samples. Time of day has been normalized to minimize potential diurnal variations in physical performance and hormonal levels. After a 10-min rest period, blood samples (10 ml) were taken from the antecubital vein. Then, each subject received 3 mg of MLT (Jamieson Laboratories, Toronto, Canada) or PLA (starch capsule, Galpharma Laboratories, Sfax, Tunisia) 40 min prior to exercise, the necessary time to detect MLT in blood before its elimination [26]. A second blood sample was taken 5 min after exercise cessation. HR was monitored throughout each exercise session using a Polar HR monitor (H7, Canada) (Fig. 1).

The HIIE protocol includes a warm up and recovery period (empty pedaling) of 3 min each, and 8 intervals of 1 min (90% of MAP) alternated with 2 min of recovery (45% of MAP) (Fig. 1).

Blood collection and biochemical analysis

Blood samples were taken, in a sitting position, before and 5 min after each experimental session (HIIE + MLT and HIIE + PLA). Blood samples were divided into 3 different tubes. The first tube (4 ml) containing lithium heparin was used to measure routine biochemistry parameters (Uric acid (UA), Total bilirubin (TBIL), ASAT (Aspartate aminotransferase), ALAT (Alanine aminotransferase), CPK (Creatine phosphokinase), CRP (C-reactive protein) and LDH (Lactate dehydrogenase)) evaluated in automated biochemistry analyzer (Beckman Coulter Synchron CX9 PRO) of the biochemistry laboratory of CHU Habib Bourguiba (Sfax). The second tube (3 ml) containing EDTA was used to determine oxidative stress parameters (malondialdehyde (MDA), advanced oxidation protein products (AOPP), thiol and catalase (CAT)). The third tube (3 ml) containing EDTA was used to measure total white blood cells (WBC), neutrophils, lymphocytes and monocytes counts, evaluated in automated biochemistry analyzer (Beckman Coulter LH 780) of the hematology laboratory of the CHU Habib Bourguiba (Sfax). Both the first and second tubes were immediately centrifuged at 2,500 rpm for 10 min at 4 °C. The plasma was separated into aliquots and held at −80 °C until analyzed.

Determination of lipid peroxidation

MDA was measured according to the procedures described by Wong et al. [29]. A mixture of 0.5 ml of plasma was incubated with 0.1 ml of tris-HCL buffer (pH 7.2) in a bathwater at 37 °C. After incubation, 9 ml of distilled water and 2 ml of 0.6% thiobarbituric acid were applied to

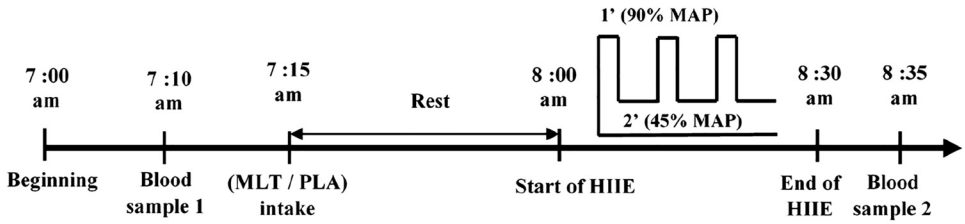

Fig. 1: Experimental sessions.
Abbreviations. MLT, melatonin; PLA, placebo; HIIE, high-intensity interval exercise; MAP, maximal aerobic power.

0.5 ml of incubated solution and then heated in a boiling bathwater for 30 min. After cooling, 5 ml of n-butanol was added and the mixture was again vigorously stirred. The n-butanol layer was separated by centrifugation at 1,000 g for 10 min, and the production amount of malonic MDA was measured at 532 nm in a spectrophotometer (Libra S21, Biochrom).

Determination of protein peroxidation

Plasma AOPP levels were measured according to Witko-Sarsat et al. [30]. 18 µl of plasma was diluted in 200 µl and mixed with 8.1 ml of PBS, 1.5 ml of acetic acid and 400 µl of potassium iodide, then centrifuged for 5 min. 200 µl of the reaction mixture was placed into a microtiter plate. The absorbance was immediately read at 340 nm on the microplate reader (PowerWave XS2, Biotek) against a blank containing 200 ml of PBS. The AOPP concentrations were expressed in µmol/L of Chloramine-T equivalents.

Determination of total thiol levels

Thiol was measured by spectrophotometric method according to the procedures described by Hu [31]. 50 µl of plasma was mixed with 1,000 µl de Tris-EDTA (0.25 M, 20 mM, pH = 8.2) and absorbance at 412 hm was read. Next, 20 µl of DTNB (10 mM) was added to the solution and the mixture was placed away from light. After 10 min the absorbance was read again. The concentration of thiols was calculated from the difference of these two results divided by the coefficient of the standard range and by the concentration of plasma proteins. the result was expressed in µmol/L.

Determination of catalase activity

Catalase activity was measured at 240 nm using a visible UV spectrophotometer (JENWAY 6105), by the variation of the optical density following the hydrolysis of H_2O_2 at an incubation temperature of 25 °C for 1 min [32].

Statistical analysis

All data were analyzed using SPSS statistical software (v.23, IBM, New York, USA). The results were presented as mean ± standard deviation (SD) in the text and tables. The normality of the distribution was verified with Shapiro-Wilk W-test and considered significant for $P > 0.05$. The effects of exercise and supplement used were assessed by two-way analysis of variance (ANOVA) with repeated measures [treatment (MLT × PLA) and exercise (pre exercise × post exercise)]. When a significant difference was detected, a Bonferroni post-hoc test was performed to locate differences in pairs. The paired *t*-test or wilcoxon test were performed to compare the percentage changes between both conditions. Effect sizes (ES) were calculated as partial eta-square (η_P^2) and interpreted as trivial $\eta_P^2 < 0.2$, small $0.2 \leq \eta_P^2 < 0.5$, moderate $0.5 \leq \eta_P^2 < 0.8$ and large $\eta_P^2 \geq 0.8$ [33]. For all tests, the significance level was set at $P \leq 0.05$.

RESULTS

All the results of ANOVA and the pairwise comparisons of the post hoc test are presented in Table 2.

Table 2. Biochemical responses to HIIE session following melatonin (MLT) and placebo (PLA) ingestion

Variable	PLA		MLT		Anova		
	Pre	Post	Pre	Post	Exercise effect $F_{(1,22)}$ (P; ηp^2)	Melatonin effect $F_{(1,22)}$ (P; ηp^2)	Interaction $F_{(1,22)}$ (P; ηp^2)
Biomarkers of inflammation							
WBC (10^3/µL)	7.8 ± 2.3	9.0 ± 2.4***	7.6 ± 2.2	8.0 ± 2.0***	55.2 ($P < 0.001$; 0.71)	4.32 ($P = 0.049$; 0.16)	18.6 ($P < 0.001$; 0.45)
Neutrophils (10^3/µL)	4.6 ± 1.7	5.4 ± 1.8***	4.3 ± 1.4	4.6 ± 1.0**	40.6 ($P < 0.001$; 0.64)	5.24 ($P = 0.032$; 0.19)	12.6 ($P < 0.01$; 0.36)
Lymphocytes (10^3/µL)	2.4 ± 0.6	2.6 ± 0.74	2.4 ± 0.7	2.6 ± 0.7	4.02 ($P = 0.057$)	0.01 ($P = 0.93$)	0.86 ($P = 0.36$)
Monocytes (10^3/µL)	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	0.6 ± 0.2*	2.77 ($P = 0.11$)	0.29 ($P = 0.59$)	1.64 ($P = 0.21$)
CRP (mg.L^{-1})	4.2 ± 2.4	4.7 ± 2.5***	4.0 ± 2.7	4.3 ± 2.9**	87.6 ($P < 0.001$; 0.79)	0.33 (P =0.56)	18.1 ($P < 0.001$; 0.45)
Biomarkers of muscle and liver damage							
ASAT (U/L)	17.0 ± 3.2	19.0 ± 3.0***	17.3 ± 3.0	18.0 ± 3.3***	53.5 ($P < 0.001$; 0.7)	0.0004 ($P = 0.98$)	7.37 ($P < 0.01$; 0.25)
ALAT (U/L)	20.7 ± 7.0	22.1 ± 7.3 ***	21.3 ± 3.9	22.0 ± 4.2*	16.8 ($P < 0.001$; 0.43)	0.06 ($P = 0.81$)	8.21 ($P < 0.01$; 0.27)
CPK (U/L)	126 ± 44.0	139 ± 52.6 ***	122 ± 38.6	127 ± 36.7***	50.6 ($P < 0.001$; 0.69)	1.17 ($P = 1.17$)	6.63 ($P = 0.02$; 0.23)
LDH (Ul/L)	185.6 ± 33	200 ± 33 ***	183 ± 34.8	193 ± 39.7 **	23.7 ($P < 0.001$; 0.51)	1.59 ($P = 0.22$)	1.27 ($P = 0.27$)
Biomarkers of radical damage							
MDA (µmol.L^{-1})	5.9 ± 3.0	7.3 ± 3.8 ***	5.4 ± 2.6	5.9 ± 2.5*	30.2 ($P < 0.001$; 0.57)	1.46 ($P = 0.23$)	5.39 ($P = 0.03$; 0.19)
AOPP (µmol. L^{-1})	49.4 ± 10.8	66 ± 15.3 ***	52 ± 10.7	55.7 ± 11.5 **	51.5 ($P < 0.001$; 0.7)	2.53 ($P = 0.12$)	27.5 ($P < 0.001$; 0.55)
Biomarkers of antioxidant system							
CAT (U/mL)	271.6 ± 82.8	167.3 ± 48.4***	248.8 ± 65.8	207.3 ± 63.9*	50.8 ($P < 0.001$; 0.69)	0.34 ($P = 0.56$)	7.73 ($P < 0.01$; 0.26)
Thiol (µmol. L^{-1})	360.4 ± 55.2	284.5 ± 75 ***	371 ± 92.8	343 ± 83*	31.4 ($P < 0.001$; 0.58)	2.52 ($P = 0.12$)	10.7 ($P < 0.01$; 0.32)
UA (µmol.L^{-1})	311.6 ± 71.5	327.8 ± 73.6***	313.7 ± 74	325.3 ± 77.4***	55.7 ($P < 0.001$; 0.71)	0.097 ($P = 0.75$)	5.8 ($P = 0.02$; 0.2)
TBIL (µmol. L^{-1})	6.7 ± 2.4	7.7 ± 2.9 ***	6.8 ± 3	7.1 ± 3*	39.4 ($P < 0.001$; 0.64)	0.41 ($P = 0.52$)	11.2 ($P < 0.01$; 0.33)

Abbreviations: WBC, White blood cells; CRP, C-reactive protein; ASAT, Aspartate aminotransferase; ALAT, Alanine aminotransferase; CPK, Creatine phosphokinase; LDH, Lactate dehydrogenase; MDA, Malondialdehyde; AOPP, Advanced oxidation protein products; CAT, Catalase ; UA, Uric acid; TBIL, Total bilirubin; Thiol: Total Thiol Levels.

*, **, ***: significant difference between pre-post training session ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

#, ##, ###: significant difference between PLA-MLT % of change ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

Inflammation and immune responses

Results showed a significant increase of WBC, neutrophils and CRP pre-post HIIE in both PLA ($P < 0.001$, for all) and MLT ($P < 0.001$, $P < 0.01$ and $P < 0.01$, respectively) sessions. However, the significant interaction (treatment × exercise) reported that the rates of increase pre-post exercise were lower for WBC, CRP ($P < 0.001$, $\eta p^2 = 0.45$; for both) and neutrophils ($P<0.01$, $\eta p^2 = 0.36$) after MLT condition.

Muscle and liver damage

Muscle and hepatic damage biomarkers increased after HIIE in PLA (ASAT, ALAT, CPK and LDH ($P<0.001$, for all)) and in MLT (ASAT and CPK ($P<0.001$; for both); LDH ($P<0.01$) and ALAT ($P<0.05$)) sessions. The significant interaction for treatment and exercise showed a lower increase for ASAT ($P < 0.01$, $\eta p^2 = 0.25$), ALAT ($P < 0.01$, $\eta p^2 = 0.27$) and CPK ($P = 0.02$, $\eta p^2 = 0.23$) in MLT compared to PLA session.

Oxidative stress

Lipid and protein peroxidation increased pre-post HIIE during PLA session (MDA and AOPP, $P < 0.001$ for both) and MLT session (MDA ($P < 0.05$) and AOPP ($P < 0.01$)), but this increase was significantly lower after MLT ingestion, observed through the significant interactions (treatment × exercise) for MDA ($P = 0.03$, $\eta p^2 = 0.19$) and AOPP ($P < 0.001$, $\eta p^2 = 0.55$).

Thiol and CAT decreased after HIIE in PLA ($P < 0.001$ for both) and in MLT ($P < 0.05$ for both) sessions. This decrease was significantly lower after MLT ingestion, Thiol ($P < 0.01$, $\eta p^2 = 0.26$) and CAT ($P < 0.01$, $\eta p^2 = 0.32$). Biomarkers of non-enzymatic antioxidant system increased pre-post exercise in both PLA (UA, TBIL ($P < 0.001$ for both)) and MLT sessions (UA ($P < 0.001$), TBIL ($P < 0.05$)). A significant interaction (treatment × exercise) was observed for UA ($P = 0.02$, $\eta p^2 = 0.2$) and TBIL ($P < 0.01$, $\eta p^2 = 0.33$).

DISCUSSION

The aim of this study was to investigate the effect of acute MLT intake on biomarkers of cellular damage, oxidative stress, and inflammation after a single HIIE session in subjects living with obesity.

In the present study, the significant increase in post-exercise inflammatory parameters (i.e., CRP and major WBC subpopulations) indicate a transient leukocytosis and inflammatory response during intense exercise. In this context, it has been found that pro-inflammatory cytokines (i.e., IL-1, IL-6 and TNF-α) and CRP were elevated immediately after strenuous exercise and associated to muscle damage [12]. Moreover, MLT administration attenuated the inflammatory response induced by exercise (CRP and WBC ($P < 0.001$, $\eta p^2 = 0.45$; for both), and Neutrophils ($P < 0.01$, $\eta p^2 = 0.36$)). This could be explained by MLT's ability to modulate immune responses [22]. Indeed, MLT is a positive regulator of the immune response and can activate innate and adaptive immunity [21]. MLT receptors are present on the cell membrane of T-lymphocytes CD4 and CD8, B-lymphocytes, monocytes, neutrophils and natural killer cells [20]. In this sense, Marzougui et al. [34] have shown that the combination of MLT with intradialytic exercise has positive immunoregulatory and anti-inflammatory effects by

improving the activity of natural killer cells and the proportion of T-lymphocytes $CD4^+$ and $CD8^+$ and decreasing the proportion of $CD14^{++}$, $CD16^+$ and monocytes. Furthermore, it has been demonstrated that MLT administration inhibits the activation of (NF-κB) in Wistar rats performing a 60-min acute running exercise [35] which may alleviate the increase of pro-inflammatory cytokines (i.e., IL-1β, IL-6 and TNF-α) [24]. Veneroso et al. [35] also found that administration of low MLT dose (1 mg/kg) in rats decreased pro-inflammatory cytokines, as well as mRNA and protein levels of inducible nitric oxide synthase and cyclooxygenase-2 after acute exercise. The lower increase of CRP in MLT condition could reflect reduced IL-6 production and the associated exacerbation of endothelial dysfunction after HIIE [36].

Otherwise, the decrease of antioxidant biomarkers (Thiol and CAT) and the increase of MDA and AOPP levels after HIIE could be explained by the enhanced oxygen consumption and catecholamine release during intense exercise which may disrupt the balance between FR and antioxidants [14]. The present findings indicate that MLT ingestion attenuated oxidative stress through decreasing FR production which confirms the protective effect of MLT against lipid and protein peroxidation (i.e., MDA ($P = 0.03$, $\eta p^2 = 0.19$) and AOPP ($P < 0.001$, $\eta p^2 = 0.55$)), in accordance with the findings of Bicer et al. [25]. Additionally, the lower rate of decrease pre-post exercise of Thiol and CAT during MLT condition may reflect the direct antioxidant and FR scavenging properties of MLT [21]. In this way, MLT can improve the mitochondrial respiratory chain by increasing the activity of complexes I and IV and inhibiting the production of reactive oxygen species (i.e., superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$)) [21]. MLT also acts as an indirect antioxidant by stimulating the activities of antioxidant enzymes, including SOD, CAT and GPX [26]. Marrin et al. [37] showed an increase in endogenous MLT level after moderate exercise which was higher in the morning than in the afternoon, suggesting that morning MLT administration could increase the amounts of MLT in blood, thus enhancing its beneficial effects. However, exogenous melatonin induces phase delays (shifts to a later time) when administered in the morning, and phase advances (shifts to a later time) when given in the afternoon/evening [38]. Ghattassi et al. [39] showed that morning MLT ingestion could affect morning cognitive performances (e.g., vigilance and reaction time) and some short-term specific performances of soccer players, but performances measured in the afternoon remained unchanged.

In the present study, the increase of aminotransferases (i.e., ALAT and ASAT), CPK and LDH levels after exercise could reflect increased muscle and hepatic damage [40]. MLT ingestion prior to HIIE reduced cellular damages (i.e., ASAT, ALAT, and CPK) probably through decreased oxidative stress and inflammation associated with this physical effort [41]. These findings are consistent with those of Cheikh et al. [42], showing that MLT was able to prevent the increase of ASAT, LDH and CPK levels after running based anerobic sprint test. Chahbouni et al. [43] reported that MLT use in subjects with Duchenne muscular dystrophy limited the elevation of ASAT, ALAT, LDH and CPK in the MLT group, which enhances the protective effect of MLT against skeletal muscle lesions during exercise [43]. In addition, several studies have mentioned TBIL and UA as two natural antioxidants that can intervene against the FR production [44]. Our results showed a lower rate of increase in TBIL and UA after exercise in MLT compared to PLA session. These findings could be explained by the anti-radical and antioxidant effects of MLT, in particular in the improvement of the enzymatic activity of SOD, CAT and GPX [12], these enzymes constitute together the first line of defense against FR production induced by exercise. In addition, the relatively strong treatment effects of MLT

observed in this experiment are likely due to the fact that the subjects with obesity performing HIIE were naïve to exercise, it was demonstrated that obese individuals may find training at maximal levels is less tolerable compared to regular exercise training [45] in order to promote body adaptations that reduce inflammatory responses and enhance antioxidant activity [46].

The present study has shown that HIIE in people with obesity can cause muscle damage associated with a strong inflammatory response by increasing the number of pro-inflammatory mediators and impairing the oxidant-antioxidant balance. Therefore, MLT ingestion before exercise reduced inflammation and muscle damage and alleviated HIIE-induced oxidative stress.

The main limitation of this study is the relatively small number of participants who completed the intervention. Furthermore, examination of a single exercise bout in this study does not suggest strong explanations. Future studies are still needed to investigate the chronic effect of MLT on inflammatory parameters, oxidative stress and muscle damage after various training programs in people with obesity.

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