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ORIGINAL ARTICLE

The effects of exercise training associated with low-level laser therapy on biomarkers of adipose tissue transdifferentiation in obese women

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Abstract

Investigations suggest the benefits of low-level laser therapy (LLLT) to improve noninvasive body contouring treatments, inflammation, insulin resistance and to reduce body fat. However, the mechanism for such potential effects in association with exercise training (ET) and possible implications in browning adiposity processes remains unclear. Forty-nine obese women were involved, aged between 20 and 40 years with a body mass index (BMI) of 30–40 kg/m^2. The volunteers were divided into *Phototherapy* (808 nm) and *SHAM* groups. Interventions consisted of exercise training and phototherapy applications post exercise for 4 months, with three sessions/week. Body composition, lipid profile, insulin resistance, atrial natriuretic peptide (ANP), WNT5 signaling, interleukin-6 (IL-6), and fibroblast growth factor-21 (FGF-21) were measured. Improvements in body mass, BMI, body fat mass, lean mass, visceral fat, waist circumference, insulin, HOMA-IR, total cholesterol, LDL-cholesterol, triglycerides, and ANP in both groups were demonstrated. Only the Phototherapy group showed a reduction in interleukin-6 and an increase in WNT5 signaling. In addition, it was possible to observe a higher magnitude change for the fat mass, insulin, HOMA-IR, and FGF-21 variables in the Phototherapy group. In the present investigation, it was demonstrated that exercise training associated with LLLT promotes an improvement in body composition and inflammatory processes as previously demonstrated. The Phototherapy group especially presented positive modifications of WNT5 signaling, FGF-21, and ANP, possible biomarkers associated with browning adiposity processes. This suggests that this kind of intervention promotes results applicable in clinical practice to control obesity and related comorbidities.

Keywords Phototherapy · Physical exercise · Obesity · Adipose tissue · Browning

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Introduction

Obesity has long been considered an alarming global epidemic. Different strategies that could promote treatment and control of this disease are being constantly investigated. Some evidence suggests the benefits of low-level laser (light) therapy (LLLT) for cellulite treatment, lipid control, and as a noninvasive body contouring treatment [1, 2]. Phototherapy has recently been considered an important tool to improve the benefits of physical exercise to control inflammatory states, cardiometabolic risks, and metabolic inflexibility in obese women [3–5].

In addition to strategies to treat and control obesity, metabolic investigations including cellular activities stand out in this research area. Research involving adipose tissue transdifferentiation has been assuming greater importance given its therapeutic potential for the browning adipose tissue processes, inducing an increase in body energy expenditure and possible benefits in terms of body mass reduction, and consequent greater control of obesity and associated comorbidities [6–8].

It is known that white adipose tissue is the most abundant fat deposit found in the human body, especially when considering brown adipose tissue. White adipose tissue is characterized by its higher energy storage capacity and is responsible for providing energy substrates. Additionally, it is an important endocrine organ responsible for secreting innumerable adipokines involved in inflammatory states present in obesity diagnoses [6, 8].

In its turn, brown adipose tissue, is classically characterized by the higher thermogenic activity in the newborn, and its gradual disappearance throughout human development. The thermogenic capacity of brown adipose tissue is due to the greater concentration and mitochondrial activity and the high prevalence of decoupling protein-1 (UCP-1) involved in the heat dissipation process [6, 8]. However, currently, the presence of brown adipose tissue in human beings in adulthood has recently been demonstrated, as well as the possible negative association between brown adipose tissue activity and body mass index [9–13]. These findings suggest an important therapeutic potential of brown adipocytes to induce the increase of the body energy expenditure, a possible benefit for reduction of body mass index and consequent control of obesity [7].

Our hypothesis is that the potential role of exercise training in human thermogenesis may increase in association with LLLT mediating changes in biomarkers of adipose tissue transdifferentiation in obese women undergoing interdisciplinary weight loss therapy.

Material and methods

Population

It was involved 49 obese women with age of 20–40 years. The inclusion criteria were the presence of primary obesity and body mass index (BMI) greater than 30 kg/m^2 and less than 40 kg/m^2. The non-inclusion criteria were the use of antiepileptic drugs, cortisone, history of renal disease, alcohol intake, and secondary obesity due to endocrine disorders. The study demonstrated a dropout of seven volunteers; major reasons for these were family and financial problems, followed by job opportunities. The study was conducted with the principles of the Declaration of Helsinki and was approved by the ethics committee on research at the Universidade Federal de São Carlos-UFSCar with the number (237.050), Clinical Trial: 231.286. All procedures were clear to the volunteers, and informed consent was obtained from all individual participants included in the study. The interdisciplinary therapy consisted of physical exercise intervention, application of phototherapy, clinical approach, and nutritional counseling (Fig. 1).

Anthropometric measurements

Weight and height were measured for all subjects who wore minimum clothing. It was calculated the BMI by dividing the weight by height squared (kg/m^2). Waist circumference was obtained at the end of normal expiration at the midpoint between the iliac crest and the last rib using a flexible and inelastic tape without compressing the skin [14]. Body fat mass (% and kg) and body lean mass (% and kg) were obtained through the bioelectrical impedance.

Serum analysis

Blood samples were collected at the outpatient clinic at approximately 8:00 A.M. after an overnight fast (12 h). Glucose and insulin concentrations were available by commercial kits. Insulin resistance was assessed using the homeostasis model assessment-insulin resistance (HOMA-IR) calculated by the fasting blood glucose (FBG) and the immunoreactive insulin (I): [FBG (in milligrams per deciliter) × I (in milliunits per liter)] 405. The cutoff value determined for Brazilian population is HOMA-IR > 2.71 for classifying the subjects with insulin resistance [15]. The assays of Human Fibroblast Growth Factor 21 (FGF-21) (RD191108200R-BIOVENDOR), Human Wingless Type MMTV Integration Site Family, Member 5A (WNT5-signaling) (DL-WNT5A-Hu-96TST—DL Develop), interleukin-6 (IL-6) (HCVD3-67CK-04 MILLIPLEX MAP Human Cardiovascular Disease), and atrial natriuretic peptide (ANP) (Human NT-ProANP DuoSet DY8247-05—R&D SYSTEMS) were determined by ELISA.

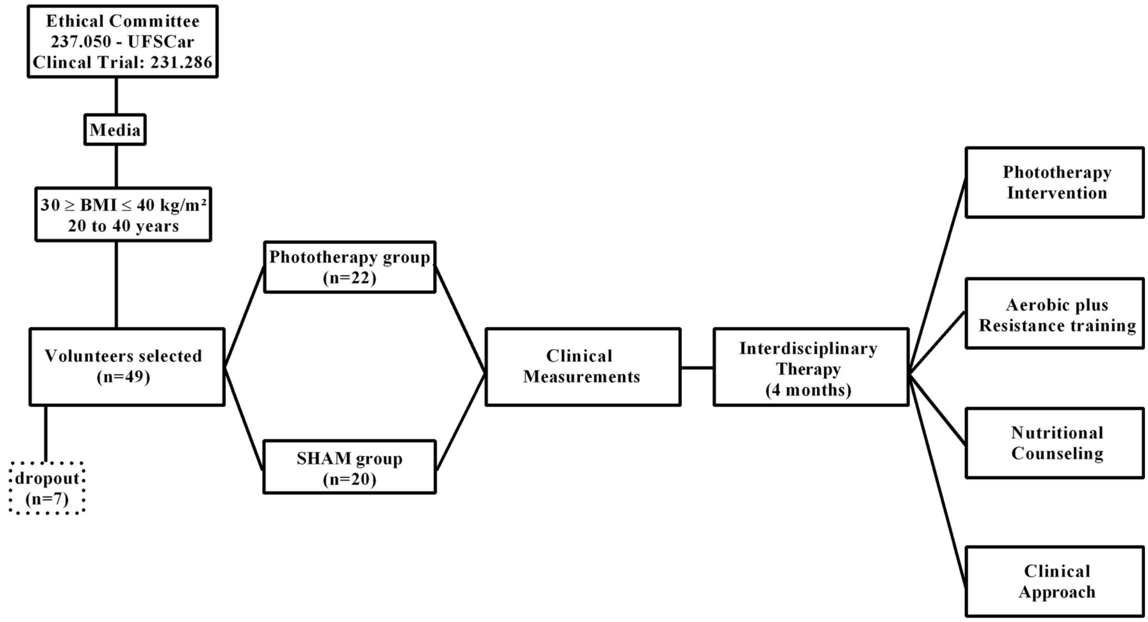

Fig. 1 Diagram of methodology

Interdisciplinary therapy

In the present investigation, the voluntaries visited the team of health professionals (endocrinologist, nutritionist, physiotherapist, and physical educator) three times during the intervention period (baseline, after 2 months of participation; and after 4 months of interventions).

Clinical approach

The medical follow-up included the initial medical history, and a physical examination of blood pressure, cardiac frequency, and body composition were checked.

Nutritional counseling

During the investigation, the voluntaries received nutritional counseling that was applied in three meetings with the nutritionist. In these sessions, the subjects had nutritional orientations. No pharmacotherapies, antioxidants and supplements were recommended.

Exercise training

The voluntaries followed an aerobic plus resistance training. The protocol was performed three times per week and included 30 min of aerobic training and 30 min of resistance training per session. The aerobic training consisted of running on a motor-driven treadmill (Movement®) at a 70–85% of cardiac frequency maximum intensity established by Bruce test adapted. The resistance training was made recruiting the muscle groups: pectoralis major, quadriceps, back, hamstrings, calf, deltoid, biceps, triceps, abdomen, and extensor muscles by performing the following exercises: chest press, leg press, lat pulldown, hamstring curls, calf raises, military press, arm curls, bench press, sit-ups, and lower back.

The first 2 weeks were used for training adaptation and to learn the movements (3 sets of 15–20 maximal repetitions [MRs]). The protocol consisted of weekly changes of the load, divided into weeks of high loads (6–8 MR), weeks of moderate loads (10–12 MR), and weeks of light loads (15–20 MR). The volunteers performed 18 sets per session, divided into three sets of each exercise, followed by rest intervals between the series and exercises: 15–20 MR = 45 s, 10–12 MR = 1 min, and 6–8 MR = 1.5 min of rest. The physical exercise intervention was based on the guidelines from the American College of Sports Medicine (ACSM) [16, 17].

Phototherapy intervention

The device prototype used was composed of four plates with 16 infrared laser emitters (per plate) totalizing 64 emitters and two electronic control box. The characteristics of phototherapy equipment are irradiation type Ga-Al-As semiconductor diode laser, with wavelength of 808 nm operating in a continuous wave mode. The irradiation information per emitter was composed by spot diameter (elliptical shape) of horizontal 0.3692/vertical 0.0582 cm, spot area of 0.0169 cm^2, output power of 100 mW and irradiance of 6.0 W/cm^2. The energy delivered per session, per point, was 96 J. The emitters were arranged perpendicularly to the skin and were allocated in the anterior region: abdominal and quadriceps simultaneously during

8 min. After this, change the position to irradiate the posterior region: gluteus and biceps femoral during 8 min, totalizing 16 min of its application (6144 J/all sessions (48) 294,912 J). Number of points irradiated were 64 (per position)/128 (total). The phototherapy equipment was developed by the Laboratory Technology Support-LAT, Center for Research in Optics and Photonics Institute of Physics in São Carlos city at Universidade de São Paulo—USP [3–5] (Fig. 2).

Statistical analysis

Statistical analysis was performed using the program STATISTICA version 7.0 for Windows. The adopted significant value was $\alpha \leq 5\%$. Data normality was verified with the Shapiro Wilk test. Parametric data were expressed as mean ± SD, and nonparametric were normality by *Z*-score values. To analyze the effects of intervention and difference between the groups, it applied ANOVA for repeated measures (ANOVA two-way) followed by Fischer post hoc. The delta values (Δ) were used for the statistical analysis obtained from the calculation: Δ = ((after intervention value − baseline value)   baseline value) × 100. Comparing the delta values between the groups was performed by t test independent by groups.

Results

In initial treatment, no statistical differences were showed between the groups for all variables. This information demonstrating that the groups were paired at the beginning of the investigation and the possible changes observed probably occur by the influence of methodology.

Analyze of interdisciplinary intervention in Phototherapy group (PHOTO)

After interdisciplinary intervention, it was showed reduction in the variables body mass (kg), body mass index (kg/m^2), body fat mass (kg/%), visceral fat (cm^2), waist circumference (cm), insulin (μU/ml), HOMA-IR, and interleukin-6 (pg/ml). In addition, an increase in body lean mass (%), WNT5 signaling (ng/ml), and atrial natriuretic peptide (pg/ml) were observed. Non-statistical changes were showed to body lean mass (kg), glucose (mg/ml), and fibroblast growth factor 21 (pg/ml) (Table 1 and Fig. 3).

Analyze of interdisciplinary intervention in SHAM group (SHAM)

In the SHAM group, it was showed reduction in body mass (kg), body mass index (kg/m^2), body fat mass (kg / %), body lean mass (kg), visceral fat (cm^2), waist circumference (cm), insulin (μU/ml), and HOMA-IR. An increase in body lean mass (%) and atrial natriuretic peptide (pg/ml) was demonstrated. It was observed a reduction in body lean mass (kg). Non-statistical changes were showed to glucose (mg/ml), and fibroblast growth factor 21 (pg/ml) (Table 1 and Fig. 3).

Analyze of interdisciplinary intervention between the groups

According the analysis of delta values, it is possible to verify statistical difference between the groups to body fat mass (%), insulin (μU/ml), HOMA-IR, and fibroblast growth factor 21 (pg/ml) (Table 1 and Fig. 4).

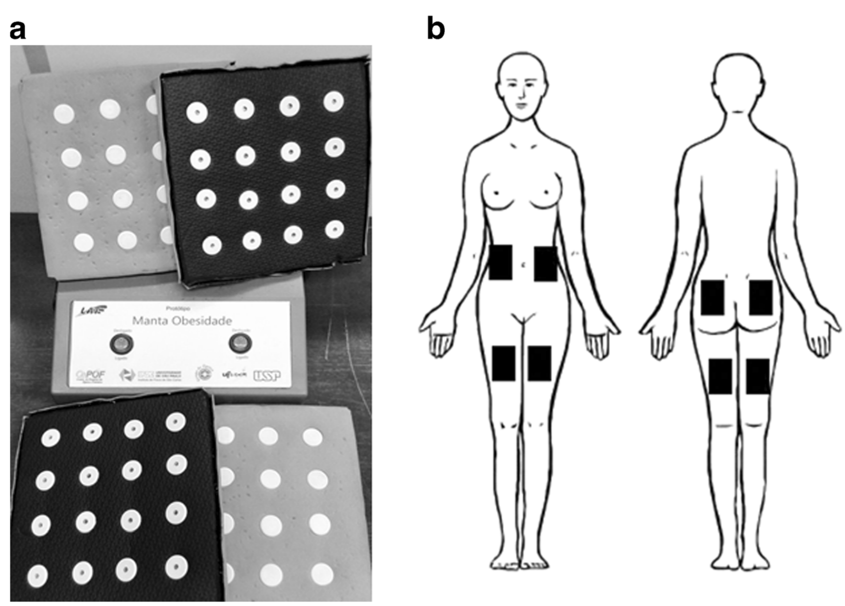

Fig. 2 **a** Illustrative of device design. **b** Illustrative regions of phototherapy application. Illustrative figures previously published in da Silveira Campos et al. 2015 [3]

Table 1 Effects of interdisciplinary therapy on anthropometric and metabolic profile

Variables	Phototherapy group ($n = 22$)			SHAM group ($n = 20$)		
	Baseline	After intervention	Δ (%)	Baseline	After intervention	Δ (%)
Body mass (kg)	93.57 ± 11.57	90.14 ± 11.62[a]	− 4.15 ± 3.7	93.29 ± 12.56	89.73 ± 12.64 [a]	− 5.39 ± 3.54
Body mass index (kg/m^2)	34.35 ± 3.86	32.78 ± 4.2[a]	− 4.42 ± 3.69	35.2 ± 4.14	33.89 ± 4.15[a]	− 5.37 ± 3.44
Body fat mass (kg)	37.47 ± 6.93	34.55 ± 7.33[a]	− 8.77 ± 7.29	37.73 ± 7.63	34.78 ± 7.58[a]	− 9.04 ± 6.52
Body fat mass (%)	39.88 ± 3.24	37.96 ± 3.79[a]	− 5.1 ± 4.59	40.23 ± 3.2	38.54 ± 3.42[a]	− 4.41 ± 3.66[b]
Body lean mass (kg)	55.9 ± 5.7	55.58 ± 5.19	− 0.82 ± 3.07	55.26 ± 5.55	54.59 ± 5.37[a]	− 1.98 ± 2.39
Body lean mass (%)	60.11 ± 3.24	66.94 ± 10.16[a]	11.15 ± 13.59	59.76 ± 3.2	65.21 ± 7.58[a]	9.05 ± 8.54
Visceral fat area (cm^2)	154.73 ± 22.43	144.19 ± 23.11[a]	− 7 ± 2.79	158.11 ± 21.3	148.96 ± 23.41[a]	− 6.03 ± 3.37
Waist circumference (cm)	107.97 ± 11.03	105.29 ± 9.84[a]	− 2.6 ± 2.98	106.69 ± 10.65	103.76 ± 11.4[a]	− 3.02 ± 3.51
Glucose (mg/ml)	91.42 ± 8.52	93.05 ± 7.28	2.29 ± 6.83	92.25 ± 8.47	94.54 ± 10.97	3.47 ± 9.19
Insulin (μU/ml)	16.25 ± 5.51	12.41 ± 6.99[a]	− 30.24 ± 19.13	17.15 ± 5.95	14.81 ± 6.51[a]	− 10.51 ± 29.22[b]
HOMA-IR	3.72 ± 1.48	2.76 ± 1.59[a]	− 31.15 ± 20.22	3.93 ± 1.56	3.34 ± 1.54[a]	− 10.16 ± 33.7[b]

Δ value: ((after intervention value − baseline value)   baseline value) × 100

[a] Statistical difference between baseline and after intervention values

[b] Statistical difference between delta values

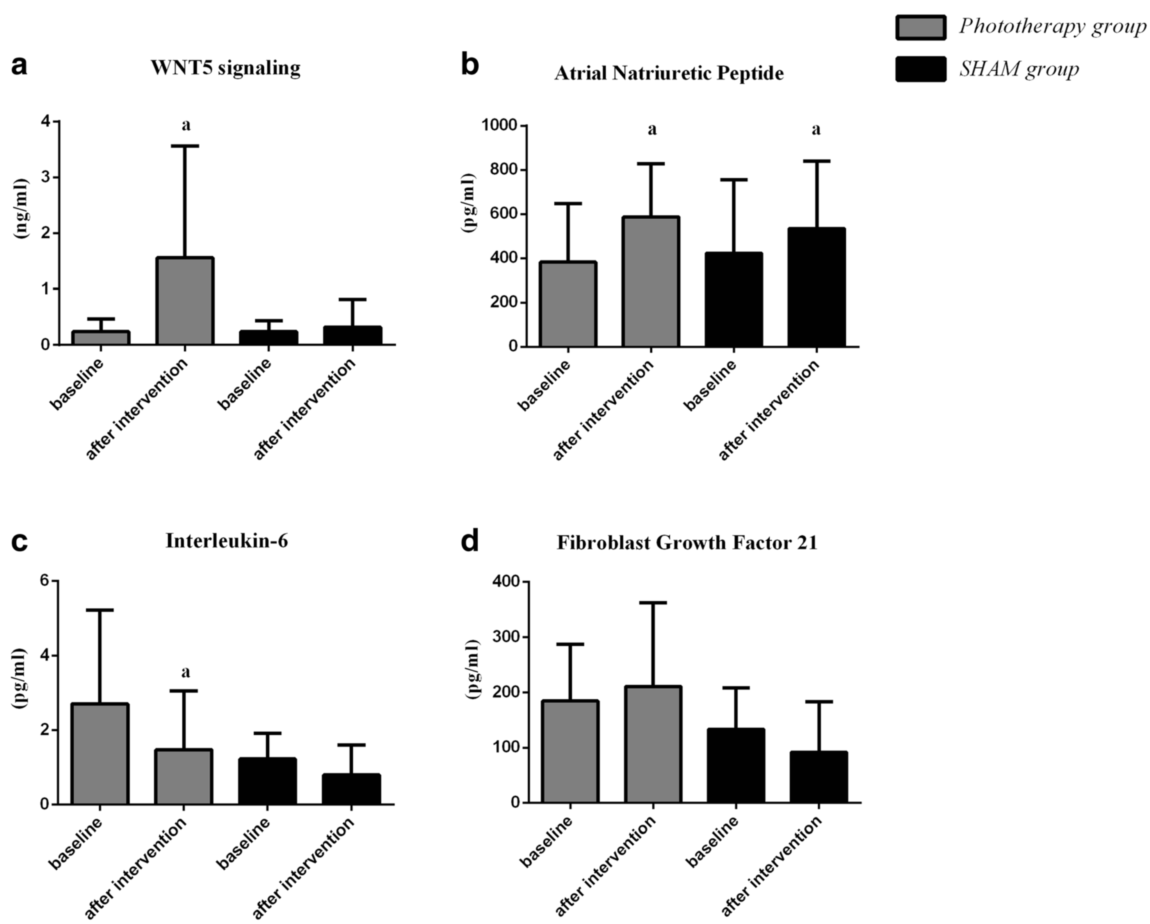

Fig. 3 Effects of interdisciplinary therapy on **a** WNT5 signaling. **b** Atrial natriuretic peptide concentration. **c** Interleukin-6 concentration. **d** Fibroblast growth factor-21 concentration. "a" statistical difference

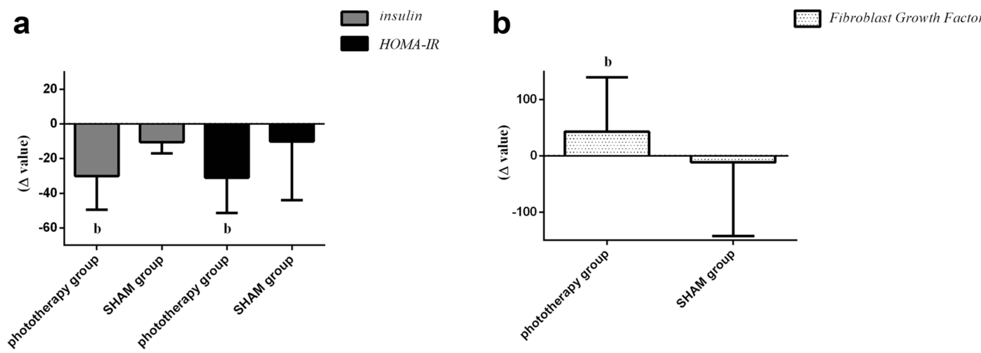

Fig. 4 Effects of interdisciplinary therapy on **a** insulin and HOMA-IR; **b** fibroblast growth factor-21. "b" statistical difference

Discussion

Investigations focusing on the transdifferentiation process of body adipose tissue have assumed increasing importance and the scientific community has shown great interest in it as a possible mediator for obesity control. In the present study, the specific focus was the possible effects of exercise training associated with phototherapy intervention on the mechanism of adipose tissue transdifferentiation biomarkers in obese women.

Low-level laser therapy (LLLT) has received greater attention in the literature and clinical interventions; given that the application of phototherapy is able to enhance the proliferation of various cell lines [18]. In general, as previously described; photobiomodulation is based on photon energy that is converted into chemical energy in the cells. Laser energy of a particular wavelength is absorbed by mitochondrial respiratory chain components, leading to an increase in ROS (reactive oxygen species) and ATP (adenosine triphosphate) levels. Therefore, this event promotes biophysical modifications in the cells and can prevent cell apoptosis, stimulating cell growth, proliferation and differentiation [18–21].

There are numerous factors involved in the adipogenesis process and in transdifferentiation of adiposity cells, which offers various mechanisms to be investigated. In the present study, an increase in WNT5 signaling only in the group receiving LLLT therapy after physical exercise intervention was specifically observed. As previously mentioned, WNTs are glycoproteins involved in the regulation of remodeling and homeostasis tissue, including cellular development, proliferation and differentiation, especially in terms of the adipocytes cells [22–27].

It is worth noting that some specific WNTs signaling is associated with adipogenesis processes. WNT10b is an endogenous factor involved in preadipocytes differentiation, specifically acting as an inhibitor of adipogenesis [23, 25, 28]. On the other hand, WNT6 and WNT10a are considered important endogenous regulators of brown adipocyte development [29, 30]. Moreover, it is suggested that brown adipocyte transdifferentiation is mediated by the relevant contribution of WNT5 that promotes adipocyte differentiation through an unknown mechanism that involves destabilizing β-catenin [31, 32].

Another important result shown in our investigation was an increase in atrial natriuretic peptide (ANP). Natriuretic peptides include atrial NP (ANP), B-type NP (BNP), and C-type NP (CNP). It is previously related that both natriuretic peptides, ANP and BNP, are secreted by cardiomyocytes; whereas CNP is mainly produced by endothelium, bones, central nervous system, and in the reproductive system [33–36].

It is known that the circulating level of ANP is reduced in obese individuals and weight loss is able to promote an increase of this natriuretic peptide [37]. It has previously been shown that in addition to the cardiac function of ANP, this peptide promotes an increase of UCP-1 transcription, a relevant mechanism associated with greater energy release, heat production with a possible role in the adiposity browning [38]. This process occurs through increased p38α signaling, and phosphorylation of the ATF2 transcription factor, and a consequent increase of the peroxisome proliferator-activated receptor (PGC-1α) coactivator 1 alpha, was previously described in the literature. Both PGC-1α and UCP-1 may promote increased mitochondrial biogenesis favoring increased energy expenditure [33, 39]. In addition, human adipocyte investigations "in vitro," demonstrated that both ANP and BNP are able to increase adiponectin secretion, an important anti-inflammatory biomarker related to obesity [40]. Additionally, ANP was related to a reduction in pro-inflammatory biomarkers, such as interlukin-6 (IL-6), tumoral necrosis factor- α (TNF-α) and hyperleptinemia state [33].

In fact, in the present study, it was demonstrated that in both groups an increase of ANP occurs, suggesting that weight loss due to exercise training and the adoption of healthy eating habits is able to promote this outcome, especially considering its importance as a cardiovascular protector function in this population. It is worth noting that specifically in the Phototherapy group, the ANP may contribute together with the WNT5 signaling to the adipocyte browning process. However, it is necessary to verify this data in future investigations in a large cohort study.

Considering the pro-inflammatory state present during obesity, one of the most relevant biomarkers to investigate in this population is interleukin-6. Our results show a reduction in IL-6 concentration in the Phototherapy group. This finding has already been observed in a prior study [3] that suggested the beneficial effects of physical exercise in association with the phototherapy intervention in this cytokine. IL-6 is secreted by adipocytes, especially from visceral adiposity tissue. It is known that visceral adipocytes produce up to three times more IL-6 compared with subcutaneous adipocytes, and in obesity, this fact is related to an increase that results in the pro-inflammatory state observed in this disease, contributing to the development of atherosclerosis and other possible comorbidities. IL-6 promotes inflammatory events through the expansion and activation of T cells, differentiation of B cells, and the induction of acute-phase reactants by hepatocytes [41]. It contributes to the development of insulin resistance when chronically elevated, as in obesity [42]. Therefore, our results suggest the relevant effect of applying low laser light therapy after exercise training.

Another positive result shown in this investigation was the increase in the delta value of the FGF-21 concentration in the Phototherapy group compared to the *SHAM* group. FGF-21 is a member of the FGF family representing a group of peptides that regulate diverse biological functions, including cell differentiation, cell growth, and angiogenesis [43]. It is mainly expressed in the liver, and it functions as a potent activator of glucose uptake on adipocytes [44, 45]. Due to its effect on glucose and lipid metabolism in hepatocytes and adipocytes, it was assumed to be a great target with potential antidiabetic properties that might be useful to treat hyperglycemia, insulin resistance, and hyperlipidemia [42]. Moreover, it was recently found that the conversion of white to brown adipose tissue is mediated by FGF-21 [46, 47]. Previous investigations highlight the importance of this hormone together with irisin, in the transdifferentiation of “beige/brite” to brown adipose tissue, reinforcing its potential in obesity treatments [48–51]. Additionally, we showed that FGF-21 can downregulate the NPY concentration after the same protocol used in obese women associated with an increase in the metabolic rate and adiponectin concentration corroborating its role in energy balance and adipogenesis in humans [52].

In agreement with previous studies [3–5], our results demonstrated the effectiveness of intervention to promote body composition improvements. It is worth noting that independent of the intervention group, both benefited from a reduction in body mass, BMI, body fat mass, visceral adiposity, and waist circumference concomitant with an increase in body lean mass. This data suggests the effects of regular physical exercise associated with nutritional counseling to control obesity.

Furthermore, both groups showed an improvement in insulin resistance status, resulting in a reduction in insulin concentration after intervention. No changes were observed in glucose concentration, although the participants presented basal moment glucose values in agreement with the standard of normality. Insulin resistance is a framework result of excess lipid stores and the activity of visceral adipocytes, which secrete high amounts of inflammatory cytokines such as tumor necrosis factor-a (TNF-a) and interleukin-6 (IL-6) [53]. This condition appears to play a central role in the development and maintenance of insulin resistance and other potential comorbidities [54]. It is important to highlight that the application of phototherapy combined with exercise training was more effective to reduce the insulin resistance index compared to the group that only realized exercise training. This data is in agreement with a previous study, where the researchers investigated the “metabolic inflexibility” developed during the obesity [5], suggesting that the regulation of body mass has been associated with a relevant link that involves adipose and muscle tissues mechanisms, considered essential for homeostasis [55]. Consequently, a reduction in body mass/adiposity, improves the lipid profile and metabolic status in obese populations [5].

Research into the use of low light laser therapy has highlighted the possible mechanisms involved in cellular homeostasis enhancement, including biochemical and structural changes in the mitochondria [56, 57]. Therefore, the application of phototherapy in association with exercise training is considered, given that LLLT may accelerate oxidative metabolism leading to an increase in ATP synthesis, optimizing the effects of exercise [58].

Finally, experimental studies explore the effects of LLLT in adipocyte derivations and proliferation showed that combined exercise training and phototherapy interventions in obese rats, enhancing the effects of exercise [59]. Moreover, in a recent publication, the authors showed that the combination of the electromagnetic field and low-level laser applied in adipose tissue-derived mesenchymal stem cells of subcutaneous fat affect various biological processes, including the growth and proliferation of cells, and especially that of stem cells; suggesting their role in stem cell tissue [60]. Corroborating this data, in a previous study, Min and colleagues demonstrated that LLLT could enhance the proliferation and viability of human adipose-derived stem cells (ADSCs) [18].

The hypothesis regarding the mechanism suggests that the laser energy of a particular wavelength is absorbed by mitochondrial respiratory chain components, leading to an increase in ROS (reactive oxygen species) and ATP (adenosine triphosphate) levels, favoring the expression of growth factors and leading to cell proliferation [61–63]. However, as previously mentioned by AlGhamdi and colleagues, it is necessary to be cautious about speculation in this respect, since the effects of LLLT on cell proliferation depends on many factors, including laser parameters such as the type of laser, wavelength, energy density, power density, and the type of cell being irradiated [64].

In conclusion, we note that the effects of LLLT on biological processes of adipose tissue-derived mesenchymal stem cells is still something new in the literature, so the present data should be further investigated with larger populations. Previously, we had been able to demonstrate the benefits of this intervention on the inflammatory profile, cardiometabolic risk, and obesity metabolic inflexibility, particularly in obese women [3–5]. In the present study, our results suggest a new perspective in the field of adipose tissue transdifferentiation and its possible markers, given that the application of LLLT combined with exercise training could improve biomarkers of browning adipose tissue in obese women thereby enhancing clinical practice and mechanisms to control obesity and related comorbidities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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