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Visceral fat decreased by long-term interdisciplinary lifestyle therapy correlated positively with interleukin-6 and tumor necrosis factor–α and negatively with adiponectin levels in obese adolescents

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Abstract

The purpose of this study was to assess the level of cytokine expression in correlation with visceral and subcutaneous fat in obese adolescents admitted to long-term interdisciplinary weight loss therapy. The study was a longitudinal clinical intervention of interdisciplinary therapy. Adolescents (18, aged 15-19 years) with body mass indexes greater than the 95th percentile were admitted and evaluated at baseline and again after 1 year of interdisciplinary therapy. Visceral and subcutaneous fat was analyzed by ultrasonography. Blood samples were collected to analyze tumor necrosis factor–α (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), and adiponectin concentrations that were measured by enzyme-linked immunosorbent assay. The most important finding in the present investigation is that the long-term interdisciplinary lifestyle therapy decreased visceral fat. Positive correlations between IL-6 levels and visceral fat ($r = 0.42$, $P < .02$) and TNF-α levels and visceral fat ($r = 0.40$, $P < .05$) were observed. Negative correlations between TNF-α levels and subcutaneous fat ($r = -0.46$, $P < .01$) and adiponectin levels and subcutaneous fat ($r = -0.43$, $P < .03$) were also observed. In addition, we found a positive correlation between TNF-α levels and the visceral to subcutaneous fat ratio ($r = 0.42$, $P < .02$) and a negative correlation between adiponectin level and the visceral to subcutaneous fat ratio ($r = -0.69$, $P < .001$). Despite the limitation of sample size, our results indicate that the observed massive weight loss (mainly visceral fat) was highly correlated with a decreased inflammatory state, suggesting that the interdisciplinary therapy was effective in decreasing inflammatory markers.

1. Introduction

Obesity is a heterogeneous condition with respect to the regional distribution of fat: *visceral obesity* refers to fat accumulation within omental and mesenteric fat depots, whereas *peripheral obesity* generally refers to subcutaneous fat accumulation [1]. The functional differences between visceral and subcutaneous adipocytes may be related to their anatomical location. Visceral adipose tissue and its adipose tissue resident macrophages produce many proinflammatory cytokines, such as tumor necrosis factor–α (TNF-α) and interleukin-6 (IL-6), and less adiponectin and interleukin-10 (IL-10) [2-4]. These cytokine changes induce insulin resistance and play a major role in the pathogenesis of endothelial dysfunction and subsequent atherosclerosis [5]. Such differences are also reflected in the contrasting roles that visceral and subcutaneous adipose tissues play in the pathogenesis of obesity-related cardiometabolic problems in both lean and obese individuals [6]. Thorne et al [7] reported that the removal of visceral adipose tissue by omentectomy resulted in decreased glucose and insulin levels in humans,

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whereas the removal of subcutaneous adipose tissue by liposuction did not always result in improvements in glucose metabolism or lipid levels [8,9].

Different strategies are adopted with the intention of restoring the inflammatory state in obese subjects. Several studies have shown that diet-induced weight loss significantly decreases the levels of markers of inflammation, such as TNF-α, IL-6, and C-reactive protein [10,11]. On the other hand, sustained aerobic exercise is recommended both for the prevention and treatment of several chronic diseases [12-14]. Moreover, endurance training seems to induce an increase in the secretion of anti-inflammatory cytokines by adipose tissue [15].

Recently, our group has shown that long-term interdisciplinary lifestyle therapy is effective in controlling the psychologic aspects, nonalcoholic fatty liver disease, body composition, bone mineral density, and hormonal alterations [16-21] commonly observed in obese patients. However, despite these promising results, few studies have addressed the effects of long-term multidisciplinary intervention on pro- and anti-inflammatory cytokine levels.

Thus, the aim of this study was to assess the effect of long-term multidisciplinary intervention on visceral and subcutaneous fat loss and cytokine levels in obese adolescents.

2. Material and methods

2.1. Population

Adolescents were invited to participate in 1-year-long multidisciplinary therapy to promote changes in their sedentary lifestyle and nutritional habits. The basic requirements for participation were motivation and high attendance at the therapy sessions.

Fifty-four adolescents were invited to participate in a 1-year-long Interdisciplinary Obesity Program of the Federal University of São Paulo-Paulista Medical School to promote changes in their sedentary lifestyle and nutritional habits. The basic requirements for participation were motivation and high attendance at the therapy sessions. Thirty-nine adolescents continued until the end of the therapy. For the present study, 18 obese adolescents who lost more than 5% fat mass (range sample: 5.4% at 22.50% fat mass) were selected.

These adolescents were evaluated at baseline and after long-term (1 year) weight loss intervention. This study was carried out in accordance with the principles of the Declaration of Helsinki and was formally approved by the Institutional Ethical Committee (#0135/04). Informed consent was obtained from all subjects and/or their parents, and agreement of the adolescents and their families to participate was voluntary.

The ages of the 18 participants ranged from 15 to 19 years (16.6 ± 1.67 years), and the average body mass index (BMI) was 37.03 ± 3.78 kg/m^2 (7 boys and 11 girls). All participants met the inclusion criteria of postpubertal stage V based on the Tanner stages [22] and of obesity (BMI >95th percentile) according to the Centers for Disease Control and Prevention reference growth charts (2004). Noninclusion criteria included identifiable genetic, metabolic, or endocrine disease or previous drug utilization [23].

2.2. Study protocol and medical screening

Subjects were medically screened, their pubertal stage was assessed, and their anthropometric measures were recorded (ie, height, weight, BMI, and body composition). The endocrinologist completed a clinical interview including questions to determine eligibility based on inclusion and exclusion criteria. Blood samples were collected and analyzed, and an ultrasound (US) was performed. The procedures were scheduled for the same time of day for all subjects to remove any influence of diurnal variations. Thereafter, obese adolescents started the interdisciplinary weight loss program (described in a later section).

2.3. Anthropometric measurements and body composition

Subjects were weighed on a Filizola scale (São Paulo-SP, Brazil) while wearing light clothing and no shoes, and weight was recorded to the nearest 0.1 kg. Height was measured using a wall-mounted stadiometer (Sanny, São Paulo-SP, Brazil; model ES 2030) to the nearest 0.5 cm. Body mass index was calculated as body weight divided by height squared. Body composition was estimated by plethysmography using the BOD POD body composition system (version 1.69; Life Measurement Instruments, Concord, CA). This is the most advanced technique for assessing body composition available today. The patented air displacement plethysmography used by the BOD POD and PEA POD is similar in principle to hydrostatic (or "underwater") weighing. The obvious difference is that air is more convenient and comfortable than water, such that air displacement plethysmography provides a much simpler and safer testing environment, better reliability, and significantly improved repeatability and accuracy [24].

2.4. Serum analysis

Blood samples were collected in the outpatient clinic at around 8:00 AM after an overnight fast. Cytokine (TNF-α, IL-6, and IL-10) and adiponectin concentrations were measured using commercially available enzyme-linked immunosorbent assay kits from eBioscience (San Diego, CA) and R&D Systems (Minneapolis, MN) according to the manufacturer's manual.

2.5. Visceral and subcutaneous adiposity measurements

All abdominal ultrasonographic procedures and the measurements of visceral and subcutaneous fat tissue were performed by the same physician, who was blinded to the subjects' assignment group. This physician was a specialist in imaging diagnostics using a 3.5-MHz multifrequency transducer (broadband), which reduces the risk of misclassification. The intraexamination coefficient of variation for US was 0.8%.

Table 1
Effect of long-term multidisciplinary lifestyle therapy on body fat and cytokines levels (n = 18)

	Before	After	% Change	*P* value
Age (y)	15±1.73	16 ±0.63	1 ± 0.51	.11
Body weight (kg)	95.03 ± 13.06	85.59±11.58	−11%	.05
BMI (kg/m^2)	34.99 ± 4.00	31.71 ± 4.00	−9.3%	.05
Percentage fat	47.50 ± 6.99	35.81 ± 9.60	−24%	<.001
Fat mass (kg)	49.6 ± 10.1	32.9 ± 12.3	−33%	<.001
Fat-free mass (kg)	55.8 ± 7.49	58.1 ± 7.71	+4%	.19
VO_{2max} (mL/[kg min])	27.5 ± 6.87	30.3 ± 8.10	+10%	.01
Visceral fat (cm)	4.19 ± 1.16	2.13 ± 0.84	−49%	<.001
Subcutaneous fat (cm)	3.61 ± 0.47	2.85 ± 0.77	−21%	.001
Visceral to subcutaneous ratio	1.17 ± 0.34	0.80±0.38	−31%	.006
Adiponectin (ng/mL)	9.70 ± 2.13	12.98 ± 2.17	+33%	<.001
TNF-α (pg/mL)	22.76 ± 29.67	20.88 ± 29.22	−8%	.59
IL-6 (pg/mL)	49.32 ± 34.91	34.53 ± 21.46	−29%	.13
IL-10 (pg/mL)	7.86 ± 9.02	14.74 ± 22.95	+87%	.16
IL-10/TNF-α ratio	0.40 ± 0.37	0.58 ± 0.65	+43%	.28

Results are expressed as mean value ± SD.

Ultrasound measurements of intraabdominal ("visceral") and subcutaneous fat were taken. The US-determined *subcutaneous fat* was defined as the distance between the skin and external face of the recto abdominis muscle, and *visceral fat* was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta. Cutoff points to define visceral obesity by ultrasonographic parameters were based on previous methodological descriptions by Ribeiro-Filho et al [25].

2.6. Clinical intervention

2.6.1. Dietary program

Energy intake was set at the levels recommended by the dietary reference intake for subjects with low levels of physical activity of the same age and sex following a balanced diet [26]. No drugs or antioxidants were recommended. Once a week, adolescents had a dietetics lesson providing information on the following: the food pyramid, diet record assessment, weight loss diets and miracle diets, food labels, dietetics, fat-free and low-calorie foods, fats (kinds, sources, and substitute foods), fast food calories and nutritional composition, good nutritional choices in special occasions, healthy sandwiches, shakes and products to promote weight loss, functional foods, and decisions on food choices. All patients received individual nutritional consultation during the intervention program.

A 3-day dietary record was collected at the beginning of the study and again at 12 months into the program. Because most obese people underreport their food consumption, each adolescent was asked to record their diet with the help of their parents [27]. The degree of underreporting may be substantial; however, this is a validated method to assess dietary consumption [28]. Portions were measured in terms of familiar volumes and sizes. The dietician taught the parents and the adolescents how to record food consumption. These dietary data were transferred to a computer by the same dietician, and the nutrient composition was analyzed by a PC program developed at the Federal University of São Paulo–Paulista Medical School (Nutwin software for Windows, 1.5 version, 2002) that used data from Western and local food tables. In addition, the parents were encouraged by a dietitian to call if they needed extra information.

2.6.2. Exercise program

During the 1-year interdisciplinary intervention period, adolescents followed a personalized aerobic training program that included a 60-minute session completed 3 times a week (180 min/wk) under the supervision of a sports therapist. Each program was developed according to the results of an initial oxygen uptake test for aerobic exercises (cycle ergometer and treadmill). The intensity was set at a workload corresponding to a ventilatory threshold of 1 (50%-70% of oxygen uptake test). Adolescents were under heart rate monitoring during the aerobic sessions. The exercise program was based on the 2009 recommendations given by the American College of Sports Medicine [29].

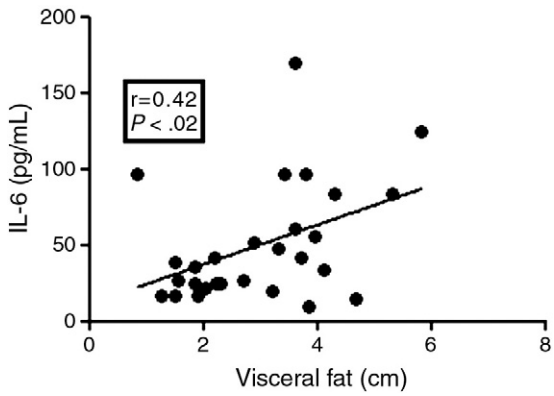

Fig. 1. Correlation of IL-6 levels and visceral fat.

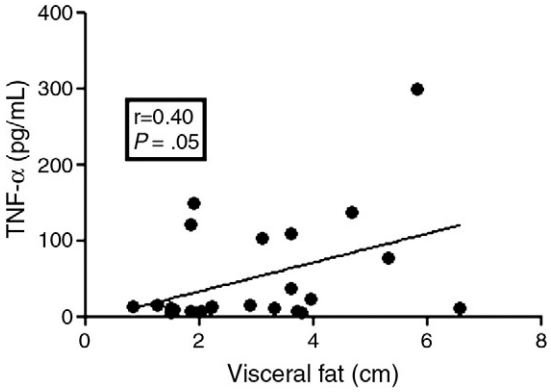

Fig. 2. Correlation of TNF-α levels and visceral fat.

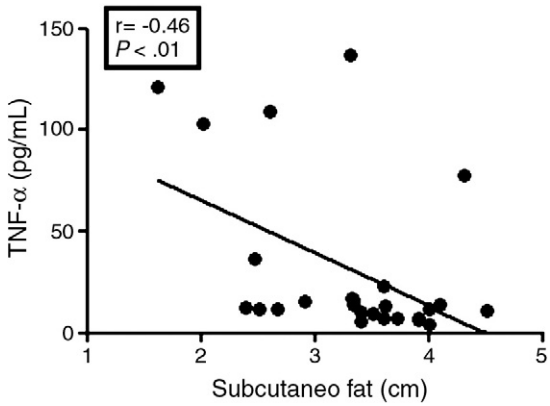

Fig. 4. Correlation of TNF-α levels and subcutaneous fat.

2.6.3. Psychologic intervention

Diagnoses of common psychologic problems associated with obesity, such as depression, disturbances of body image, anxiety, and decreased self-esteem, were established by validated questionnaires. During the interdisciplinary intervention, the adolescents had weekly psychologic support group sessions. During these sessions, the adolescents discussed the following topics: body image; alimentary disorders including bulimia, anorexia nervosa, and binge eating; the signs, symptoms, and health consequences of these disorders; the relationship between feelings and food; and family problems such as alcoholism, among other topics. Individual psychologic therapy was recommended if individuals were found to have nutritional or behavioral problems [19].

2.7. Statistical analysis

The data distribution was checked by the Bartlett test for equal variances, and the data are reported as means ± SD. Statistical outliers within each treatment group were identified using a Grubbs test (GraphPad Software, San Diego, CA) and subsequently removed. All remaining data were analyzed by GraphPad Prism (version 5.00). The differences between groups for all parameters were assessed by a paired Student *t* test. The Pearson correlation coefficient was calculated to assess the relationship between variables. The analysis was carried out with the significance level set at $P < .05$.

3. Results

Long-term therapy was effective in reducing body weight (−11%), BMI (−9.3%), percentage fat (−24%; range before, 32.3%-58.4% and after, 10.2%-55.5%), visceral fat (−49%; range before, 2.2-6.5 cm and after, 0.8-3.7 cm), and subcutaneous fat (−21%; range before, 2.6-4.5 cm and after, 1.6-3.9 cm). Long-term therapy increased fat-free mass (+4%; range before, 44.1-71.2 kg and after, 44.2-74.9 kg) and VO_{2max} (+10%; range before, 21.2-34.1 mL/[kg min] and after, 22.7-38.4 mL/[kg min]). A reduction in TNF-α (−8%; range before, 4.13-109.45 pg/mL and after, 6.31-121.26 pg/mL) and IL-6 (−29%; range before, 9.47-111.41 pg/mL and after, 16.56-96.45 pg/mL) was observed after long-term therapy, as was an increase in IL-10 (+87%; range before, 0.16-38.59 pg/mL and after, 0.16-67.13 pg/mL). On the other hand, the ratio of IL-10 to TNF-α (+43%; range

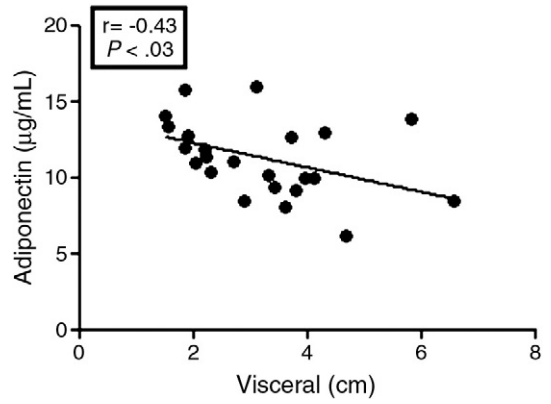

Fig. 3. Correlation of adiponectin levels and visceral fat.

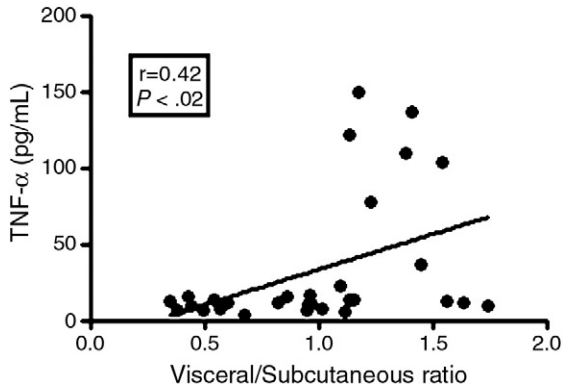

Fig. 5. Correlation of TNF-α levels and visceral to subcutaneous ratio.

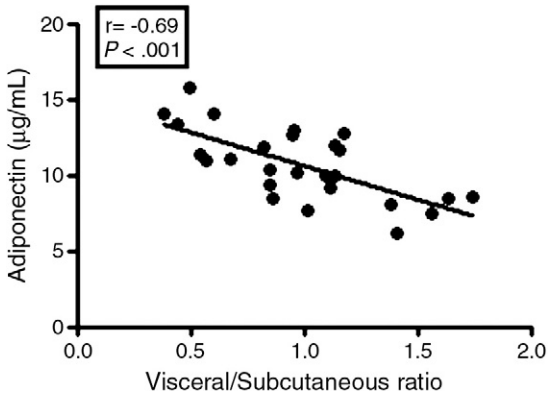

Fig. 6. Correlation of TNF-α levels and visceral to subcutaneous ratio.

before, 0.011-1.10 and after, 0.019-2.06 ratio) was increased; but the difference was not statistically significant ($P > .05$) (Table 1). Indeed, adiponectin levels were increased (+33%; range before, 6.12-13.86 μg/mL and after, 8.43-15.99 μg/mL; $P < .0001$). These results are shown in Table 1.

The most important findings in the present investigation are the observed positive correlation between IL-6 levels with visceral fat ($r = 0.42$, $P < .02$, Fig. 1), TNF-α levels with visceral fat ($r = 0.40$, $P < .05$, Fig. 2), and the negative correlations between TNF-α and adiponectin levels and TNF-α and subcutaneous fat ($r = −0.46$, $P < .01$, Fig. 3; $r = −0.43$, $P < .03$, Fig. 4). In addition, there was a positive correlation between TNF-α levels and the ratio of visceral to subcutaneous fat ($r = 0.42$, $P < .02$, Fig. 5) and a negative correlation between adiponectin levels and the ratio of visceral to subcutaneous fat ($r = −0.69$, $P < .001$, Fig. 6). Based on the theoretical equation, adiponectin concentration was a predictive factor of the ratio of visceral to subcutaneous fat (equation: $y = −4.445x + 15.07$, $r = 0.4809$, Fig. 7).

No statistical differences were observed in TNF-α, IL-6, IL-10, or adiponectin levels between boys and girls either before or after therapy. However, there was a tendency for proinflammatory cytokines levels to be higher in the boys than in the girls. The visceral fat depot and the ratio of visceral to subcutaneous fat pads were higher in boys than girls, and the subcutaneous fat pad was lower in boys than girls (data not shown).

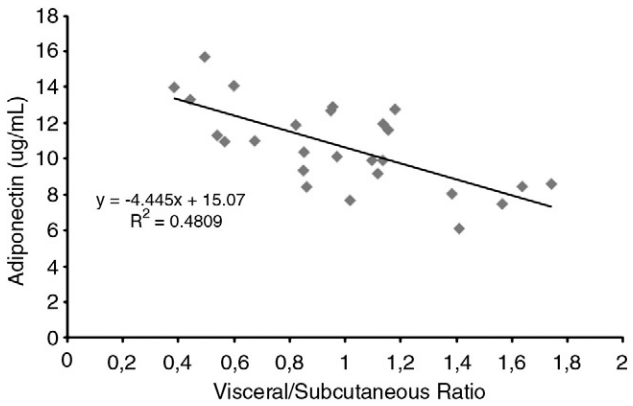

Fig. 7. Theoretical equation: adiponectin concentration is a predictive factor to visceral to subcutaneous ratio.

4. Discussion

In the present study, we examined the relationship between circulating IL-6, TNF-α, IL-10, and adiponectin concentrations and direct measures of visceral and subcutaneous adiposity. The results indicate that IL-6 and TNF-α levels were positively correlated with visceral fat and negatively correlated with adiponectin levels. Previous studies have shown that visceral fat in obese adolescents correlates with fatty liver, neuroendocrine alterations, and insulin resistance [17,30]. Our data are consistent with those of Park et al [31], which showed that circulating IL-6 levels were significantly associated with visceral adiposity. These data are also consistent with Fontana et al [32], who reported in massively obese subjects that plasma IL-6 concentrations were much higher in the portal vein than in systemic arterial blood; these results suggest that visceral fat is an important source of IL-6 production in obese people. Many studies have shown that visceral obesity is associated with a higher expression of cytokines than subcutaneous obesity [6,33,34]. Cao et al [1] found a significant increase in TNF-α expression in omental adipose tissue as compared with subcutaneous adipose tissue in obese individuals. These data show that omental TNF-α expression is highly correlated with insulin sensitivity, and the authors suggest that visceral fat is associated with a decrease in insulin sensitivity that could lead to an increased risk of cardiovascular disease. Van der Poorten et al [33] reported that central obesity, in which fat mass is predominantly intraabdominal, is more strongly associated with insulin resistance, dyslipidemia, and atherosclerosis than peripheral obesity, in which fat is predominantly gluteofemoral.

In the present study, long-term interdisciplinary lifestyle therapy was effective in decreasing visceral fat (49%) and increasing adiponectin levels (33%). Despite the lack of a statistically significant difference in the mean levels of TNF-α, IL-6, and IL-10 before and after therapy, there was an observed decrease of 8% and 29% and an increase of 87%, respectively.

A previous study [16] demonstrated that interdisciplinary lifestyle therapy was efficient in restoring the parameters associated with reduced visceral fat in obese adolescents with metabolic syndrome and insulin resistance. Our data suggest that visceral fat is correlated with a marked inflammatory state, whereas subcutaneous fat showed the opposite effect. In fact, TNF-α was significantly negatively correlated with subcutaneous fat. In addition, when the ratio of visceral to subcutaneous fat was assessed in relation to cytokine levels,

we observed a positive correlation with TNF-α levels and a negative correlation with adiponectin levels.

Klein et al [8] stated that abdominal liposuction should not, by itself, be considered a clinical therapy for obesity. The aspiration of large amounts of subcutaneous abdominal fat in women with abdominal obesity may have cosmetic benefits; but the procedure does not significantly improve insulin sensitivity in the liver, skeletal muscle, or adipose tissue; serum concentrations of inflammatory markers; or other risk factors for coronary heart disease. However, Porter et al [35] demonstrated that, whereas abdominal adiposity (visceral and subcutaneous fat) is associated with a higher absolute risk of metabolic and cardiovascular disease, subcutaneous abdominal fat is not associated with a linear increase in the prevalence of risk factors among the obese. Indeed, subcutaneous adipose tissue may actually be a protective fat depot in obese individuals in the case of high triglycerides. In their review, Chaston and Dixon [36] related that preferential loss of visceral fat compared with subcutaneous fat is greatest with modest weight loss; the effect is attenuated, and possibly lost completely, with increasing weight loss.

It has been hypothesized that a reduction in visceral fat without substantial weight loss is effective in ameliorating obesity-related comorbidity. In this sense, the "portal hypothesis" suggests that the proximity of visceral fat to the liver increases the fatty acid, hormone, and cytokine delivery from adipose tissue to the liver, exacerbating hepatic insulin resistance and increasing glucose output [37].

An important observation in the present research was the beneficial effects of lifestyle intervention on visceral fat depot and inflammatory state in obese adolescents. Many studies have demonstrated benefits from aerobic training and diet leading to an anti-inflammatory state in obese rats and human models [11-13,38]. Classically, aerobic exercise training is adopted as a weight loss program, inducing an increase in the mobilization of fatty acid from adipose tissue and leading principally to fat oxidation by skeletal muscle that contributes to obesity control [13,15,29,38].

Adiponectin may also have antiatherogenic and anti-inflammatory properties, and high circulating levels have been related to a lower risk of coronary heart disease [4]. A transcriptional mechanism leading to decreased adiponectin plasma levels in obese women has been previously demonstrated. In addition, low levels of adiponectin have been associated with high levels of C-reactive protein and IL-6 [39]. However, Borges et al [40] found that a weight loss greater than 5% improved inflammatory status in adult women by decreasing C-reactive protein and insulin resistance, regardless of changes in adiponectin or TNF-α levels.

In the present study, it was verified that the theoretical equation from the correlation between adiponectin and the visceral to subcutaneous ratio was useful in predicting the visceral to subcutaneous ratio from the adiponectin serum concentration.

Although the small number of participants could be a limitation of our study, the results contribute to the understanding of the mechanisms linking obesity and cytokines to the inflammatory state and the importance of lifestyle interdisciplinary therapy intervention as clinical practice for obesity treatment.

Although the small numbers of boys and girls made it difficult to explore our data for sex differences, girls showed lower levels of TNF-α and a lower visceral to subcutaneous ratio when compared with boys (before and after experimental protocol). These results corroborate the study carried out by Cartier et al [41], which showed that premenopausal women had lower TNF-α levels when compared with men, thus reinforcing the idea that visceral fat greatly contributes to an inflammatory state in obese patients.

Borges et al [40] and Cartier et al [41], using a large number of subjects and a long-term follow-up, investigated the roles of other inflammatory biomarkers, such as C-reactive protein, to better elucidate the beneficial effects of the lifestyle interdisciplinary therapy in adolescent obese patients.

Future studies are needed to better understand pathway contributes for high cytokines levels and relation with fat depot. In addition, these findings should be confirmed in other populations to prevent the development of many obesity comorbidities at a young age.

In summary, the present study demonstrated that decreasing the visceral fat in obese adolescents showed an anti-inflammatory effect and that this reduction was accompanied by a decreased proinflammatory and an increased anti-inflammatory state.

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