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European Journal of Sports and Exercise Science, 2014, 3 (1):5-17
(http://scholarsresearchlibrary.com/archive.html)

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ISSN: 2278 – 005X

Aerobic *plus* resistance training on pro-anti-inflammatory adipokinesin obese adolescents

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ABSTRACT

In order to investigate the effects of aerobic plus resistance training as part of a long-term interdisciplinary therapy on pro-anti-inflammatory biomarkers in obese adolescents, a total of 86 obese adolescents (42 in bicycle and 44 in treadmill training groups) were enrolled for 1 year of interdisciplinary weight-loss therapy.The most important finding was that Δ submaximum and maximum oxygen consumption (VO_2) was inversely correlated to Δ leptin/adiponectin ratio in obese adolescents after weight loss intervention. In addition, both groups presented significant reduction in thepro-inflammatory biomarkers leptin and leptin/adiponectin ratio. Also, an increase of adiponectin, adiponectin/leptinratio was observed. In conclusion, aerobic plus resistance training as part of a long-term interdisciplinary therapy was effective in improving pro-anti-inflammatory adipokines in obese adolescents. Additionally, the improvement of work capacity and physical fitness observed contributed directly to the improvement of pro-anti-inflammatory biomarkers and may also contribute to enhance exercise tolerance in obese adolescents.

Key-Words: interdisciplinary therapy, physical exercise, leptin, adiponectin, obesity.

INTRODUCTION

With an increasing prevalence, pediatric obesity is often a prelude to adulthood obesity, and represents a major public health issue. Since comorbidities are very common and severe in obese adults, therapeutic strategies such as lifestyle interventions in early childhood obesity appear all the more necessary, optimally including both exercise and diet because of their known effects on inflammatory and oxidative stress markers[1].Obesity is characterized as an inflammatory state associated with a modification in the pattern of adipokine secretion and is related to disorders including metabolic syndrome risk factors (visceral obesity, hypertension, type 2 diabetes, dyslipidemia, nonalcoholic fatty liver diseases), asthma, sleep apnea, psychological disturbances, psychosocial difficulties and lower health related quality of life scores even in adolescents [2].

Adipokines are polypeptides secreted in the adipose tissue in a regulated manner. While some of these molecules are expressed only by adipocytes, resident and infiltrating macrophages and components of the vascular stroma markedly contribute to expression of other adipokines. As a result, adipose tissue inflammation is associated with a

modification in the pattern of adipokine secretion. Leptin, adiponectin, and resistin are the best-studied molecules in this class, but other cytokines are also secreted at high levels by the adipose tissue [3].

Leptin is an adipokine that is primarily expressed by adipose tissue and is considered to be involved in neuroendocrine control of energy balance. Aside from its effect on inhibiting food intake and increasing energy expenditure at the central level, leptin appears to play a pro-inflammatory function mediating atherogenic processes [4, 5].On the other hand, adiponectin is the most abundant hormone secreted by adipose tissue and has potent anti-inflammatory effects that have been shown to be inversely correlated with insulin resistance (IR). Obesity (mainly visceral adiposity) is a chronic inflammatory disease of low intensity and has been shown to enhance leptin and to reduce adiponectin[6, 7].A small amount of available data suggest that acute exercise does not increase circulating adiponectin concentrations in adolescents; however, it is very possible that more rigorous exercise protocols could acutely affect circulating adiponectin levels [8].

Ghrelin is an orexigenic peptide secreted mainly from the stomach and proximal small intestine and it is currently the only known circulating hormone that stimulates appetite and promotes food intake. Ghrelin is unique in that it is the only substance that is secreted in response to a reduction in gastrointestinal contents, and it is suppressed by eating. Ghrelin exerts its action on appetite and food intake largely through central processes [9].Investigations have demonstrated that exercise training increases total ghrelin levels in adolescents and that ghrelin is sensitive to reductions in body fat or increases in energy expenditure in this population [8].

Signaling of circulating ghrelin is mediated by neurons of the arcuate nucleus of the hypothalamus. In particular, neurons expressing two potent orexigenic neuropeptides, neuropeptide Y (NPY) and agouti-related protein (AgRP), have been demonstrated to reduce the activity of proopiomelanocortin (POMC) neurons via ghrelin. Therefore, NPY and AgRP are mediators of the orexigenic effect of circulating ghrelin via inhibition of melanocortin signaling [9].On the other hand, leptin, melanocortins and alpha-melanocortin-stimulating hormone (α-MSH) are involved in satiety (anorexigenic factors) [4, 5].The role of α-MSH in the peripheral regulation of body weight in humans is unknown, especially in the pediatric population. However, recently it was demonstrated that changes of weight status are associated with changes of peripheral α-MSH [10].

Different strategies are adopted with the intention of restoring the inflammatory state in obese subjects. Aerobic training is the most prescribed exercise modality for the management of pediatric obesity. There is strong evidence that it decreases waist circumference, percent body fat and visceral fat, increases cardiorespiratory fitness, and decreases blood pressure in obese adolescents [11]**.**Caranti et al [12] showed that aerobic training as part of a long-term multidisciplinary therapy was effective in promoting beneficial changes in some predictors and decreasing the prevalence of metabolic syndrome in obese adolescents. Moreover, Carnier et al [13] showed that aerobic training as part of an interdisciplinary therapy is more effective than aerobic *plus* resistance training to improve secretion of anorexigenic/orexigenic factors in obese adolescents. On the other hand, some authors have shown that aerobic *plus* resistance training is more effective than aerobic training alone to improve body composition [14], metabolic profiles, adiponectinemia[15] and nonalcoholic fatty liver disease [16]in the same population.

However, despite these promising results, few studies have addressed the effects of a long-term multidiscliplinary intervention on pro-and anti-inflammatory adipokines in obese adolescents. Thus, the aim of the present study was to investigate the effects of aerobic *plus* resistance training as part of a long-term interdisciplinary therapy on pro-anti-inflammatory adipokines in obese adolescents.

MATERIALS AND METHODS

Study Subjects

A total of 108 adolescents were selected to participate of the present study. They were selected from GEO (Interdisciplinary Obesity Program) of the Universidade Federal de São Paulo- UNIFESP in recent years. The GEO project has occurred every year since 2004 in São Paulo, Brazil. At the beginning of each year, the project is published in newspapers and magazines from São Paulo to recruit adolescents. Of these 108 participants, we excluded those who did not complete therapy for reasons such as having found work, changes in school hours, lack of motivation and lack of money for transportation, as well as patients who did not perform all necessary examinations for this study in three stages of evaluation. Then, a total of86 obese adolescents were evaluated in this study. Obese adolescents (BMI>95th percentile of the CDC reference growth charts) [17], aged from 15 to 19 years (16.41±2.34 years), who reported not having had experience of exercise training before the study, including 56 girls and 30 boys, were recruited for a long-term (one year) weight loss intervention study. The inclusion criteria for the postpubertal stage were based on Tanner (stage five) for boys and girls [18]. The non-inclusion criteria were: endocrine diseases, chronic alcohol consumption, pregnancy and previous use of drugs which may affect appetite

regulation, such as anabolic-androgenic steroids or psychotropics. Informed parental consent and adolescents' assent to participate as volunteers in an interdisciplinary weight loss program were obtained. This study was conducted in accordance with the principles of Helsinki Declaration and was formally approved by the ethics committee of the Universidade Federal de São Paulo - EscolaPaulista de Medicina (Number: 0135/04) and registered in the Clinical Trial: Clinicaltrials.govNCT 0135/7883.This study meets the ethical standards of the journal [19].

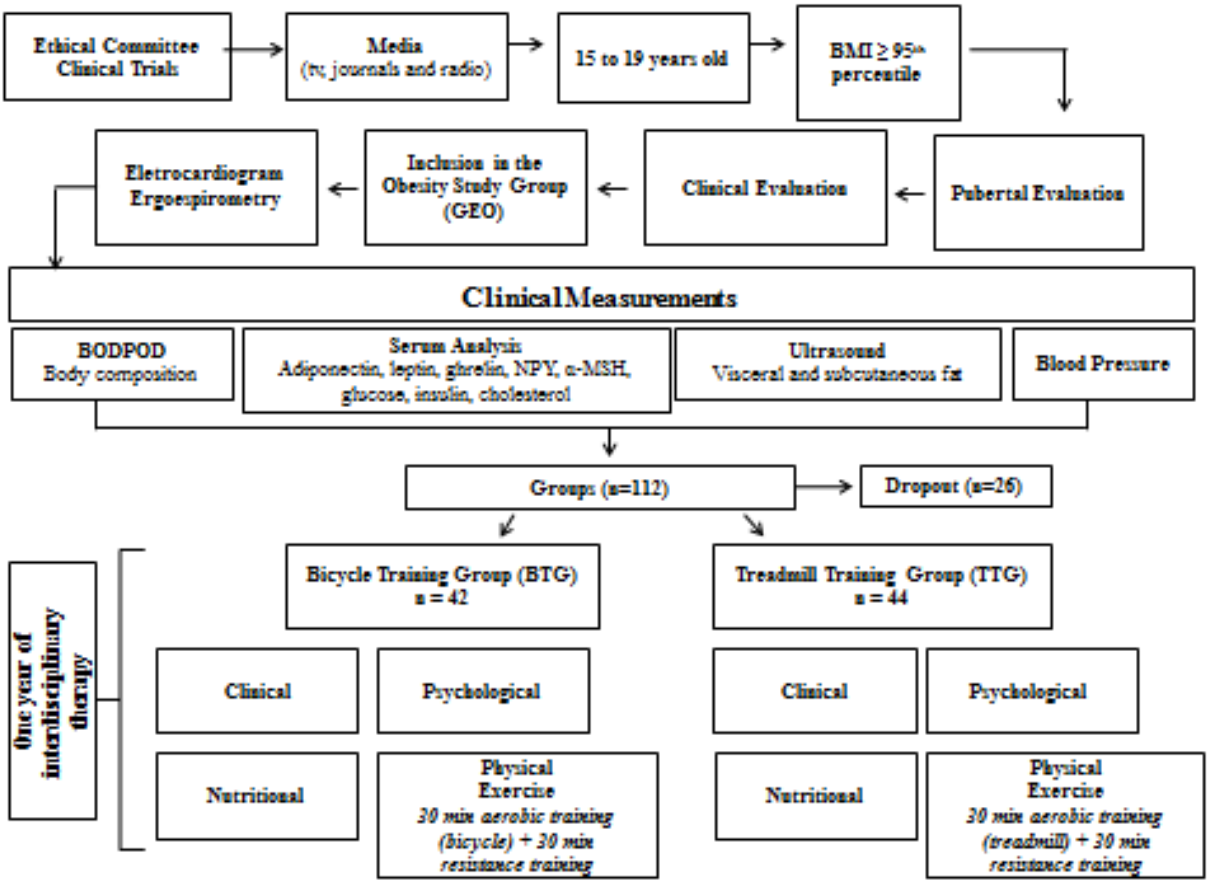

Figure 1: Description of the methodology adopted to develop the study.

The complete methodology adopted to develop the study is described in Figure 1. Subjects were randomized into two groups: 1) bicycle training as part of an aerobic plus resistance training (BTG: n = 42; 12 boys and 30 girls) and 2) treadmill training as part of an aerobic plus resistance training (TTG: n = 44; 18 boys and 26 girls).All subjects reported not having had experience of exercise training before the study. Volunteers were instructed to not participate in any physical activity the day before the experiments (pre and post-training). All subjects were completely familiarized with all testing procedures before the experiment to reduce the influence of any learning effects, solely due to the mechanics of performing the test protocol. After evaluations, but before the periodization, BTG and TTG groups performed two weeks of training for adaptation, totaling one year of protocol.

Anthropometric Measurements and Body Composition

Subjects were weighed wearing light clothing and bare footed on a Filizola scale to the nearest 0.1 kg. Stature was measured to the nearest 0.5 cm by using a wall-mounted stadiometer (Sanny, model ES 2030). BMI was calculated as body weight divided by height squared (wt/ht^2). Body composition was measured by plethysmography in the BOD POD body composition system (version 1.69; Life Measurement Instruments, Concord, CA) [20]. All volunteers were encouraged to adopt a balanced diet throughout treatment, including before and after assessments. Before the body composition assessment, adolescents were instructed to not consume foods with caffeine.

Visceral and subcutaneous adiposity measurements

Abdominal ultrasonographic measurements of visceral and subcutaneousfat tissues were performed by the same physicianblinded to subject assignment groups at baseline and after intervention.This physician was a specialist in imaging diagnostics.A 3.5-MHz multifrequency transducer (broad band) was used toreduce the risk of misclassification. The intra-examination coefficientof variation for ultrasound (US) was 0.8%.Subcutaneous fat was

defined as the distance between the skinand superficial plane of the rectus abdominal muscle. Visceralfat was defined as the distance between the deep plane of thesame muscle and the anterior wall of the aorta [21].

Blood pressure
Blood pressure was measured on the right arm using a mercury-gravity manometer with an appropriately sized cuff. Two measurements were made after the subjects had been seated for at least 5 min, and the mean value was used for analyses.

Serum Analysis
Blood samples were collected in the outpatient clinic around 8 h after an overnight fast. After collection, the blood was centrifuged for 10 min at 5000 rpm and stored at -70^{o}C for future analysis. The materials used for collection were disposable and adequately labeled. Blood was collected by a skilled and qualified technician. The serum concentrations of glucose, insulin, triglycerides, total cholesterol (T-cholesterol), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were determined by enzymatic colorimetric methods (CELM, Barueri, Brazil). Insulin resistance was assessed by homeostasis model assessment of insulin resistance index (HOMA-IR). HOMA-IR was calculated by the product of blood glucose (fasting blood glucose) and the immunoreactive insulin (I): (fasting blood glucose (mg/dl) × I (mU/l))/405. The HOMA-IR data were analyzed according to reference values reported by Keskin et al. The HOMA-IR cutoff point for insulin resistance adopted for adolescents was 3.16 [22]. Insulin sensitivity was determined by the Quantitative Insulin Sensitivity Check Index – QUICKI: [1/(log fasting insulin (lU/ml) + log fasting glucose (mg/dl)] [23].Adiponectin and leptin were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. For this study, the leptin reference values were described by Gutin et al [24] and Dâmaso et al [25]. Serum total ghrelin, NPY and α-MSH concentrations were measured using a commercially available enzyme-linked immune sorbent assay (ELISA) kit from Phoenix Pharmaceuticals (Belmont, CA) according to the manufacturer's instructions.

The evaluations and data collection were made at baseline and one year after interdisciplinary therapy, both after an overnight fast in the Sleep Institute.

Research Design
The interdisciplinary obesity intervention consisted of physical training (aerobic plus resistance training) associated to clinical, nutritional and psychological therapy. The use of interdisciplinary therapy has been suggested by World Health Organization [26]. All measurements were performed at baseline and after one year of therapy.

Exercise Protocol
Exercise protocol consisted of aerobic plus resistance training performed three times per week for one year, including 30 minutes of aerobic training plus 30 minutes of resistance training per session (180-minute/week) for both groups. The volunteers were oriented to invert the order of the exercises at each training session: in one session the adolescent started the training session with aerobic exercises and in the subsequent session the same adolescent started with the resistance training.

The aerobic training mode was running performed on a motor-driven treadmill or pedaling on a cycle-ergometer for treadmill training group (TTG) or bicycle training group (BTG), respectively.Both groups performed aerobic training at the cardiac frequency intensity of the ventilatory threshold I (±4 bpm), according with the results of an initial oxygen uptake test for aerobic exercises (treadmill or cycle-ergometer, respectively). The physiologists controlled the cardiac frequency, which was measured with a cardiometer at intervals of 5 min during all training sessions (Polar Model FS1 dark blue). The exercise program was based on the 2001 recommendations provided by the American College of Sports Medicine (ACSM) [27]and adapted by Foschini and colleagues [28].

Resistance training was divided following the recommendations of ACSM [27]. It were used exercises for the main muscular groups: bench press, leg press, sit-ups, latpull-down, hamstring curls, lower back, military press, calf raises, arm curls, triceps pushdown. The exercise program was based on the 2001 recommendations provided by the American College of Sports Medicine (ACSM) [27] and adapted by Foschini and colleagues [28].

The first two weeks of the resistance training had as main purpose the learning of the movements (three sets of 15-20RM for each exercise). Succeeding, the load of training was adjusted, modifying volume and intensity inversely, decreasing the number of repetitions to between 6 and 20 repetitions for three sets. The rest interval between series and exercises were: 15-20RM = 45s; 10-12RM = 1 minute and 6-8RM = 1.5 minutes. The training loads were adjusted in each training session and evaluated according to the increase in participants' strength. Therefore, the

training was conducted with maximal repetitions (RM).

Psychological Therapy
Psychological therapy was established by validated questionnaires, taking into account some of the psychological problems caused by obesity, as described in the literature, including depression, eating disorders, anxiety, decreased self-esteem and body-image disorders. During the interdisciplinary therapy, the adolescents received psychological orientation for 1h in a weekly group session. The psychologist discussed: body image and eating disorders, such as bulimia and anorexia nervosa, and binge eating disorders as well as their signs, symptoms, and consequences for health; the relationship between feelings and food; familiar problems, such as alcoholism and other issues. Individualized psychological therapy was recommended when weight problems or poor-dietary habits were found.

Nutritional Therapy
Energy intake was set at the levels recommended by the dietary reference for subjects with low levels of physical activity of the same age and gender, following a balanced diet [29]. No drugs or antioxidants were recommended. Once a week, adolescents had a dietetics lessons (providing information on 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 the weight loss; functional foods; decision on food choices). All patients received individual nutritional consultation during the intervention program.

At the beginning of the study and six months into the program, a 3-day dietary record was collected. Once most obese people under-report their food consumption, each adolescent was asked to record their diet with the help of their parents [30].The degree of under-reporting may be substantial, but this is a validated method to assess dietary consumption [31]. 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 Universidade Federal de São Paulo - EscolaPaulista de Medicina (Nutwin software, for windows, 1.5 version, 2002), that use data from western and local food tables. In addition, the parents were encouraged by a dietitian to call if they needed extra information. All volunteers were encouraged to adopt a balanced diet throughout treatment, including before and after assessments.

Clinical Therapy
To accomplish the health and clinical parameters, obese adolescents of all groups analyzed visited the endocrinologist once each month. Medical follow-up and treatment is based on patient's characteristics, familiar history, physical examination and also intervention on unhealthy problems that were developed along the therapy.

Statistical Analysis
All data were analyzed using STATISTICA version 7 (StatSoft) for Windows, with the significance level set at 5%. A Kolmogorov-Smirnov test to normality was performed. Dependent variables were transformed into Z scores to check outliers. Data were expressed as the mean ± standard deviation for parametric variables and median (minimum-maximim) for non-parametric variables. Comparisons between measures at baseline and after weight-loss intervention in each group and between groups were made using an analysis of variance (ANOVA) for repeated measures and Tukey post hoc testfor parametric variables. Mann-Whitney (baseline conditions between groups) and Wilcoxon signed rank tests were performed for non-parametric variables. Dependent Student´s *t*-tests were used to compare maximum and anaerobic threshold speeds for treadmill training group and maximum workload for bicycle training group while, Wilcoxon test were used to compare anaerobic threshold workload for bicycle training group. Independent Student´s *t*-tests were used to compare deltas (Δ) between groups. Pearson´s or Spearmann´s correlations were performed to verify the relationship between Δ of the variables for the entire population (bicycle + treadmill training groups).

RESULTS

Table 1 shows anthropometric and body composition data of obese adolescents before and after weight loss interventions. It was observed that both groups presented significant improvement for body mass (kg), height (cm), body mass index (kg/m^2), body fat mass (kg and %), body lean mass (%), waist circumference (cm), visceral and subcutaneous fat (cm) after interventions. Body lean mass (kg) and visceral to subcutaneous ratio were improved only in bicycle training group. Additionally, delta values were significant different between bicycle and treadmill training groups for body mass (kg), body fat mass (% and kg), body lean mass (%) and the best improvements were observed in the bicycle training group.

Table 1: Anthropometric and body composition data of obese adolescents before and after weight loss interventions.

	Bicycle training (n=42)			Treadmill training (n=44)		
	Baseline	**Afterintervention**	**Δ**	**Baseline**	**Afterintervention**	**Δ**
Bodymass (kg)	107.22±16.34	95.44±15.48**	-11.78±8.80	101.85±15.63	94.12±18.00**	-7.73±6.36 &
Height (cm)	1.68±0.08	1.69±0.09**	0.007±0.010	1.68±0.09	1.69±0.09**	0.009±0.012
BMI (kg/m^2)	37.68 (30.18-48.06)	32.10 (25.06-44.13)**	-3.72 (-10.81-2.04)	34.28 (29.90-48.51) #	31.83 (23.06-48.80)**	-2.81 (-7.31-2.98)
Body fat mass (%)	46.78±6.23	38.44±7.53**	-8.69±5.40	45.90±5.72	39.83±6.71**	-6.07±4.27 &
Body lean mass (%)	53.50±6.02	61.56±7.53**	8.50±5.31	54.10±5.72	60.18±6.71**	6.08±4.26 &
Body fat mass (kg)	49.99±11.34	36.75±12.38**	-13.41±8.17	47.09±11.27	37.91±11.35**	-9.18±5.72 &&
Body lean mass (kg)	56.05±7.66	59.30±9.11**	2.26±4.22	54.78±7.99	56.05±9.30	1.27±3.90
Waist circumference (cm)	105.26±10.60	94.26±12.24**	-10.85±12.10	101.13±10.58	95.12±10.93**	-4.32±21.31
Visceral fat (cm)	4.44±1.50	2.61±1.06**	-1.67±1.11	4.57±1.31	3.29±1.34**	-1.31±0.94
Subcutaneous fat (cm)	4.05±0.89	3.31±0.78**	-0.76±0.94	3.95±0.70	3.24±0.83**	-0.76±0.67
Visceral to subcutaneous ratio	1.16±0.49	0.82±0.35**	2.07±9.52	1.18±0.32	1.05±0.41	2.00±5.30

Abbreviations: BMI, body mass index; Δ, change; Data are presented as mean ± SD or median (minimum-maximum).
**comparison of baseline vs. after intervention, p≤0.05; **comparison of baseline vs. after intervention, p≤0.01; # baseline comparison of both groups, p≤0.05; & delta comparison of both groups, p≤0.05; && delta comparison of both groups, p≤0.01.*

Table 2: Clinical characteristics of obese adolescents before and after weight loss interventions.

	Bicycle training (n=42)			Treadmill training (n=44)		
	Baseline	**Afterintervention**	**Δ**	**Baseline**	**Afterintervention**	**Δ**
Glucose (mg/dl)	89.00±6.25	87.85±6.43	-1.12±9.41	91.20±7.03	90.56±7.71	-0.53±6.63
Glucose (µU/ml)	4.94±0.35	4.88±0.36	-0.06±0.52	5.06±0.39	5.03±0.43	-0.03±0.37
Insulin (µU/ml)	17.58±5.58	10.79±5.07**	-6.68±5.61	14.38±6.16	12.20±7.54*	-2.86±7.17 &&
HOMA-IR	3.87±1.27	2.36±1.17**	-1.48±1.38	3.23±1.44	2.59±1.49	-0.63±1.74 &
QUICKI	0.31±0.014	0.34±0.028**	0.028±0.026	0.33±0.019	0.34±0.029**	0.015±0.027 &
Total cholesterol (mg/dl)	167.38±29.44	153.59±28.07**	-13.41±23.13	171.36±36.28	160.82±28.59**	-10.55±16.41
HDL-c (mg/dl)	43.55±7.21	45.32±7.33	1.83±5.93	46.32±10.15	46.44±9.07	0.77±4.79
LDL-c (mg/dl)	103.95±27.15	91.32±24.28**	-12.46±17.76	103.72±30.08	95.66±24.29	-8.63±17.04
VLDL-c (mg/dl)	18.50 (7-47)	15 (6-43)*	-2 (-33-22)	18 (7-50)	15 (6-43)*	-1 (-23-9)
Triglycerides (mg/dl)	92 (35-235)	75 (32-215)*	-8 (-92-110)	88 (37-252)	73 (30-183)*	-7 (-116-45)
SBP (mm Hg)	120 (100-155)	120 (100-140)*	-10 (-45-20)	120 (100-150)	110 (100-140)**	-10 (-25-20)
DBP (mm Hg)	80 (70-90)	70 (70-90)**	-5 (-20-10)	80 (70-90)	70 (60-90)*	0 (-80-20)

Abbreviations: DBP, diastolic blood pressure; HDL, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment insulin resistance index; LDL-c, low-density lipopotein-cholesterol; SBP, systolic blood pressure; VLDL-c, very-low-density lipoprotein-cholesterol; Δ, change.
Data expressed as mean±SD or median (minimum-maximum)
Reference values: Glucose (60–110 mg/dL), Insulin (<20 µU/mL), HOMA-IR (<2.0), QUICKI (>0.339); Total cholesterol (<17 mg/dL), Triglycerides (33–12 mg/dL), HDL-cholesterol (>3 mg/dL), LDL-cholesterol (<13 mg/dL), VLDL-cholesterol (10–5 mg/dL) [32].
**comparison of baseline vs. after intervention, p≤0,05; **comparison of baseline vs. after intervention, p≤0,01; # comparison of baselines of both groups, p≤0,05; & delta comparison of both groups, p≤0.05; && delta comparison of both groups, p≤0.01.*

Table 3 shows adipokines and neuropeptides of obese adolescents before and after weight loss interventions. Adiponectin(µg/l), leptin (ng/ml), adiponectin to leptin ratio and leptin to adiponectin ratio presented significant improvements for both groups after interventions. On the other hand, it was not observed significant changes for ghrelin (ng/ml), NPY (ng/ml), α-MSH (ng/ml) for both groups. Additionally, delta values were not significantly different between bicycle and treadmill training groups.

Clinical characteristics of obese adolescents before and after weight loss interventions are presented in Table 2. Insulin (µU/ml), QUICKI, total cholesterol (mg/dl), VLDL-cholesterol (mg/dl), triglycerides (mg/dl), systolic and diastolic blood pressures (mmHg) were improved in both groups after interventions. However, HOMA-IR and LDL-cholesterol (mg/dl) improved significantly only in bicycle training group. Finally, it was not observed significant changes for glucose (mg/dl and µU/ml) and HDL-cholesterol (mg/dl) in both groups of interventions. Additionally, delta values were significant different between bicycle and treadmill training groups for insulin (µU/ml), HOMA-IR and QUICKI and the best improvements were also observed in the bicycle training group.

Table 3: Adipokines and neuropeptides of obese adolescents before and after weight loss interventions.

	Bicycle training group (n=42)			Treadmill training group (n=44)		
	Baseline	**Afterintervention**	**Δ**	**Baseline**	**Afterintervention**	**Δ**
Adiponectin (µg/l)	7.31 (0.26-19.40)	9.44 (2.54-31.20)**	1.75 (-2.76-7.46)	4.29 (1.87-23.90) #	4.92 (2.49-31.18)*	0.87 (-8.86-11.35)
Leptin (ng/ml)	42.07±26.28	24.51±13.38**	-13.76±20.40	45.39±23.53	26.86±12.88**	-17.29±20.50
Adiponectin to leptin ratio	0.25 (0.04-1.53)	0.38 (0.08-3.97)**	0.11 (-0.15-3.69)	0.10 (0.03-6.53)	0.22 (0.06-3.61)**	0.09 (-2.92-1.86)
Leptintoadiponectinratio	4.10 (0.65-25.88)	2.55 (0.17-13.02)**	-1.61 (-19.10-3.53)	10.18 (0.15-31.11)	4.46 (0.28-17.40)**	-3.14 (-18,76-2.54)
Ghrelin (ng/ml)	1.15±0.26	1.16±0.23	-0.002±0.204	1.05±0.31	1.10±0.29	0.032±0.157
NPY (ng/ml)	1.02 (0.24-9.61)	1.32 (0.18-7.60)	0.04 (-7.46-0.91)	1.22 (0.22-4.85)	1.19 (0.44-3.05)	-0.09 (-2.28-1.18)
Alpha-MSH (ng/ml)	1.15±0.72	1.11±0.54	-0.02±0.55	1.16±0.80	1.06±0.66	-0.14±0.54

Abbreviations: Δ, change.
Data are presented as mean ±SD or median (minimum- maximium)
**comparison of baseline vs. after intervention, p≤0,05; **comparison of baseline vs. after intervention, p≤0,01; # comparison of baselines of both groups, p≤0,05; & comparison of deltas of both groups, p≤0,05; && delta comparison of both groups, p≤0.01.*

Table 4: Exercise parameters of obese adolescents before and after weight loss interventions.

	Bicycle training group (n=42)			Treadmill training group (n=44)		
	Baseline	**Afterintervention**	**Δ**	**Baseline**	**Afterintervention**	**Δ**
Maximum VO_2 (ml/kg/min)	24.11±5.47	26.30±6.02*	2.19±4.54	28.58±4.56 #	32.59±6.10**	4.01±5.02
Maximum VO_2 (L/min)	2.59±0.62	2.53±0.61	-0.07±0.39	2.86±0.54	3.04±0.60	0.17±0.45 &
Maximum HR (bpm)	184.43±10.26	182.55±13.16	-1.88±10.31	186.64±11.27	188.59±11.62	1.95±8.92
Maximum pulmonary ventilation (L/min)	90.99±23.76	95.69±23.06	3.72±16.75	100.28±17.09	108.52±17.67*	5.87±14.42
Maximum speed (km/h)	-	-	-	8.28±1.14	9.56±1.31**	1.30±0.98
Maximum workload (W)	179.88±44.45	197.44±40.47**	17.11±26.70	-	-	-
VO_2 at AT (ml/kg/min)	15.26±2.81	17.20±3.43**	1.94±2.96	16.54±3.02	19.66±4.48**	2.82±3.91
VO_2 at AT (L/min)	1.64±0.37	1.66±0.35	0.01±0.32	1,66±0,33	1,85±0,41**	0.18±0.40 &
HR at AT (bpm)	143.31±14.00	146.02±13.42	2.71±13.93	143.23±16.47	143.59±15.76	0.81±15.14
Speed at AT (km/h)	-	-	-	5.22±1.03	6.09±1.02**	0.88±0.78
Workload at AT (W)	75 (25-150)	100 (25-200)**	25 (-25-75)	-	-	-

Abbreviations: Δ, change; AT, anaerobic threshold.
Data are presented as mean ±SD or median (quartile range)
**comparison of baseline vs. after intervention, p≤0,05; **comparison of baseline vs. after intervention, p≤0,01; # comparison of baselines of both groups, p≤0,05; & comparison of deltas of both groups, p≤0,05; && delta comparison of both groups, p≤0.01.*

Exercise parameters of obese adolescents before and after weight loss interventions are presented in Table 4. Both groups improved maximum oxygen uptake (VO_2) and VO_2 at anaerobic threshold (AT) in ml/kg/min. Moreover, bicycle training group improved submaximum (at AT) and maximum workloads (W) as well as treadmill training group improved submaximum (at AT) and maximum speed (km/h) reached on treadmill after weight loss intervention. Treadmill training group also improved significantly VO_2 at AT (L/min). It was observed significant differences between bicycle and treadmill training groups in delta only for submaximum (at AT) and maximum VO2 (L/min).

Significant correlation coefficients of delta values are presented in Tables 5, 6 and 7. Table 5 shows significant Pearson correlation coefficient of delta variables analyzed for the entire population of the study. Delta (Δ) QUICKI correlated positively with Δ body lean mass (%) and negatively with Δ body fat mass (%). Additionally, Δ leptin correlated positively with Δ body fat mass (kg).

Table 5: Significant Pearson Correlation coefficients of delta variables analyzed in the entire population of obese adolescents after weight loss intervention (bicycle + treadmill training groups altogether).

	Δ QUICKI	**Δ Leptin**
Δ Bodyfatmass %	r = - 0.52*	-
Δ Body fat mass (kg)	-	r = 0.54*
Δ Bodyleanmass %	r = 0.52*	-

** p≤0.05*

Tables 6 and 7 show significant Spearman correlation coefficient of delta variables analyzed for the entire population of the study. Delta (Δ) waist circumference correlated positively with Δ body fat mass (%) and negatively with Δ body lean mass (%). Moreover, Δ alpha-MSH correlated positively with Δ body lean mass (%) and negatively with Δ body fat mass (%). Additionally, it was observed that Δ leptin to adiponectin ratio correlated positively with Δ body fat mass (% and kg) and negatively with Δ VO_2submaximum (AT) and maximum (ml/kg/min). Finally, Δ adiponectin to leptin ratio correlated positively with Δ body lean mass (%) and Δ QUICKI and negatively with Δ body mass, Δ body fat mass (%), Δ waist circumference, Δ visceral fat, Δ subcutaneous fat and Δ HOMA-IR.

Table 6: Significant Spearman Correlation coefficients of delta variables analyzed in the entire population of obese adolescents after weight loss intervention (bicycle + treadmill training groups altogether).

	Δ Waistcircumference (cm)	**Δ α-MSH**
Δ Bodyfatmass %	r = 0.62*	r = - 0.32*
Δ Bodyleanmass %	r = - 0.62*	r = 0.32*

** p≤0.05*

Table 7: Significant Spearman Correlation coefficients of delta leptin to adiponectin ratio and adiponectin to leptin ratio with other variables analyzed in the entire population of obese adolescents after weight loss intervention (bicycle + treadmill training groups altogether).

	ΔLeptin to adiponectin ratio	**Δ Adiponectin to leptin ratio**
Δ Body mass	-	r = - 0.41*
Δ Body fat mass %	r = 0.25*	r = - 0.48*
Δ Body fat mass kg	r = 0.35*	-
Δ Body lean mass %	-	r = 0.47*
Δ Waist circumference	-	r = - 0.33*
Δ Visceral fat	-	r = - 0.30*
Δ Subcutaneous fat	-	r = - 0.30*
Δ HOMA	-	r = - 0.33*
Δ QUICKI	-	r = 0.41*
Δ VO_2 max (ml/kg/min)	r = - 0.44*	-
Δ VO_2 at AT (ml/kg/min)	r = - 0.28*	-

** p≤0.05*

DISCUSSION

The aim of the present study was to investigate the effects of aerobic *plus* resistance training as part of an interdisciplinary therapy on pro-anti-inflammatory biomarkers in obese adolescents. Therefore, the most important finding of the present investigation was that Δ submaximum (at AT) and maximum oxygen consumption (VO_2) (ml/kg/min) was inversely correlated toΔ leptin/adiponectin ratio in obese adolescents after weight loss intervention (Table 7).

Leptin is an adipokine that is primarily expressed by adipose tissue and is considered to be involved in neuroendocrine control of energy balance.Aside from its effect on inhibiting food intake and increasing energy expenditure at the central level, leptin appears to play a pro-inflammatory function mediating atherogenic processes [4, 5].On the other hand, adiponectinis the most abundant hormone secreted by adipose tissue and has potent anti-inflammatory effectsthat have been shown to be inversely correlated with insulin resistance (IR).

Although obesity (mainly visceral adiposity) is a chronic inflammatory disease of low intensity and has been shown to enhance leptin and to reduce adiponectin [6, 7]we were able to show an improvement of pro-anti-inflammatory response in the present study sinceboth bicycle and treadmill training groups increased adiponectin and adiponectin/leptinratio and reduced leptin and leptin/adiponectin ratio in obese adolescents after weight loss intervention (Table 3).

These finding was corroborated by the positive correlation observed for the entire population (bicycle + treadmill training groups) between Δ body fat mass (kg) and Δ leptin (Table 5). Additionally, Δ leptin/adiponectin ratio was also directly correlated toΔ body fat mass (kg and %)(Table7). On the other hand, Δ adiponectin/leptin ratio was directly correlated to Δ body lean mass (%) and Δ QUICKI and inversely correlated to Δ body mass, Δ body fat mass (%), Δ waist circumference, Δ visceral fat, Δ subcutaneous fat and Δ HOMA-IR (Table 7).

In fact, an earlier study showed thatboth leptin and leptin/adiponectin ratio were decreased significantlywith low-to-moderate (5.8-10.9 kg) weight loss as well as adiponectin/leptin ratio was increased.However, adiponectin was increased only with massive weight loss (>15.9 kg) [33].In addition, in the present study, both groups (bicycle and treadmill training groups) showed reduced levels of leptin and leptin/adiponectin ratio and increased levels of adiponectin and adiponectin/leptin ratio although the weight loss observed in the bicycle training group (moderate weight loss) was higher than treadmill training group (low to moderate weight loss) (Table 1). Corroborating, all correlations presented in the present paper were observed in the entire group that showed a low to moderate weight loss[Δ body mass (mean±SD)= -9.71±7.86 kg, data not shown].

In another previous study, Dâmaso et al [25] showed significant increase in adiponectin after both short and long-term therapies only in non-hyperleptinemic patients. However, inhyperleptinemic patients, adiponectin increased only after short-term therapybut returned to baseline values after long-term therapy. Corroborating with this finding, it was shown that ahyperleptinemic statemay impair the attenuation of inflammation in obese adolescents undergoing interdisciplinary therapy, particularly by impeding the increase in adiponectin concentration, which is directly involved in vascular protection [34].However, in the present investigation it was showed that both bicycle and treadmill training groups increased adiponectin levels after long-term intervention, althoughwe did not compare hyperleptinemic patients with non-hyperleptinemic patients. Therefore, it needs to be confirmed in future research.

The energy balance of the body is mediated by anumber of factors, including the expression andparacrine secretion of hypothalamic neuropeptidesthat regulate this process by altering food intake andenergy expenditure [35].The signaling networkunderlying hunger, satiety and metabolic statusincludes the hormonal signals leptin and insulinfrom energy stores, cholecystokinin, glucagon-likepeptide-1, ghrelin and peptide YY3-36 from the gastrointestinaltract, as well as neuronal influences viathe vagus nerve from the digestive tract. This signaling is routed to the arcuate nucleus and the solitarytract nucleus, which activate distinct neuronalnetworks [4]. Neuropeptide Y(NPY), agouti gene-relatedpeptide (AgRP), ghrelin, melanin-concentrating hormone (MCH) and orexin stimulate appetite (orexigenic factors), while leptin, melanocortinsand alpha-melanocortin-stimulating hormone (α-MSH) are involved in satiety (anorexigenic factors) [4, 5].Most obese individuals display increased levels ofcirculating leptin, indicating a state of leptin resistance [36].

In the present study, it was not observed significant changes for NPY, ghrelin and α-MSH after one year of weight loss intervention for both bicycle and treadmill training groups (Table 3). An earlier study showed that NPY presented a significant increase after short-term therapy but decreased after long-term intervention suggesting that long-term interventions are necessary to promote better neuroendocrine regulation of food intake and energy balance [37].In another study, NPY increased after short-term therapy in obese adolescents who presented a lower weight loss (<2.5kg) but did not change after long-term intervention compared to baseline condition [38].Damaso et al [25]showed an increase in NPY levels after short-term therapy in hyperleptinemic obese adolescents but after long-term intervention NPY was similar to baseline condition. On the other hand, it was not observed in non-hyperleptinemic obese adolescents.The authors suggested that the hyperleptinemic state affects the neuroendocrine energetic balance, stimulating the orexigenic pathways, which make weight loss difficult in obese adolescents.

Carnier et al [37] showed that α-MSH decreased after short-term therapy but, on the other hand, Oyama et al [38] showed that α-MSH was significantly increased with massiveweight loss(>14kg), a change that is important in

attenuating the NPY-elicitedincrease in food intake and modulating feeding behavior [39, 40, 41].Although weight loss intervention did not change α-MSH levels in the present study, Δ α-MSH for the entire population was positively correlated to Δ body lean mass (%) and negatively correlated to Δ body fat mass (%) (Table 6) which reinforces that α-MSH changes in expression seems to be correlated to weight status change [38,42].Finally, the moderate levels of weight loss observed in both treadmill (low to moderate weight loss) and bicycle (moderate weight loss) training groups could be the reason for the absence of change in α-MSH levels in the present study since these obese adolescents did not achieve a massive weight loss during therapy.

The effect of weight loss on ghrelin concentrations is still controversial in scientific literature. Some studies have reported an increase in ghrelin concentrations [43, 44] whereas other studies have found no change in ghrelin concentrations [45, 46].Our findings of stable ghrelin concentrations for both treadmill and bicycle training groups after overweightreduction (Table 3) corroborated with others authors that showed no significant changes in ghrelinconcentrations after one year of weight loss intervention [38, 47, 48].This seems to be important for the maintenance of weight loss since increased levels of ghrelinduring weight reduction are regarded as being responsible for the difficulties encountered in long-term weight loss, being previouslydemonstrated [49].Moreover, Dâmaso et al [25] showed an increase in ghrelin concentration after one year of therapy only in non-hyperleptinemic patients and supposed that such a change is considered an adaptative function of ghrelin in response to negative energy balance [50]. These data reinforced the concept of leptinresistance in leptin excess status, as observed in obesity, as it was previously demonstrated that leptin inhibits ghrelin efflux from the stomac and reduced ghrelin-induced feeding [51, 52, 53].

As mentioned above, the main finding of the present study was that Δ submaximum (at AT) and Δ maximum VO_2 (ml/kg/min) was inversely correlated to Δ leptin/adiponectin ratio in obese adolescents after weight loss intervention (Table 7). Obese adolescents seems to presentpoor physical fitness and consequently, low tolerance to physical exercise [54, 55]which may impair the practice of exercise as an adjuvant to diet intervention in the treatment of obesity in this population. As it was demonstrated in the results section of the present paper (Table 4), it was observed a significant improvement of submaximum (at anaerobic threshold) and maximum VO_2 (ml/kg/min) after training for both groups as well as an improvement of submaximum and maximum workload and speed achieved after intervention for both bicycle and treadmill training groups, respectively.This last finding was indicative of an improvement of workratecapacity and suggested that the exercise trainingprogram caused typical and efficient adaptations (probablyperipheral) while the significant improvements of VO_2 mainly indicated a central cardiovascular benefit of bicycle/treadmill training. It is important to note that obese adolescents engaged in physical training may improve exercise tolerance and consequently increase physical training time which has a clinical importance in the treatment of obesity for this population.Moreover, it was demonstrated in the present study that obese adolescents engaged in physical training may enhance submaximum and maximum VO_2 (ml/kg/min) and this benefit was inversely correlated to Δ leptin/adiponectin ratio which may improve pro-anti-inflammatory state.

The present study also demonstrated the efficacy of an aerobic *plus* resistance training (bicycle or treadmill trainings) as part of an interdisciplinary therapy to promote weight loss. It was observed that both bicycle and treadmill trainings significantly improved anthropometric and body composition parameters of obese adolescents after weight loss intervention, except for body lean mass (kg) and visceral to subcutaneous ratio for treadmill training (Table 1).Additionally, Δwaist circumference was directly correlated to Δ body fat mass (%) and indirectly correlated to Δ body lean mass (%) for the entire population of this study (Table 6).This last finding corroborated with others described in scientific literature that showed the importance of waist circumference as a marker of obesity [56, 57].

Moreover, the efficacy of the interdisciplinary therapy adopted in the present study was also demonstrated in Table 2. Both treadmill and bicycle trainings promoted improvements of clinical characteristics which may contribute to the control of metabolic syndrome in obese adolescents after weight loss intervention.Since, insulin, QUICKI, total cholesterol, VLDL-cholesterol, triglycerides, systolic and diastolic blood pressureswere improved in both groups. However, HOMA-IR and LDL-cholesterol improved only in bicycle training group while glucose and HDL-cholesterol did improve neither bicycle nor treadmill training groups. Additionally, Δ QUICKI was directly correlated to Δ body lean mass (%) and inversely correlated to Δ body fat mass (%) for the entire population (Table 5). Altogether, these findings are of particular importance in this context since visceral fat stores and cholesterol are related to inflammatory process and the development of chronic diseases associated to obesity such as metabolic syndrome, hypertension, type 2 diabetes, dyslipidemia, nonalcoholic fatty liver diseases, asthma and sleep apnea [2]. Additionally, we interestingly observed significant differences between deltas (Δ) of some variables analyzed when comparing bicycle to treadmill training groups. Although our results have shown significant improvements in a lot of parameters analyzed for both groups (comparing beforeto after interventions), as discussed earlier, bicycle

training group had significantly higher changes throughout the intervention in body mass, body fat mass (% and kg), body lean mass (%), insulin, HOMA-IR and QUICKI compared to treadmill training group.Recently, Carnier et al [13] showed that aerobic training as part of an interdisciplinary therapy is more effective than aerobic *plus* resistance training to improve secretion of anorexigenic/orexigenic factors in obese adolescents. On the other hand, some authors have shown that aerobic *plus* resistance training is more effective than aerobic training alone to improve body composition [14],metabolic profiles,adiponectinemia [15] andnonalcoholic fatty liver disease [16] in the same population. As far as we know, this is the first study to compare bicycle to treadmill training as part of an aerobic *plus* resistance training in obese adolescents.Finally, the small sample size used can be considered as a limitation of the present study. Therefore, it needs to be confirmed in a large cohort study.

In conclusion, we were able to show that aerobic *plus* resistance training as part of a long-term interdisciplinary therapy was effective in improving pro-anti-inflammatory adipokines in obese adolescents and bicycle training seems to promote better benefits in body composition, insulin, HOMA-IR and QUICKI than treadmill training for this population. Another finding of great value in this investigation was that the improvement of work capacity and physical fitness observed contributed directly to the improvement of pro-anti-inflammatory biomarkers and may also contribute to enhance exercise tolerance in obese adolescents.

Acknowledgements
We would like to thank the patients who participated in this study, Francieli Ruiz da Silva for statistical assistance and the following funding sources of support: CAPES (AUX-PE-PNPD 2566/2011), FAPESP (CEPID/Sleep #9814303-3 S.T.; 2011/50356-0; 2011/50414-0; 2013/041364), AFIP, CNPqand UNIFESP-EPM, which supported the CEPE-GEO Interdisciplinary Obesity Intervention Program.

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