Peptides 32 (2011) 1384–1391

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Hyperleptinemia in obese adolescents deregulates neuropeptides during weight loss

Ana R. Dâmaso[a,b,*], Aline de Piano[c], Priscila L. Sanches[c], Flávia Corgosinho[c], Lian Tock[c], Lila M. Oyama[d], Luciana Tock[e], Claudia M. Oller do Nascimento[d], Sérgio Tufik[f], Marco Túlio de Mello[f]

[a] Biosciences Department, Federal University of São Paulo – Paulista Medicine School – UNIFESP-EPM, São Paulo, Brazil
[b] Post Graduate Program of Interdisiciplinary Health Sciences, Federal University of São Paulo – Paulista Medicine School – UNIFESP-EPM, São Paulo, Brazil
[c] Post Graduate Program of Nutrition, Federal University of São Paulo – Paulista Medicine School – UNIFESP-EPM, São Paulo, Brazil
[d] Physiology Department, Federal University of São Paulo – Paulista Medicine School – UNIFESP-EPM, São Paulo, Brazil
[e] Endocrinology Department, Federal University of São Paulo – Paulista Medicine School – UNIFESP-EPM, São Paulo, Brazil
[f] Psychobiology Department, Federal University of São Paulo – Paulista Medicine School – UNIFESP-EPM, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 15 March 2011
Received in revised form 28 April 2011
Accepted 28 April 2011
Available online 6 May 2011

Keywords:
Leptin
Obesity
Neuropeptides
Adolescent

ABSTRACT

Leptin has emerged over the past decade as a key hormone not only in energy balance regulation but also in neuroendocrine and inflammatory processes. The aim of the present study was to evaluate whether hyperleptinemia deregulates neuropeptides during weight loss. A total of 86 post-pubertal obese adolescents (with or without hyperleptinemia) participated in one year of interdisciplinary weight loss therapy (clinical, nutritional, psychological and exercise-related). Adipokine and neuropeptide concentrations were measured by ELISA, visceral fat was measured by ultrasound and body composition was measured by pletismography. The hyperleptinemic patients presented a lower alpha-MSH concentration and higher NPY/AgRP ratio while the adiponectin/leptin (A/L) ratio was lower compared with the non-hyperleptinemic group. After therapy, significant improvements in BM, BMI, body fat mass, visceral and subcutaneous fat, HOMA-IR, QUICKI, total cholesterol and triglycerides were observed in both groups. Indeed, we observed significant increases in adiponectin and A/L as well as reductions in leptin and NPY/AgRP ratio in the hyperleptinemic group. In the stepwise multiple linear regression analysis with leptin concentration as the dependent variable, α-MSH and body fat mass (%) were the independent predictors to explain leptin concentration. For the entire group, we found positive correlations between leptinemia and BMI and body fat mass (%) as well as a negative correlation with free fat mass (%) and alpha-MSH. Finally, we verified negative correlations between adiponectin/leptin ratio with total cholesterol and LDL-c, only in hyperleptinemic patients. In conclusion, the hyperleptinemia in obese adolescents deregulates neuropeptides during weight loss.

1. Introduction

Leptin, an adipokine that is primarily expressed by adipose tissue, is considered to be involved in neuroendocrine control of energy balance. However, in human obesity states of hyperleptinemia, central and peripheral leptin insensitivity is suggested. Indeed, recent studies showed that the hypothalamus is not leptin resistant in hyperleptinemia conditions. Leptin deficiency results from decreased leptin transport across the blood brain barrier [18,27,29].

In the context of obesity and co-morbidities, states of hyperleptinemia began as an idea that the body's biomolecular milieu decreases overall sensitivity to leptin effects such that normal or, classically, increased levels produce an inadequate response [18,27]. This concept is reinforced by the observation that most obese individuals, including adolescents have increased serum leptin concentrations, causing hyperleptinemia, as recently demonstrated in the literature and by our research group [24,27,43].

In a previous study, it was demonstrated that the prevalence of hyperleptinemia was 25.92% among obese adolescents [11] and that 20% of postmenopausal women presented hyperleptinemia [2]. Since its discovery more than a decade ago, leptin has been established as a key regulator of energy balance [7,38]; however, recent evidence indicates that leptin deficiency is a pivotal link in obesity-related diseases [3,14].

* Corresponding author at: Rua Professor Francisco de Castro, no 93, Vila Clementino – São Paulo/SP, Postal Code: 04020-050, Brazil. Tel.: +55 11 5572 0177; fax: +55 11 55720177.
E-mail address: ana.damaso@unifesp.br (A.R. Dâmaso).

0196-9781 © 2011 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.peptides.2011.04.025

As mentioned above, hyperleptinemia is commonly observed in obese humans and animals [4,42,45]. Inversely, a decrease in adiponectin concentration was demonstrated by several investigations in obese adolescents and adults. However, the potential mechanisms for diminished adiponectinemia and hyperleptinemia as related to inflammation remain to be investigated in obese adolescents [9,37]. Thus, the interplay among adipokines, leptin and adiponectin may be an important contributor in the pathogenesis of obesity and other co-morbidities.

In the central nervous system, NPY, AgRP and α-MSH produced by neurons in the hypothalamus act locally to regulate energy balance. NPY exerts a physiologically important role in energy homeostasis [25,41]. However, blood NPY levels will reflect its secretion from the adrenal gland, sympathetic nervous system and adipocytes, which may contribute to adiposity and its metabolic consequences [13,26].

α-MSH exerts an important role in the energy balance in obese adolescents; changes in expression were correlated to weight status changes [24,31]. However, previous authors did not investigate this association with changes in leptin concentration, as related to hyperleptinemic status. Because the melanocortin (MC) system lies downstream of leptin sensitivity, it is important to understand this interaction during clinical weight loss intervention to optimize the clinical approach to improve energy balance as a key strategy for obesity control.

Several studies have shown a relationship between leptin levels and energy balance in obese youngsters; however, the results are inconclusive, with leptin levels that either decrease or remain unchanged after exercise or dietary intervention [5].

Therefore, the role of a long-term interdisciplinary weight loss program on pro-anti-inflammatory pathways and the central regulation of energy balance were analyzed in obese adolescents with or without hyperleptinemia. The aims of the present study were to investigate the role of hyperleptinemia during weight loss therapy on energy balance in obese adolescents and to determine whether a state of hyperleptinemia could adversely deregulate neuropeptides.

2. Materials and methods

2.1. Subjects

A total of 86 obese adolescents (39 boys and 47 girls) who entered the Interdisciplinary Obesity Program of the Federal University of São Paulo – Paulista Medical School were assigned to two sub-groups: hyperleptinemic (H) or non-hyperleptinemic (n-H). Those who were considered hyperleptinemic presented baseline values above 20 ng/ml for boys and 24 ng/ml for girls, as based on reference values cited by Gutin et al. [12] and Whatmore et al. [44]. These patients were submitted to weight loss therapy. The evaluations were performed at baseline, after 6 months and after 1 year of an interdisciplinary approach.

The ages of the participants ranged from 15 to 19 years (16.6 ± 1.67 years). BMI was $37.03 \pm 3.78\ kg/m^2$. All participants were confirmed as meeting the inclusion criteria of post-pubertal Stage V [40] (based on the Tanner stages of obesity (BMI >95th percentile of the CDC reference growth charts)) [6]. Exclusion criteria were identified genetic, metabolic or endocrine disease and previous drug utilization. Informed consent was obtained from all subjects and/or their parents, including agreement of the adolescents and their families to participate as volunteers. This study was performed in accordance with the principles of the Declaration of Helsinki and was formally approved by the Institutional Ethical Committee (#0135/04).

2.2. Study protocol and medical screening

The subjects were medically screened; their pubertal stages and their anthropometric measures were assessed (height, weight, BMI and body composition). The endocrinologist completed a clinical interview, including questions to determine eligibility based on inclusion and exclusion criteria. A blood sample was collected and analyzed, and ultrasound (US) was performed to measure visceral and subcutaneous fat. All subjects underwent an ergometric test. Indeed, the procedures were scheduled for the same time of day to remove any influence of diurnal variations.

2.3. Anthropometric measurements and body composition

Subjects were weighed wearing light clothing and no shoes on a Filizola scale to the nearest 0.1 kg. Height 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. Body composition was estimated by plethysmography in the BOD POD body composition system (version 1.69, Life Measurement Instruments, Concord, CA) [10].

2.4. Serum analysis

Blood samples were collected in the outpatient clinic around 8 h after an overnight fast. Insulin resistance was assessed by the homeostasis model assessment-insulinesistance (HOMA-IR) index and the quantitative insulin sensitivity check index (QUICKI). HOMA-IR was calculated using the fasting blood glucose (FBG) and immunoreactive insulin (I): [FBG (mg/dL) × I (mU/L)]/405; QUICKI was calculated as 1/(log I + log FBG). Total cholesterol, TG, HDL, LDL and VLDL were analyzed using a commercial kit (CELM, Barueri, Brazil). The HOMA-IR data were analyzed according to reference values reported by Schwimmer et al. [35]. The a-MSH, NPY, AgRP, total ghrelin, total adiponectin and leptin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from Phoenix Pharmaceuticals, Inc. (Belmont, CA, USA) according to the manufacturer's instructions. The coefficient of variation (CV) for the adipokines and neuropeptide procedure was calculated: a-MSH (CV = 6.48%), NPY (CV = 11.91%), AgRP (CV = 13.47%), ghrelin (CV = 6.82%), adiponectin (CV = 4.5%) and leptin (CV = 4.07%). For this study, the leptin data were analyzed according to reference values described by Gutin et al. [12] and the ghrelin reference value adopted was 10–14 ng/ml. according to Whatmore et al. [44].

2.5. Visceral and subcutaneous adiposity measurements

All abdominal ultrasonographic procedures and measurements of visceral and subcutaneous fat tissue were performed by the same physician, who was blinded 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 to reduce the risk of misclassification. The intra-examination coefficient of variation for ultrasound (US) was 0.8%.

US measurements of intra-abdominal (visceral) and subcutaneous fat were obtained. US-determined subcutaneous fat was defined as the distance between the skin and external face of the rectus 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. Cut-off points to define visceral obesity by ultrasonographic parameters were based on previous methodological descriptions by Ribeiro-Filho et al. [30].

2.6. 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 gender following a balanced diet [22]. No drugs or antioxidants were recommended. Once a week, adolescents had dietetic lessons (providing information on 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 substitutes), fast-food calories and nutritional composition, good nutritional choices on 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.

At the beginning of the study and at 6 months and 12 months into the program, a 3-day dietary record was collected. 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 software program developed at the Federal University of São Paulo – Paulista Medical School (Nutwin version 1.5 for Windows, 2002) that used data from Western and local food tables. In addition, the parents were encouraged by a dietician to call if they needed extra information.

2.7. Exercise program

During the 1-year interdisciplinary intervention period, adolescents followed a personalized aerobic training program including a 60-min session three times a week (180 min/week) under the supervision of an exercise physiologist. 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). At the end of 6 months, aerobic tests were performed to assess physical capacity, and physical training intensity was adjusted for each individual. During the aerobic sessions, adolescents were submitted to heart-rate monitoring. The exercise program was based on the 2001 recommendations provided by the American College of Sports Medicine [1].

2.8. Psychological intervention

Diagnoses of common psychological 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 psychological support group sessions where they discussed body image and alimentary disorders, such as bulimia and anorexia nervosa, binge eating; their signs, symptoms and health consequences; the relationship between their feelings and food; problems in the family, such as alcoholism, and other topics. Individual psychological therapy was recommended when we found individuals with nutritional and behavioral problems.

2.9. Statistical analysis

All data were analyzed using STATISTICA version 6 for Windows, with the significance level set at $p < 0.05$. Data are expressed as the mean ± SD unless otherwise stated. Distributional assumptions were verified by the Kolmogorov–Smirnov test, and non-parametric methods were performed when appropriate. Adipokines and neuropeptides were analyzed with non-parametric tests and expressed as median, minimum and maximum values. Comparisons between measures at baseline and after weight-loss

Table 1
Anthropometric and clinical data among obese adolescents with hyperleptinemia and without hyperleptinemia before and after weight loss intervention.

Variables/time	Hyperleptinemic patients ($n = 57$)			Non-hyperleptinemic patients ($n = 29$)		
	Baseline	6 months	After intervention	Baseline	6 months	After intervention
Body weight (kg)	102.84 ± 15.10	94.85 ± 13.97[b]	90.16 ± 13.43[c]	99.28 ± 12.60	91.56 ± 11.25[b]	89.93 ± 11.65[c]
BMI (kg/m^2)	36.89 ± 4.98	33.76 ± 4.58[b]	32.03 ± 4.64[c]	34.89 ± 4.27	32.15 ± 4.27[b]	31.57 ± 4.69[c]
Body fat (%)	45.75 ± 6.76	40.50 ± 8.33[b]	38.00 ± 9.18[c]	39.89 ± 7.27	35.55 ± 7.88[b]	33.76 ± 8.92[c]
Body fat (kg)	46.97 ± 11.15	38.60 ± 11.26[b]	34.77 ± 11.16[c,d]	39.70 ± 9.29	32.90 ± 9.79[b]	30.79 ± 10.73[c]
Fat free mass (%)	54.47 ± 6.60	59.49 ± 8.33	62.07 ± 9.25[c,d]	60.17 ± 7.34	64.44 ± 7.88[b]	66.23 ± 8.92[c]
Fat free mass (kg)	55.47 ± 8.66	55.51 ± 8.50	55.47 ± 8.27	59.60 ± 9.82	58.65 ± 7.96	59.46 ± 9.01
Visceral fat (cm)	4.17 ± 1.34	3.24 ± 0.97[b]	2.71 ± 1.11[c]	4.75 ± 1.46	3.95 ± 1.29[b]	2.89 ± 1.13[c]
Subcutaneous fat (cm)	3.81 ± 0.84	3.13 ± 0.61[b]	2.87 ± 0.76[c,d]	3.54 ± 0.70	3.12 ± 0.75[b]	2.72 ± 0.83[c]
Glucose (mg/dL)	89.31 ± 6.83	89.19 ± 6.85	89.90 ± 7.22	91.20 ± 4.82	91.65 ± 6.5	88.28 ± 6.57
Insulin (μU/mL)	16.44 ± 7.79	12.17 ± 7.45[b]	11.23 ± 6.88[c]	16.95 ± 8.13	16.85 ± 10.63	14.88 ± 9.88
HOMA-IR	3.64 ± 1.90	2.77 ± 1.95[b]	2.53 ± 1.67[c]	3.80 ± 1.74	3.84 ± 2.74	3.29 ± 2.24
QUICKI	0.32 ± 0.01	0.33 ± 0.03[b]	0.34 ± 0.03[c]	0.32 ± 0.01	0.32 ± 0.02	0.33 ± 0.02
Total cholesterol (mg/dL)	165.26 ± 30.64	153.41 ± 27.98[b]	153.98 ± 28.53[c]	171 ± 34.47	160.55 ± 25.04	160.50 ± 35.81
Triglycerides (mg/dL)	104.17 ± 51.17	82.25 ± 37.71[b]	84.18 ± 41.94[c]	105.06 ± 46.68	85.34 ± 34.39	89.03 ± 45.11
HDL (mg/dL)	44.94 ± 9.42	44.23 ± 8.30	46.94 ± 9.82	44.62 ± 10.12	45.03 ± 9.95	45.32 ± 11.56
LDL (mg/dL)	99.38 ± 28.01	92.69 ± 26.01	90.14 ± 25.74	105.37 ± 32.18	98.51 ± 32.18	97.28 ± 23.85
VLDL (mg/dL)	20.85 ± 10.24	17.33 ± 9.38	16.89 ± 8.41	21.03 ± 9.27	17 ± 6.83	17.89 ± 9.12

Values expressed by mean ± SD.
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) [35].
[a] Comparison of the hyperleptinemic group *vs* Non-hyperleptinemic group at the same study period, $p \leq 0.05$.
[b] Comparison of baseline *vs* 6 months, $p \leq 0.05$.
[c] Comparison of baseline *vs* after one year of intervention, $p \leq 0.05$.
[d] Comparison of 6 months *vs* after one year of intervention, $p \leq 0.05$.

Table 2
Adipokines and neuropeptides among obese adolescents with hyperleptinemia and without hyperleptinemia before and after weight loss intervention.

Variables/time	Hyperleptinemic patients			Non-hyperleptinemic patients		
	Baseline	6 months	After intervention	Baseline	6 months	After intervention
Adiponectin (ug/l)	5.46 (1.82–13.86)	5.89 (1.40–15.06)[b]	5.46 (2.48–15.99)	5.26 (2.71–10.65)	5.63 (2.65–16.13)[b]	6.77 (2.78–13.23)[c]
Leptin (ng/ml)	43.50 (24.37–69.44)[a]	28.37 (1.29–55.57)[a,b]	31.24 (1.10–51.75)[a,c,d]	7.93 (1.23–23.77)	5.15 (1.24–49.94)	5.05 (1.26–38.41)[c]
Adiponectin/leptin ratio	0.13 (0.03–0.53)[a]	0.22 (0.05–21.88)[a,b]	0.23 (0.06–10.24)[a,c]	0.72 (0.14–6.52)	1.46 (0.13–5.41)[b]	1.35 (0.09–4.90)
Ghrelin (ng/ml)	1.19 (0.31–13.30)[a]	1.31 (0.18–13.30)	1.22 (0.52–23.47)[a]	3.12 (0.90–42.66)	1.97 (0.68–11.61)	3.89 (0.79–23.47)[c]
NPY (ng/ml)	0.89 (0.21–6.71)[a]	1.17 (0.27–27.21)[b]	0.94 (0.17–19.67)[a,d]	1.17 (0.23–15.43)	1.17 (0.75–14.36)	1.27 (0.18–4.68)
AgRP (ng/ml)	0.25 (0.11–1.02)	0.28 (0.13–0.76)[a]	0.34 (0.02–0.88)[a,c,d]	0.31 (0.13–0.78)	0.40 (0.21–0.85)	0.49 (0.23–15.43)[c,d]
NPY/AgRP ratio	3.74 (0.33–30.49)[a]	3.01 (1.83–68.11)[b]	3.08 (0.18–19.05)[c]	2.88 (0.36–42.08)	5.30 (1.52–64.46)	2.55 (0.22–57.76)
Alpha-MSH (ng/ml)	0.71 (0.24–14.40)[a]	0.64 (0.28–17.13)[a]	0.74 (0.30–13)	7.72 (0.64–28.36)	2.01 (0.60–15.41)[b]	1.41 (0.72–7.48)[c]

Values expressed by median (minimum–maximum).
Reference values: leptin (1–20 ng/ml for boys and 4, 9–24 ng/ml for girls) [12;44].
[a] Comparison of the hyperleptinemic group *vs* Non-hyperleptinemic group at the same study period, $p \leq 0.05$.
[b] Comparison of baseline *vs* 6 months, $p \leq 0.05$.
[c] Comparison of baseline *vs* after 1 year of intervention, $p \leq 0.05$.
[d] Comparison of 6 months *vs* after 1 year of intervention.

intervention were made using an analysis of variance (ANOVA) for repeated measures or the Wilcoxon signed rank test of non-parametric variables. Comparisons between groups were performed using a one-way ANOVA or the Mann–Whitney test (non-parametric variables). Pearson's correlation was performed to test the direction and strength of the relationship between leptin concentration and the variables of interest and to select those variables that did not present collinearity, to select the predictors in the multiple regression. Stepwise multiple linear regression analysis was performed to estimate the association with parameters known to influence leptin concentration.

3. Results

At the beginning of therapy, 86 obese adolescents were enrolled in the program. The results are presented for the whole population studied: we did not find significant gender differences in BMI at baseline. Indeed, the subjects were paired according to BMI, then divided and analyzed according to leptinemic state.

3.1. Normoleptinemic patients

After weight loss intervention, we observed significant improvements in BM, BMI, body fat mass (% and kg), visceral and subcutaneous fat, insulin concentration, HOMA-IR, QUICKI, total cholesterol and triglycerides. Indeed, short- and long-term interventions increased the free fat mass (%) (Table 1 Table 1).

Based on the adipokine and neuropeptide data, we verified increases in adiponectin concentration and adiponectin/leptin ratio with a concomitant reduction in alpha-MSH concentration. The leptin concentration decreased, while the orexigenic factors (ghrelin and AgRP) increased after 1 year. When we analyzed the AgRP from 6 months to 1 year of intervention, a significant increase was observed (Table 2 Table 2).

3.2. Hyperleptinemic patients

After weight loss intervention, we observed significant improvements in BM, BMI, body fat mass (% and kg), visceral and subcutaneous fat, insulin concentration, HOMA-IR, QUICKI, total cholesterol and triglycerides, similar to the trend observed in the normoleptinemic patients. Only long-term (1 year) treatment was able to promote a significant increase in free fat mass (%) (Table 1).

The group with hyperleptinemia exhibited a significant increase in adiponectin, NPY concentration and adiponectin/leptin ratio as well as a reduction in leptin and NPY/AgRP ratio with short-term intervention. After one year, this group presented a significant increase in adiponectin/leptin ratio and in AgRP concentration, with a reduction in the NPY/AgRP ratio (Table 2).

3.3. Comparison between the groups

It is important to note that hyperleptinemic patients presented lower adiponectin/leptin ratio, alpha-MSH and ghrelin concentration at baseline. Indeed, the NPY/AgRP ratio was higher compared with that observed in the non-hyperleptinemic group.

We found positive correlations between leptin concentrations and BMI and body fat mass (%) at baseline, in the entire group (Fig. 1a and b). On the other hand, the leptin concentrations were negatively correlated with free fat mass (%) and alpha-MSH (Fig. 2a and b). Negative correlations between adiponectin/leptin ratio and total cholesterol and LDL-c were confirmed at baseline only in hyperleptinemic patients (Fig. 3a and b).

As shown in Table 3 Table 3, stepwise multiple linear regression analysis was performed with leptin concentration as the depen-

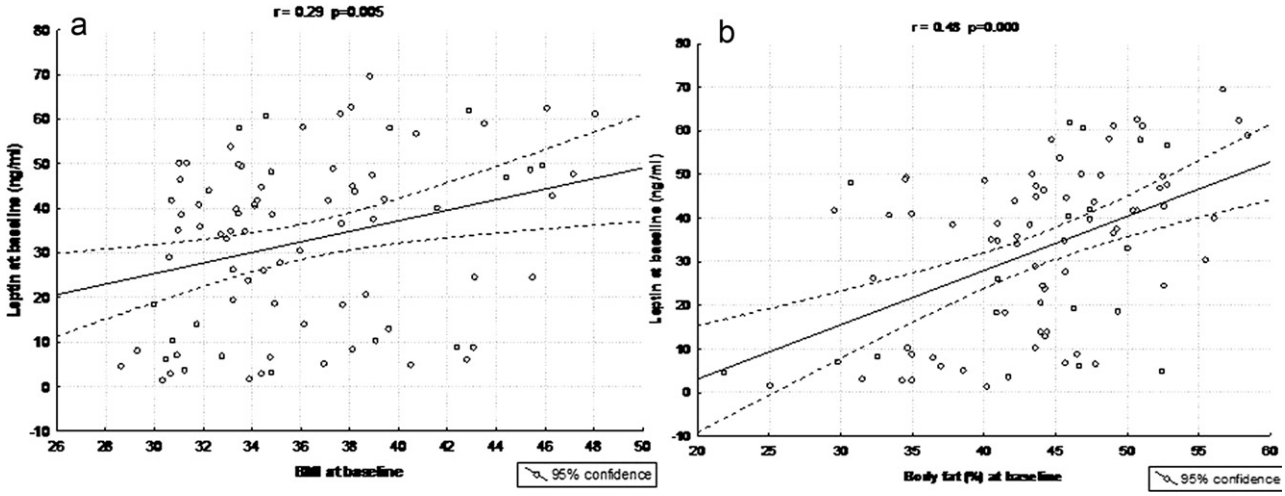

Fig. 1. Positive correlation between leptin concentration with BMI (a) and body fat (%) (b) at baseline conditions in the entire group.

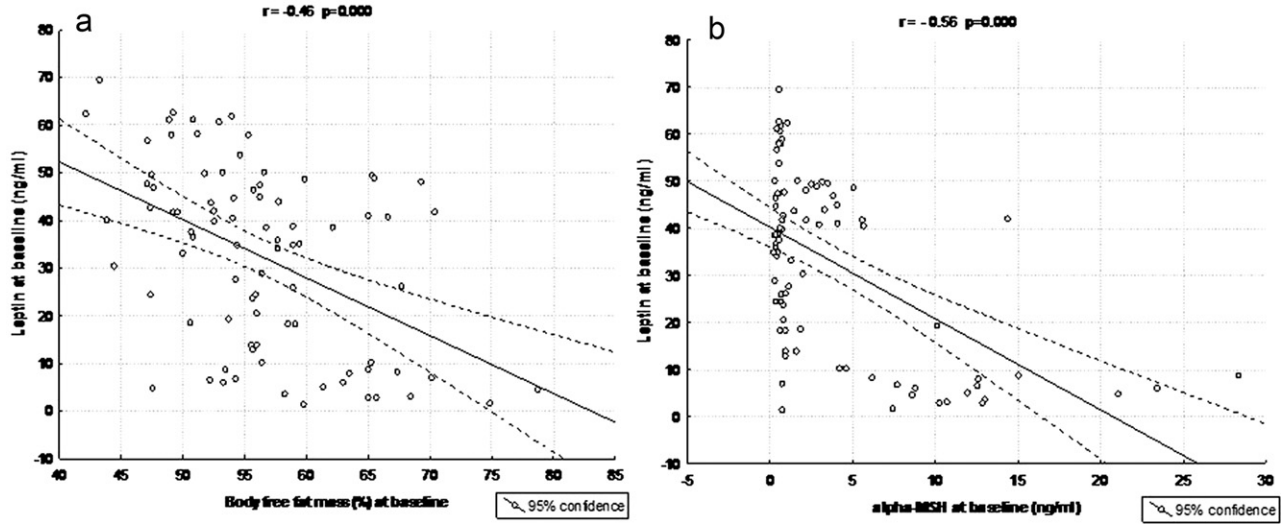

Fig. 2. Negative correlation between leptin concentrations with free fat mass (%) (a) and alpha-MSH (b) at baseline conditions in the entire group.

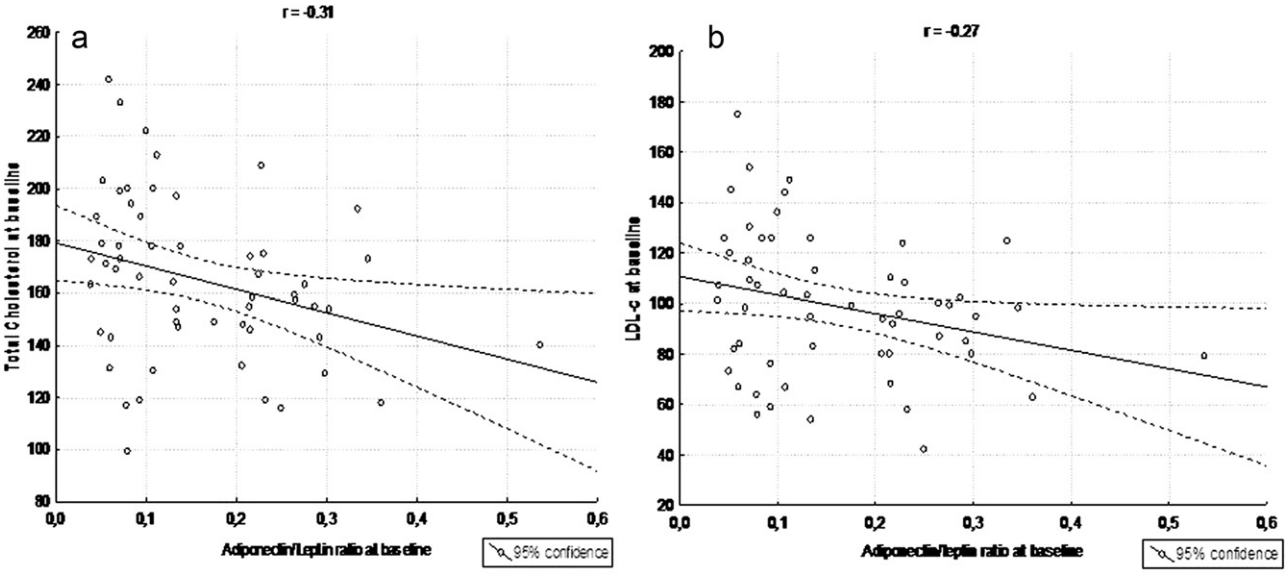

Fig. 3. Negative correlations between adiponectin/leptin ratio with cholesterol total (a) and LDL-c (b) in hyperleptinemic patients at baseline conditions.

Table 3
Multiple regression analysis for the leptin concentration in the entire group.

	Leptin	
	Regression coefficient β	p
Age	0.08	0.29
Alpha-MSH (ng/ml)	−0.47	0.001
Body fat mass (%)	−0.33	0.001
Visceral fat (cm)	−0.04	0.60
Subcutaneous fat (cm)	0.08	0.39

dent variable. α-MSH and body fat mass (%) were the independent predictors to explain leptin concentration in the present study.

4. Discussion

Obesity has been shown to cause resistance or reduced sensitivity to several hormones, including leptin and adiponectin. Obese individuals appear to have higher sympathetic nervous system (SNS) activity; however, the metabolic response to SNS stimulation appears reduced in this population. This finding suggests that, in obesity, any compensatory effect of the SNS on metabolism to increase energy expenditure may not occur, rendering weight loss more difficult [8,33].

We verified that, after the therapy, obese adolescents have decreased body weight, total body fat, visceral fat and leptin concentration; however, some of them remain in a hyperleptinemic state (Table 1).

Studies have reported hyperleptinemia in insulin-resistant individuals independently of the level of obesity. Indeed, they reported cross-sectional associations between hyperleptinemia and insulin resistance independently of body mass index in a population-based cohort. These studies indicate that leptin and insulin are involved in a complex regulatory loop and highlight the pivotal role of leptin in glucose homeostasis, acting as an insulin sensitizer when leptin levels are at low and normal levels and possibly contributing to insulin resistance when leptin is chronically elevated [32,36].

In addition, in the non-hyperleptinemic group, there was a significant increase in free fat mass (%) after short-term therapy. In the hyperleptinemic patients, this increase occurred after only one year of intervention. In fact, evidence derived from animal and human studies suggests that the ability of leptin and adiponectin to stimulate fat acid (FA) oxidation in muscle is impaired in obesity. Thus, leptin deficiency and adiponectin resistance may be initiating factors in the accumulation of intramuscular lipids. This finding may partially explain why the fat free mass (%) was significantly increased only after long-term intervention in the hyperleptinemic group [8].

In the present study, hyperleptinemic patients presented higher values of orexigenic factors. This fact suggests that the leptinemic state affects the neuroendocrine energetic balance, stimulating the orexigenic pathways, which make weight loss difficult in obese adolescents.

One of the most important findings in the present investigation was the lower alpha-MSH concentration at baseline, which was maintained after weight loss in the volunteers with hyperleptinemia (Table 2). We also showed that at baseline, leptin concentration was negatively correlated with alpha-MSH, reinforcing the concept that a disruption between the mechanisms involved in energy balance occurs in obese adolescents, rendering weight loss difficult and ultimately predisposing these individuals to weight regain [33].

However, at the end of therapy, alpha-MSH was similar in both analyzed groups. In addition, we verified that the hyperleptinemia decreased significantly after weight loss intervention, suggesting the important role of this type of therapy in providing superior neuroendocrine regulation of energy balance.

Animal experiments recently showed that the complexity of melanocortin (MC) system effects varies with the nutritional state and that responsiveness to the effects of alpha-MSH may be maintained even in leptin-resistant animals, suggesting that the MC system (receptors and post-receptor signal transduction pathways) is operant even in the absence of leptin input [33]. Similarly, in multiple regression analyses, alpha-MSH was a negative independent predictor of leptin concentration (Table 3).

Therefore, it is necessary to confirm these findings in different populations because age-related obesity in the long-term regulation of body weight is known to be associated with leptin resistance [34,39] and alterations in body weight and composition. These findings may be, at least partly, caused by changes in the activity of anorexigenic and orexigenic neurohumoral systems. Components of the MC system in the hypothalamus are considered to be major players in the regulation of energy metabolism and body weight [28].

In agreement with the literature, we observed that in hyperleptinemic status, the ghrelin concentration was lower during the intervention in comparison with the non-hyperleptinemic group. An increase in ghrelin concentration at the end of therapy was observed only in the non-hyperleptinemic patients. Such a change is considered as an adaptive function of ghrelin in response to negative energy balance [7]. These data reinforce the concept of leptin resistance in leptin excess status, as observed in obesity, as it was previously demonstrated that leptin inhibits ghrelin efflux from the stomach and reduced ghrelin-induced feeding [15,21,23].

Important evidence in the present investigation is that the NPY/AgRP ratio was significantly higher at baseline in the hyperleptinemic group. This finding could be explained by impaired leptin function in maintaining energy homeostasis, restraining the release of NPY, in the hyperleptinemia group [15]. However, both groups presented a reduction of this ratio in the course of weight loss therapy, showing similar values at the end of the intervention. These data reinforce the role of circulating levels of these peptides in energy homeostasis in obese adolescents. Previously, it was demonstrated that NPY and leptin form a loop system responsible for providing feedback to the central nervous system on the state of the peripheral energy stores. The suggested mechanism includes nitric oxide-mediated regulation of leptin and NPY during food intake in mice [19,20]. However, these mechanisms need to be fully investigated in humans in future research efforts.

Recent studies showed that elevated circulating NPY levels and leptin were observed in patients with cardiovascular diseases, such as acute myocardial infarction, angina pectoris, heart failure and hypertension where sympathetic nerve activity is increased, indicating the clinical importance of NPY in regulating vessel function [16,26]. Moreover, the interactions between NPY and the release of inflammatory cytokines, such as leptin, in an atherosclerotic milieu may play a major role in the cardiovascular system [26].

Adiponectin levels improved significantly after short- and long-term therapies in the normoleptinemic group; however, the hyperleptinemic patients showed an increase in this variable only after long-term therapy. The slight change in adiponectin levels could be linked to the time of intervention and may not be sufficient for detection. In a study with obese children, after lifestyle intervention, adiponectin levels, together with several other metabolic parameters, were significantly improved, potentially due to weight loss, improvement of metabolic status, or both [5].

Leptin and adiponectin are involved in the regulation of metabolic homeostasis and inflammatory process in a constellation of chronic diseases. Several studies have reported the association of adipokines, especially A/L ratio, with the presence of metabolic syndrome [14,17,46]. In agreement, Jung et al. [14] showed in adults that the A/L ratio was decreased in the presence of metabolic syndrome (MS) and that changes are related to the number of MS

components. Our study corroborated these findings, revealing a negative correlation between the A/L ratio and total cholesterol and LDL cholesterol in the hyperleptinemic group.

Thus, one important finding from the present study is that the A/L ratio was significantly lower throughout the intervention in those with hyperleptinemia compared with non-hyperleptinemic patients. However, weight loss therapy was effective in improving this ratio in both analyzed groups.

Our study presented some limitations, such as a reduced number of subjects, and we measured total ghrelin rather than acyl ghrelin, although the acylation of this peptide is necessary to cross the blood brain barrier to release GH and exert others endocrine functions.

However, we demonstrate in obese adolescents that the A/L ratio was negatively correlated with total cholesterol and LDL cholesterol and higher values of NPY/AgRP in hyperleptinemic patients. All together, these data reinforce the role of hyperleptinemia in the deregulation of energy balance in obese adolescents, suggesting that this pivotal interplay of leptin in energy balance and inflammation needs to be considered in a clinical intervention.

5. Conclusions

In conclusion, our study reveals that long-term interdisciplinary therapy promotes significant improvement in the disruption of homeostatic cross-talk between the afferent hormonal signals from the periphery and the hypothalamic network of NPY, observed mainly in hyperleptinemic obese patients. Finally, these data can elucidate the interplay between hyperleptinemic status and increased NPY/AgRP ratio with a concomitant decrease in alpha-MSH, factors implicated in impaired weight loss control.

Grant support

AFIP, FAPESP 2008/53069-0 and 2006/00684-3, FAPESP (CEPID/Sleep #9814303-3 S.T) CNPq, CAPES, CENESP, FADA, and UNIFESP-EPM, supported the CEPE-GEO Interdisciplinary Obesity Intervention Program.

Disclosure statement

There is no conflict interest.

References

[1] ACSM. ACMS position stand on the appropriate intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 2001;33(12):2145–56.
[2] Barrios Y, Díaz N, Meertens L, Naddaf G, Solano L, Fernández M, et al. Leptina Sérica, su relación con peso y distribuición de grasa corporal en mujeres posmenopáusicas. Nutr Hosp 2010;25(1):80–4.
[3] Beltowski J. Leptin atherosclerosis. Atherosclerosis 2006;189:47–60.
[4] Blüher S, Mantzoros CS. Leptin in humans: lessons from translational research. Am J Clin Nutr 2009;89(3):991S–7S.
[5] Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V, et al. Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. J Clin Endocrinol Metab 2008;93(8):3051–7.
[6] Centers for Disease Control and Prevention. Prevalence of overweight among children and adolescents: United States; 1999–2000. http://www.cdc.gov/nchs/products/pubs/pubd/hestats/overwght99.htm [accessed 2010].
[7] Delhanty PJ, van der Lely AJ. Ghrelin and glucose homeostasis. Peptides 2011 [Epub ahead of print].
[8] Dyck DJ, Heigenhauser GJ, Bruce CR. The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. Acta Physiol 2006;186(1):5–16.
[9] Faraj M, Havel PJ, Phélis S, Blank D, Sniderman AD, Cianflone K. Plasma acylation-stimulating protein, adiponectin, leptin, and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects. J Clin Endocrinol Metab 2003;88(4):1594–602.
[10] Fields DA, Goran MI. Body composition techniques and the four-compartment model in children. J Appl Physiol 2000;89(2):613–20.
[11] Foschini D, Santos RV, Prado WL, de Piano A, Lofrano MC, Martins AC, et al. Platelet and leptin in obese adolescents. J Pediatr (Rio J) 2008;84(6):516–21.
[12] Gutin B, Ramsey L, Barbeau P, Cannady W, Ferguson M, Litaker M, et al. Plasma leptin concentrations in obese children: changes during 4-mo periods with and without physical training. Am J Clin Nutr 1999;69:388–94.
[13] Ilhan A, Rasul S, Dimitrov A, Handisurya A, Gartner W, Baumgartner-Parzer S, et al. Plasma neuropeptide Y levels differ in distinct diabetic conditions. Neuropeptides 2010;44(December (6)):485–9.
[14] Jung CH, Rhee EJ, Choi JH, Bae JC, Yoo SH, Kim WJ, et al. The relationship of adiponectin/leptin ratio with homeostasis model assessment insulin resistance index and metabolic syndrome in apparently healthy Korean male adults. Korean Diabetes J 2010;34(4):237–43.
[15] Kalra SP. Disruption in the leptin-NPY link underlies the pandemic of diabetes and metabolic syndrome: new therapeutic approaches. Nutrition 2008;24(9):820–6.
[16] Kshatriya S, Liu K, Salah A, Szombathy T, Freeman RH, Reams GP, et al. Obesity hypertension: the regulatory role of leptin. Int J Hypertens 2011:270624.
[17] Lee JM, Kim SR, Yoo SJ, Hong OK, Son HS, Chang SA. The relationship between adipokines, metabolic parameters and insulin resistance in patients with metabolic syndrome and type 2 diabetes. J Int Med Res 2009;37:1803–12.
[18] Martin SS, Qasim A, Reilly MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. J Am Coll Cardiol 2008;52(15):1201–10.
[19] Morley JE, Alshaher MM, Farr SA, Flood JF, Kumar VB. Leptin and neuropeptide Y (NPY) modulate nitric oxide synthase: further evidence for a role of nitric oxide in feeding. Peptides 1999;20(5):595–600.
[20] Morley JE, Farr SA, Sell RL, Hileman SM, Banks WA. Nitric oxide is a central component in neuropeptide regulation of appetite. Peptides 2011;32(4):776–80.
[21] Myers MG, Cowley MA, Münzberg H. Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 2008;70:537–56.
[22] NRC. Dietary reference intake: applications in dietary assessment. Washington (DC): National Academic Press; 2001.
[23] Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Kocełak P, Janowska J, Semik-Grabarczyk E. The effect of weight reduction on plasma concentrations of ghrelin and insulin-like growth factor 1 in obese women. Endokrynol Pol 2008;59(4):301–4.
[24] Oyama LM, do Nascimento CM, Carnier J, de Piano A, Tock L, Sanches Pde L, et al. The role of anorexigenic and orexigenic neuropeptides and peripheral signals on quartiles of weight loss in obese adolescents. Neuropeptides 2010;44(6):467–74.
[25] Palou M, Sánchez J, Rodríguez AM, Priego T, Picó C, Palou A. Induction of NPY/AgRP orexigenic peptide expression in rat hypothalamus is an early event in fasting: relationship with circulating leptin, insulin and glucose. Cell Physiol Biochem 2009;23(1–3):115–24.
[26] Pankajakshan D, Jia G, Pipinos I, Tyndall SH, Agrawal DK, Neuropeptide Y. receptors in carotid plaques of symptomatic and asymptomatic patients: effect of inflammatory cytokines. Exp Mol Pathol 2011;90(3):280–6.
[27] Paz-Filho GJ, Volaco A, Suplicy HL, Radominski RB, Boguszewski CL. Decrease in leptin production by the adipose tissue in obesity associated with severe metabolic syndrome. Arq Bras Endocrinol Metabol 2009;53(9):1088–95.
[28] Petervari E, Balasko M, Garami A, Soos S, Szekely M. Suppression of food intake by intracerebroventricular injection of alpha-MSH varies with age in rats. Acta Physiol Hung 2009;96:483–7.
[29] Reinehr T, Kratzsch J, Kiess W, Andler W. Circulating soluble leptin receptor, leptin, and insulin resistance before and after weight loss in obese children. Int J Obes (Lond) 2005;29(10):1230–5.
[30] Ribeiro-Filho FF, Faria AN, Azjen S, Zanella MT, Ferreira SR. Methods of estimation of visceral fat: advantages of ultrasonography. Obes Res 2003;11:1488–94.
[31] Roth CL, Kratz M, Ralston MM, Reinehr T. Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children. Metabolism 2010 [Epub ahead of print].
[32] Ruige JB, Dekker JM, Blum WF, Stehouwer CD, Nijpels G, Mooy J, et al. Leptin and variables of body adiposity, energy balance, and insulin resistance in a population-based study. The Hoorn Study. Diabetes Care 1999;22(7):1097–104.
[33] Sainsburya A, Zhanga L. Role of the arcuate nucleus of the hypothalamus in regulation of body weight during energy deficit. Mol Cell Endocrinol 2010;316:109–19.
[34] Scarpace PJ, Matheny M, Shek EW. Impaired leptin signal transduction with age-related obesity. Neuropharmacology 2000;39(10):1872–9.
[35] Schwimmer JB, Deutsch R, Rauch JB, Behling C, Newbury R, Lavine JE. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. J Pediatr 2003;143(4):500–5.
[36] Segal KR, Landt M, Klein S. Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. Diabetes 1996;45(7):988–91.
[37] Shaibi GQ, Cruz ML, Weigensberg MJ, Toledo-Corral CM, Lane CJ, Kelly LA, et al. Adiponectin independently predicts metabolic syndrome in overweight Latino youth. J Clin Endocrinol Metab 2007;92(5):1809–13.
[38] Simpson KA, Martin NM, Bloom SR. Hypothalamic regulation of food intake and clinical therapeutic applications. Arq Bras Endocrinol Metab 2009;53(2):120–8.
[39] Soos S, Petervari E, Szekely M, Jech-Mihalffy A, Balasko M. Complex catabolic effects of central alpha-MSH infusion in rats of altered nutritional states: differences from leptin. J Mol Neurosci 2010 [Epub ahead of print].
[40] Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight velocity and stages of puberty. Arch Dis Child 1976;51:170–9.

[41] Trayhurn P, Bing C. Appetite energy balance signals from adipocytes. Philos Trans R Soc Lond B Biol Sci 2006;361(1471):1237–49.
[42] Tschöp MH, Hui DY, Horvath TL. Diet-induced leptin resistance: the heart of the matter. Endocrinology 2007;148(3):921–3.
[43] Vendrell J, Broch M, Vilarrasa N, Molina A, Gómez JM, Gutiérrez C, et al. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. Obes Res 2004;12(6): 962–71.
[44] Whatmore AJ, Hall CM, Jones J, Westwood M, Clayton PF. Ghrelin concentrations in healthy children and adolescents. Clin Endocrinol 2003;59:649–54.
[45] Yu F, Deng C, Huang X-F. Obese reversal by a chronic energy restricted diet leaves an increased Arc NPY/AgRP, but no alteration in POMC/CART, mRNA expression in diet-induced obese mice. Behav Brain Res 2009:50–6.
[46] Zhuo Q, Wang Z, Fu P, Piao J, Tian Y, Xu J, et al. Comparison of adiponectin, leptin and leptin to adiponectin ratio as diagnostic marker for metabolic syndrome in older adults of Chinese major cities. Diabetes Res Clin Pract 2009;84:27–33.