Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 613–619
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Negative correlation between neuropeptide Y/agouti-related protein concentration and adiponectinemia in nonalcoholic fatty liver disease obese adolescents submitted to a long-term interdisciplinary therapy

Aline de Piano[a,*], Lian Tock[a], June Carnier[a], Lila M. Oyama[a], Cláudia M. Oller do Nascimento[a], Aniella C. Martinz[a], Denis Foschini[a], Priscila L. Sanches[a], Regina M.Y. Ernandes[b], Marco Túlio de Mello[c], Sérgio Tufik[b], Ana R. Dâmaso[a,d,*]

[a]*Post Graduate Program of Nutrition, Federal University of São Paulo, Paulista Medicine School, UNIFESP-EPM, 04020-060 Sao Paulo/SP, Brazil*
[b]*Association Found of Incentive to Physicobiology, Federal University of São Paulo, Paulista Medicine School, UNIFESP-EPM, 04020-060 Sao Paulo/SP, Brazil*
[c]*Department of Psychobiology, Federal University of São Paulo, Paulista Medicine School, UNIFESP-EPM, 04020-060 Sao Paulo/SP, Brazil*
[d]*Department of Biosciences, Federal University of São Paulo, Paulista Medicine School, UNIFESP-EPM, 04020-060 Sao Paulo/SP, Brazil*

Received 23 June 2009; accepted 3 September 2009

Abstract

The complexity pathogenesis in the nonalcoholic fatty liver disease (NAFLD) involves an interplay between adipokines and neuroendocrine regulation of energy balance, including the role of neuropeptide Y (NPY)/agouti-related protein (AgRP) system. The first aim of this study was to assess the effect of long-term interdisciplinary intervention on NAFLD in obese adolescents, and the second objective was to establish the relationship between NPY/AgRP ratio and adiponectinemia. Fifty-five postpuberty obese adolescents were submitted to interdisciplinary intervention. The group was divided between subjects with and without NAFLD (n = 19 and 36, respectively). Blood samples were collected to measure glycemia, hepatic transaminases, lipid profile, insulin resistance, and sensitivity. Adiponectin, NPY, and AgRP concentrations were measured by enzyme-linked immunosorbent assay. Food intake was measured using 3-day diet records. It was observed at baseline that important clinical parameters including body weight, body mass index, visceral fat, homeostasis model assessment of insulin resistance, quantitative insulin sensitivity check index, triglycerides, very low-density lipoprotein cholesterol, and hepatic transaminases were more altered in NAFLD patients. After the intervention, these parameters, total energy, and macronutrient intake were reduced significantly in both groups. The most important finding was the positive correlation between AgRP and visceral fat in all patients and the negative correlation between NPY/AgRP and adiponectinemia only in NAFLD obese adolescents. The NAFLD patients presented more altered clinical parameters than the non-NAFLD subjects, including the negative correlation between adiponectinemia and NPY/AgRP. These results suggested that NAFLD obese adolescents presented an inflammatory profile that can influence the neuroendocrine regulation of energy balance, suggesting an additional impairment in the weight loss therapy.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) affects 10% to 39% of the world population, 50% of people with diabetes, 57% to 74% of obese people, and up to 90% of people with morbid obesity [1,2]. In obese adolescents specifically, the estimates of the prevalence reported in the literature are from 22.5% to 52.8% [3]. A number of variables have been associated with NAFLD in the pediatric population, which offer potential clues to the

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

Disclosure Statement: There is no conflict interest.

* Corresponding authors. Ana R. Dâmaso and Aline de Piano are to be contacted at Post Graduate Program of Nutrition, Federal University of São Paulo, Paulista Medicine School, UNIFESP-EPM, 04020-060 Sao Paulo/SP, Brazil. Tel.: +55 5511 5572 0177; fax: +55 5511 55720177.

E-mail addresses: aline.depiano@gmail.com (A. de Piano), ana.damaso@unifesp.br (A.R. Dâmaso).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.09.003

pathogenesis of NAFLD, including visceral adiposity, insulin resistance, and the presence of other features of metabolic syndrome [4,5].

Although the disease mechanisms in pediatric NAFLD need to be more investigated, insulin resistance and visceral fat accumulation appear to be critical [6]. In fact, it was recently reported that each 1-cm increase in visceral adiposity was associated with a 2-fold greater risk of NAFLD in obese adolescents [7]. Indeed, the association of insulin resistance with visceral obesity has prompted some to suggest that NAFLD should also be considered part of the metabolic syndrome [8].

Recently, evidences have indicated that white adipose tissue–derived adipokines also contribute to the pathogenesis of NAFLD, including hypoadiponectinemia in development of the insulin resistance and hepatic fat accumulation [9,10]. Adiponectin is an insulin sensitizer, and the serum levels present decreased in obesity and NAFLD and were negatively associated with homeostasis model assessment of insulin resistance (HOMA-IR) [11,12]. Importantly, this adipokine may be a direct signal in appetite and the control of body weight [13]. There is some evidence that adiponectin inhibits food intake and reduces body weight, concomitantly with improvement of insulin sensitivity and reduction of serum lipid levels, in diet-induced obese rats [14]. Therefore, it was proposed by Trayhurn and Bing (2006) [13] in a recent review that adiponectin could act centrally in the regulation of appetite and energy balance by stimulating hypothalamic corticotrophin-releasing hormone synthesis.

In the central nervous system, the arcuate nucleus of hypothalamus is crucial for feeding control and contains 2 interconnected groups of "first-order" neurons producing neuropeptide Y (NPY) and agouti-related protein (AgRP), both involving the orexigenic pathways, and proopiomelanocortin, cocaine, and amphetamine-regulated transcript peptide, which correspond to the anorexigenic pathways. These hypothalamic circuits also have effects on secretion of fat metabolism regulating hormones. In return, hormones from fat stores and other tissues, as well as other peripheral circulating signals, can regulate the response of NPY/AgRP [13].

Neuropeptide Y is the most powerful central enhancer of appetite, and 90% of NPY neurons coexpress AgRP. Indeed, NPY and AgRP appear to be implicated in the pathogenesis of obesity [15]. Although these neuropeptides might provide a pathophysiologic link between obesity, the interplay between the NPY/AgRP ratio and adipokines in the pathogenesis of NAFLD has not yet been reported in obese adolescents.

Thus, the first aim of this study was to assess the effect of long-term interdisciplinary intervention on clinical and nutritional parameters in NAFLD obese adolescents. The second objective was to establish if NPY/AgRP ratio is modulated by hypoadiponectinemia concentration, commonly associated with obesity and NAFLD.

2. Materials and methods

2.1. Population

A total of 55 obese adolescents (25 boys and 30 girls) who entered the Interdisciplinary Obesity Program of the Federal University of São Paulo–Paulista Medical School in January 2008 were recruited for a long-term (1 year) weight loss intervention study.

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 on a voluntary basis.

The ages of the 55 participants ranged from 15 to 19 years (mean = 16.55 ± 1.75 years); mean body mass index (BMI) (±SD) was 35.81 ± 4.06 kg/m^2. All participants were confirmed to meet the inclusion criteria of postpubertal stage V based on the Tanner and Whitehouse [16] stages and of obesity (BMI >95th percentile of the Centers for Disease Control and Prevention reference growth charts) [17]. Exclusion criteria were as follows: identified genetic, metabolic, or endocrine disease; chronic alcohol consumption (≥20 g/d); viral hepatic diseases; previous drug use; and other causes of liver steatosis [1].

2.2. Study protocol and medical screening

Subjects were medically screened, and their pubertal stage and anthropometric measures were assessed (ie, height, weight, BMI, and body composition). Blood samples were collected and analyzed. Ultrasound (US) was performed to assign subjects to the NAFLD or non-NAFLD groups. The endocrinologist reassessed their health and clinical parameters monthly. The patients were assigned to 2 groups based on US screening: with NAFLD (n = 19) and non-NAFLD (n = 36). For all subjects, the procedures were scheduled for the same time of day to remove any influence of diurnal variations. Thereafter, obese adolescents started the interdisciplinary weight loss program, as described below.

2.3. Anthropometric measurements and body composition

Subjects were weighed wearing light clothing and no shoes on a Filizola (São Paulo-SP, Brazil) 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, São Paulo-SP, Brazil). Body mass index 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) [18].

2.4. Serum analysis

Blood samples were collected in the outpatient clinic at around 8:00 AM after an overnight fast. After centrifugation, the plasma and serum samples were separated in small

aliquots and then frozen at −70°C until use. Insulin resistance was assessed by the HOMA-IR and the quantitative insulin sensitivity check index (QUICKI). The HOMA-IR was calculated by the fasting blood glucose (FBG) and the immunoreactive insulin (I): [FBG (in milligrams per deciliter) × I (in milliunits per liter)]/405; QUICKI was calculated as 1/(log I + log FBG). Total cholesterol, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and the hepatic transaminases (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and γ-glutamyl transferase [GGT]) were analyzed using a commercial kit (CELM, Barueri, Brazil). The HOMA-IR, serum lipid, and ALT data were analyzed according to reference values described by Schwimmer et al (2003) [19]. The adiponectin, NPY, and AgRP were measured at baseline conditions using a commercially available enzyme-linked immunosorbent assay kit from Phoenix Pharmaceuticals (Belmont, CA) according to manufacturer's manual.

2.5. Hepatic steatosis, and visceral and subcutaneous adiposity measurements

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

Ultrasound measurements of intraabdominal ("visceral") and subcutaneous fat were taken. Ultrasound-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. Cutoff points to define visceral obesity by ultrasonographic parameters were based on previous methodological descriptions by Ribeiro-Filho et al (2003) [20].

The definition of fatty liver on ultrasound was based on previously reported diagnostic criteria [21,22].

2.6. Intervention procedures

The interdiscipinary obesity intervention consisted of clinical, nutritional, and psychologic therapy and exercise program. The use of interdiscipinary therapy as a criterion has been suggested by the World Health Organization (2000) [23].

2.7. Dietary program

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 sex following a balanced diet [24]. No drugs or antioxidants were recommended. Once a week, adolescents had 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 at 6 and 12 months into the program, a 3-day dietary record was collected. Because most obese people underreport their food consumption, each adolescent was asked to record their diet with the help of their parents [25]. The degree of underreporting may be substantial; however, this is a validated method to assess dietary consumption [26]. 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 Medicine 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.

2.8. Exercise program

During the 1-year interdisciplinary intervention period, adolescents followed a personalized aerobic training program including a 60-minute session 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). At the end of 6 months, aerobic tests were performed to assess physical capacities; and physical training intensity was adjusted for each individual. During the aerobic sessions, adolescents were under heart rate monitoring. The exercise program was based on the 2001 recommendations given by the American College of Sports Medicine [27].

2.9. Psychologic intervention

Diagnoses of common psychologic problems associated with obesity, such as depression, disturbances of body image, anxiety, and the decrease of self-esteem, were established by validated questionnaires. During the interdisciplinary intervention, the adolescents had weekly psychologic support group sessions where they discussed body image and alimentary disorders such as bulimia and anorexia nervosa, binge eating, and their signs, symptoms, and the consequences for health; the relation between the feelings and food; problems in the family such as alcoholism; and

Table 1
Anthropometric and clinical data among NAFLD and non-NAFLD obese adolescents before and after weight loss intervention

Variables	Non-NAFLD patients		NAFLD patients	
	Baseline	After intervention	Baseline	After intervention
Age (y)	16.19 ± 1.51	16.96 ± 1.51	17.17 ± 1.99	17.89 ± 1.99
Body weight (kg)	95.03 ± 13.06	85.59 ± 11.58#	105.00 ± 12.09*	98.87 ± 10.91*#
BMI (kg/m^2)	34.99 ± 4.00	31.71 ± 4.00#	37.37 ± 3.75*	34.59 ± 5.23*#
Visceral fat (cm)	4.09 ± 1.24	2.90 ± 1.22#	5.50 ± 1.49*	3.69 ± 1.29*#
Subcutaneous fat (cm)	3.40 ± 0.84	2.83 ± 1.02#	3.55 ± 0.75	3.16 ± 0.97
Glucose (mg/dL)	90.46 ± 5.7	88.82 ± 7.01	91.23 ± 5.85	92.45 ± 8.01
Insulin (μU/mL)	15.25 ± 5.14	12.14 ± 4.66#	23.15 ± 14.08*	19.51 ± 12.27*
HOMA-IR	3.37 ± 1.16	2.66 ± 1.05#	5.22 ± 3.20*	4.46 ± 2.81*#
QUICKI	0.32 ± 0.01	0.33 ± 0.02#	0.30 ± 0.02*	0.31 ± 0.03*#
Total cholesterol (mg/dL)	156.81 ± 26.96	146.56 ± 27.83#	167.73 ± 43.31	157.15 ± 36.75
TG (mg/dL)	95.44 ± 42.26	75.68 ± 36.07#	135.84 ± 73.42*	106.85 ± 50.77*
HDL (mg/dL)	49.38 ± 10.93	48.68 ± 11.36	47.11 ± 9.90	48.00 ± 11.89
LDL (mg/dL)	88.20 ± 24.36	82.70 ± 24.84	93.46 ± 34.88	87.75 ± 32.45
VLDL (mg/dL)	19.14 ± 8.44	15.17 ± 7.28#	27.15 ± 14.75*	21.40 ± 10.22*
AST (U/L)	23.34 ± 42.26	26.12 ± 23.88	30.23 ± 10.32*	27.65 ± 8.98#
ALT (U/L)	32.40 ± 9.86	28.26 ± 10.53#	48.26 ± 28.32*	42.25 ± 17.22*#
GGT(U/L)	21.64 ± 10.05	17.61 ± 7.4#	32.50 ± 26.81*	29.00 ± 12.26*#

Reference values: glucose (60-110 mg/dL), insulin (<20 μU/mL), HOMA-IR (<2.0), QUICKI (>0.339), total cholesterol (<170 mg/dL), TG (33-129 mg/dL), HDL cholesterol (>38 mg/dL), LDL cholesterol (<130 mg/dL), VLDL cholesterol (10-50 mg/dL), AST (10-40U/L), ALT (10-35 U/L), and GGT (17-30U/L) (Schwimmer et al [19]).

* Comparison of the group with NAFLD vs group without NAFLD at the same study period; $P \leq .05$.

\# Comparison of baseline vs after intervention; $P \leq .05$.

other topics. An individual psychologic therapy was recommended when we found individuals with nutritional and behavioral problems.

2.10. Statistical analysis

All data were analyzed using STATISTICA (StatSoft, Tulsa, OK) version 6 for Windows, with significance level set at $P < .05$; and data are expressed as means ± SD unless otherwise stated. Distributional assumptions were verified by Kolmogorov-Smirnov test, and nonparametric methods were performed when appropriate. Visceral fat, insulin, HOMA-IR, QUICKI, VLDL, TG, AST, ALT, and GGT were analyzed with nonparametric tests. Comparisons between measures at baseline and after weight loss program were made using paired *t* tests or Wilcoxon signed rank test in nonparametric variables. Comparisons between groups were made using independent *t* tests or Mann-Whitney tests (nonparametric variables). The correlations between the visceral fat, neuropeptides, and adiponectin were evaluated by Pearson correlation.

3. Results

At baseline, 35% of all subjects presented NAFLD; and after 1 year of interdisciplinary intervention, the NAFLD prevalence was reduced to 25.4%.

Comparing the 2 groups at baseline, the NAFLD patients had significantly higher values for body weight, BMI, visceral fat, HOMA-IR, TG, VLDL cholesterol, and hepatic transaminases and lower value of QUICKI. After 1 year of interdisciplinary intervention, in these NAFLD patients, body weight, BMI, visceral fat, HOMA-IR, ALT, and AST were reduced, whereas QUICKI increased significantly. However, when compared with non-NAFLD patients, the NAFLD patients continued to have significantly higher

Table 2
Energy and nutrients intake measured at baseline and after interdisciplinary weight loss program among NAFLD and non-NAFLD obese adolescents

Nutrients	Non-NAFLD patients		NAFLD patients	
	Baseline	After intervention	Baseline	After intervention
Energy intake (kcal)	2018.50 ± 441.86	1481.43 ± 242.26*	2120.80 ± 899.84	1535.14 ± 455.07*
Carbohydrate (g)	264.66 ± 63.00	186.63 ± 43.28*	262.48 ± 106.40	203.38 ± 75.15*
Carbohydrate (%)	52.89 ± 7.00	52.83 ± 8.49	49.83 ± 7.89	53.12 ± 8.62
Protein (g)	86.22 ± 22.19	69.04 ± 19.14*	104.01 ± 53.52	75.21 ± 26.38*
Protein (%)	17.58 ± 6.10	19.88 ± 5.66*	19.79 ± 4.30	19.38 ± 3.96
Lipid (g)	67.67 ± 23.07	43.36 ± 12.06*	72.99 ± 34.88	45.90 ± 14.95*
Lipid (%)	29.51 ± 6.09	27.73 ± 5.14	30.36 ± 7.20	27.39 ± 7.09*

* Comparison of baseline vs after intervention; $P \leq .05$.

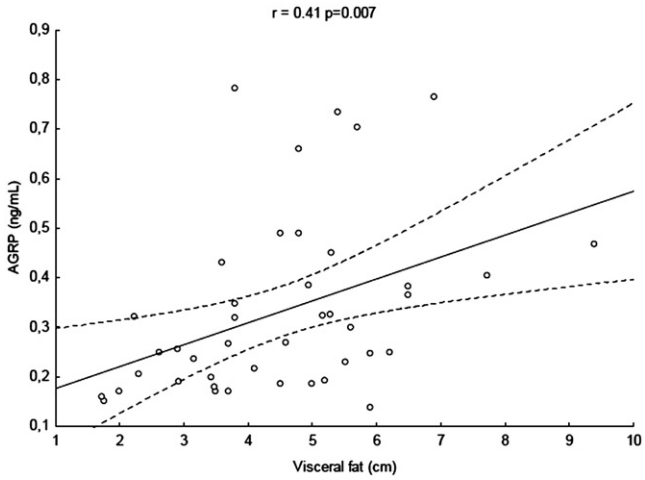

Fig. 1. Positive correlation between visceral fat (in centimeters) and AgRP (in nanograms per deciliter) of obese adolescents with and without NAFLD.

values of body weight, BMI, visceral fat, insulin levels, HOMA-IR, TG, VLDL cholesterol, ALT, and GGT and lower QUICKI after the long-term interdisciplinary intervention. In non-NAFLD obese adolescents, after the intervention, there were significant decreases in body weight, BMI, visceral and subcutaneous fat, insulin levels, HOMA-IR, total cholesterol, TG, VLDL cholesterol, ALT, and GGT and a significant increase in QUICKI values (Table 1).

At baseline, no significant differences were observed between the NAFLD and non-NAFLD obese patients for all nutrient intakes. After the intervention, non-NAFLD patients reduced their intake of total energy and macronutrients (in grams) and increased the protein percentage intake. Similarly, NAFLD patients showed significant decreases in energy, macronutrients (in grams), and lipids percentage (Table 2).

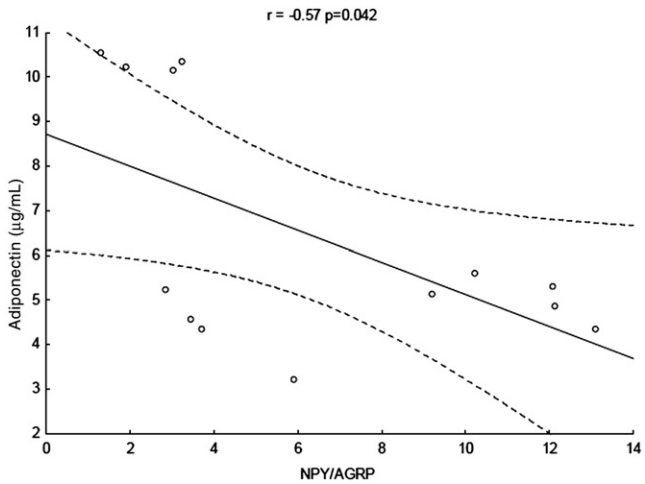

Fig. 2. Negative correlation between adiponectin (in micrograms per milliliter) and NPY/AgRP in NAFLD obese adolescents.

We verified a positive correlation between visceral fat and AgRP concentration in all obese patients ($r = 0.41$, $P = .007$) (Fig. 1), and only in NAFLD patients was a negative correlation between adiponectin and NPY/AgRP observed ($r = -0.57$, $P = .042$) (Fig. 2).

4. Discussion

Associations between NAFLD and the features of the metabolic syndrome have been extensively reported [3,5,28-30]. In the present study, NAFLD patients had significantly higher values for body weight, BMI, visceral fat, HOMA-IR, TG, VLDL cholesterol, and hepatic transaminases and lower values for QUICKI.

Subjects with more pronounced visceral obesity appear to be at greater risk of fatty liver; besides that, insulin resistance plays a key role in genesis of NAFLD. Insulin resistance causes alterations in the uptake, degradation, or secretion of lipid molecules, which lead to the accumulation of lipid in the hepatocytes [1]. According to Park et al (2007) [30], patients with central adiposity and insulin resistance presented higher risk to develop NAFLD, providing evidence of a profound and dose-dependent association between these parameters. In fact, a study with 181 obese adolescents showed that the expansion of visceral fat was a determining factor in NAFLD development and that visceral fat measured by ultrasound might be a good predictor of NAFLD in obese adolescents [7].

Recent reviews showed the importance of a long-term interdisciplinary intervention for weight loss to improvement in fatty liver, mainly by decreasing the predictive factors of NAFLD development and metabolic syndrome components, such as BMI, visceral fat, insulin resistance, and hepatic enzymes [1,5,31,32]. Elevated aminotransferases in nonalcoholic subjects were used as a surrogate for NAFLD; and weight gain preceded high values of aminotransferases and other insulin resistance–related features, which appear sequentially in the order of low HDL cholesterol, hypertriglyceridemia/hypertransaminasemia/hypertension, and glucose intolerance, demonstrating the chronological ordering and an association between development of elevated aminotransferases and risk factors of NAFLD. This way, it is important to observe that, in the present study, the NAFLD group presented ALT and GGT mean values that were altered according to reference values proposed by Schwimmer et al (2003) [19] and that the long-term interdisciplinary intervention promoted an improvement in all these parameters. This finding supports the notion that it is possible to control some worsened parameters commonly observed in NAFLD patients [33].

There is evidence that diet composition can influence the development and improvement of NAFLD. Dietary macronutrients significantly affect hepatic de novo lipogenesis [34]. Studies suggest that a hypocaloric diet promotes a reduction of 5% to 10% in body weight and decreases intake

of lipids and simple carbohydrate, besides increasing antioxidants contributing positively to the treatment of NAFLD [35,36]. Previous research showed a positive correlation between lipid ingestion percentage and accumulated visceral fat, which has a key role in the pathogenesis of NAFLD [37]. Thus, reduction of energy intake, mainly from reduction of lipid ingestion, is essential to promote an improvement of NAFLD.

In fact, it was observed in the present investigation that long-term interdisciplinary therapy was effective to reduce significantly the intake of total energy and the percentage of macronutrients and lipid, reinforcing the importance of nutritional counseling in NAFLD treatment.

Indeed, a positive correlation between visceral fat and AgRP levels in all obese patients was observed. This neuropeptide is involved in feeding behavior inducing hyperphagia. This way, the reduction of visceral fat may be considered an important strategy in the energetic balance control. These data were corroborated Katsuki et al (2001) [38], who observed the same positive correlation between visceral fat and AgRP levels ($r = 0.478$, $P < .01$). Altogether, the results suggest the importance of these measurements to support clinical therapies and to reach best practices for NAFLD associated with obesity and related comorbidities during growth.

In the literature, the association of hypoadiponectinemia with additional fat deposition in NAFLD obese patients is well known [9,39]. The impaired action of adiponectin in NAFLD patients must be carefully considered in a clinical approach mainly because the adiponectin is the most abundant adipocyte-derived hormone with established anti-inflammatory and insulin-sensitizing properties. The mechanisms included decreased levels of mitochondrial lipid peroxidation products through regulating hepatic mitochondrial functions and the attenuation of proinflammatory cytokine production mediated in part by attenuating the translocation of nuclear factor–κB to the nucleus, which might represent a common mechanism underlying the multiple beneficial activities of this hormone in various obesity-related pathologies.

Moreover, the adiponectin ameliorates nonalcoholic steatohepatitis and liver fibrosis through suppressing the activation of Kupffer cells and hepatic stellate cells, inhibits platelet-derived growth factor, and reduces the secretion of monocyte chemoattractant protein–1. In addition, adiponectin has been demonstrated to increase fatty acid oxidation and decrease TG storage in muscle, which may explain, in part, the insulin-sensitizing effect of this cytokine [40]. However, the relationship between this anti-inflammatory adipokine and NPY/AgRP system in NAFLD obese adolescents is not clear.

The highlight of the present investigation was the evidence of interplay between the hypoadiponectinemia and higher NPY/AgRP ratio, showing impairment in the neuroendocrine energy balance and other inflammatory processes in NAFLD patients, as well as the important role of the long-term therapy to control body weight. In fact, although the NAFLD obese patients presented significant reduction in the clinical parameters analyzed after the long-term interdisciplinary intervention, they maintain higher values compared with non-NAFLD subjects, demonstrating a worse metabolic profile.

Hypoadiponectinemia is associated with insulin resistance and histologic nonalcoholic steatohepatitis [9]. Neuropeptide Y is up-regulated by insulin in vitro, although release of the adipogenic NPY in response may, in part, explain the weight gain associated with hyperinsulinemia [15]. This way, lower levels of adiponectin could be taken to develop insulin resistance and hyperinsulinemia, modulating the increase in NPY secretion. This mechanism could be hypothesized to explain the link between hypoadiponectinemia and higher NPY/AgRP concentration observed in the present investigation only in NAFLD obese adolescents. Indeed, a recent study suggested that resistin action on NPY neurons is an important regulator of hepatic insulin sensitivity [41]. It can be considered in future research in the pediatric population.

The small size of participants can be considered as a limitation of our study. Nevertheless, we showed the negative correlation between adiponectinemia and NPY/AgRP in NAFLD obese adolescents for the first time.

A greater understanding of the mechanisms linking obesity, cytokines, and neuropeptides to NAFLD is critical to the development of preventive and treatment strategies. In conclusion, NAFLD patients presented clinical parameters more altered than non-NAFLD patients; and the negative correlation between adiponectinemia and NPY/AgRP concentrations suggested that these patients presented an inflammatory profile that can cause important influence in the neuroendocrine energy balance, stimulating the orexigenic neuropeptides; however it needs to be confirmed in future researches. Altogether, these data emphasize the importance of long-term interdisciplinary weight loss intervention that can be effective to improve associated metabolic abnormalities in NAFLD obese adolescents.

Acknowledgment

Our acknowledgements to all development agencies that supported the CEPE-GEO Interdisciplinary Obesity Intervention Program, and special thanks to patients and their parents.

References

[1] Nobili V, Marcellini M, Devito R, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. Liver Dis 2006;44:458-65.
[2] Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. J Clin Gastroenterol 2006;40:S5-S10.
[3] Schwimmer JB, Deutsch R, Kahen T, et al. Prevalence of fatty liver in children and adolescents. Pediatrics 2006;118:1388-93.

[4] Patton HM, Sirlin C, Behling C, et al. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. J Pediatr Gastroenterol Nutr 2006;43:413-27.
[5] Sartorio A, Del Col A, Agosti F, et al. Predictors of non-alcoholic fatty liver disease in obese children. Eur J Clin Nutr 2007;61:877-83.
[6] Roberts EA. Pediatric nonalcoholic fatty liver disease (NAFLD): a "growing" problem? J Hepatol 2007;46:113-42.
[7] Dâmaso AR, do Prado WL, de Piano A, et al. Relationship between nonalcoholic fatty liver disease prevalence and visceral fat in obese adolescents. Dig Liver Dis 2008;40:132-9.
[8] Loguercio C, De Simone T, D'Auria MV, et al. Non-alcoholic fatty liver disease: a multicentre clinical study by Italian Association for the Study of the Liver. Dig Liver Dis 2004;36:398-405.
[9] Jarrar MH, Baranova A, Collantes R, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2008;27:412-21.
[10] Tasci I, Erdem G, Sonmez A, et al. Hepatic steatosis, visceral adiposity, insulin resistance, adiponectin, and inflammation. Metabolism 2009;58:141.
[11] Kotronen A, Seppälä-Lindroos A, Bergholm R, Yki-Järvinen H. Tissue specificity of insulin resistance in humans: fat in the liver rather than muscle is associated with features of the metabolic syndrome. Diabetologia 2008;51:130-8.
[12] Rubin DA, McMurray RG, Harrell JS, et al. The association between insulin resistance and cytokines in adolescents: the role of weight status and exercise. Metabolism 2008;57:683-90.
[13] Trayhurn P, Bing C. Appetite and energy balance signals from adipocytes. Philos Trans R Soc Lond B Biol Sci 2006;361:1237-49.
[14] Shklyaev S, Aslanidi G, Tennant M, et al. Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. Proc Natl Acad Sci USA 2003;100:14217-22.
[15] Kos K, Harte AL, James S, et al. Secretion of neuropeptide Y in human adipose tissue and its role in maintenance of adipose tissue mass. Am J Physiol Endocrinol Metab 2007;293:E1335-40.
[16] Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight velocity and stages of puberty. Arch Dis Child 1976;51:170-9.
[17] Centers for Disease Control and Prevention. Prevalence of overweight among children and adolescents: United States, 1999-2000. www.cdc.gov/nchs/products/pubs/pubd/hestats/overwght99.htm. Accessed 2004.
[18] Fields DA, Goran MI. Body composition techniques and the four-compartment model in children. J Appl Physiol 2000;89:613-20.
[19] Schwimmer JB, Deutsch R, Rauch JB, et al. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. J Pediatr 2003;143:500-5.
[20] Ribeiro-Filho FF, Faria AN, Azjen S, et al. Methods of estimation of visceral fat: advantages of ultrasonography. Obes Res 2003;11:1488-94.
[21] Sabir N, Sermez Y, Kazil S, et al. Correlation of abdominal fat accumulation and liver steatosis: importance of ultrasonographic and anthropometric measurements. Eur J Ultrasound 2001;14:121-8.
[22] Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745-50.
[23] World Health Organization. Obesity: preventing and managing the global epidemic. WHO obesity technical report series 894. Geneva: World Health Organization; 2000.
[24] Institute of Medicine dietary reference intakes: applications in Dietary Assessment. Washington, DC: National Academic Press; 2001.
[25] Hill RJ, Davies PS. The validity of self-reported energy intake as determined using the doubly labeled water technique. Br J Nutr 2001;85:415-30.
[26] Stanton RA. Nutrition problems in an obesogenic environment. Med J Aust 2006;184:76-9.
[27] ACSM. Position stand on the appropriate intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 2001;33:2145-56.
[28] Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. J Clin Endocrinol Metab 2006;91:4287-94.
[29] Targher G, Bertolini L, Scala L, et al. Non-alcoholic hepatic steatosis and its relation to increased plasma biomarkers of inflammation and endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. Diabet Med 2005;22:1354-8.
[30] Park JW, Jeong G, Kim SJ, et al. Predictors reflecting the pathological severity of non-alcoholic fatty liver disease: comprehensive study of clinical and immunohistochemical findings in younger Asian patients. J Gastroenterol Hepatol 2007;22:491-7.
[31] Utzschneider KM, Kahn SE. Review: the role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2006;91:4753-61.
[32] Caranti DA, de Mello MT, Prado WL, et al. Short- and long-term beneficial effects of a multidisciplinary therapy for the control of metabolic syndrome in obese adolescents. Metabolism 2007;56:1293-300.
[33] Suzuki A, Angulo P, Lymp J, et al. Chronological development of elevated aminotransferases in a nonalcoholic population. Hepatology 2005;41:64-71.
[34] Solga S, Alkhuraishe AR, Clark JM, et al. Dietary composition and nonalcoholic fatty liver disease. Dig Dis Sci 2004;49:1578-83.
[35] Huang MA, Greenson JK, Chao C, et al. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. Am J Gastroentorol 2005;100:1072-81.
[36] Tendler D, Lin S, Yancy Jr WS, et al. The effect of a low-carbohydrate, ketogenic diet on nonalcoholic fatty liver disease: a pilot study. Dig Dis Sci 2007;52:589-93.
[37] de Piano A, Prado WL, Caranti DA, et al. Metabolic and nutritional profile of obese adolescents with non-alcoholic fatty liver disease (NAFLD). J Pediatr Gastroenterol Nutr 2007;44:446-52.
[38] Katsuki A, Sumida Y, Gabazza EC, et al. Plasma levels of agouti-related protein are increased in obese men. J Clin Endocrinol Metab 2001;86:1921-4.
[39] Watanabe S, Yaginuma R, Ikejima K, et al. Liver diseases and metabolic syndrome. J Gastroenterol 2008;43:509-18.
[40] Wang YU, Mingyan Z, Karen SLL, et al. Protective roles of adiponectin in obesity-related faty liver diseases: mechanisms and therapeutic implications. Arq Bras Endocrinol Metabol 2009;53:201-12.
[41] Singhal NS, Lazar MA, Ahima RS. Central resistin induces hepatic insulin resistance via neuropeptide Y. J Neurosci 2007;27:12924-32.