

# The role of nutritional profile in the orexigenic neuropeptide secretion in nonalcoholic fatty liver disease obese adolescents

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**Background** Little progress has been made to identify the central neuroendocrine pathway involved in the energy intake control in nonalcoholic fatty liver disease (NAFLD) patients.

**Objective** To assess the influence of orexigenic neuropeptides in the nutritional aspects of NAFLD obese adolescents submitted to a long-term interdisciplinary approach.

**Methods** Fifty adolescents aged 15–19 years, with body mass index at least 95th percentile, consisting of 25 patients without NAFLD and 25 with NAFLD. The NAFLD diagnosis was determined by ultrasonography. Blood samples were collected to analyze glycemia, hepatic transaminases, and lipid profile. Insulin resistance was estimated by Homeostasis Model Assessment Insulin Resistance Index. Neuropeptide Y (NPY) and agouti related protein concentrations were measured by enzyme-linked immunosorbent assay. Analyses of food intake were made by 3 days recordatory inquiry.

**Results** At baseline conditions, the patients with NAFLD had significantly higher values of body mass, body mass index, visceral fat, triglycerides, VLDL-C, and hepatic transaminases. After the long-term intervention, they presented a significant reduction in these parameters. In both the groups, it was observed a significant decrease

in energy intake, macronutrients and dietetic cholesterol. Only the patients with NAFLD presented a positive correlation between the saturated fatty acids intake and the orexigenic neuropeptides NPY and agouti related protein, and carbohydrate with NPY. Indeed, it was observed a positive correlation between energy intake, lipid (%) and saturated fatty acids with visceral fat accumulation.

**Conclusion** Our findings showed an important influence of diet composition in the orexigenic system, being essential consider that the excessive saturated fatty acids intake could be a determinant factor to increase nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 22:557–563 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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## Introduction

The emergent increase of obesity leads to a substantial prevalence of nonalcoholic fatty liver disease (NAFLD) in pediatric population [1]. The NAFLD pathogenesis is unclear, however, there are evidences that the insulin resistance and visceral fat exerts an important role in the NAFLD development [2,3]. Given the close relations between the metabolic syndrome (MS) and NAFLD, an adequate treatment must be included in all MS parameters, consisting of gradual body mass reduction, glycemia, and lipid control [4].

A cornerstone of the management strategy in NAFLD obese adolescents is the long-term interdisciplinary intervention, including exercise, clinical, psychological,

and nutritional therapy [5]. There is a bewildering array of diets that have been recommended for the prevention and treatment of all MS components [6].

Studies have shown that the restrictive diets can lead to a worsening of NAFLD, mainly it is associated with higher inflammation and fibrosis degree of the liver due to these diets that promote a quick and intense weight loss through high influx of free fatty acids to the liver [7]. Indeed, excessive carbohydrate and fat intake could play a role in increasing blood glucose, free fatty acids, and insulin concentrations, independently or together [6].

The high-saturated fat intake is associated with liver dysfunction caused by an increased production of oxygen

reactive species, which damages mitochondria of hepatocyte. In addition, saturated fat intake of more than 10% of total energy, promotes the insulin resistance, which represents a key role in the NAFLD genesis [8].

Another layer of complexity in the pathogenesis of NAFLD involves interplay between neuroendocrine regulations of energy balance, including the role of diet in the neuropeptide Y (NPY)/agouti related protein (AgRP) system. The appetite control and energy balance are key biological processes in higher animals, and unraveling the complex system of peripheral and central signals involved represents a continuing challenge in fundamental physiology. Much recent progress has been made to identify the central neuroendocrine pathways involving both in the energy intake and expenditure control [9].

In the central nervous system, the arcuate nucleus of hypothalamus is crucial for feeding control and contains two interconnected groups of 'first order' neurons producing neuropeptide (AgRP), both involving the orexigenic pathways, and pro-opiomelanocortin and cocaine and amphetamine regulated transcript peptide corresponding to 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 [9,10].

As described in the literature, the fat metabolism and hormones are altered in NAFLD patients [7,11]. Although several studies have shown that the nutritional profile influences the NAFLD development and treatment, the effect of diet in the orexigenic neuropeptides secretion in NAFLD remains unclear [4,8,12]. This way, the aim of this study was to assess the role of nutritional profile in the NPY/AgRP system in NAFLD obese adolescents submitted to a long-term interdisciplinary approach.

## Materials and methods

### Population

A total of 50 obese adolescents, including 22 boys and 28 girls who entered the Interdisciplinary Obesity Program of the Federal University of São Paulo-Paulista Medical School 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 participants and/or their parents, and agreement of the adolescents and their families to participate was on a voluntary basis.

The ages of the 50 participants ranged from 15 to 19 years ( $16.72 \pm 1.77$  years), body mass index (BMI) was  $33.73 \pm 9.42$  kg/m<sup>2</sup>. All participants were confirmed to meet the inclusion criteria of postpubertal stage V based on the Tanner stages [13] and of obesity (BMI > 95th

percentile of the Centers for Disease and Control and Prevention reference growth charts) [14]. Exclusion criteria were: identified genetic, metabolic or endocrine disease, chronic alcohol consumption ( $\geq 20$  g/day), viral hepatic diseases, previous drug utilization, and other causes of liver steatosis [4].

### Study protocol and medical screening

Participants were medically screened, their pubertal stage was assessed, and their anthropometric measures were assessed (i.e. height, weight, BMI, and body composition). The endocrinologist completed a clinical interview, including the question to determine the eligibility based on inclusion and exclusion criteria, particularly for NAFLD. Blood sample was collected and analyzed and ultrasound (US) was performed. The patients were divided into two groups: NAFLD ( $n=25$ ) and nonNAFLD ( $n=25$ ) according to US screening. For all participants, 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 (described in a later section).

### Anthropometric measurements and body composition

Participants 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, São Paulo, Brazil). BMI was calculated as body weight divided by height squared (weight/height<sup>2</sup>). Body composition was estimated by Pletismography in the BOD POD body composition system (version 1.69, Life Measurement Instruments, Concord, California, USA) [15].

### Serum analysis

Blood samples were collected in the outpatient clinic around 08:00 h after an overnight fast. Insulin resistance was assessed by the Homeostasis Model Assessment Insulin Resistance Index (HOMA-IR). HOMA-IR was calculated by the fasting blood glucose and the immunoreactive insulin: [fasting blood glucose (mg/dl)  $\times$  immunoreactive insulin (mU/l)]/405. Total cholesterol, triglycerides (TG), HDL, LDL, VLDL and the hepatic transaminases [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and  $\gamma$  glutamil transferase (GGT)] were analyzed using a commercial kit (CELM, Barueri, Brazil). The HOMA-IR, serum lipid and hepatic transaminases data were analyzed according to reference values described by Schwimmer *et al.* [16]. The NPY and AgRP concentrations were measured using a commercially available ELISA kit from Phoenix Pharmaceuticals Inc. (Belmont, California, USA) according to manufacturer's manual.

### Hepatic steatosis, visceral and subcutaneous adiposity measurements

All abdominal ultrasonographic procedures and measurements of visceral, subcutaneous fat tissue and fatty liver,

before and after intervention, were performed by the same physician who was blinded to Participants' assignment group. This physician was a specialist in imaging diagnostics using a 3.5-MHz multifrequency transducer (broadband), which reduces the risk of misclassification. The intra-examination coefficient of variation for US was 0.8%.

US measurements of intraabdominal (visceral) and subcutaneous fat were taken. US-determined subcutaneous fat was defined as the distance between the skin and external face of the recto abdominis muscle, and visceral fat was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta. Cutoff points to define visceral obesity by ultrasonographic parameters were based on previous methodological descriptions by Ribeiro-Filho *et al.* [17].

The diagnosis of ultrasonic fatty liver was based on criteria reported earlier by Sabir *et al.* [18] and Saadeh *et al.* [19].

### Dietary program

Energy intake was set at the levels recommended by the dietary reference intake for participants with low levels of physical activity of the same age and sex following a balanced diet [20]. No drugs or antioxidants were recommended. Once in 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, 6 and 12 months into the program, a 3-day dietary record was collected. As most obese people underreport their food consumption, each adolescent was asked to record their diet with the help of their parents [21]. The degree of underreporting may be substantial; however, this is a validated method to assess dietary consumption [22]. 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; São Paulo - Sp, Brazil) 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.

### 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 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 [23].

### Psychological intervention

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

### Statistical analysis

All data were analyzed using STATISTICA (Statsoft, Tulsa, Oklahoma, USA) version 6 for Windows, with significance level set at *P* value less than 0.05 and data are expressed as means  $\pm$  SD unless otherwise stated. Distributional assumptions were verified by Kolmogorov–Smirnov and nonparametric methods were performed when appropriate. Insulin, HOMA-IR, VLDL, TG, AST, ALT, GGT, AgRP, and NPY 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 in nonparametric variables. Comparisons between groups were made using independent *t*-tests or Mann–Whitney (nonparametric variables). Pearson's correlation was performed to test the direction and strength of the relationship between the NAFLD and the variables of interest.

### Results

At baseline conditions, the patients with NAFLD had significantly higher values of body mass, BMI, visceral fat, TG, VLDL-C and hepatic transaminases. It was verified in NAFLD patients, that the long-term interdisciplinary intervention could promote a significant reduction in body mass, BMI, fat mass (%), visceral adipose tissue and AST. In the same way, body mass, fat mass (%), visceral

and subcutaneous fat, TG, VLDL-C, and GGT decreased significantly in patients without NAFLD (Table 1).

According to nutritional profile data, no significant differences in all nutrient values were observed between the studied groups. After 1 year of interdisciplinary intervention, both the groups presented a significant decrease in energy intake, carbohydrate (g), protein (g), lipids and saturated fatty acids (g and %), monounsaturated fatty acids consumption in percentage and dietetic cholesterol. However, only the NAFLD patients reduced the carbohydrate (%), mono and polyunsaturated fatty acids intake (g) (Table 2).

It was verified as positive correlation between energy intake (Fig. 1a) and saturated fatty acids intake with visceral fat accumulation only in NAFLD obese adolescents

(Fig. 1b). Indeed, we observed a positive correlation between saturated fatty acids intake, AgRP ( $r=0.54$ ) and NPY ( $r=0.48$ ) and between carbohydrate intake and NPY concentration ( $r=0.55$ ) (Figs 2a, b and 3) only in NAFLD obese adolescents.

**Discussion**

In this study, NAFLD patients had significantly higher values for body mass, BMI, visceral fat, TG, VLDL-C, and hepatic transaminases, corroborating the literature findings that showed the strict association between MS parameters and NAFLD [12,24–26].

The important role of interdisciplinary therapy on the NAFLD treatment is well known. Studies showed that the long-term interdisciplinary intervention was effective in improving the MS components in NAFLD patients

**Table 1 Clinical and laboratorial data measured at baseline and after long-term interdisciplinary therapy in obese adolescents with and without NAFLD**

Variables/time	Patients without NAFLD			Patients with NAFLD		
	Baseline	Long-term intervention	P value	Baseline	Long-term intervention	P value
Body mass (kg)	91.77 ± 11.50	81.58 ± 8.91	0.0000	107.89 ± 9.02*	97.44 ± 10.71*	0.004
BMI (kg/m <sup>2</sup> )	32.45 ± 8.70	30.41 ± 3.18	0.29	37.89 ± 3.80*	34.34 ± 5.29*	0.0004
Fat mass (%)	40.32 ± 6.46	34.23 ± 8.47	0.00001	42.00 ± 6.03	35.94 ± 10.58	0.002
Visceral fat (cm)	3.88 ± 1.24	2.83 ± 0.99	0.001	6.22 ± 1.33*	3.72 ± 1.33*	0.000002
Subcutaneous fat (cm)	3.53 ± 0.97	2.67 ± 1.01	0.00003	3.4 ± 0.84	3.18 ± 1.00	0.19
Glucose (mg/dl)	90.00 ± 5.25	87.87 ± 8.25	0.21	91.42 ± 5.74	92.31 ± 8.21	0.56
Insulin (mU/dl)	17.02 ± 15.02	11.75 ± 4.7	0.11	24.85 ± 15.4	19.33 ± 12.58*	0.12
HOMA-IR	3.82 ± 3.58	2.54 ± 1.04	0.10	5.60 ± 3.44	4.41 ± 2.88*	0.12
Total cholesterol (mg/dl)	153 ± 25.95	147.20 ± 25.16	0.06	157.94 ± 41.71	156.73 ± 37.71	0.80
Triglycerides (mg/dl)	93.29 ± 43.86	72.29 ± 31.73	0.003	120.63 ± 70.2*	106.47 ± 52.13*	0.19
HDL (mg/dl)	49.83 ± 11.64	50.20 ± 10.25	0.80	46.05 ± 8.95	48.52 ± 11.98	0.11
LDL (mg/dl)	84.87 ± 22.47	82.54 ± 24.01	0.38	87.84 ± 36.35	86.89 ± 33.18	0.80
VLDL (mg/dl)	18.75 ± 8.77	14.45 ± 6.44	0.003	24.05 ± 14.05*	21.31 ± 10.49*	0.21
AST	22.20 ± 4.70	27.58 ± 30.97	0.40	31.47 ± 9.55*	26.15 ± 6.17	0.003
ALT	30.12 ± 6.76	26.83 ± 9.11	0.09	47.92 ± 28.85*	41.37 ± 17.22*	0.38
GGT	19.30 ± 5.51	16.26 ± 5.55	0.003	37.00 ± 29.58*	29.21 ± 12.55*	0.20

Reference values: glucose (60–100 mg/dl), insulin (<20 mg/dl), HOMA-IR (<2.0), total cholesterol (<170 mg/dl), triglycerides (33–129 mg/dl), HDL (>38 mg/dl) and LDL (<130 mg/dl) [16].

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT,  $\gamma$  glutamil transferase; HDL, high-density lipoprotein; HOMA-IR, Homeostasis Model Assessment Insulin Resistance Index; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; VLDL, very low-density lipoprotein.

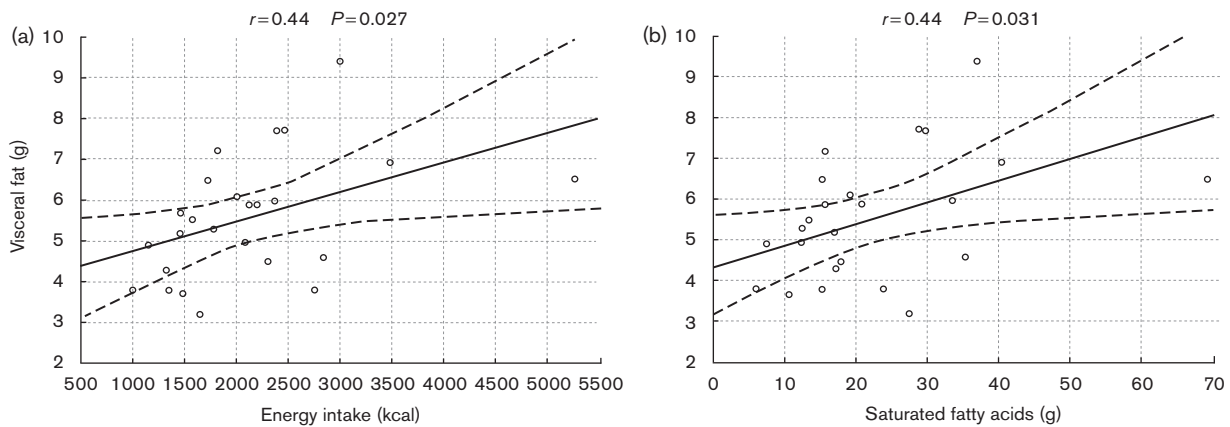
\*Group with NAFLD versus group without NAFLD for the same time  $P \leq 0.05$ .

**Table 2 Characteristics of the food intake measured at baseline in obese adolescents with and without NAFLD submitted to an interdisciplinary weight loss program**

Variables/time	Patients without NAFLD			Patients with NAFLD		
	Baseline	Long-term intervention	P value	Baseline	Long-term intervention	P value
Energy intake (kcal)	1913.90 ± 375.87	1395.90 ± 287.48	0.0000	2184.58 ± 962.69	1469.48 ± 471.65	0.0012
Carbohydrate (g)	246.12 ± 56.90	184.80 ± 39.63	0.0000	266.40 ± 114.34	190.30 ± 72.24	0.004
Carbohydrate (%)	51.23 ± 6.76	54.07 ± 7.47	0.11	48.50 ± 8.78	52.59 ± 7.43	0.08
Protein (g)	86.18 ± 20.20	70.45 ± 22.25	0.01	110.75 ± 61.81	76.26 ± 30.56	0.002
Protein (%)	18.94 ± 8.14	20.07 ± 5.19	0.59	19.79 ± 4.30	20.16 ± 4.51	0.79
Lipids (g)	66.37 ± 23.55	40.50 ± 14.00	0.00001	78.95 ± 38.07	42.95 ± 16.2	0.0004
Lipid (%)	29.80 ± 6.39	26.44 ± 4.96	0.03	31.35 ± 7.44	26.76 ± 7.00	0.04
Saturated fatty acids (g)	21.55 ± 9.85	11.68 ± 4.77	0.00003	23.11 ± 14.25	11.15 ± 6.43	0.003
Saturated fatty acids (%)	10.81 ± 3.48	8.14 ± 1.93	0.0007	10.20 ± 2.54	7.42 ± 3.45	0.01
Monounsaturated fatty acids (g)	16.21 ± 8.53	9.27 ± 6.39	0.11	19.95 ± 13.49	8.46 ± 6.25	0.005
Monounsaturated fatty acids (%)	7.82 ± 2.76	5.91 ± 3.59	0.03	8.65 ± 3.27	6.11 ± 3.35	0.0027
Polyunsaturated fatty acids (g)	6.99 ± 5.38	4.00 ± 3.29	0.03	7.79 ± 4.94	4.11 ± 2.30	0.02
Polyunsaturated fatty acids (%)	3.05 ± 1.12	2.80 ± 1.62	0.54	3.43 ± 1.72	3.15 ± 1.73	0.67
Cholesterol (mg)	232.12 ± 76.30	156.90 ± 91.83	0.002	302.44 ± 226.79	196.27 ± 122.76	0.004

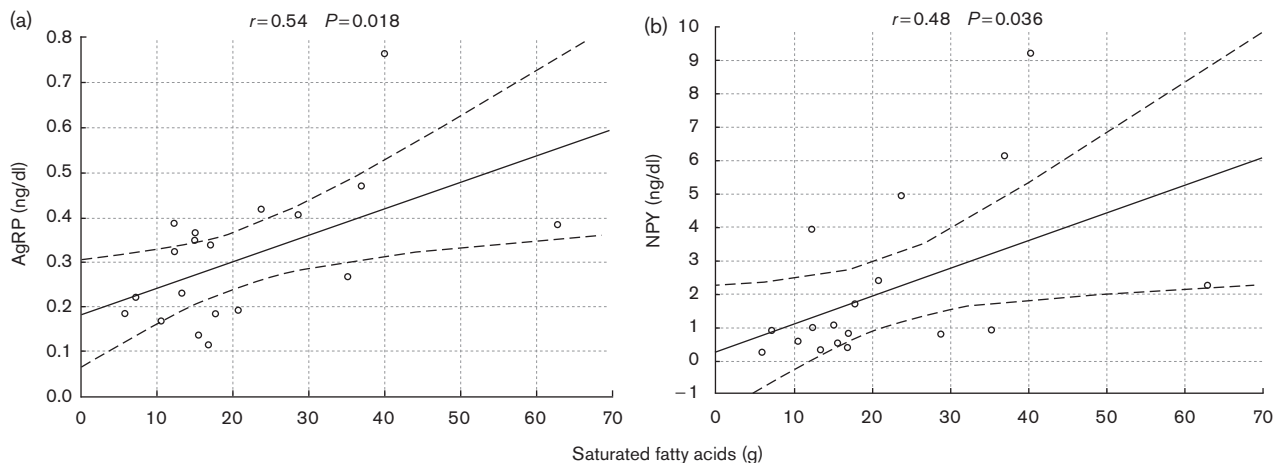
NAFLD, nonalcoholic fatty liver disease.

Fig. 1



Positive correlation between visceral fat, energy (a) and saturated fatty acids intake (b) in nonalcoholic fatty liver disease obese adolescents.

Fig. 2



Positive correlation between saturated fatty acids intake, agouti related protein (AgRP) (a) and neuropeptide Y (NPY) concentrations (b) in nonalcoholic fatty liver disease obese adolescents.

[12,24,27–30]. In fact, it was observed that long-term interdisciplinary intervention could promote a significant reduction in body mass, BMI, visceral adipose tissue and AST, which are considered important parameters of NAFLD development, as well improved the nutritional profile decreasing the energy intake, carbohydrate, lipids, mainly saturated fatty acids (Tables 1 and 2).

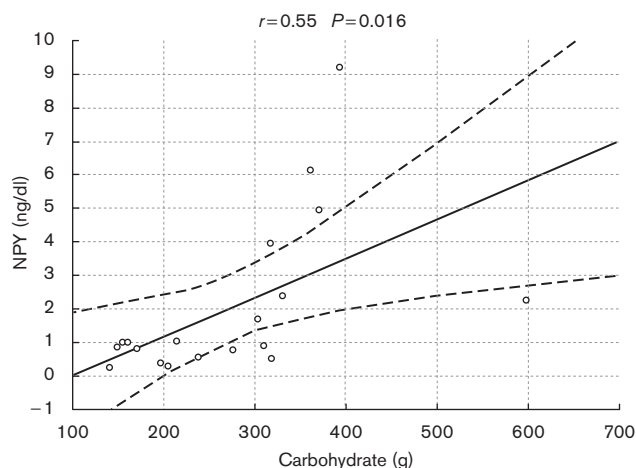
Elevated aminotransferases in nonalcoholics were used as a surrogate for NAFLD and weight gain preceded high values of aminotransferases and other insulin resistance-related features, showing the chronological ordering and an association between development of elevated aminotransferases and risk factors of NAFLD [27].

In a study with 108 obese women, the logistic regression analysis, which computed the association between the

risk factors (independent variables) and NAFLD (dependent variable), verified that only ALT was significantly associated with NAFLD. In addition, this study shows that obese women with similar metabolic alterations exhibit different hepatic outcomes, suggesting that the presence of additional cofactors of obesity are a necessity for the triggering of hepatic inflammation and developing NAFLD. In this regard, they found that elevation of ALT was significantly different between the case and control groups, supporting that inflammation is the main pathogenic factor involved in the development of NAFLD [25].

The association between high-saturated fat and cholesterol intake with NAFLD was shown earlier [31–34]. The saturated fatty acids promote endoplasmic reticulum stress as well as hepatocyte injury. Accumulation of saturated fatty acids in the liver resulted in an increase in

Fig. 3



Positive correlation between carbohydrate intake (g) and neuropeptide Y (NPY) (ng/dl) concentration in nonalcoholic fatty liver disease obese adolescents.

markers associated with endoplasmic reticulum stress and liver dysfunction. Indeed, it was verified that a positive correlation between saturated fatty acids intake with insulin resistance, represents a key role in the NAFLD genesis, suggesting that saturated fat intake must be limited as a nutritional strategy in NAFLD prevention and treatment [8].

Papandreou *et al.* [34] demonstrated that the saturated fatty acids intake were proportionally increased to the degree of hepatic steatosis. Indeed, they observed in multiple regression analysis of factors associated with fatty liver that only HOMA-IR and saturated fatty acids were the most significant after adjustment for age, sex and diet. Diets rich in fatty acids mainly saturated and trans-fatty acids, as well as carbohydrate-rich diets, favor an acute increase in insulin resistance independent of adiposity. High-saturated fatty acids intake may also promote steatohepatitis directly by modulating hepatic TG accumulation and oxidative activity as well as indirectly by affecting insulin sensitivity and postprandial triglyceride metabolism.

In this study, a positive correlation between the energy, saturated fatty acid intake and visceral fat in NAFLD patients (Fig. 1a and b) was shown in accordance to previous research [12]. In addition, Katsuki *et al.* [35] found a strict relation between visceral fat and AgRP concentrations, reinforcing that elevated saturated fatty acids consumption have implications in visceral fat, orexigenic systems and in some important parameters of NAFLD development.

One of the most important findings of our results is the positive correlation between saturated fatty acids intake and the orexigenic neuropeptides (NPY and AgRP) in

NAFLD obese adolescents (Fig. 2a and b). High amounts of saturated fat and cholesterol increase the risk of diabetes elevating insulin secretion, and this hormone could mediate NPY release [36,37].

In addition to energy intake *per se*, macronutrient composition of the diet also influences NPY abundance in the hypothalamus. Each dietary preference presumably reflects the result of a balance between the actions of NPY and those of other hormones and neuropeptides. NPY neurons in the arcuate nucleus are connected with another orexigenic system: the orexins, which are sensitive to fat ingestion. This way, dietary preference for either carbohydrate or fat is associated with NPY status [38].

Solga *et al.* [39] demonstrated that high carbohydrate intake was associated with greater levels of inflammation in obese adolescents with NAFLD. High carbohydrate intake, mainly sucrose, glucose, fructose and foods with high-glycemic index, cause an increase of *de novo* lipogenesis, which leads to an increased conversion of glucose to fatty acids. Zelber-Sagi *et al.* [40] verified that the higher intake of soft drinks is associated with an increased NAFLD development risk. Indeed, the high-glycemic index carbohydrate leads to a quick increase with a subsequent decrease in insulin levels and an increase in glucagon and ghrelin, which are orexigenic hormones [6]. Our data corroborate this finding, showed by the positive correlation between carbohydrate intake (g) and NPY (ng/ml), stimulating an orexigenic pathway in these NAFLD patients (Fig. 3).

This way, to choose low-glycemic index carbohydrate produces small fluctuations on blood glucose and insulin levels and could be considered an important co-adjuvant tool to long-term health, since this would reduce the NAFLD risk [41].

The key observation in this clinical study is the positive correlation between the saturated fatty acids intake and the orexigenic neuropeptides in NAFLD obese adolescents, reinforcing that the nutritional plan must be based on a balanced and individualized diet, prioritizing the complex carbohydrate, including fibers, decreasing lipid consumption, mainly the saturated fatty acids intake.

### Conclusion

Our data suggested that the diet composition exerts an important role in the NAFLD genesis and treatment, as well as, in the orexigenic pathways of these patients, being essential consider that the excessive saturated fatty acids intake was a determinant risk factor to increase the NAFLD development. Indeed, the long-term interdisciplinary intervention was effective to improve important NAFLD parameters in a pediatric population.

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Conflicts of interest statement: none declared.

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