Multidisciplinary treatment reduces visceral adiposity tissue, leptin, ghrelin and the prevalence of non-alcoholic fat liver disease (NAFLD) in obese adolescents

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ABSTRACT

The aim of this study was to assess the changes promoted by a multidisciplinary therapy in ghrelin and leptin concentrations, visceral adiposity and non-alcoholic fat liver disease-NAFLD, in obese adolescents. A total of 28 obese adolescents, 16 girls (BMI 34.58 ± 3,86 wt/ht^2) and 12 boys (BMI 37.08 ± 3.17 wt/ht^2), aged between 15 and 19 years old, was evaluated to leptin, ghrelin and insulin concentrations, visceral adiposity and NAFLD through ultrasonography. The results showed a significant decrease in ghrelin, leptin concentrations and visceral adiposity ($p < 0.01$). Moreover, a decrease in the NAFLD prevalence was observed. It is an important result, since this disease can progress to cirrhosis, not only in children but also in obese adolescents. This kind of treatment can be efficient to improve metabolic and hormonal profile, as well as, to control obesity and related co-morbidities in obese adolescents.

Keywords: Obesity. Life-style. Leptin. Ghrelin. Non-alcoholic fat liver disease.

INTRODUCTION

The prevalence of obesity in childhood and adolescence has drastically increased, making these individuals susceptible to become obese adults, perpetuating and aggravating the epidemic[1].

Recently, obesity has been associated with alterations in the circulating concentrations of leptin, with an exacerbated increase in obese people in relation to observed indices in eutrophics. Such fact means that the bigger the fat deposition, the mote intense its secretion and circulating concentration will be. On the other hand, the reduction in the adiposity decreases its secretion[2].

Previous studies have demonstrated that physical exercise performed in high intensity may decrease the circulating concentration of this hormone; however, when derived from exercises performed in low intensity, the same behavior was not observed[3-7]. This evidence suggests that identifying the leptinemia adaptations in response to exercise may generate subsides for clinical strategies and in public health.

Ghrelin, a polypeptide hormone predominantly produced by the stomach, is involved in the central regulation of the food ingestion and the energetic balance, stimulating the appetite, the lypogenesis, the adipogenesis and decreasing the metabolic rate[8]. The ghrelin circulating concentration is high in patients with anorexia nervosa and reduced in obese people[9]. Some researchers have suggested that the ghrelin action may lead to a decrease in adiposity, since it stimulates the growth hormone secretion (GH), being this hormone widely known by its lipolitic effect[10].

Nonetheless, a recent study demonstrated that the central or peripheral administration of ghrelin, independently from the growth hormone, decreases the oxidation of fats and increases the food intake and the adiposity, suggesting that the low concentration of ghrelin in obese individuals is an effect that should be further investigated[11]. Other studies have demonstrated that the circulating concentration of ghrelin increases in response to the body mass reduction, even when no reduction in the food intake is observed. Such fact may be a specific adaptation and seems to be a compensatory response to changes in the energetic homeostasis in young healthy people, showing specific sensibility to the changes of body mass[9,12].

In this context, Morpurgo *et al.*[13], verified that three weeks of intervention for the reduction of body mass, through food limitations, physical exercise and clinical and psychological orientation were not sufficient in order to stabilize the ghrelin indices in obese people.

Another relevant aspect to be observed, is the increase in the incidence of non-alcoholic fat liver disease (NAFLD), which is becoming an emerging clinical problem among the obese, even in children and adolescents, and may lead to hepatic cirrhosis[14-15]. Tominagata *et al.*[16] verified that 20% of the obese patients with hepatic steatosis developed cirrhosis or hepatocellular carcinoma.

The NAFLD affects 2,6% of children and 10% to 25% of adolescents; however, it varies from 22,5% to 52,8% in obese children. Specifically, the high visceral adiposity may aggravate the degree of the hepatic lesion[16]. Although there is not specific pharmacological treatment for the NAFLD control, all patients are encouraged to reduce the fats intake and engage in regular physical exercising, with the purpose to increase the daily energetic use[14-17].

The leptin significance in the NAFLD is not clear. Nevertheless, the hyperleptinemia commonly observed in obese individuals may promote insulin resistance. It was previously observed that leptin increases with the hepatocytes lesion severity[18-19]. However, another research did not demonstrate such effect[20]. We did not find publications in the national literature approaching this topic in an associated means.

Thus, the aim of the present study was to evaluate the possible alterations promoted by multidisciplinary intervention in a short term, in the plasmatic concentrations of ghrelin and leptin, visceral adiposity and NAFLD prevalence in obese adolescents.

MATERIALS AND METHODS

Population

28 obese adolescents were selected for this study; 16 girls and 12 boys, with age range between 15 and 19 years, who participated in a non-medicated multidisciplinary obesity treatment devel-

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oped in the CAAA/CEPE (Center of Treatment and Counseling to the Adolescent/Center of Psychobiology and Exercise Studies) UNIFESP-EPM, for a 12-week period, including clinical, psychological and nutritional treatment. The inclusion criterion was the BMI above the 95 percentage of the Growth Curve of the *Center for Disease Control* (CDC)[21].

The study was conducted according to the Helsinki Declaration and was previously approved by the Ethics Committee of the São Paulo Federal University – Paulista Medicine School (#0135/04). All patients and their family members received information about the protocol, and the consent form was later signed.

Clinical intervention

The adolescents went through a diagnostic clinical evaluation of the general health state (family and obesity history), their sexual maturity was evaluated as well. Only the post-pubescent adolescents selected by the pediatricians according to the Tanner criterion participated in the study[22]. Later, the adolescents were followed every two weeks.

Psychological intervention

It was conducted every two weeks through appointments, counseling and individual psychotherapy and in small groups, according to the patient's approval and will. Psychological questionnaires were applied in order to identify mood swings (Beck Inventory, VAMS – Visual Analogical Mood Swings Scales, POMS, Idate, SF-36 – Health Research) Data not presented.

Nutritional intervention

A 3-day record questionnaire was used in order to estimate the food intake[23]. From this diagnosis the patients had individual nutritional appointments, where specific topics of the quantitative and qualitative aspects of the diet that should be altered were raised. This orientation was performed every 3 weeks with the specific aim of eating reeducation. Moreover, the nutritionists were available to the family for additional information (via telephone). The energetic consumption followed the *Recommended Dietary Allowances* – RDAs recommendations[24], for individuals with low level of physical activity for the same age and gender.

Intervention – Physical activity

The volunteers of this study reported a sedentary lifestyle, that is, without routine physical activity, and the criteria used for this determination was based on interview, using for this determination the routine physical activity[25], and later analysis of the maximal oxygen consumption.

During the treatment period, the adolescents were submitted to 2 weekly sessions of moderate physical activity. Each session had duration of 60 minutes and consisted of recreational sports activities (soccer, basketball, handball, volleyball, etc.), gymnastics and walking. Information about alterations in the lifestyle were provided, and the adolescents were also encouraged to perform spontaneous physical activities during the week and on weekends as well (walking, climbing up the stairs, etc.).

Anthropometrical measurements

The adolescents were weighted barefoot and wearing light clothes in a Filizola® scale with precision scale of approximately 0,1 kg. The height was obtained using an assembled wooden stadiometer, with precision scale of approximately 0,5 cm. The Body Mass Index (BMI – Kg/m^2) was calculated dividing the body mass (Kg) by the height square (m) (table 1).

Plasmatic analyses

Hormonal evaluations and glycemia

After night fasting of 12 hours, the plasma was collected and stored at –70ºC. All the samples were double evaluated. The leptin and ghrelin hormones were determined through radioimmunoassay (Linco Research, St. Charles, Missouri, United States), with sensibility of 0,5 ng/ml for leptin and of 1ng/ml for ghrelin. The intra and inter variations evaluations were smaller than 5% and 10%, respectively. The reference indices used for obese adolescents concerning the leptin and ghrelin concentrations were previously described by Gutin *et al.*[26] and Whatmore *et al.*[27], respectively. The insulin was measured through radioimmunoassay [^{125}I]-insulin and [^{125}I], by Amersham (Aylesbury, UK). The reagents kit was obtained from the Molecular Research Center, Inc. (Cincinnati, OH). The glucose circulating concentration was measured through enzymatic method and the reading performed through spectophometer, model UV-1601PC (Shimadzu Corp., Kyoto, Japan).

HOMA-IR and QUICKI calculation

The formulas and the reference indices previously described by Schwimmer *et al.*[28] were used for the calculation of the insulin resistance indices (HOMA-IR) and the insulin sensibility (QUICKI).

Visceral and subcutaneous adipose tissue

The abdominal ultrasound method was used according to the criterion previously described by Ribeiro-Filho *et al.*[29] in order to measure the visceral and subcutaneous adipose tissue of the obese adolescents.

Non-alcoholic fat liver disease

It was evaluated through the hepatic ultrasound method in the right and left lobes of the liver. According to the diagnosis, the prevalence was classified in degrees: Light (I), Moderate (II) and Severe (III), according to the one previously described by Saadeh *et al.*[30].

Statistical analysis

All the data were analyzed through the *Statsoft* program, with significance index set at $p \leq 0,05$ and expressed in Average ± Standard Deviation. Comparisons between the basal indices and after the multidisciplinary intervention program in the lifestyle were performed, using *Test-T* for dependent measures. Descriptive statistics was performed for the analysis of the prevalence data of the NAFLD.

TABLE 1
Body mass, height, BMI, visceral and subcutaneous fats of obese adolescents evaluated at the beginning and end of multiprofessional treatment

Variables	Boys			Girls		
	Initial	Final	p	Initial	Final	p
Body mass (kg)	115,78 ± 12,4	111,32 ± 11,37	0,005	91,55 ± 11,65	88,31 ± 11,20	0,0001
Height (cm)	1,76 ± 0,07	1,77 ± 0,08	0,02	1,62 ± 0,05	1,63 ± 0,05	0,002
BMI (kg/m^2)	37,08 ± 3,17	35,60 ± 3,39	0,003	34,58 ± 3,86	33,20 ± 3,73	0,0001
Visceral fat (cm)	4,96 ± 1,62	3,97 ± 1,17	0,005	3,21 ± 1,22	2,64 ± 1,04	0,001
Subcutaneous fat (cm)	3,40 ± 0,50	2,79 ± 0,72	0,006	3,45 ± 0,86	3,03 ± 0,77	0,00001

TABLE 2
Circulating concentration of leptin, ghrelin, insulin, glycemia indices, and the HOMA-IR and QUICKI indices of obese adolescents evaluated in the beginning and end of multiprofessional treatment

Variables	Boys			Girls		
	Initial	Final	p	Initial	Final	p
Glycemia (70-100 mg/dl)	94,91 ± 7,25	90,50 ± 5,99	0,001	93,50 ± 7,42	86,94 ± 5,89	0,002
Insulin (< 17 µIU/ml)	19,07 ± 7,10	15,38 ± 5,37	0,006	18,35 ± 7,87	12,55 ± 5,26	0,0002
HOMA-IR (< 2)	4,28 ± 1,83	3,67 ± 1,30	0,041	4,05 ± 2,09	2,83 ± 1,89	0,003
QUICKI (> 0,339)	0,315 ± 0,01	0,316 ± 0,01	0,794	0,318 ± 0,02	0,33 ± 0,01	0,023
Leptin* (1-20 e 4,9 a 24 ng/dl)	27,94 ± 10,96	23,97 ± 9,92	0,006	51,25 ± 15,69	44,78 ± 14,02	0,0009
Ghrelin (10-14 ng/dl)	5,42 ± 2,15	3,48 ± 1,71	0,0008	4,44 ± 1,71	3,04 ± 1,27	0,003

* Reference indices of leptin for boys and girls, respectively.

RESULTS

It was observed that the multidisciplinary treatment reduced significantly the indices obtained for body mass, BMI and subcutaneous and visceral adipose tissue, both in boys and girls (table 1).

In table 2, one may observe that there was significant decrease in the glycemia indices (remained within the reference indices), insulin, leptin and ghrelin of the treated obese adolescents (boys and girls). However, only the insulinemia was stabilized. Concerning the HOMA-IR, significant reduction in the indices observed at the end of the study was verified, not only for boys, but also for girls; however, these indices were not stabilized. The QUICKI index increased significantly for girls, while no modification was observed for boys.

Non-alcoholic fat liver disease

The prevalence of non-alcoholic fat liver disease reduced in percentage in both groups, for the Right and Left Lobes of the Liver; however, these reductions were not significant.

TABLE 3
Prevalence of non-alcoholic fat liver disease in obese adolescents evaluated in the beginning and end of multiprofessional treatment

Variables	Boys			Girls		
	Initial	Final	p	Initial	Final	p
Right lobe	75%	34%	ns	48%	25%	ns
Left lobe	67%	34%	ns	32%	25%	ns

(ns) non-significant indices.

The nutritional intervention was effective in order to reduce the total energetic intake (2010 Kcal ± 881.01 for 1650 Kcal ± 480.30), the lipids intake (32.87% for 30.57%) and increased the protein intake (15.80% for 18.25%). However, there was no alteration in the carbohydrates intake.

DISCUSSION

In the present study, significant reduction in the circulating concentration of leptin in response to recreational physical activities, performed for 60 minutes/day, two weekly sessions, associated with the non-medicated multiprofessional treatment was observed. However, such reduction was not sufficient in order to adequate its indices to the normality standards.

Kraemer *et al.*[31] verified that physical exercise performed with duration lower than 60 minutes did not change the leptin concentration in healthy men and women; however, when performed for more than 60 minutes, the physical exercises stabilized the concentration of this hormone, suggesting that leptin is not changeable in short duration exercises. Another fact to be considered is the intervention time, since exercise programs with duration lower than 12 weeks do not promote reduction in the adiposity, and consequently do not alter the circulating indices of leptin.

Despite of that, it is important to mention that in the study by Gutin *et al.*[26], they observed reduction in the leptin concentration after systematized exercises for 4 months, corroborating the findings of the present study. Although results about adaptations in the leptin concentration are inconclusive and many times conflicting[7], we believe that this hormone is more responsive to the long term interventions (more than 12 weeks), with exercise sessions duration for longer than 60 minutes.

It is relevant to highlight the importance of the physical exercise intensity, once after exertion test until exhaustion (85% maximal $\dot{V}O_2$) on treadmill, decrease in the circulating concentration of leptin is observed. Such fact is probably due to the increase in the production of non-ester fatty acids during exercise, reflecting a catabolic state of the adipose tissue, and hence, lower secretion of leptin[32-33]. The authors suggested a decrease in the circulating concentration of this hormone in acute response to exercise (~3ng/ml), even in the lack of weight loss.

Therefore, four hypotheses are suggested to explain our results: 1) non-systematized physical activity – recreational activities; 2) session duration of the trainings – 60 minutes; 3) short term intervention (*short term-therapy)* and 4) the exacerbated initial hyperleptinemia in some patients. However, due to the obtained results, it is believed that this kind of physical activity is a good option for long term multidisciplinary treatments (including changes in the lifestyle).

The present non-medicated multidisciplinary intervention model seemed efficient in the improvement of the glycidic metabolism and the metabolic health of the volunteers, once it reduced the fasting glycemia and the circulating concentration of insulin. According to Fischer *et al.*[33], the circulating concentration of glucose and insulin explains 86% of the variation in the circulating concentration of leptin in hierarchic regression model. As a result, leptinemia is strongly changeable, depending on the glycemic homeostasis and insulinemia.

Concerning ghrelin, in the present study it was observed that despite the initial indices below normal, there was decrease in the concentration of this hormone. According to Van der Lely *et al.*[8], reductions in the concentration of this hormone after treatment for body mass control are important, since in a certain extent, they could contribute in the reoccurrence of weight gain that occurs after treatment interruption. Therefore, according to Leidy *et al.* (2004)[9], ghrelin could increase after changes in the energetic balance and in the body set point in order to supply what was depleted.

Obesity is the main risk factor for the development of the non-alcoholic fat liver disease (NAFLD) in childhood and adolescence. Thus, changes in the lifestyle are desirable, since they reduce the prevalence of this disease and its co-morbidities[34].

When the data of the present study are confronted with the ones in the literature, it is verified that the prevalence of NAFLD

was higher in boys and lower in girls[16]. After the 12-week intervention there was a percentage decrease of the initial indices, demonstrating the importance of this kind of treatment in the prevention and control of this pathology in obese adolescents. However, it is important to mention that despite these adaptations, some obese subjects did not present the same improvement, which suggests that there are responsive and non-responsive individuals to the treatment[35].

It is known that the increase in the NAFLD prevalence may be derived from several alterations in the metabolism, namely: in the capitation, synthesis, degradation or secretion of lipids, which result in insulin resistance[15]. Facing this insulin action resistance, the lipolysis is increased in the adipocyte resulting in increase in the release of free fatty acids (FFA), which will be picked by the hepatocites, where the lipogenesis will occur[36], Hence, the relation between the insulin resistance and the development of NAFLD is partially explained[34,37-39].

Actually, the average indices of insulin and HOMA-IR of the obese adolescents were increased in the beginning of the study; however, the QUICKI index was decreased, corroborating this hypothesis. At the end of the study, the normal insulin, the reduced HOMA-IR and the increased QUICKI in relation to the initial indices (table 2), caused hormonal and metabolic changes which possibly contributed for the reduction of NAFLD prevalence.

According to Scheen and Luyckx[34], the adipose mass reduction decreases the degree of hepatic lesion and its consequences. However, the abrupt reduction of body mass, derived from bariatric surgery in extreme obese individuals, for instance, may promote undesirable hepatic alterations such as toxicity. Finally, Bouneva and Kirby[39] mentioned that the exercise adaptability for each patient specifically, may be essential to stimulate the inactive obese person to exercise. Moreover, exercises in high intensities are not necessary in order to prevent and control this disease. Therefore, multidisciplinary treatments for lifestyle changes are desirable, reinforcing the importance of the model studied in the present study.

It is relevant mentioning that one of the limitations of our study refers to the determination of the exercise intensity applied, and the evolution of the cardiorespiratory ability, which enables us to assert that such alterations were promoted by physical exercise. Nonetheless, the aim of the present study was to investigate the effect of the changes in the lifestyle through the program here proposed.

CONCLUSION

The results of the present study suggest that the non-medicated multidisciplinary treatment was efficient in order to reduce the visceral adiposity. Moreover, it promoted desirable alterations in the ghrelin and leptin profile, and decreased the prevalence of non-alcoholic fat liver disease in obese adolescents. Nonetheless, further studies are needed, especially long term ones, using other kinds of physical exercises, as well as medication treatment for the insulin resistance reduction, helping the control of the non-alcoholic fat liver disease in this specific population.

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