Alterations in Downstream Mediators Involved in Central Control of Eating Behavior in Obese Adolescents Submitted to a Multidisciplinary Therapy

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ABSTRACT

Objective: The aim of this study was to verify the effects of a multidisciplinary therapy (24 weeks) on neurohormonal control of food intake, specifically in orexigenic (total ghrelin, agouti-related protein [AgRP], neuropeptide Y [NPY], and melanin-concentrating hormone) and anorexigenic factors (leptin, insulin, and alpha-melanocyte stimulating hormone [α-MSH]), in obese adolescents.

Methods: A total of 88 adolescents (38 boys and 50 girls), including 62 obese and 26 normal-weight, aged 15–19 years were recruited. Obese adolescents were submitted to a 24-week multidisciplinary therapy. AgRP, NPY, melanin-concentrating hormone, leptin, insulin, glucose, α-MSH, total ghrelin, and food intake were measured at three stages (at baseline, after 12 weeks, and after 24 weeks).

Results: At baseline, obese adolescents showed hyperleptinemia (circulating leptin levels, which were, in boys and girls, 40 and 35 times higher than in normal-weight subjects, respectively). After 24 weeks, these values decreased in all obese patients. Our results showed no differences in ghrelin levels between obese and normal-weight adolescents, in both genders. However, obese boys reduced their plasma ghrelin concentration after 24 weeks of therapy (p < .05). The multidisciplinary therapy decreased NPY and AgRP values and increased α-MSH; simultaneously with these changes there was a decrease in total food intake after 24 weeks of therapy.

Conclusions: We can conclude that the multidisciplinary therapy was efficient to modulate neurohormonal control of food intake in obese adolescents.

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Over the past decades, global trends have shown an alarming increase in the prevalence of overweight/obesity among children and adolescents [1,2]. Fundamentally, the etiology of obesity in children and adolescents, which is similar to adults, is attributable to positive energy balance [3]. There has been a long-standing fascination with the biochemistry of energy homeostasis, and currently, there is a pressing need to understand the biochemical pathways that control energy intake (EI) and expenditure [4] to improve the quality of obesity interventions.

Both short- and long-term regulations operate in the control of quantity food intake. Short-term regulation is concerned primarily with preventing overeating at each meal and long-term regulation is primarily related to the maintenance of energy stores in the form of fat in the body [5]. Signals reflecting a long-term energy balance are centrally processed, leading to the modulation of daily EI and energy expenditure.
There are several gut hormones and other peripheral and central signals that influence the hypothalamic, limbic, and brainstem circuits, which control food intake and energy expenditure. These include glucose, insulin, leptin, ghrelin, cholecystokinin, glucagon-like peptide 1, and peptide YY [6].

Specific areas in the hypothalamus and in the brainstem are responsible for coordinating these signals. The arcuate nucleus in the hypothalamus contains two distinct subsets of neurons that control food intake. One group contains neurons which express orexigenic peptides, neuropeptide Y (NPY), and agouti-related protein (AgRP). The other group expresses anorexigenic peptides derived from pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) [7].

Over the past decade, our knowledge of the homeostatic system has increased dramatically. Important advances have been made in the characterization of hypothalamic neuronal networks and neuropeptide transmitters, along with the discovery of circulating peptides that signal to the brain regarding the nutritional status of the body [8]. The understanding of the physiological systems, which regulate food intake and body weight, would open avenues to address obesity prevention, treatment, and control [9].

The treatment of adolescent obesity is classically based on three approaches—increased physical activity, improved diet, and lifestyle changes; however, dieting in overweight or obese human beings has a very high failure rate [10]; similarly, exercise alone has not shown positive effects [11]. These negatives results, especially in weight maintenance, are associated with changes in signals involved in the control of food intake (e.g., leptin and ghrelin).

A multidisciplinary approach to obesity therapy, including clinical, nutritional, and psychological therapies, as well as regular physical activity, has been previously proven to reduce obesity-related morbidity [12]. We have shown that a long-term multidisciplinary therapy can result in decreased visceral fat stores, a reduction in the prevalence of nonalcoholic fatty liver disease [13], improved metabolic syndrome predictors [14], and improved quality of life [15].

A further interesting feature of such programs is that they appear to be more effective in children than in adults [16,17]. Although the multidisciplinary therapy seems to have beneficial results, its effects on the hormonal mechanisms involved in the control of food intake remain to be elucidated.

Therefore, the aim of this study was to verify the effects of a multidisciplinary long-term therapy (24 weeks) on neurohormonal control of food intake, specifically in orexigenic (ghrelin, AgRP, NPY, and melanin-concentrating hormone [MCH]) and anorexigenic factors (leptin, insulin, and alpha-melanocyte stimulating hormone [α-MSH]), in obese adolescents.

Methods

Population

A total of 88 adolescents (38 boys and 50 girls) were recruited between January 2005 and February 2006 by means of television, newspapers, radio, and schools, in São Paulo, Brazil. The volunteers were divided into two groups: obese (n = 62) and nonobese (n = 26). The inclusion criteria were the following: age between 15 and 19 years; and pubertal stage, as assessed by means of Tanner criteria [18], in stage 3 or above. According to Cole et al (2000) [19], participants from the nonobese group were normal-weight and physically active (engaged in >6 hours of physical exercise per week), whereas the obese group included subjects with primary obesity (according to Cole et al, 2000) [19] who agreed to participate in a weight loss multidisciplinary program. The exclusion criteria were pregnancy—for both groups, and <75% compliance in all nutritional, psychological, clinical, and exercise sessions for obese subjects.

This study was performed in accordance with the principles of the declaration of Helsinki and was formally approved by the ethical committee of the Federal University of São Paulo—Paulista Medicine School (#0135/04). Informed consent was obtained from all subjects and/or their parents.

Study protocol

During the first visit, subjects underwent a medical screening, which included assessment of pubertal stage and measurement of anthropometric profile (height, weight, body mass index [BMI], and body composition). For each subject, the procedures were scheduled at the same time of the day and a minimum of 15 hours after the last training session so as to avoid diurnal variations. Thereafter, obese adolescents started the multidisciplinary therapy, composed of medical (once a month), dietary (once a week), exercise (three times a week), and psychological (once a week) programs, as described previously [14,20] and also later in the text. All volunteers performed the evaluations at baseline, and obese adolescents repeated the same procedures after 12 and 24 weeks of the multidisciplinary therapy.

Clinical therapy

Subjects visited an endocrinologist once a month to accomplish healthy clinical parameters. Medical follow-up included initial medical history, physical examination, and appropriate tests followed by regular clinical surveillance.

Psychotherapy

Adolescents participated in weekly psychological orientation group sessions, in which they discussed body image, eating disorders (bulimia and anorexia nervosa, binge eating, as well as their signals, symptoms, and consequences for health), the relation between food and feelings, and family problems. Individual psychological therapy was performed when either nutritional or emotional problems (e.g., history of physical or sexual abuse, suicide risk, depression, and emotional instability) were identified.

Nutritional therapy

Adolescents participated in weekly nutritional classes (food pyramid; recorded inquiry; weight loss diets; diet vs. light; fat and cholesterol; and nutrition facts). As fixed intake of calories was not prescribed, the subjects were only encouraged to reduce their food intake and follow a balanced diet.

Physical therapy

Adolescents underwent a 60-min/session of personalized aerobic training on a treadmill (50% to 70% of maximum oxygen uptake), three times a week (180 min/wk), under the supervision of a sports physiologist. Each program was developed according
approximately 8:00 A.M., after an overnight fast (12 hours), and serum assays performed at Life Measurement Instruments, Concord, CA [22]. Position in the BOD POD body composition system (version 1.69, no shoes. Height was measured to the nearest .5 cm by using a wall-mounted stadiometer (Sanny, model ES 2030, São Paulo, Brazil). BMI was calculated by dividing body weight (kg) by squared height (m²). Plethysmography measured body composition in the BOD POD body composition system (version 1.69, Life Measurement Instruments, Concord, CA) [22].

**Anthropometric measurements and body composition**

Standing height and weight were measured on a Filizola scale to the nearest .1 kg, with volunteers wearing light clothing and no shoes. Height was measured to the nearest .5 cm by using a wall-mounted stadiometer (Sanny, model ES 2030, São Paulo, Brazil). BMI was calculated by dividing body weight (kg) by squared height (m²). Plethysmography measured body composition in the BOD POD body composition system (version 1.69, Life Measurement Instruments, Concord, CA) [22].

**Serum assays**

Blood samples were collected in the outpatient clinic at approximately 8:00 A.M., after an overnight fast (12 hours), and plasma was stored in a refrigerator at −70°C. Fasting blood glucose was determined and plasma insulin was measured by a radioimmunoassay (125I)-insulin and (125I), with commercial kits (Molecular Research Center, Inc. Cincinnati, OH). Serum total ghrelin, AgRP, NPY, MCH, leptin, and α-MSH were assessed by enzymatic immunoassay, using commercial enzyme-linked immunosorbent assay kits (Phoenix pharmaceutical, INC, CA).

**Statistical analysis**

All data were analyzed by means of STATISTICA version 6 (StatSoft) for Windows, with significance set at p < .05 and expressed as means ± standard deviation unless otherwise stated. First, a Komolgorov–Smirnov test to normality was performed. Comparisons between measures at baseline, and then after 12 and 24 weeks of multidisciplinary therapy were made using analysis of variance for repeated measures, and Duncan’s post hoc test or Kruskal–Wallis test were performed for nonparametric variables. Comparisons between genders and groups were made using independent Student’s t-tests. Pearson’s correlation was performed to verify the relationship between the variables. Sample size was based on previous studies and the statistical power of the sample was determined (power = .995).

**Results**

Of all patients admitted for treatment, only 56.45% adhered to the 24-week multidisciplinary therapy. Table 1 shows the anthropometric characteristics of the 26 normal-weight adolescents (15 girls and 11 boys), as well as the 35 obese adolescents (21 girls and 14 boys) who completed all 24 weeks of treatment. As expected, obese adolescents had higher BMI, body weight, and fat mass (%) when compared with normal-weight adolescents. As observed in Table 1, the 24-week therapy was efficient to reduce BMI, body weight, and fat mass (%) in the obese group; nevertheless, it is noteworthy that they remained obese (BMI = 33.46 ± 3.26 and 31.95 ± 6.28, for boys and girls) after 24 weeks of the intervention program.

At baseline, obese adolescents showed hyperleptinemia, and circulating leptin levels were 40 times higher in obese than in nonobese girls. This value was approximately 35 times higher in boys. After 24 weeks, leptin circulating values decreased in all obese patients, but not enough to reach a normal range. The same patterns were observed for insulin values, as hyperinsulinemia in obese adolescents improved after the 24-week therapy. Our results showed no differences in total ghrelin levels between obese and normal-weight adolescents of both genders, but obese boys reduced their plasma ghrelin concentration after 24 weeks of therapy (p < .05). For girls, no changes were observed in circulating values but ghrelin had a delta percent increase after 24 weeks (Table 2 and Figure 1). It is noteworthy that all volunteers showed normal ghrelin values during all evaluations.

A comparison between obese and normal-weight individuals revealed that normal-weight adolescents had higher plasma concentration of α-MSH, and in response to the multidisciplinary therapy, obese adolescents improved the circulating levels of this peptide, but yet lower than nonobese values. Regarding orexigenic neuropeptides, obese adolescents showed lower levels of NPY and AgRP than normal-weight adolescents in all evaluations, and the therapy was efficient to decrease these values, mainly after 24 weeks. Only MCH shows no differences between groups and genders; however, obese girls presented lower levels of this neuropeptide after the 24-week therapy (Table 2 and Figure 1).

Figure 2 shows the positive correlation between leptin and fat mass (%) (A) and BMI (B) in all adolescents at baseline. The same patterns were observed for absolute fat (kg) and leptin concentration (p = .000; r = .765).

### Table 1

Body composition and anthropometric profile of normal-weight and obese adolescents submitted to a multidisciplinary long-term therapy

<table>
<thead>
<tr>
<th></th>
<th>Girls Normal-weight</th>
<th>Obese Basal</th>
<th>12 Weeks</th>
<th>24 Weeks</th>
<th>Boys Normal-weight</th>
<th>Obese Basal</th>
<th>12 Weeks</th>
<th>24 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>15.75 ± .93</td>
<td>16.49 ± 1.82</td>
<td>16.72 ± 1.81</td>
<td>17.17 ± 1.83</td>
<td>15.09 ± 1.04</td>
<td>16.62 ± 1.93</td>
<td>17.06 ± 1.92</td>
<td>17.33 ± 1.93</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.60 ± .04</td>
<td>1.60 ± .06</td>
<td>1.61 ± .06</td>
<td>1.60 ± .07</td>
<td>1.74 ± .05b</td>
<td>1.69 ± .06b</td>
<td>1.69 ± .06b</td>
<td>1.69 ± .06b</td>
</tr>
<tr>
<td><strong>Body mass (kg)</strong></td>
<td>57.15 ± 7.97</td>
<td>93.66 ± 14.73</td>
<td>90.00 ± 14.12</td>
<td>86.49 ± 11.43</td>
<td>68.36 ± 13.58b</td>
<td>104.86 ± 9.77b</td>
<td>97.01 ± 11.83b</td>
<td>93.25 ± 15.50bc</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>22.25 ± 1.94</td>
<td>36.13 ± 4.38</td>
<td>34.61 ± 4.26</td>
<td>33.46 ± 3.26</td>
<td>22.22 ± 2.19</td>
<td>36.77 ± 4.24a</td>
<td>33.87 ± 4.68b</td>
<td>31.95 ± 6.28bc</td>
</tr>
<tr>
<td>**Free fat mass (%)</td>
<td>75.80 ± 6.21</td>
<td>53.44 ± 5.22</td>
<td>55.82 ± 4.71</td>
<td>59.08 ± 6.02</td>
<td>86.61 ± 10.60b</td>
<td>61.77 ± 6.28b</td>
<td>66.84 ± 10.33b</td>
<td>70.65 ± 11.19bc</td>
</tr>
<tr>
<td>**Fat mass (%)</td>
<td>24.19 ± 6.21</td>
<td>46.55 ± 5.23</td>
<td>44.17 ± 4.71</td>
<td>40.91 ± 6.02</td>
<td>13.38 ± 10.60b</td>
<td>38.22 ± 6.28b</td>
<td>33.15 ± 10.33b</td>
<td>29.35 ± 11.19bc</td>
</tr>
</tbody>
</table>

BMI, Body Mass Index.

* Normal-weight versus obese; * Boys versus girls; * Versus basal; * Versus 12 weeks. p < .05.
Discussion

Classic nutritional feedback signals such as leptin, insulin, ghrelin, and circulating nutrients themselves were originally considered to act mainly on a few areas of the brain, that is specific parts of the hypothalamus and brainstem. However, recent studies have suggested that these metabolic signals have a much broader influence on brain functions [3] and these pathways could be disturbed in obesity [23].

The main finding of this study is the improvements in leptin sensitivity, followed by a decrease in AgRP and NPY and an increase in α–MSH concentrations, which reflect dynamic alterations in leptin downstream mediators on the POMC/CART and AgRP/NPY subset of neurons, which could explain the physiological pathway involved in the changes observed after multidisciplinary therapy.

Obese subjects demonstrate lower fasting ghrelin levels [23] and postprandial ghrelin suppression [24]. In the present study, no differences were found in ghrelin levels in obese and non-obese adolescents, but more important than basal values, is the fact that fasting plasma levels of ghrelin are high in subjects with diet-induced weight loss [25]. This higher ghrelin values could be associated with weight regain; however, 24 weeks of multidisciplinary therapy was able to prevent this increase in ghrelin levels after weight loss, which could be seen as a strong point of this kind of therapy.

Obese human beings show elevated levels of leptin in serum and adipocytes, and this exposure of the hypothalamus to high leptin levels may have damaging effects on the hypothalamus [26]. As a result, the hypothalamus becomes less sensitive to leptin, and therefore, many researchers suggest obese human beings to be leptin-resistant [26–28]. This leptin resistance will, at least, act in a different way in POMC and CART neurons, stimulating orexigenics and inhibiting anorexigenics neuropeptides.

As expected, in the present study, a decrease in leptin and insulin levels was found after 12 and 24 weeks of therapy, respectively, probably as a result of a decrease in fat mass. Together with this leptin/insulin reduction, obese male adolescents showed alterations in downstream mediators of leptin/insulin in hypothalamus (decrease in NPY, AgRP, and an increase in α–MSH).

Katsuki et al (2001) [29] found elevated AgRP levels in overweight elderly individuals. Our data show that obese adolescents had AgRP levels that were lower than those in normal-weight adolescents at baseline; this controversial result could be attributed to the differences in leptin levels, once the leptin levels are negatively correlated to AgRP, and in the present study subjects presented with hyperleptinemia, which was not observed in the previous study.

When obese subjects undergo a diet-induced weight loss, there is deterioration in the central transport of leptin and subsequent worsening of leptin resistance. This could be one of the mechanisms that explain the well-known problem in maintaining negative energy balance during weight loss [30].

Table 2

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<tbody>
<tr>
<td>Leptin (ng/mL)</td>
<td></td>
<td>44.75 ± 9.84⁴</td>
<td>36.66 ± 14.84⁴</td>
<td>31.83 ± 15.12⁴</td>
<td></td>
<td>36.51 ± 15.97⁴</td>
<td>20.01 ± 22.15⁴</td>
<td>22.64 ± 20.39⁴</td>
</tr>
<tr>
<td>Ghrelin (ng/mL)</td>
<td></td>
<td>6.92 ± 3.64</td>
<td>6.80 ± 2.72</td>
<td>9.76 ± 8.96</td>
<td></td>
<td>11.88 ± 15.79</td>
<td>9.12 ± 9.87</td>
<td>8.63 ± 1.91</td>
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<tr>
<td>NPY (ng/mL)</td>
<td></td>
<td>2.67 ± 1.84⁴</td>
<td>3.32 ± 2.20⁴</td>
<td>2.58 ± 2.32⁴</td>
<td></td>
<td>2.54 ± 1.71⁴</td>
<td>1.96 ± 1.35⁴</td>
<td>1.16 ± 1.64⁴</td>
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<tr>
<td>α–MSH (ng/mL)</td>
<td></td>
<td>3.86 ± 1.80⁴</td>
<td>4.36 ± 2.37⁴</td>
<td>7.53 ± 2.78⁴</td>
<td></td>
<td>5.30 ± 3.77⁴</td>
<td>5.88 ± 3.70⁴</td>
<td>9.09 ± 6.34⁴</td>
</tr>
<tr>
<td>MCH (ng/mL)</td>
<td></td>
<td>12.53 ± 13.54</td>
<td>7.68 ± 2.94</td>
<td>5.50 ± 2.40⁴</td>
<td></td>
<td>15.90 ± 8.90</td>
<td>15.11 ± 9.63</td>
<td>8.79 ± 6.37</td>
</tr>
<tr>
<td>AgRP (ng/mL)</td>
<td></td>
<td>2.36 ± .61⁴</td>
<td>2.31 ± .37⁴</td>
<td>2.16 ± .36⁴</td>
<td></td>
<td>2.83 ± .96⁴</td>
<td>2.60 ± .66⁴</td>
<td>2.16 ± .85⁴</td>
</tr>
</tbody>
</table>

NPY, neuropeptide Y; α–MSH, alpha-melanocyte stimulating hormone; MCH, melanin-concentrating hormone; AgRP, agouti and related protein.

⁴ Versus basal; ⁵ Versus 12 weeks; ⁶ Normal-weight versus obese; ⁷ Boys versus girls. p < .05.

Figure 1. Changes in hormones and neuropeptides in (A), girls and (B), boys after 12 and 24 weeks of multidisciplinary therapy. NPY, neuropeptide Y; α–MSH, alpha-melanocyte stimulating hormone; MCH, melanin-concentrating hormone; AgRP, agouti and related protein.
Leptin resistance is a complex and as yet poorly understood process which is believed to occur and may be targeted at different levels, broadly divided into central leptin transport and impairment of leptin receptor function and signaling. Related to the first hypothesis, Hollman and Struder (2000) [31] postulated that blood-brain barrier, being more permeable to plasma proteins in response to physical exercise at moderate intensity is possibly caused by the effect of hemoconcentration.

Other point to be addressed is that reduced body weight and fat mass in response to physical exercise is associated with increased sensitivity to leptin’s anorectic and thermogenic effects. These behavioral and physiological improvements were associated with increased leptin receptor binding and increased leptin expression in the arcuate nucleus [32]. Thus, we suggest that the incorporation of physical exercise in therapy, and not only diet, will improve leptin/insulin signaling in the central nervous system, avoiding long-term weight regain.

The nutritional imbalance and psychological disturbance can be also responsible for eating behavior, modulating the neurohormonal pathways. However, these variables were not assessed in the present study, and therefore, the lack of these results can be seen as a weakness.

Previously, Semjonous et al (2009) [33] showed that an increase in NPY and AgRP produced a significant increase in food intake, with the effect lasting throughout the remaining 24-hour measurement period. By contrast, an elevation in α-MSH rapidly decreased food intake in food-deprived rats. After the therapy, the obese adolescents studied in the present study presented a decrease in NPY and AgRP and an increase in α-MSH. A recent study showed that peripheral NPY is analogue to NPY that is expressed in the brain and has a dynamic role in regulating energy homeostasis [34].

Sex steroids are also involved in the regulation of adiposity and energy homeostasis, females being more sensitive to the appetite-inhibiting effects of leptin than males [35]. Then, the differences observed between boys and girls in the present study could be partly attributed to sex hormones, and there are evidences showing that the peripheral factors (e.g., ghrelin) are dependent on stages of puberty [36].

Specially for ghrelin, NPY, and AgRP, the results presented in this study are different from the previously published data [29,37,38]. However, all these studies used different populations (different age groups, body mass, and also individuals with anorexia nervosa). In all previous researches, individuals presented leptin values that were lower than the levels found in this study (four or five-folds). Once leptin is a potent determinant of ghrelin, NPY, and AgRP, we strongly believe that the higher leptin levels could explain this controversial result.

Blher and Mantzoros (2009) [39] have suggested that therapies aimed to sensitize the body to leptin’s actions may provide better weight-loss outcomes. Thus, we can conclude that a 24-week multidisciplinary therapy, composed of physical exercise and nutritional, psychological, and clinical support, is effective to enhance the neurohormonal control of food intake in obese adolescents.

Acknowledgments

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