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ORIGINAL ARTICLE

Platelet and leptin in obese adolescents

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Abstract

Objective: To analyze the influence of obesity status on immune cell count and concentration of the hormones cortisol and leptin, in order to establish a relationship among the variables analyzed.

Methods: We recruited 27 obese [body mass index (BMI) ≥ 95th percentile] and 21 non-obese (BMI ≤ 75th percentile) adolescent boys and girls, aged 15-19 years at the post-pubertal stage. BMI was calculated as body weight divided by height squared, and body composition was estimated by plethysmography in the Bod Pod™ system. Blood samples were collected to analyze leukocytes, neutrophils, lymphocytes, monocytes, platelets, cortisol, and leptin. The Kolmogorov-Smirnov test was performed, followed by the independent Student *t* test in case of normal distribution. Significance values were set at $p < 0.05$ and expressed as means ± standard deviation. The statistical package SPSS for Windows version 12.0 was used.

Results: There was no difference between obese and non-obese adolescents in terms of leukocyte, neutrophil, lymphocyte, monocyte and cortisol serum concentrations. The group of obese adolescents presented higher platelet and leptin concentrations ($p < 0.01$). The prevalence of hyperleptinemia was 25.92% in the obese adolescents (15.38% in boys and 35.7% in girls).

Conclusions: Obese adolescents have higher platelet and leptin concentrations in comparison with non-obese adolescents. It was also found that obese girls presented a higher prevalence of hyperleptinemia than obese boys.

J Pediatr (Rio J). 2008;84(6):516-521: Adolescents, obese, non-obese, leukocytes, cortisol, leptin.

Introduction

The incidence of obesity and its associated disorders is increasing markedly worldwide. Obesity predisposes individuals to an increased risk of developing many diseases, including atherosclerosis, diabetes, nonalcoholic fatty liver disease, certain cancers, and some immune-mediated disorders, such as asthma.[1]

These pathologies seem to be associated with the high amount of fat found in obese individuals. Adipocytes secrete several adipokines and acute-phase proteins, which directly or indirectly increase production and circulation of factors

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related to inflammation.[2] Currently obesity is characterized by chronic inflammation, in parallel with other complications.[2]

Adipokines may have antagonist actions in the inflammatory process. There are the typically pro-inflammatory ones, the so-called interleukines IL-1, IL-6, IL-8, the TNFα and those produced by Th1 cells (IL-2 and interferon-γ),[3] leptin and resistin.[4] However, adipokines with anti-inflammatory action are the antagonists of the IL-1 (IL-1ra) receptor, transformation growth factor-β (TGF-β), cytokines produced by Th2 cells (IL-4, IL-5 and IL-10)[3] and adiponectin.[5] Leptin is the most abundant one[6] and has influence on the immune system and on platelet activation and segregation.

Data in the literature are controversial with regard to the reduction in fat mass and alteration in pro-inflammatory cytokine concentration. Xenachis et al.[7] observed that the circulating concentration of TNF-α diminished with the reduction in body mass, although other studies failed to find this reduction.[8]

Another factor that could alter the function of immune cells is the hormone cortisol. Cortisol is recognized as a potent hormone responsible for the suppression of various inflammatory and immune reactions.[9] Obesity seems to be followed by various signs of hypothalamic dysfunctions, similar to those observed in rodents, but usually to a lesser degree.[10] Alterations in cortisol concentrations in obesity are related to central obesity.[11] Rosmond et al.[12] found positive correlations with abdominal circumference and sagittal-abdominal diameter, and more importantly, with some metabolic variables, such as triglycerides, insulin, HDL cholesterol (inverse proportion), IGF-1 and with blood pressure, i.e., with parameters indicative of the metabolic syndrome (MS). To sum up, central-visceral obesity, the probable major mechanism of MS, is related to an increase in the activity of the hypothalamus-hypophysis-adrenal axis and a decrease in its suppressability. In fact, this evidence was observed in previously investigation developed by our research group.[13]

In obese individuals the cortisol concentration is increased,[14] which may occur due to the action of leptin in the hypothalamus-hypophysis-adrenal axis.

Obesity is a chronic metabolic disorder associated with cardiovascular disease and atherosclerosis. Platelet activation and aggregation are central processes in the pathophysiology of cardiovascular disease. Mean platelet volume (MPV), a determinant of platelet activation, is a newly emerging risk marker for atherothrombosis.[15]

A possible factor that induces platelet activation and segregation is leptin, increasing cardiovascular risks. The long form of the leptin receptor was detected in human platelets and high concentrations of leptin were reported to promote platelet aggregation in vitro. These latter observations raise the possibility that leptin may also promote thrombosis and contribute to other disorders in obesity.[16] As in adults, obese adolescents present high leptin concentration.[17] Viso González et al.[18] demonstrated a state of hyperleptinemia in obese adolescents.

Therefore, the aim of this study was to analyze the influence of the obesity status on the immune cell count and concentration of the hormones cortisol and leptin.

Methods

Population

In the Ambulatory for Obesity Intervention (CEPE-GEO) 27 obese (BMI ≥ 95th percentile) and 21 non-obese (BMI ≤ 75th percentile) adolescents boys and girls of the CDC reference growth charts[19] were recruited, aged 15-19 years and pubertal stage as assessed by means of the Tanner.[20]

This is a cross-sectional screening study designed to evaluate the leukocytes, cortisol and leptin serum levels in obese and non-obese adolescents. The study was carried out in accordance with the principles of the Declaration of Helsinki and was formally approved by the Research Ethics Committee of the Universidade Federal de São Paulo (protocol no. 0135/04). Informed consent was obtained from all subjects and/or their parents. All individuals were subjected to a medical evaluation by a physician, including a full medical history and physical examination.

Study protocol

During the first visit, subjects were medically screened, had their pubertal stage assessed and their anthropometric profile measured (height, weight, BMI, and body composition were measured by Bod Pod™).

Anthropometric measurements

Subjects wearing light clothing and no shoes were weighed on a Filizola scale to the nearest 0.1 kg. Height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer (Sanny, model ES 2030). BMI was calculated as body weight divided by height squared. Body composition was estimated by plethysmography in the Bod Pod™ body composition system (version 1.69; Life Measurement Instruments, Concord, CA)[21].

Serum analysis

Blood samples were collected in the outpatient clinic around 08:00 a.m. after an overnight fast. After collection, the blood was centrifuged for 10 minutes at 5,000 rpm and stored at -20 °C for future analyses. The materials used for collection were disposable, adequately labeled and of recognized quality. Blood was collected by a skilled and qualified technician.

Hemogram

Blood to perform the hemogram was collected in vacuum tubes with EDTA anticoagulant. Afterwards, a smear was

Table 1 - Anthropometric variables of obese and non-obese groups: comparison of the biochemical parameters between obese and non-obese adolescents (values expressed by the mean ± standard deviation from the mean)

	Non-obese (n = 21)	Obese (n = 27)
Age (Y)	15.37±1.25	15.83±0.87
Body mass (kg)	60.45±8.91	107.95±10.34*
Height (m)	1.72±0.06	1.74±0.07
BMI (kg/m^2)	20.41±2.0	35.83±3.88*
Body fat (%)	8.06±4.86	35.20±8.72*
Lean body mass (kg)	55.28±5.38	69.26±9.38*
Neutrophil (1,000/mm^3)	3.85±1.06	3.93±1.66
Lymphocytes (1,000/mm^3)	2.48±0.55	2.25±0.69
Monocytes (1,000/mm^3)	0.43±0.12	0.44±0.29
Cortisol (ug/dL)	9.17±5.09	12.84±5.23
Platelet (1,000/mm^3)	212.45±59.72	279.57±43.81*

BMI = body mass index.
* Group obese vs. group non-obese, $p \leq 0.01$.

taken and the material was stained by the panoptic technique. The same tube was submitted to an automated system (Sysmex SF-3000, Roche Diagnostics, Sydney, Australia), which uses the flow cytometry methodology, obtaining the parameters of the red series (hemacias, hemoglobin, hematocrit and the hematimetric indexes), white series (total and differential leukocytes) and platelets.

Statistical analysis

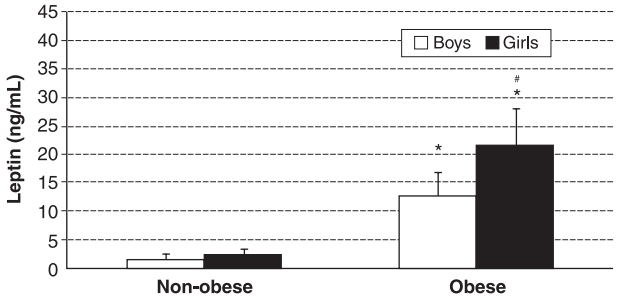
The Kolmogorov-Smirnov test was used to verify the hypothesis of normal distribution, followed by the independent Student *t* test in case of normal distribution. Significance values were set at $p < 0.05$ and expressed as means ± standard deviation unless otherwise stated. The statistical package SPSS for Windows version 12.0 was used.

Results

The characteristics of each group are shown in Table 1. The body mass, BMI and fat presented significantly higher values for obese group ($p < 0.01$). In the present study the obese adolescent group had 78.4% more body mass (kg) in comparison with the non-obese group (107.95±10.34 and 60.45±8.91 kg, respectively). In the same context, the obese group obtained a 75.5% higher body fat (8.06±4.86 in the non-obese group and 35.20±8.72% in the obese group) and the fat-free mass was significantly higher in the obese group (69.26±9.38 kg) than in the non-obese group (55.28±5.38 kg). On the other hand, the entire group did not differ significantly in age and height, when comparison was made between the groups.

In Table 1, note that there was no difference between obese and non-obese in the leukocyte, neutrophil, lymphocyte, monocyte and cortisol serum concentrations. The group of obese adolescents presented a higher platelet concentration ($p < 0.01$).

The mean and standard deviation concentrations of leptin were all significantly higher in the obese groups than in the non-obese groups. Interestingly, the obese girls showed higher concentration in leptin than boys (21.52±6.58 and 12.58±4.17 ng/mL, respectively) ($p < 0.05$). In contrast,



*Obese vs. non-obese, p<0.01
#Girls vs. boys, p<0.05

Figure 1 - Leptin concentration of adolescent girls and boys: comparison between obese and non-obese groups

comparison of leptin in non-obese group was not significant (Figure 1). The prevalence of hyperleptinemia was 25.92% in the obese adolescents (15.38%, boys and 35.7%, girls).

Discussion

To date, this was the first study to the compare immune cell profile between obese and non-obese adolescents. The main finding of the present research was that obese individuals presented a higher platelet count when compared with non-obese individuals.

Although epidemiological data has shown that obese individuals have higher white blood counts than lean individuals, the absolute leukocyte count of obese individuals is generally within the normal range.[22] However, there are almost no reports that focused on immune cell counts in obesity and specifically in obese adolescents.

Another study showed that obese children have high circulating leukocyte counts, in particular neutrophils, monocytes, and lymphocytes.[23] Although the mechanisms of these increases are not clear, obesity in children, as in adults, is related to increased circulating cytokines, like IL-6 and TNF-α,[24] which may contribute to the increased numbers of circulating leukocytes.[25] It is difficult to explain the difference between the results of the present study and the above-mentioned studies. Despite the higher leukocytosis observed in obese child and adult subjects, these leukocytes levels were within accepted clinical values.

Differently, in our study, no differences were observed for white immune cell counts between obese and non-obese adolescents. Although an increase in leptin was found in the present study, which could be related to immune alterations, this seems to alter the function of cells more than it alters their counts. In this context, leptin seems to participate in responses related to immune function, such as: inflammatory responses.[26]

The function and count of immune cells is also influenced by cortisol. Classically, an increase in the secretion of glucocorticoids is found in obese children, adolescent and adult.[15]

In this study, the comparison of cortisol concentration between obese and non-obese groups was not significant. This can be justified because the non-obese group practiced systematic physical exercises. The human organism decodes physical exercise as a stressful stimulus produced by means of the hypothalamus, a strong sympathetic adrenal discharge, resulting in cortisol release by the cortex of the supra-renal gland.[27]

Obesity is a chronic metabolic disorder associated with cardiovascular disease and atherosclerosis. Platelet activation and aggregation are central processes in the pathophysiology of cardiovascular disease. MPV, a determinant of platelet activation, is a newly emerging risk marker for atherothrombosis.[28] Little is known about the relation between BMI and MPV levels in obese patients. Toplak & Wascher[29] reported that after weight loss, the MPV was significantly decreased to initial values. Coban et al.[28] found that MPV showed positive correlations with BMI level in obese group. The data of the present study support this hypothesis, showing that obese adolescents present higher platelet counts in comparison with non-obese adolescents.

Since the literature has concluded that elevation of platelet counts and MPV are significantly associated with obesity and they contribute to the development of several diseases, significant efforts must be implemented to keep adolescents' weight within normal limits or to reduce their weight when they are obese, in order to reduce the potentially harmful effects of the obesity-induced inflammatory state and atherothrombosis risks.

Leptin is a factor that induces platelet activation and aggregation, thus increasing cardiovascular risks. In addition, an association of leptin with thrombosis and hemostatic balance disorders in obesity has been suggested.[16]

The results of the present study showed that obese adolescents have higher platelet counts in comparison with non-obese adolescents. In adults, this relationship has already been presented by Nakata,[30] however, in obese adolescents, this has not been documented in the literature, which leaves the subject inconclusive.

The prevalence of hyperleptinemia was higher in obese girls, 35.7%, vs. 15.38% in obese boys. In the non-obese groups there was no prevalence of hyperleptinemia. Nevertheless, the platelet count was similar between the genders. These data suggest that if the hyperleptinemia condition is not controlled, girls could be more susceptible to cardiovascular risks associated with leptin-dependent platelet activation and aggregation.

Based on the results found in the present study, it could be concluded that obese adolescents have higher platelet and

leptin concentrations in comparison with non-obese adolescents, a stat that could favor functional alterations and represent higher risk for disorders associated with cardiovascular diseases. It was also found that obese girls presented higher prevalence of hyperleptinemia than obese boys. Therefore, it is reasonable to suggest that treatment for obesity control in adolescence is relevant to promote and maintain the state of health and quality of life of this population, and those therapies, such as physical exercise, nutritional, psychological and medical follow-up could be used as prophylactic tools.

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References

1. Tilg H, Moschen RA. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006;6: 772-83. Epub 2006 Sep 22.
2. Trayhurn P. Adipocyte biology. Obes Rev. 2007;8 Suppl 1:41-4.
3. Kelly DA. The use of anti-interleukin-2 receptor antibodies in pediatric liver transplantation. Pediatr Transplant. 2001;5: 386-9.
4. Holcomb IN, Kabakoff RC, Chan B, Baker TW, Gurney A, Henzel W, et al. FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. EMBO J. 2000;19:4046-55.
5. Neumeier M, Weigert J, Schäffler A, Wehrwein G, Müller-Ladner U, Schölmerich J, et al. Different effects of adiponectin isoforms in human monocytic cells. J Leukoc Biol. 2006;79:803-8. Epub 2006 Jan 24.
6. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112:1785-98.
7. Xenachis C, Samojlik E, Raghuwanshi MP, Kirschner MA. Leptin, insulin and TNF-alpha in weight loss. J Endocrinol Invest. 2001; 24:865-70.
8. Nicklas BJ, You T, Pahor M. Behavioural treatments for chronic systemic inflammation: effects of dietary weight loss and exercise training. CMAJ. 2005;172:1199-209.
9. Stites DP, Terr AL. Basic human immunol. New York: Prentice Hall; 1991.
10. Bjorntorp P, Rosmond R. Obesity and cortisol. Nutrition. 2000; 16:924-36.
11. Stewart PM, Tomlinson JW. Cortisol, 11 beta-hydroxysteroid dehydrogenase type 1 and central obesity. Trends Endocrinol Metab. 2002;13:94-6.
12. Rosmond R, Holm G, Bjorntorp P. Food-induced cortisol secretion in relation to anthropometric, metabolic and hemodynamic variables in men. Int J Obes Relat Metab Disord. 2000;24:416-22.
13. Dâmaso AR, do Prado WL, de Piano A, Tock L, Caranti DA, Lofrano MC, et al. Relationship between nonalcoholic fatty liver disease prevalence and visceral fat in obese adolescents. Dig Liver Dis. 2008;40:132-9. Epub 2007 Dec 21.
14. Reinehr T, Hinney A, de Sousa G, Austrup F, Hebebrand J, Andler W. Definable somatic disorders in overweight children and adolescents. J Pediatr. 2007;150:618-22, 622.e1-5.
15. Coban E, Yilmaz A, Sari R. The effect of weight loss on the mean platelet volume in obese patients. Platelets. 2007;18:212-6.
16. Konstantinides S, Schäfer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. J Clin Invest. 2001;108:1533-40.
17. Dubey S, Kabra M, Bajpai A, Pandey RM, Hasan M, Gautam RK, et al. Serum leptin levels in obese Indian children: relation to clinical and biochemical parameters. Indian Pediatr. 2007;44: 257-62.
18. Viso González ME, Solano L, Sánchez A, Portillo Z, Llovera D. Serum leptin in eutrophic and overweight Venezuelan children and adolescents. Arch Latinoam Nutr 2005;55:47-54.
19. National Center for Health Statistics. Prevalence of overweight among children and adolescents: United States, 1999-2002. http://www.cdc.gov/nchs/products/pubs/pubd/hestats/overwght99.htm. Access: 10/08/2006.
20. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976;51:170-9.
21. Fields DA, Goran MI. Body composition techniques and the four-compartment model in children. J Appl Physiol. 2000;89: 613-20.
22. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diabetes Care. 1999;22:1971-7.
23. Zaldivar F, McMurray RG, Nemet D, Galassetti P, Mills PJ, Cooper DM. Body fat and circulating leukocytes in children. Int J Obes (Lond). 2006;30:906-11.
24. Nemet D, Wang P, Funahashi T, Matsuzawa Y, Tanaka S, Engelman L, et al. Adipocytokines, body composition, and fitness in children. Pediatr Res. 2002;53:148-52.
25. Moriguchi S, Oonishi K, Kato M, Kishino Y. Obesity is a risk factor for deteriorating cellular immune function decreased with aging. Nutr Res. 1995;15:151-60.
26. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. FASEB J. 1998;12:57-65.
27. Kraemer WJ, Ratamess NA. Hormonal responses and adaptations to resistance exercise and training. Sports Med. 2005;35:339-61.

28. Coban E, Ozdogan M, Yazicioglu G, Akcit F. The mean platelet volume in patients with obesity. Int J Clin Pract. 2005;59:981-2.

29. Toplak H, Wascher TC. Influence of weight reduction on platelet volume: different effects of a hypocaloric diet and a very low calorie diet. Eur J Clin Invest. 1994;24:778-80.

30. Nakata M, Yada T, Soejima N, Maruyama I. Leptin promotes aggregation of human platelets via the long form of its receptor. Diabetes. 1999;48:426-9.

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