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Research Article

Comparison of Anthropometric, Inflammatory and Metabolic Markers in 8-Year-Old Children from the *Brasil Sul Cohort* Study

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Abstract

Aim of study: Compare anthropometric, inflammatory and metabolic characteristics in eutrophic and obese 8-year-old schoolchildren attending at a cohort in Palhoça/SC, Brazil.

Methods: Cross-sectional analytical study involving 34 8-year-old students selected for convenience using the anthropometric measurements from obese or eutrophic individuals according to their BMI. Anthropometric measurements were repeated at the clinic (weight, height, waist circumference and tricipital fold), and a clinical examination was also performed. Biochemical measurements were performed for glucose, insulin, total cholesterol and HDL-cholesterol, triglycerides, ultra-sensitive C reactive protein (us-CRP), interleukin (IL)-6, IL-1β, IL-10, tumor necrosis factor (TNF) and adiponectin. For the comparison of independent samples, Student's t-test was used for parametric data or the Mann-Whitney test for non-parametric data, after normality analysis by Shapiro-Wilk.

Results: Waist circumference (p= 0.002), the tricipital fold (p= 0.009), as well as the serum levels of fasting insulin (p= 0.04) and us-CRP (p= 0.002) were increased in obese group.

Conclusions: These biomarkers may be helpful in the premature detection for obesity in children and could lead to early prevention of complications.

ABBREVIATIONS

WHO: World Health Organization; IBGE: Brazilian Institute of Geography and Statistics; ELISA: enzyme-linked immunosorbent assay; us-CRP: ultra-sensitive C reactive protein; SBP: Brazilian Society of Pediatrics; P90: the 90th percentile

INTRODUCTION

The World Health Organization (WHO) has been warning about the considerable increase in the number of obese children and adolescents worldwide [1], in 2016, this number reached 134 million in the age range between 5 and 19 years [2]. In Brazil, prevalence increased from 4% in 1975 to 18% in 2016 [3]. According to the latest Household Budget Surveys 2008-2009 from the Brazilian Institute of Geography and Statistics (IBGE) [4], although the height deficit has diminished in this period in relation to the 1974-1975 period, overweight (overweight and obesity) prevalence reached values of 51.4% and 43.8% among boys and girls, respectively, showing the importance of this condition as a public health problem in our country. Still, according to data from IBGE overweight and obesity rates in the 10 to 19 age group were lower than in the 5 to 9 age group, in both genders, but are nevertheless considered high [4].

In Florianópolis, capital city of the Brazilian southern state of Santa Catarina, the region where this study was performed, Bernardo and colleagues (2012) [5], found, among school children aged seven to 10 years enrolled in primary schools in 2007, an overweight prevalence of 36.2% among boys and 32.7% among girls. This overweight growing increase among

children and adolescents is especially important if we consider the complications that obesity can cause already in childhood, as well as the consequences in the future. Among children, the onset of hypertension that is associated with increased morbidity and mortality due to cardiovascular problems [6], is indeed noteworthy. In addition, the development of metabolic complications associated with obesity during childhood may predispose adults to an increased risk of diseases such as type 2 diabetes mellitus, early cardiovascular diseases [7], among others.

These changes observed in the different systems associated with obesity, may result in the inflammatory changes that are currently blamed for the genesis of obesity [8], in addition to its endogenous or exogenous causes. Although the hypothesis of an inflammatory cause for obesity is recent, several researchers have investigated possible differences in inflammatory markers serum concentrations among children and adolescents from different countries. Thus, Landgraf and collaborators (2015) [9], did not observe statistically significant differences in the levels of IL-6, IL-1ß, IL-10 and TNF between the eutrophic or obese groups when working with 171 individuals aged 0-18 years in the Leipzig cohort. The concentration of IL-6 was also not altered in the work of Caballero and colleagues (2008) [10], with individuals aged 10 to 18 years. Other authors worked with Australian children/adolescents and found no change in the serum levels of IL-6, IL-8 and IL-10 of individuals with eight years of age; however, when the same children were reassessed at the age of 15, it was possible to observe a statistically significant increase in these markers in females [11]. Finally, adiponectin (an adipocytokines), concentrations were inversely associated with visceral adipose tissue in prepubertal and pubertal children from Mexico [12].

Given these results, it seems clear that a problem related to this type of analysis is due to the fact that most studies evaluating inflammatory markers in children/adolescents use a population of a wide age range, involving both children, pre-adolescents and teenagers. Thus, it is difficult to identify at what point in childhood and/or adolescence the dysfunctional trajectory begins in this system, as well as which age group is at greatest risk for developing comorbidities associated with obesity that, once identified, could serve as a useful tool for interventions that would lead to a better prognosis. Thus, the monitoring of a narrow age group children, for a long period could be a strategy to answer such questions.

In 2015, a cohort study named *Brasil Sul Cohort* was started by investigators in the municipality of Palhoça/SC, Brazil [12]. At first, this study retrospectively evaluated children born in 2009 and who in 2014 were enrolled in public or private schools in the municipality; their parents or guardians were interviewed regarding aspects of the child and family's general health conditions history, the child's oral health, eating habits [14], children allergic diseases [15], family socioeconomic conditions at the time of the child's birth, conditions related to childbirth and children's physical activity, among others, to establish the influence of the first thousand days of life on these factors. Thereafter, children of this study were evaluated for complementary measures to assess children overweight [16].

In the present study, developed during 2017, eutrophic or obese children (8 years old), from database of individuals in this cohort were surveyed with the objective of evaluating and comparing the serum levels of inflammatory markers, metabolic characteristics, adiposity and insulin resistance among these children, also thinking about their longitudinal monitoring within the *Brasil Sul Cohort* Study in the future.

MATERIALS AND METHODS

Study Design

A total of 34 children aged 8 years, participants of the *Brasil Sul Cohort* Study living and enrolled in schools in the municipality of Palhoça/SC, Brazil were selected. They had previously been measured and weighed in their schools. From the cohort database, subjects were chosen for convenience; an equal number of girls and boys were selected to participate and were allocated in two groups: the group of eutrophic children (BMI Z score >-2 and <+ 1SD), and obese (BMI Z score > ≥ DP + 2). For the calculation of the sample, a 95% of confidence level and 80% of power of the test were adopted. The value of the difference to be detected in the present study used the same parameters from a previous study that evaluated the same variables in subjects of similar age range [10]. The minimum sample size required was 32 children, 16 in each group.

Anthropometric Measures

To calculate the BMI (weight divided by the squared height), a digital anthropometric scale (Welmy®, Santa Bárbara D'Oeste/SP, Brazil) was used, placed on a flat surface to collect the weight; children were measured barefoot and wearing light clothing. Height was measured in an upright position, with feet placed together and using a stadiometer with a resolution of 1 mm. The waist circumference was checked with an inelastic measuring tape, with the child in an upright position; the measure was taken at the midpoint between the lower margin of the last rib and the upper border of the iliac crest. Triceps skin fold was measured with a scientific plicometer (Cescorf®, Porto Alegre/RS, Brazil).

Biochemical Assays

Blood samples were collected in two Vacutainer® tubes, one containing a separating gel without anticoagulant, to obtain the serum and the other containing EDTA, for blood count. After collection, the samples were centrifuged for 10 minutes at 3500 rpm to obtain the serum. The supernatant was collected and divided into 150 µL aliquots, being stored at -80ºC until analyzed. The quantification of us-CRP and insulin was performed by the Bioclínico laboratory (MG, Brazil), by radioimmunoassay, using a chemiluminescence system for ultra-sensitive analysis. Glucose, total cholesterol, HDL-c and triglycerides were measured using commercial enzymatic kits from Labtest® (Lagoa Santa/MG, Brazil).

To detect cytokines IL-6, IL-1β, TNF, IL-10 and adiponectin in serum samples, the enzyme-linked immunosorbent assay (ELISA), was performed using DuoSet ELISA kits (R&D Systems, Minneapolis/MN, USA) according to the manufacturer's instructions. The values of the colorimetric assay were obtained by reading the ELISA plates in the spectrophotometer (Perlong

DNM-9602, Nanjing Perlove Medical Equipment Co, Nanjing, China), measured at 450 nm, with the correction at 540 nm wavelength. The readings were interpolated with the standard curve values for each cytokine and are expressed in pg/mL. The blood count of all children was also performed, aiming at identifying possible infectious changes to be considered as exclusion criterion, in order to avoid possible biases that could alter the levels of cytokines and us-CRP. The results were compared to the reference values published by Brazilian Society of Pediatrics (SBP), in the guidance manual on childhood obesity.

Homa-Ir Determination

The Homa-ir index was obtained by multiplying fasting plasma insulin (μU/mL), with fasting glycemia (mmol/L) divided by 22.5. The cut-off point was ≥3. Normal values were considered: fasting blood glucose ≤100mg/dL; fasting insulin ≤15 μU/mL; total cholesterol ≤170mg/dL; HDL-c ≥ 45mg/dL; triglycerides ≤130mg/dL and us-CRP ≤1mg/dL.

Ethical Aspects

The study was approved by the Research Ethics Committee of Southern Santa Catarina University under number 2,113,661. Data collection was performed after the signature of the Free and Informed Consent Form by the subjects' guardians, and the Consent Form by the children themselves.

Statistical Analysis

The data were inserted in a database developed in the GraphPad Prism software, version 6.0. The variables were subjected to the Shapiro-Wilk test to verify normal distribution. For the data considered as parametric, the Student's t test was used, and the results were presented as mean ± standard deviation of the mean. For non-parametric data, the Mann-Whitney test was used, with the results presented as median ± interquartile range. For all analyses, $p < 0.05$ was considered as statistically significant.

RESULTS

A total of 34 children were assessed, 13 belonging to the eutrophic group and 21 to the obese group, all 8 years old. The characteristics of the children evaluated in the study are shown in Table 1, while the average values for the differences in anthropometric, inflammatory and metabolic characteristics between obese and eutrophic children are shown in Table 2.

Table 1: Characteristics of children assessed in the study.

Characteristic	Obese	Eutrophic	p-value
Female	10	6	
Male	11	7	
BMI (kg/m^2)	24.61 ± 0.72 kg/m^2	16.14 ± 0.53 kg/m^2	0.042*
*Student t test			

Table 2: Anthropometric, inflammatory and metabolic characteristics of children assessed in the study.

Characteristics	Obese	Eutrophic	p value
Anthropometric			
waist circumference (cm)$^{\$}$	74.0 ± 1.54	55.0 ± 0.8	0.002*
tricipital fold (mm)$^{\&}$	25 (20-30)	15 (10-20)	0.009**
Inflammatory			
IL-1β (pg/mL)$^{\&}$	5.8 (0.9-43.6), n=7	11.1 (3.2-11.5), n=8	0.230
IL-6 (pg/mL)$^{\&}$	n.d.	n.d.	-
IL-10 (pg/mL)$^{\&}$	48.0 (35.9-63.0), n=10	39.3 (32.1-59.3), n=10	0.615
TNF (pg/mL)$^{\$}$	40.8 ± 6.3, n=10	42.4 ± 8.0, n=11	0.406
us-CRP (pg/mg protein)$^{\$}$	4.1 ± 1.4, n=19	1.2 ± 0.5, n=14	<0.001*
Adiponectin (pg/mL)$^{\$}$	1.715,4 ± 143.3, n=8	2.111,1 ± 208.6, n=13	0.199
Metabolic			
glucose (mg/dL)$^{\$}$	88.7 ± 1.9, n=20	91.7 ± 2.4, n=13	0.855
insulin (mU/ml)$^{\$}$	8.9 ± 0.7, n=19	3.4 ± 0.4, n=13	0.047*
Homa-ir$^{\$}$	1.8 ± 0.1, n=18	0.8 ± 0.1, n=13	0.112
TC (mg/dL)$^{\$}$	176.9 ± 6.9, n=20)	172.8 ± 7.7, n=13	0.828
HDLc (mg/dL$^{\$}$	45.9 ± 2.3, n=17	52.3 ± 2.3, n=12	0.544

Legend: $^{\$}$mean ± standard deviation of the mean; $^{\&}$median ± interquartil interval; BMI= body mass index; WC= waist circumference; TF= tricipital fold; IL-1ß= interleucin 1ß; IL-6= interleucin 6; IL-10= interleucin 10; TNF: tumoral necrosis factor; us-CRP = ultrasensitive C-reactive protein; Homa-ir= Homeostasis model assessment-insulin resistance; TC= total cholesterol; HDLc= HDL cholesterol; TG= triglycerides; n.d= not detected (values outside the kit curve); *Student t test; ** Mann-Whitney test.

The waist circumference showed a statistically significant increase in the obese group, although in 8 (38%), subjects the values were below the 90th percentile (P90), for the measurement. All children in the eutrophic group exhibited waist circumference values below P90 for age and gender. The tricipital fold also showed a statistically significant increase in the obese group, where 17 (80.9%), had values higher than the P90, a fact that did not occur with any child in the eutrophic group.

As for the metabolic markers, the values of fasting insulin were significantly increased in the obese group in relation to the eutrophic group, although still with values considered within the normal range. In turn, fasting blood glucose was slightly increased in only one obese child (4.7%), and one eutrophic child (7.7%). The total cholesterol values were increased in 13 of the obese (61.9%), and in six of the eutrophics (46.1%) children; in contrast, HDL-c levels were reduced in four obese children (19.0%), and one eutrophic child (7.7%), and triglycerides levels were increased in three obese children (14.2%) and in no eutrophic. Finally, among the obese, only three (14.2%), had normal us-CRP, 12 children (57.1%), showed borderline values and four (19.0%), had increased values, while among the eutrophic children only one exhibited a change (7.7%).

Finally, regarding the serum inflammatory markers analyzed by ELISA, no differences were observed between the two groups for any of the cytokines evaluated in the study; it is worth noting that there was a variation in the number of subjects for whom it was possible to determine the serum levels of these markers and, in this specific case, of IL-6. In all samples analyzed the values of this marker were non-compliant with the detection limits of the kit, as recommended by the manufacturer, which prevented comparison of their values between the two groups.

DISCUSSION

This study showed that in the group of 8 years obese children both waist circumference and triceps skin fold were increased (61% and 80.9%, respectively), in relation to the eutrophic group, meaning greater deposition of adipocytes. Fasting insulinemia was also significantly increased in the obese group, although the mean values were not above the values considered normal for the age studied. Furthermore, it is noteworthy that the children evaluated in the obese group already had levels of us-CRP statistically increased in relation to the control group (eutrophic), with values already suggest a low degree of inflammation (us-CRP ≥ 1,04 mg/L).

The association between high body mass indexes in children and the increase in waist circumference and triceps skin fold measurements are already expected due to visceral fat deposition; however, in the case of waist circumference, a fact that draws attention in the present study is that 61.9% (data not shown), of obese children had values higher than the P90 for age and gender, a condition that puts them at greater risk for metabolic disorders such as dyslipidemia and hyperinsulinemia [17], which is even more worrying if we consider the young age of the participants. Regarding the triceps skin fold measurement, 80.9% of the children in the obese group showed values higher than the P90 for their age and gender, suggesting excessive adiposity [18], while in the eutrophic group, no child presented such alteration.

With regard to fasting insulinemia, recent studies have associated high body mass indexes in children, even at moderate levels, with metabolic changes and cardiovascular risk in different populations such as Swedish population [19]. In this last study, although fasting insulin levels were higher in the obese group, no child reached clinical levels for the diagnosis of hyperinsulinemia which is in line with other authors [20], and it is important for the age group evaluated since the increase in insulin is an initial event that already denotes the difficulty of target tissues to respond to the action of insulin and therefore greater difficulty in of glucose intake by the cells [21]. In addition, fasting blood glucose was slightly altered in only one child in the obese group and no child had a Homa-ir index higher than the cut-off value for the diagnosis of insulin resistance. These factors are important to be monitored, since they can indicate the onset of insulin resistance in individuals and, although our findings regarding the Homa-ir index are different from those found by other authors [21,22], again we underscore the homogeneity and the low age of the individuals evaluated in the present study in relation to other individuals.

Considering the increase in waist circumference in children with values above P90 for age and gender, as well as the results regarding fasting insulinemia in the obese group, it is important to also monitor the HDLc values in connection with the possibility of developing metabolic syndrome. This is because, according to the SBP, which adopts the criteria of the International Diabetes Federation, even if there is no consensus in the case of children, among adolescents aged 10 to 16 this syndrome can be characterized when there is an increase in waist circumference (greater than the P90 for gender and age) plus at least two of four abnormalities, including HDL-c (> 40mg/dL), and fasting blood glucose (> 100mg/dL), or presence of type 2 diabetes mellitus, in addition to hypertriglyceridemia (>150mg/dL), or arterial hypertension (systolic >130 mmHg and diastolic >85 mmHg). In the present study, total HDL-c was shown to be increased in 65% of obese individuals, but the same happened to 46% of eutrophic individuals. Although this can be explained by the familial etiology of dyslipidemia, regardless of nutritional status, this finding disagrees with that of other authors who have also seen a reduction of HDL-c in obese individuals, which did not occur in our study [9,22].

However, it is noteworthy that at age 8 years, children evaluated in this study are still very busy running, jumping and moving around a lot, which differs substantially from what occurs with adolescents, for example, who are included in other studies together with younger children. In addition, the children in the present study belonged to a low socioeconomic level and attended mainly government schools close to their homes in the studied municipality where children come and go walking; both factors may influence the progression to overweight and obesity in children [23], increasing HDL-c.

In addition to the anthropometric and metabolic parameters, in the present study were evaluated pro-inflammatory (us-CRP, IL-6, IL-1β, IL-10 and TNF) or anti-inflammatory (IL-10 and adiponectin), markers of interest during obesity. Adiponectin was found to be significantly lower, while TNF, glucose, insulin and Homa-ir score were significantly higher in obese children compared with controls [24]. IL-1β appears to affect glucose

homeostasis and insulin sensitivity through central and peripheral mechanisms, decreasing the expression and activity of the lipoprotein lipase in the adipocyte, with increased lipolysis [25]. IL-6 is another pro-inflammatory cytokine associated to with obesity in in Brazilian schoolchildren [26], which can contribute to hypertriglyceridemia associated with visceral obesity and affect the lipid profile that contributes to the pathogenesis of atherosclerotic disease. In addition to suppressing adiponectin expression and altering insulin signaling in the hepatocytes, IL-6 contributes to the resistance to the action of adiponectin in the tissue [9]. On the other hand, IL-10 and adiponectin appear to be secreted under conditions of obesity to modulate such deleterious effects of the inflammatory response, with adiponectin increasing sensitivity to insulin and showing an inverse correlation with obesity [23].

In the present study, no statistically significant differences were observed between the eutrophic and obese groups with regard to most of the inflammatory markers. For IL-6 it was not possible to determine serum levels in the samples under the conditions employed in this study (technical difficulties). Once again, we emphasize the importance of performing this analysis in narrower age groups, since it may result in differences. Guedes et al. (2015) [27], performed a bibliographic review analyzing 21 articles that investigated the relationship between the increase in serum levels of these markers and childhood obesity in children from different parts of the world; in this review, it was found that in 73.3% of the studies an increase in IL-6 and in 80% of the studies an increase in TNF were observed. On the other hand, Tam et al. (2010) [11], working with eight-year-old children in Australia, did not observe differences in blood levels of IL-6, IL-8 and IL-10; however, in the same study, when these same children were reevaluated at 15 years of age, significantly increased values were found for those three markers among girls in the overweight/obesity group, suggesting that the levels of these markers may vary according to gender and age in association with weight.

Thus, our findings reproduce the results obtained by the latter author in earlier age children regarding cytokines, enhancing the need for long-term follow-up of children in order to monitor possible similarities in different populations. In the present study, one of the main changes found was in connection with the statistically significant increase in us-CRP in the obese group in relation to the control group. When assessed individually, only three children exhibited normal values in the obese group, while 12 children showed borderline values and four had increased levels, while in the eutrophic group, only one child had an increased us-CRP level while the others exhibited normal values. This finding is different from that found by Tam et al. (2010) [11], with Australian children aged eight years, in which no differences were found between the groups, which may reflect the ethnic differences already mentioned by other authors [27], since a recent study with Brazilian children aged 8-9 years also identified changes in us-CRP levels associated with changes in cardiometabolic measures found by the authors who evaluated this parameter exclusively in children aged eight years [28].

CONCLUSION

In conclusion, increased abdominal circumference and triceps skin fold measures as well as fasting serum insulin levels and us-CRP were found in eight years of age obese children, compared with eutrophic children; these data enhance the suggestion of other authors that changes in insulin resistance measures occur before changes in pro or anti-inflammatory cytokine levels. In addition to the data found by other authors (Aygun et al. 2005), despite the small sample size of the present study being a limitation, considering that the us-CRP has been shown to be increased in the children assessed in the present study at only the age of eight, it is possible to further suggest that the dysfunctionality caused by this mediator may be responsible for the beginning of changes in the levels of cytokines in other tissues.

Finally, the results of the present study have as strong points the fact that it was carried out with children of the same age, in relatively early childhood, as well as for presenting data on metabolic parameters associated with pro and anti-inflammatory cytokines in this age group in a Brazilian population, which to the knowledge of the authors, are still scarce.

As future perspectives, studies with a larger number of individuals of the same age may confirm these findings and, especially, they would serve as a starting point for monitoring individuals within the *Brasil Sul Cohort*, which will attempt to identify how the changes observed in the metabolic markers found in the present study correlate with the inflammation process that increases during the time of exposure to obesity, until more significant changes can occur in the individual's body, with consequent changes in the serum levels of pro and anti-inflammatory cytokines. Understanding these aspects will allow us to outline better strategies to avoid the dysfunctional trajectory that represents a greater risk for the development of comorbidities associated with obesity.

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