Original article (full paper)

Association of inflammation, dyslipidemia, obesity and physical activity status in children

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Abstract—The aim of this study was to verify the association between inflammatory biomarkers, dyslipidemia, obesity and physical activity status in 10-years old children. Ninety-four children participated in this study and were classified into eutrophic (n=36), overweight (n=34) or obese (n=24) according to their body mass index (BMI). The genic expression of interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and chemokine C-C motif ligand 2 (CCL-2) mRNA; the serum concentration of high-density lipoprotein cholesterol (HDL-c) and triglycerides; BMI, percentage of body fat (% BF) and waist circumference; and the number of steps per day were determined. The expression of IL-6, TNF-α and CCL-2 were associated ($p < 0.05$) positively with serum triglycerides, BMI, % BF and waist circumference, and negatively with serum HDL-c. No association ($p > 0.05$) between pro-inflammatory biomarkers and number of steps per day was found.

Keywords: child, inflammatory biomarkers, physical activity status, obesity

Introduction

Over 2.8 billion people die in consequence of overweight and obesity worldwide (World Health Organization [WHO], 2014). In Brazil, obesity triggers serious public health problems and demands high social costs (Duncan *et al*., 2012). Inflammation plays a key role in the pathophysiology of obesity (Geraldo & Alfenas, 2008). The white adipocytes exhibit altered physiology due to excessive fat storage and release of pro-inflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and chemokine C-C motif ligand 2 (CCL-2), also referred to as monocyte chemotactic protein-1 (Leite, Rocha, & Brandão-Neto, 2009). Such framework results in a state of chronic low-grade inflammation (Calder *et al.,* 2011; Herder *et al.,* 2007; Tam, Clement, Baur, & Tordjman, 2010).

Chronic low-grade inflammation is directly related to health problems such as insulin resistance, dyslipidemia, type 2 diabetes and atherosclerosis in obese children (Codoner-Franch *et al.,* 2011; Elshorbagy, Valdivia-Garcia, Refsum, & Butte, 2012; Garanty-Bogacka *et al.,* 2011; Kim *et al.,* 2010). Indeed, a recent study showed that body mass index (BMI) and fasting insulin correlated positively with insulin resistance, serum lipids, and pro-inflammatory biomarkers, but negatively with adiponectin, an anti-inflammatory mediator (Chang *et al*., 2015). Despite the positive associations between obesity and chronic low-grade inflammation, the expression of IL-6, TNF-α and CCL-2 in obese children shows conflicting results (Breslin *et al.,* 2012; Dixon, Meng, Goldberg, Schneiderman, & Delamater, 2009; Kim *et al.,* 2010; Maffeis *et al.,* 2007).

Regular physical activity has been stimulated, in order, to promote a healthy lifestyle as well as to counteract overweight and obesity in the childhood. Indeed, regular physical activity reduces obesity, the production of pro-inflammatory biomarkers as well as the prevalence of cardiovascular diseases in children (Ben Ounis *et al.,* 2010; Chae *et al.,* 2010; Garanty-Bogacka *et al.,* 2011; Vos, Wit, Pijl, & Houdijk, 2011). To achieve the benefits of physical activity, it was proposed that 13000 to 15000 steps per day for boys and 11000 to 12000 steps per day for girls should be performed by 7 to12-years old children (Tudor-Locke *et al.,* 2011). To our knowledge, no study tested the associations between IL-6, TNF-α, CCL-2 with the number of steps per day in 10-years old children.

Taking into consideration that the prevalence of overweight and obesity in children and adolescents is high (Espin Rios, Perez Flores, Sanchez Ruiz, & Salmeron Martinez, 2013; Ferreira, 2006; Instituti Brasileiro de Geografia e Estatísitca(IBGE), 2004), the early diagnostic of the association of pro-inflammatory biomarkers with modifiable lifestyle factors are of interest as well as could preventing the outcomes of chronic diseases in the future. Therefore, we designed this cross-sectional study to examine the association between inflammatory biomarkers, dyslipidemia, obesity and physical activity status in 10-years old children.

Methods

Participants

Ninety-four children (44 boys and 50 girls) aged 10.03 ± 0.74 years (mean ± SD) were selected and classified as eutrophic (n = 19 girls, n = 17 boys), overweight (n = 18 girls, n = 16 boys) and obese (n = 13 girls, n = 11 boys), according to their BMI. This study was approved by the Federal University of Triângulo Mineiro Research Ethics Committee (CEP/UFTM n° 2388) and authorized by the Municipal Secretary of Education and Culture of Uberaba, Minas Gerais, Brazil (CRAFT GAB / SEMEC / No. 0898). All participants agreed to take part in the study, and either parents or legal guardians signed the statement of consent.

Anthropometric measures

Body mass index calculated using the formula [BMI = body mass (kg)/height (m^2)]. Waist circumference (WC) was measured at the midpoint between the iliac crest and the last rib (WHO, 2005). Triceps (TS) and subscapular (SS) skinfolds were measured in triplicate using a caliper (Lange Skinfold Caliper, Cambridge, USA) according to Guedes (2006) protocol. The mean value of the three measures was used. The percentage of body fat percentage (% BF) was determined according to Slaughter *et al.* (1998). Boys and girls were classified as eutrophic, overweight and obese using BMI values according to Cole, Bellizzi, Flegal and Dietz (2000).

Physical activity status

The habitual physical activity status was determined by measuring the number of steps per day using the uni-axial motion sensor, pedometer (Yamax SW200 Digiwalker, Japan). After explanations and familiarization with the pedometer, the participants registered the number of steps for five consecutive days (Brusseau *et al.,* 2011). Participants were instructed to position the pedometer above the iliac crest in the midline of the right thigh, attached to an elastic waistband during all wake time. They were advised to perform their routine activities and remove the pedometer only when lying down, riding a bicycle, bathing or practicing water activities. The participants were told that the pedometer should be placed back to the waist immediately after these activities. Children were instructed to record the number of steps shown on the pedometer on a paper given at the end of each day before lying down to sleep. On the next day, after wake up, children were told to reset the pedometer and verify if the display showed zero before placed back on the waist. For analyzes, the average of steps performed during the 5 days were considered. For classification the physical activity status, we adopted the cut-offs of 11000 steps/day for girls and 13000 steps/day for boys (Tudor-Locke *et al.,* 2011).

Serum lipid profile

After 12-14 hours fasting, blood samples were collected in appropriate tubes (BD Vacutainer®, São Paulo, Brazil) containing gel and clot activator. Serum, plasma and buffy-coat were separated from other blood components by centrifugation at 3,400 rpm for eight minutes and stored in Eppendorf tubes (1.5 mL) at -80°C. Serum triglycerides and HDL-c were analyzed by an enzymatic colorimetric method using a semiautomatic biochemical analyzer BIO 200F (Bioplus, São Paulo, Brazil) and commercial kits (LABTEST, System Diagnostics Ltda. Lagoa Santa, Brazil). The cut-offs used for dyslipidemia diagnosis were: triglycerides ≥ 150 mg / dL and HDL-c ≤ 35 mg / dL (Kavey *et al.,* 2014).

Gene expression of inflammatory biomarkers

The buffy-coat aqueous and organic phases were separate by adding 100 µL of phosphate buffered saline (PBS) and 750 µL of Trizol (Invitrogen - Sao Paulo, Brazil). After incubation at room temperature for five minutes, it was added 200 µL of chloroform (Merck, Rio de Janeiro, Brazil) and centrifuged for 15 minutes at 4° C. The supernatant (aqueous phase RNA) was then transferred to another eppendorf tube in which was added 380 µL of isopropanol. Subsequently, the samples were centrifuged for 4 minutes at 4° C and stored at -80° C for 12 hours to precipitate the RNA. Then, the precipitate was washed with 750 µL of ethanol (75%) and stored for 10 minutes at room temperature to dry. Thereafter, it was added 50 µL of ultrapure water, free RNAse. The concentration of purified total RNA was quantified by spectrophotometer (Nanodrop 2000c, Wilmington, North Carolina - United States) at the concentrations of 260/280 ratio nM.

For the synthesis of c-DNA reverse transcription reaction was performed from total RNA purified. A mixture of 1 g of RNA, 1 ml of enzyme DNase and 12 µl of ultrapure H_2O was homogenized and incubated at 65° C for 5 minutes and, subsequently, cooled on ice for 1 minute. Then, it was added 2 µl of physiological buffer (10X concentrate), 2 µl of 10 mM dNTP Mix, 1 ml of the enzyme reverse transcriptase (Vivantis) and 2 µl of ultrapure H_2O. The samples were incubated again at 42° C for 60 minutes.

For the analysis of IL-6, TNF-α and CCL-2, the ECO Real Time PCR was carried out (Polymerase Chain Reaction System, Uniscience, São Paulo - Brazil).

For amplification of inflammatory biomarkers gene, it was added in a solution of 0.4 μL of c-DNA 400 nm of each primer (IL-6, TNF-α and CCL-2) and 10 μL of EVAgreen master mix (Invitrogen - Sao Paulo, Brazil). The gene expressions of IL-6, TNF-α and CCL-2 were demonstrated in values of standard dissociation curve obtained through relative endogenous control gene β-actin. The sequence of the primers is shown in Table 1.

Table 1. Primers used for the analysis of inflammatory biomarkers.

Biomarker	primer sense sequence primer anti-sense sequence
IL-6	TCCAGTTGCCTTCTTGGGAC GTACTCCAGAAGACCAGAGG
TNF - α	AAGCCTGTAGCCCATGTTGT CAGATAGATGGGCTCATACC
CCL2	AGGAAGATCTCAGTG CAGAGG AGTCTTCGGAGTTTGCCTTTG
Beta-actin	ATGTTTGAGACCTTCAACAC CACGTCADACTTCATGATGG

Legend: IL-6 – Interleukin 6; TNF-α – Tumor necrosis factor alpha; CCL-2 – Chemokine (C-C motif.) ligand 2.

Statistical analysis

The Shapiro-Wilk test was used to test data normality. For comparisons, one-way analysis of variance (ANOVA) followed by post-hoc Tukey test was used. Pearson's correlation test was used to test associations between inflammatory biomarkers, triglycerides, HDL-c, BMI, % BF, waist circumference and physical activity status. We adopted confidence interval (CI) of 95% and significance level of $\alpha \leq 5\%$.

Results

Anthropometric measurements

The obese group had higher measures for body mass, BMI, waist circumference, TS, SS, Σ 2SF (TS + SS) and %BF as compared with eutrophic and overweight (Table 2). Likewise, the overweight group differed statistically from the eutrophic group in all anthropometric variables analyzed. No gender differences were found ($p > 0.05$) in all anthropometric measurements.

Physical activity status

The number of steps per day was not statistically different among eutrophic, overweight and obese children (Figure. 1). Likewise, no gender difference between groups was observed. The recommendation of 11000 and 13000 steps/day was not reached by 58.8% of girls and 83.6% of boys, respectively. Among these individuals, 42.2% were eutrophic, 32.8% overweight and 25% obese.

Table 2. Anthropometric measures and body composition.

	Eutrophic			Overweight			Obesity		
	Boys (n = 19)	Girls (n = 17)	Total (n=36)	Boys (n = 18)	Girls (n = 16)	Total (n=34)	Boys (n = 13)	Girls (n = 11)	Total (n=24)
Height (cm)	137 ± 10	137 ± 9	137 ± 9,5	137 ± 10	138 ± 8	137,5 ± 9	137 ± 9	137 ± 8	137 ± 8,5
BM (kg)	29,7 ± 2,0	29,3 ± 2,04	29,5 ± 2,03	39,1 ± 2,8	39,7 ± 3,4	39,4 ± 3,1*	53,9 ± 3,0	53,7 ± 3,6	53,8 ± 3,3*†
BMI (kg/m^2)	16,1 ± 1,7	15,8 ± 1,8	15,9 ± 1,7	20,2 ± 1,1	21,6 ± 0,9	20,9 ± 1,0*	26,4 ± 1,4	27,1 ± 2,0	26,9 ± 1,7*†
WC (cm)	58,1 ± 1,9	56,9 ± 1,1	57,7 ± 2,6	65 ± 1,5	63± 1,9	64 ± 2,4*	79 ± 3,2	77 ± 2.6	78 ± 5,9*†
TS (mm)	10,9 ± 3,8	12,1 ± 4,0	11,08 ± 3,9	19,1 ± 4,0	20,1 ± 4,4	19,6 ± 4,2*	27 ± 3,9	27,6 ± 5,1	27,3 ± 4,5*†
SS (mm)	8 ± 3,3	8,4 ± 4,3	8,2 ± 3,8	16,2 ± 6	16,6 ± 6,4	16,4 ± 6,2*	29,6 ± 5,0	30,4 ± 6,4	30 ± 5,8*†
Σ 2SF (mm)	18,9 ± 7,0	20,5 ± 7,6	20 ± 7,3	35,3 ± 7,1	36,7 ± 13,1	36,04± 10,1*	56,6 ± 8,2	60 ± 9,8	57,3 ± 9,0*†
BF (%)	20,9 ± 7,5	21,1 ± 7,9	21 ± 7,7	32,1 ± 4,0	32,9 ± 5,4	32,5 ± 4,7*	41,9 ± 3,4	43,7 ± 4,4	42,8 ± 3,9*†

Data are expressed as mean ± Standard deviation (SD). Legend: BM = body mass; BMI = Body mass index; WC = Waist circumference; TS = Triceps skinfold; SS = Subscapular skinfold; Σ2SF= Sum of two skinfold; BF = Body fat; Test: Analysis of variance (ANOVA) plus Tukey.
* Significantly different from eutrophic individuals ($p < .05$);
† Significantly different from overweight individuals ($p < .05$).

Lipid profile

The concentration of HDL-c was higher in eutrophic children as compared with overweight and obese (Figure. 2A). However, no differences were found ($p < 0.05$) in HDL-c between overweight and obese children. Obese children had higher ($p > 0.05$) triglycerides concentrations as compared with eutrophic and overweight (Figure. 2B).

Gene expression of inflammatory biomarkers

The genic expressions of IL-6 (Figure. 3A), TNF-α (Figure. 3B) and CCL-2 (Figure. 3C) were higher in obese children as compared with eutrophic group. However, no statistical difference in the mRNA gene expressions of these biomarkers was found between overweight and obese group.

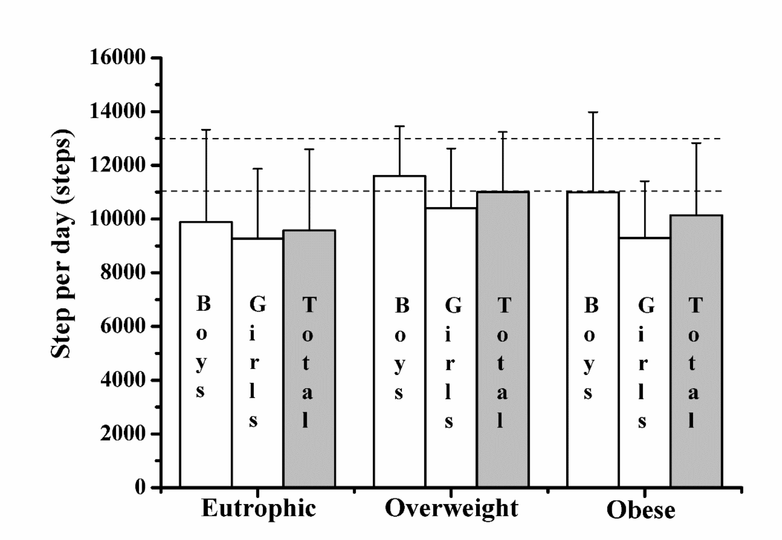

Figure 1. Number of steps per day. Data expressed as mean ± standard deviation. Eutrophic (n=19 boys and 17 girls). Overweight (n=18 boys and 16 girls). Obese (13 boys and 11 girls). *, significantly different from eutrophic individuals (p <.05)

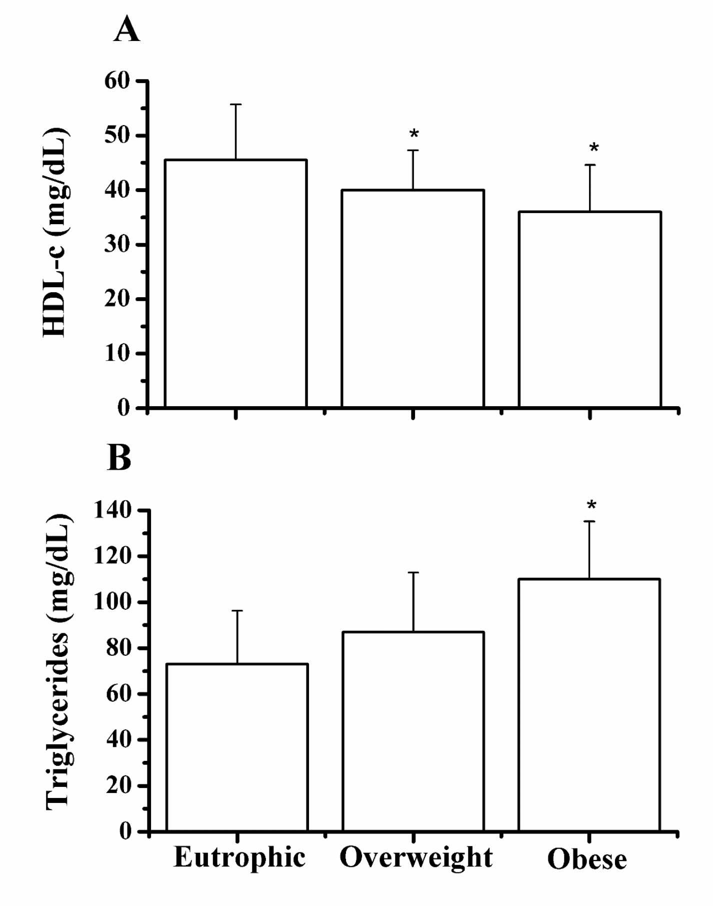

Figure 2. Serum lipid profile. (A) High-density lipoprotein cholesterol (HDL-c). (B) Triglycerides. Data expressed as mean ± standard deviation. Eutrophic (n = 36). Overweight (n = 34). Obese (n = 24).*, significantly different from eutrophic individuals ($p < 0.05$).

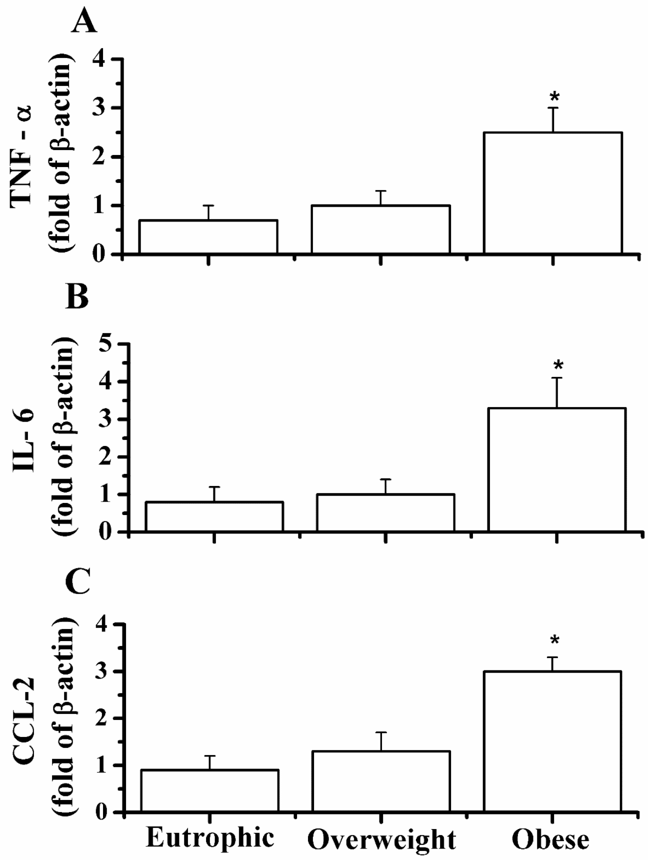

Figure 3. Genic expression of inflammatory biomarkers. (A) Tumor necrosis factor alpha (TNF-α). (B) Interleukin 6 (IL-6). (C) Chemokine C-C motif ligand 2 (CCL-2). Data are expressed as mean ± standard deviation. Eutrophic (n = 36). Overweight (n = 34). Obese (n = 24). *, significantly different from eutrophic individuals (p <.05).

Associations analysis

Table 3 shows the correlations analysis. IL-6, TNF-α and CCL-2 presented strong, positive and statistically significant associations with BMI and waist circumference. In addition, IL-6, TNF-α and CCL-2 showed moderate, positive and statistically significant associations with triglycerides and %BF; but moderate, negative and statistically significant association with HDL-c. Moreover, there was a weak, but not statistically significant association between inflammatory biomarkers and the number of steps per day. However, there was no gender difference in these correlation analysis.

Table 3. Correlation between inflammatory biomarkers, triglycerides and HDL-c, anthropometric measures, body composition and physical activity status.

	IL- 6			TNF- α			CCL- 2		
	Boys (n = 19)	Girls (n = 17)	Total (n=36)	Boys (n = 18)	Girls (n = 16)	Total (n=34)	Boys (n = 13)	Girls (n = 11)	Total (n=24)
IL-6	0.894*	0.913*	1*	0.304*	0.473*	0.929*	0.378*	0.478*	0.884*
TNF- α	0.304*	0.473*	0.929*	0.918*	0.944*	1*	0.421*	0.437*	0.908*
Triglycerides	0.394*	0.397 *	0.377 *	0.368 *	0.392 *	0.407 *	0.321 *	0.363 *	0.398 *
HDL-c	- 0.306*	- 0.394 *	- 0.324 *	- 0.412 *	- 0.394 *	- 0.348 *	- 0.385 *	- 0.348 *	- 0.359 *
Body Mass Index	0.626 *	0.785 *	0.718 *	0.669 *	0.809 *	0.735 *	0.677 *	0.841 *	0.784 *
Waist circumference	0.876*	0.815*	0.833*	0.894*	0.795*	0.851*	0.860*	0.776*	0.813*
Percentage of body fat	0.586 *	0.720 *	0.620 *	0.648 *	0.743 *	0.593 *	0.655 *	0.767 *	0.592 *
Steps per day	0.045	- 0.029	0.046	0.191	0.038	0.046	0.128	- 0.036	0.052

IL-6 = Interleukin 6; TNF-α = Tumor necrosis factor alpha; HDL-c = High-density lipoprotein cholesterol; CCL2= chemokine C-C motif ligand 2 ; * = statistically significant (p < 0.05).

Discussion

In the present study, we tested the association of inflammatory biomarkers with dyslipidemia, obesity and physical activity status in 10 years-old children. Our data showed that the genic expressions of IL-6, TNF-α, CCL-2 and serum triglycerides concentrations were higher in obese than in eutrophic and overweight children while serum HDL-c was higher in eutrophic children. In addition, it was observed that these pro-inflammatory biomarkers were positively associated with triglycerides, %BF, BMI and waist circumference; but negatively associated with HDL-c. Nevertheless, no association of these biomarkers with physical activity status was found.

Positive association of dyslipidemia (i.e. LDL-C, VLDL-C and triglycerides) with IL-6 (Alvarez *et al.,* 2009; Bugge *et al.,* 2012a; Chirico *et al.,* 2013; Elshorbagy *et al.,* 2012; Garanty-Bogacka *et al.,* 2011; Kim *et al.,* 2010; Pyrzak *et al.,* 2009; Roth, Kratz, Ralston, & Reinehr, 2011) and TNF-α (Breslin *et al.,* 2012; Bugge *et al.,* 2012a; Codoner-Franch *et al.,* 2011; Dixon *et al.,* 2009; Jermendy *et al.,* 2010; Kim *et al.,* 2010; Pyrzak, Wisniewska, Popko, Demkow, & Kucharska, 2010; Roth *et al.,* 2011) in children have been demonstrated by others. However, either positive (Breslin *et al.,* 2012; Roth *et al.,* 2011) or negative (Kim *et al.,* 2010; Wasilewska, Tenderenda, Taranta-Janusz, Tobolczyk, & Stypulkowska, 2012) associations between CCL-2 and dyslipidemia has been reported.

High serum triglycerides and low HDL-c concentrations have been found in obese children which increased risk of cardiovascular disease in the adulthood (Duncan *et al*., 2012; Friedemann *et al*., 2012). According to Morrison (2003), serum cholesterol values are similar in boys and girls in the childhood, but in post-menarche girls the sex hormones, particularly estradiol, deeply influence the levels of HDL-c and LDL-c. cholesterol. Thus, it appears that sex hormones do not have great influences the relationship between inflammatory biomarkers and obesity in boys and girls before pubertal phase.

The associations of IL-6, TNF-α, CCL-2 with BMI, waist circumference and %BF observed in the present study support the global concern for the control of obesity, especially during childhood. An increase in pro-inflammatory biomarkers triggers the chronic low grade inflammation that is linked to cardiovascular disorders such as atherosclerosis (Dessi *et al.,* 2013; Gustafson, 2010; Poitou *et al.,* 2011), insulin resistance (Charles *et al.,* 2011; Elshorbagy *et al.,* 2012; Kim *et al.,* 2010) and diabetes (DeBoer, 2013; Donath & Shoelson, 2011; Sobti, Kler, Sharma, Talwar, & Singh, 2012). Therefore, obesity in the childhood can lead to chronic low-grade inflammation as well as increase of susceptibility to cardio metabolic diseases.

Concerning gender, it was observed that the genic expressions of pro-inflammatory biomarkers were not different between genders in 10-years old children. A longitudinal study by Bugge *et al.* (2012b) showed no significant differences between gender for IL-6, TNF-α and CCL-2 concentrations. However, for insulin resistance, the concentrations of IL-6 in 9-years old and 13-years old were associated with girls and not with boys.

Regarding obesity, positive associations between obesity, IL-6, TNF-α (Alvarez *et al.,* 2009; Tsaoussoglou *et al.,* 2010; Chirico *et al.,* 2013) and CCL-2 (Breslin *et al.,* 2012; Roth *et al.,* 2011) have been shown in 10-years old children. Nevertheless, no association of obesity with IL-6, TNF-α (Andersen *et al.,* 2010; Dixon, Meng, Goldberg, Schneiderman & Delamater*,* 2009) and CCL-2 (Elshorbagy *et al.,* 2012; Kim *et al.,* 2010) was found by others in this population.

Our data showed that the number of steps per day did not differ significantly between boys and girls and among eutrophic, overweight and obese children. The study by Rosa *et al.* (2011) found no association of the number of steps per day with BMI and %BF in children and adolescents 10 to18-years old. However, higher number of steps per day in boys than in girls was reported by others (Raustorp, Svenson, & Perlinger, 2007; Tudor-Locke, Ainsworth, & Popkin, 2001). It seems that boys generally have higher family support and encouragement to practice moderate to vigorous physical activities than girls (Goncalves, Hallal, Amorim, Araujo, & Menezes, 2007). Therefore, the family can be instrumental in influencing the physical activity status in the childhood. It is noteworthy that in the present study more than 50% of girls and 80% of boys did not reach the recommended 11000 and 13000 steps per day, respectively. In this sense, individuals studied here exhibited a low physical activity status.

Unexpectedly, our data showed that the number of steps day was not associated with the pro-inflammatory biomarkers. It has been reported that low physical activity status did not lead to decreased pro-inflammatory biomarkers, whereas moderate to vigorous physical activity status promoted reductions in pro-inflammatory and increases in anti-inflammatory biomarkers (Park, Schwarz, Willoughby & koh, 2015). Thus, the absence of association between number of steps per day with pro-inflammatory biomarkers in the present study is consistent with the low physical status of the studied individuals. It is worthy to note that children with high physical activity status as well as low concentrations of pro-inflammatory biomarkers presents low risks for cardiovascular diseases such as obesity (Sobieska, Gajewska, Kalmus & Samborski, 2013; Seabra *et al*., 2015).

The cut-off points for BMI classification were made according to Cole *et al*. (2000) and based on international studies. It is important to mention that BMI has been widely used in many studies with children in both national (Chiara, Sichieri, & Martins, 2003; Vieira, Alvarez, Marins, Sichieri, & Veiga, 2006; Vitolo, Campagnolo, Barros, Gama, & Lopez, 2007) and international investigations (Conde & Monteiro, 2006; Freedman *et al.,* 2004; Neovius, Linne, Barkeling, & Rossner, 2004; Zimmermann, Gubeli, Puntener, & Molinari, 2004). In addition, BMI shows strong associations with gold standard methods (Jimenez-Pavon, Kelly, & Reilly, 2010). According to WHO (1995), the use of BMI is appropriate inasmuch as it has good correlation with %BF and allows comparisons to other studies. However, the determination of specific cut-off points to classify the obesity degree is complex since there are physiological differences between populations and lack of validation for the adopted criteria (DaSilva, Lopes, & DaSilva, 2010).

Finally, our results have clinical relevance as the early detection of high concentrations of pro-inflammatory biomarkers and dyslipidemia in 10-years old children pointing out the need for the adoption of preventive strategies.

Study limitations: pedometers are limited devices to measure walking because they capture movement only of the lower body in the vertical plane and cannot distinguish the intensity of walking, load carriage as well as between walking on different surfaces or gradients. This cross-sectional study design does not allow to affirm that the reported associations are causal.

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