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Overweight and Obesity Contribute to Inflammation and Reduction in Mean Corpuscular Volume and Mean Corpuscular Hemoglobin in Schoolchildren

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Abstract: Background: The inflammation associated with overweight and obesity seems to alter iron metabolism, but there are few studies evaluating those conditions in children. Thus, we aimed to evaluate leukometric, immunological and hematimetric parameters of overweight and obese schoolchildren. **Methods:** This is a cross-sectional study, in which 39 children living in Chonim de Cima (Brazil), underwent anthropometric, hematological and immunological assessments. The evaluated parameters were compared between the study group (overweight/obesity, n=15) and the control group (n=24). Unpaired t-test, Mann-Whitney test and linear regression were used for statistical tests and the panoramic profile was used to illustrate differences between groups. **Results**: The study group had lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and higher TNF levels compared to the control group. Positive correlations were observed between BMI-for-age percentile and total leukocytes (r=0.1493; p=0.0151) or neutrophils (r=0.1395; p=0.0192). Negative correlations between the BMI-for-age percentile and MCV (r=0.1464; p=0.0162) and MCH (r=0.1460; p=0.0164) were found. Furthermore, through the panoramic profile it was noted that the study group had a higher frequency of individuals with high levels of TNF and lower frequencies of individuals with increased hemoglobin and serum iron. **Conclusions:** Our data suggests that overweight and obesity contribute to a pro-inflammatory context (leukocytes, neutrophils and TNF) and MCV and MCH reduction in schoolchildren.

Keywords: schoolchildren; overweight; obesity; inflammation; hematimetric parameters

1. Introduction

Obesity in children and adolescents is a global health issue with high prevalence in many high-income countries, and increasing prevalence in low- and middle-income countries (LMICs)[1]. The rate of childhood obesity has increased in parallel with adult obesity, resulting in pediatric patients presenting diseases traditionally associated with adulthood such as dyslipidemia, nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes[2]. Recent studies have evaluated the presence of subclinical inflammation in obesity through different laboratory parameters (leukocytes, lymphocytes, neutrophils, C-reactive protein) and immunological (interleukin-6 and tumor necrosis factor alpha)[3,4]. The inflammatory state present in obesity promotes the constant infiltration of macrophages in adipose tissue and changes in the production of interleukin 1 and 6 (IL-1, IL-6) and tumor necrosis factor (TNF)[5]. The increase in those cutokines release can also be caused by the hypoxic state induced by reduced blood supply to the multiplying adipocytes[6]. Schoolchildren, in addition to being more vulnerable to anemia, may have such risk increased when they are with

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obesity, as their inflammatory state modifies iron metabolism, which further increases the chance of anemia associated with chronic diseases[7,8]. Iron levels senns to be improved when the body weight is reduced, and consequently, inflammation is controled [9]**.** In this sense, some research groups have proposed that inflammation in obesity can modify the concentrations of hepcidin and ferroportin[10-12]. Other studies point to the relationship between obesity and anemia, which may exist either due to insufficient iron intake in the diet or inflammation of adipose cells [13-15]. However, some studies show controversial results about the relationship between anemia and obesity[16,17]. Considering the small number of studies that aim to investigate the hematological consequences in childhood obesity among schoolchildren living in Brazil, in this work we propose to evaluate the hematimetric and immunological profile of schoolchildren with obesity living in this country.

2. Methods

2.1. Study's Design and Ethical Aspects

We performed a cross-sectional study of 39 children aged between 7 and 13 years, regularly attending elementary school, in Chonim de Cima, Governador Valadares - Brazil, between June 2022 and March 2023. All children, so as their parents/guardians, selected for the study were invited to sign the Free and Informed Consent Form (FICF), in order to meet the ethical aspects of the research (CAAE: 22348619.2.0000.5157). The exclusion criteria for the present study were: Being under 7 years old, schoolchildren and/or parents who did not sign FICF, or having been absent from any of the research stages (anthropometric assessment, peripheral blood collection). When considering possible hypotheses and the criteria described previously, our study sample was divided according to the nutritional diagnosis established by the Guidelines for Collection and Analysis of Anthropometric Data in Health Services [18] . Thus, participants were organized into two groups according to their percentile values for Body Mass Index percentile by age (BMI-for-age percentile). The study group consisted of 15 children with overweight (BMI-for-age percentile ≥ 85 and < 95) and obesity (BMI-for-age percentile ≥ 95) while the control group consisted of 24 eutrophic schoolchildren (BMI-for-age percentile < 85) [18,19].

2.2. Anthropometric Assessment

Children's body weight and height measurements were recorded to calculate anthropometric indicators. Body weight was measured using a digital platform scale, with a precision of 100 g, and height was obtained using a portable anthropometer (millimetric scale). During this assessment, all children were dressed in school uniforms and without shoes. Age, height, and weight records were used to calculate BMI and BMI-for-age percentile through the Growth Charts application (http://www.slideshare.net/osamaorabi/growth-chart-app). The nutritional diagnosis was performed based on the values obtained in the anthropometric assessment and as established by the Guidelines for Collection and Analysis of Anthropometric Data in the Brazilian Ministry of Health[18].

2.3. Hematimetric and Leukocyte Evaluation

Venous blood samples were collected in test tubes, containing EDTA anticoagulant, for hematological tests. It was evaluated serum iron, ferritin, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), absolute leukocyte counting and their subpopulations. Those measures were performed using a hematology analyzer (ABX Pentra XL 80, Horiba, London Ontario, Canada).

2.4. Cytokine Measurement

Blood samples were collected, serum was separated and used to determine TNF, IFN-γ and IL-10 concentrations using ELISA assay kits (R&D Systems, MN, USA). Measurements were made using

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a Multiskan™ FC Microplate Photometer (Thermo Scientific™, USA) always following the manufacturer's recommendations.

2.5. Statistical Analysis

The data were analyzed using GraphPad Prism 8 software (La Jolla, CA, USA). Analyses were made using unpaired T test for parametric data, Mann–Whitney for non-parametric data and linear regression to evaluate possible relationships between variables. The panoramic profile of the groups evaluated was constructed based on the characterization of individuals with lower levels (LL) and higher levels (HL) of cytokines (TNF, IFN-γ and IL10), serum iron and hemoglobin. Individuals whose concentration of the evaluated parameters is less than or equal to the global median were classified as LL, while those with a greater concentration were classified as HL. The entire universe of data obtained for the groups was considered to calculate the global median, thus establishing a cutoff point (TNF = 2.1 pg/mL, IFN-γ = 6.1 pg/mL, IL-10 = 15.9 pg/mL, Fe = 92.0 mcg/dL, Hb = 13.0 g/dL). For comparison purposes between groups, the frequencies (%) of individuals with higher levels (HL) for each parameter will be used.

3. Results

Participants were classified according to nutritional assessment, with the study group consisting of 15 children with overweight (BMI-for-age percentile ≥85 and <95) or obesity (BMI-for-age percentile ≥95) while the control group consisted of 24 eutrophic schoolchildren (BMI-for-age percentile < 85). Comparisons were made between the anthropometric, leukometric, immunological and hematimetric variables presented between the groups, and the details of these analyses are represented in **Table 1**. From the analyses performed to compare the parameters identified in each group, it was observed that individuals in the study group presented lower values of Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) (**Table 1 and Figure 1**). Furthermore, plasma cytokine analysis showed that the study group presented a higher TNF level than the control group **(Figure 2).**

Table 1. - Anthropometric and hematologic parameters presented by the study and control groups.

Parameters	Study Group (n =15) Mean ± SD (Median)	Control Group (n = 24) Mean ± SD (Median)	p value
Age (years)	10.13 ± 2.59 (10.00)	9.25 ± 2.42 (9.00)	0.2869
Weight (kg)	47.24 ± 18.40 (41.30)	30.54 ± 10.41(27.80)	*0.0009
Height (cm)	140.60 ± 13.48 (136.00)	133.40 ± 12.45 (132.80)	0.1084
BMI/age percentile	94.90 ± 4.10 (95.16)	45.77 ± 26.94 (48.84)	*<0.0001
Iron (mcg/dL)	78.80 ± 24.73 (72.00)	89.42 ± 20.52 (98.00)	0.1548
Ferritin (ng/mL)	51.55 ± 27.46 (49.00)	55.74 ± 30.38 (50.60)	0.6670
Hemoglobine (g/dL)	12.77 ± 1.03 (12.80)	13.01 ± 0.91 (13.10)	0.4540
Hematocrit (%)	38.97 ± 2.82 (12.80)	39.4 ± 2.67 (39.55)	0.6354
Mean Corpuscular Volume (fL)	78.97±2.87 (78.80)	81.90 ± 3.82 (81.20)	*0.0099
Mean Corpuscular Hemoglobin (Pg)	25.88 ± 1.34 (25.70)	27.05 ± 1.32 (27.10)	*0.0125
Mean Corpuscular Hemoglobin Concentration (%)	32.78 ± 0.66 (32.70)	33.29 ± 1.30 (33.30)	0.1671
Red Cell Distribution Width (%)	12.69 ± 1.34 (12.60)	12.61 ± 1.70 (11.85)	0.8766
Total leukocytes (cells/mm^3)	6513.00 ± 1599.00 (6600.00)	5642.00 ± 1130.00 (5650.00)	0.0784
Eosinophils (cells/mm^3)	445.20 ± 281.30 (405.00)	333.80 ± 231.00 (313.80)	0.1862
Neutrophils (cells/mm^3)	3064.00 ± 1341.00 (3004.00)	2552.00 ± 726.80 (2371.00)	0.1896
Lymphocytes (cells/mm^3)	2662.00 ± 678.90 (2406.00)	2428.00 ± 629.20 (2423.00)	0.2905
Monocytes (cells/mm^3)	338.20 ± 114.10 (308.00)	304.30 ± 104.30 (283.10)	0.3469
TNF (Pg/mL)	8.13 ± 10.82 (4.44)	2.47 ± 1.73 (1.95)	**0.0429
IFN – Y (Pg/mL)	15.63 ± 33.01 (5.29)	11.39 ± 23.23 (6.62)	0.3950
IL-10 (Pg/mL)	14.75 ± 12.98 (12.46)	20.16 ± 13.22 (19.48)	0.1402

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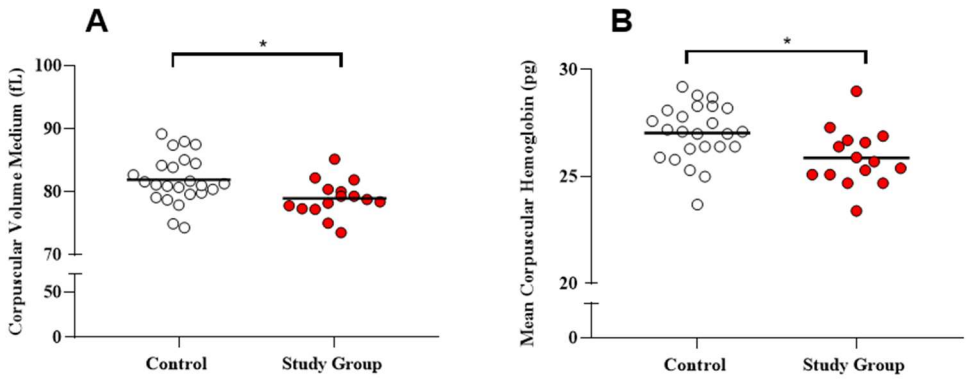

Figure 1. The study group presents differences in hematimetric indices compared to the control group. The study group (overweight and obese) has lower Mean Corpuscular Volume (A) and lower Mean Corpuscular Hemoglobin (B) compared to the control group. The differences between the groups (A and B) were considered statistically significant if $p < 0.05$ (Unpaired t test).

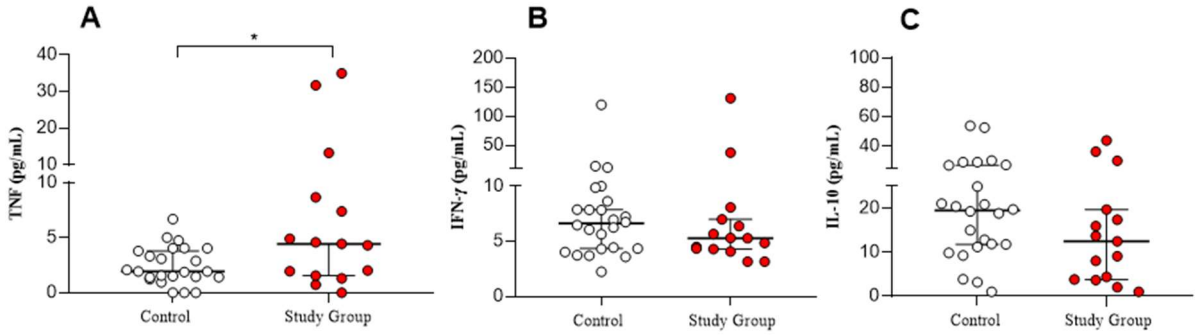

Figure 2. Comparison of serum levels of TNF, IFN-γ and IL-10 between the control group and the study group (overweight and obese). Serum TNF levels are higher in schoolchildren in the study group (overweight and obese) compared to the control group (A). There were no statistically significant differences between serum levels of IFN-γ and IL-10 between the groups evaluated (B and C).The Mann-Whitney test was used for the statistical evaluation. *Values of $p<0,05$ were considered significant.

To understand the relationship between the BMI-for-age percentile and hematimetric and leukometric parameters, the values of each variable were subjected to linear regression analysis (**Table 2**). The analyses showed a positive correlation between the BMI-for-age percentile and the number of total leukocytes (**Figure 3A**) and neutrophils (**Figure 3B**). Interestingly, the analyses also showed the existence of negative correlation between the BMI-for-age percentile and VCM (**Figure 4A**) and HCM (**Figure 4B)**. The panoramic profile allowed us to observe that the study group presents a higher frequency of individuals with HL for TNF and a lower frequency of individuals with HL for serum iron, IL-10, IFN-γ and hemoglobin compared to the profile presented by the control group (**Figure 5)**.

Table 2. – Correlations of hematimetric or leukometric parameters with BMI-for-age.

Parameters	Percentile		
	r coefficient	95% Confidence Interval (CI)	p-value
Mean Corpuscular Volume (fL)	0.1464	-0.0803 to 0.0087	**0.0162**
Mean Corpuscular Hemoglobin (Pg)	0.1460	-0.0307 to – 0.0033	**0.0164**
Mean Corpuscular Hemoglobin Concentration (%)	0.0006	-0.0124 to 0.0107	0.8839
Red Cell Distribution Width (%)	0.0107	-0.0209 to 0.0109	0.5300

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Total leukocytes (cells/mm^3)	0.1493	3.398 to 29.76	**0.0151**
Eosinophils (cells/mm^3)	0.0023	-2.250 to 3.010	0.7714
Neutrophils (cells/mm^3)	0.1395	2.054 to 21.73	**0.0192**
Lymphocytes (cells/mm^{33})	0.0395	-2.580 to 10.63	0.2246
Monocytes (cells/mm^{33})	0.0558	-0.2935 to 1.882	0.1475

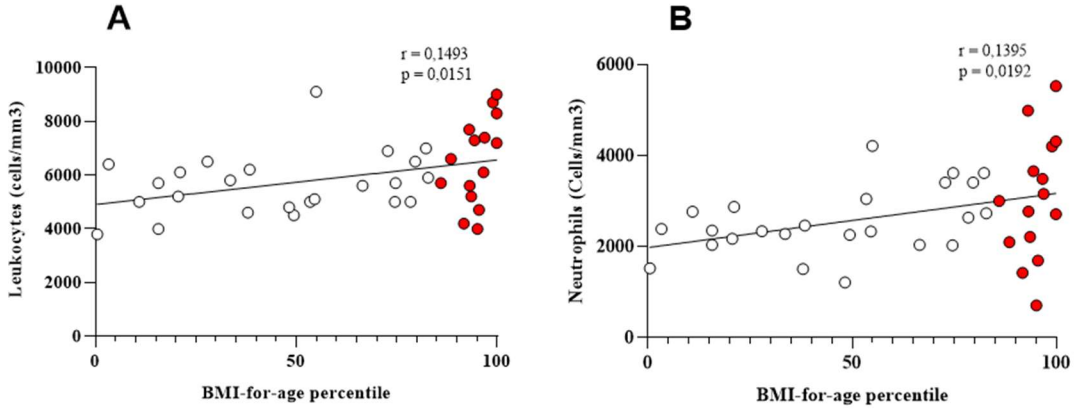

Figure 3. Positive correlation between BMI-for-age percentile and leukocytes in schoolchildren. A) Positive correlation between BMI-for-age percentile and total leukocytes in schoolchildren. B) Positive correlation between BMI-for-age percentile and neutrophils in schoolchildren. White circles represent individuals in the control group and red circles represent individuals in the study group (overweight and obese). P values < 0.05 indicate a statistically significant correlation (linear regression).

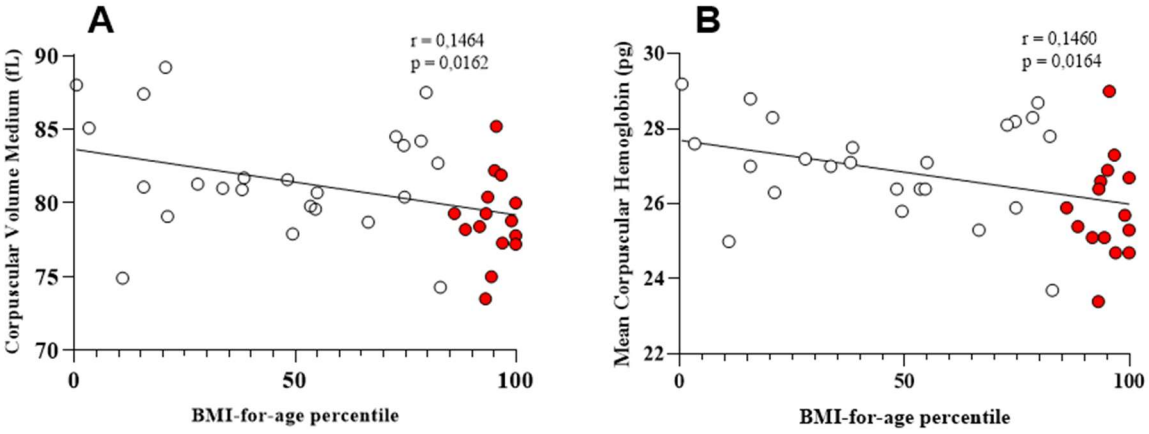

Figure 4. Negative correlation between BMI-for-age and hematimetric parameters. Negative correlation between BMI-for-age percentile and Mean Corpuscular Volume (A) and Mean Corpuscular Hemoglobin (B) in schoolchildren. The correlations (A and B) were statistically significant if p< 0.05 (linear regression).

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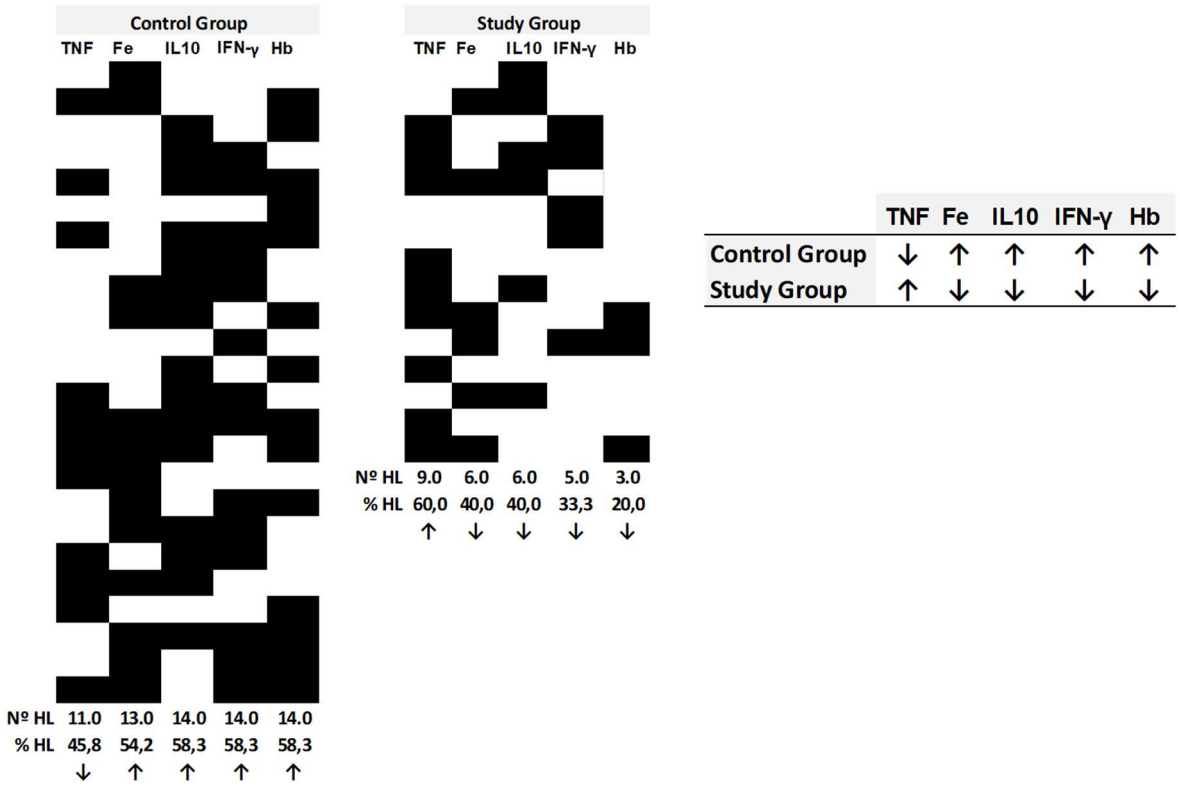

Figure 5. Panoramic profile of the control group and study group. A) Matrix for individuals with low levels - LL (white boxes) and high levels - HL (black boxes) of the evaluated parameters (TNF, Fe, IL-10, IFN-γ e Hb). Below each matrix (Control group and study group) the number of HL (Nº HL) and frequency of HL (% HL) individuals are observed (black boxes). B) On the side you can see a table of arrows for comparing the profiles presented by the groups, where the upward arrow (↑) indicates that the frequency of HL was greater than 50% and the downward arrow (↓) indicates that the frequency of HL is less than 50%.

4. Discussion

Childhood obesity is a complex disease considered a silent pandemic in the 21st century since the prevalence of childhood excess body weight is increasing year by year and has a global geographical distribution[20]. This disease is characterized by a mild, chronic, systemic inflammation due to excessive fat accumulation in the adipose tissue, with adipocyte stress [21,22]. Obesity alters the production of hormones (adipokines) and inflammatory mediators (cytokines, chemokines and lipid mediators) by adipocytes, which influences local and systemic immune responses[23]. Our findings reinforce the inflammatory nature of overweight and obesity, also among schoolchildren, since it was observed the a positive correlation between BMI-for-age percentile and the number of white blood cells (WBC) and neutrophils (**Figure 3**), in addition to the higher levels of TNF on plasma (**Figure 2**). The positive correlations identified are probably because neutrophils are the first cells of the immune system to reach adipose tissue, which implies the increasing circulating levels of such cell[24]. This fact is highly relevant to childhood obesity since other studies show that the number of neutrophils may be directly proportional to the severity of obesity and may be associated with carotid atherosclerosis, impaired glucose tolerance, and microvascular and macrovascular complications of type II diabetes[24-29].

In addition to neutrophils, macrophages may play an important role in obesity, through changes in the local production of pro-inflammatory cytokines, such as interleukin 1 and 6 (IL-1, IL-6) and TNFα[5]. Although our results did not show statistically significant results regarding the number of monocytes **(Table 1)**, it was observed that the group of overweight and obese schoolchildren showed greater production of TNF **(Figure 2).** TNF, along with IL-6 and IL-1 are increased in obese

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individuals with metabolic problems accompanied by a decrease in adiponectin[30]. Due to the behavior of TNF, this cytokine came to be known as na adipokine, which led to the appreciation of the inflammatory nature of obesity and associated metabolic diseases[31]. Other studies have shown the importance of TNF in obesity, and have highlighted the possible relationships of this cytokine with other diseases, like the positive association with the risk of type 2 diabetes in humans[32,33] and with the hepatic inflammation presente in hepatocellular carcinoma[34,35]**.**

IFN-γ has been associated with low-grade inflammation in obesity and may be related to both, general obesity (determined by BMI) and central obesity (determined by waist-to-hip ratio)[36]**.** IL-10 may have a regulatory role in childhood obesity[37]**.** Although our results showed no differences in IFN-γ and IL-10 production between the evaluated groups (**Figure 2**), the panoramic profile revealed that the study group showed lower frequency of individuals with HL of IL-10 and IFN-γ (**Figure 5**). It is not clear why the inflammatory profile of the children evaluated is more related to high TNF, and not IFN-γ, but this aspect, so as the involvment of other inflammatory mediators should be better investigated.

The relationship between obesity and changes in iron metabolism has been widely discussed[13,38-39]. In this aspect, our results showed that overweight and obesity are associated with changes in hematimetric parameters, with lower MCV and HCM **(Figure 1)** and a negative correlation between the BMI-for-age percentile and MCV and HCM (**Figure 4**). Previous studies have demonstrated lower values of MCV, adiponectin and transferrin saturation in people with obesity, but they did not find significant differences in serum hemoglobin, ferritin and iron[40]. Furthermore, **Bertinato et al. 2014** [41] found that diet-induced obese rats have lower mean concentrations of HCM and iron in the liver.

Changes related to iron metabolism in obesity can be explained by dietary iron deficiency, the greater need for iron due to a greater blood volume, the reduced number of myoglobins in muscle associated with the absence of physical activity, and especially the greater production of hepcidin[42]. In obesity, hepcidin seem to alter the function of ferroportin in the small intestine, which leads to a reduction in the release of iron into the blood[43]. Furthermore, higher levels of this hormone associated with inflammatory status may increase the risk of developing anemia[44].

Through the analysis of the panoramic profile **(Figure 5)**, a high frequency of individuals with high levels of TNF (60%) and low frequencies of individuals with high levels of iron (40%) and especially Hb (20%) were noted among schoolchildren overweight and children with obesity. Several studies have sought to understand the relationship between chronic inflammatory diseases, including obesity, and changes in iron metabolismo[45-47]. Such studies highlight that anemia of inflammation or anemia of chronic disease have become the second most common type of anemia after iron deficiency anemia.

Anemia of inflammation has been associated with chronic infections and autoimmune diseases, diseases in which inflammation is easily detectable and sustained[48,49]. In this aspect, TNF seems to act directly in the duodenum, contributing to the reduction of hepcidin-independent duodenal iron absorption[50]. Our results point to an inverse relationship between the production of TNF and the levels of iron and hemoglobin **(Figure 5)**. This can be explained by the acute or chronic immune activation in patients with inflammatory diseases associated with greater production of TNF[51-53]. Thereby, Lucendo et al. 2020 [54] presented the first evidence that TNF inhibition can be considered an effective treatment for anemia in patients with inflammatory bowel disease. The literature also reveals the benefits of anti-TNF therapy for improving hemoglobin levels in patients with rheumatoid arthritis[55,56], psoriatic arthritis, ankylosing spondylitis[57]and among children with malaria[58].

In summary, overweight and obesity require special attention among schoolchildren due to the possible consequences generated by the low-grade inflammation, specially related to high TNF levels, present in an early stage of life. Furthermore, we reinforce that schoolchildren must be frequently monitored in regard to hematimetric parameters and nutritional health, once our data show the association of BMI with the decrease of MCV and MCH. Schoolchildren's follow-up will contribute to prevent medium and long-term complications, such as anemia. New studies with a

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larger number of participants are necessary to consolidate the evidence about the impacts of overweight and obesity on schoolchildren's health.

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Conflicts of Interest: The authors declare no conflict of interest.

Impact Statement: This research evaluates how overweight and obesity affect the immune response and hematologic parameters in Brazilian children. The results show that a high BMI reduces the amount of hemoglobin and the volume of red blood cells. The study highlights the need to further investigate the effects of chronic inflammation on children's health.

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