Physical exercise intervention at school improved hepcidin, inflammation, and iron metabolism in overweight and obese children and adolescents

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BACKGROUND: Obesity is often associated with iron deficiency in children and adolescents. We aimed to study the effect of an 8-month physical exercise (PE) intervention on hepcidin and other markers of inflammation and on iron status in overweight/obese children and adolescents.

METHODS: Seventy-three overweight/obese children and adolescents participated in the 8-month-long longitudinal study. They were divided into two groups according to their participation in an after-school PE program: the PE group ($n = 44$) and the control group ($n = 29$). Hepcidin, interleukin (IL)-6, C-reactive protein (CRP), iron, ferritin, transferrin, and soluble transferrin receptor (sTfR) were evaluated.

RESULTS: At baseline, IL-6 correlated positively with hepcidin and negatively with iron and transferrin saturation, suggesting that increasing adiposity associates with increasing IL-6 and hepcidin synthesis, reducing iron availability. After 8 months, the PE group showed a decrease in BMI *z*-score ($P = 0.003$), body fat mass ($P = 0.012$), CRP ($P = 0.002$), IL-6 ($P = 0.048$), ferritin ($P = 0.013$), hepcidin ($P = 0.040$), and sTfR ($P = 0.010$), and an increase in iron concentration ($P = 0.002$). Moreover, the PE group, when compared with the control group, showed lower weight ($P = 0.026$), BMI ($P = 0.040$), waist circumference ($P = 0.010$), and waist-to-height ratio ($P = 0.046$).

CONCLUSION: We showed that an 8-month-long intervention at school allowed a reduction in BMI *z*-score and an improvement in inflammation, reducing hepcidin levels and the disturbances in iron status.

Obesity is a critical public health problem and its prevalence has increased significantly over the past three decades. Iron deficiency is one of the most predominant worldwide nutritional problems, being prevalent in both developing and industrialized countries (1). Several studies have confirmed an association between obesity and hypoferremia in adult men, women, and post-menopausal women, as well as in children and adolescents (2–8). Obesity is considered a potential independent risk factor for developing iron deficiency (3,4,7,9). Different mechanisms have been proposed to explain the association between obesity and iron deficiency (10), and the most widely accepted is that hypoferremia is a consequence of the chronic low-grade inflammation state that characterizes obesity (11). Inflammation enhances hepcidin production, the major regulator of iron availability for erythropoiesis. Hepcidin is predominantly expressed in the liver, but it can be also expressed in other tissues, such as the heart, kidney, adipose tissue (also an adipokine), pancreas, and hematopoietic cells (12). In inflammatory conditions, its concentration increases, triggering endocytosis and proteolysis of ferroportin. Thus, the efflux of ferrous iron from major iron-transporting tissues—namely, duodenal enterocytes, iron-recycling macrophages, and iron-storing hepatocytes—into plasma is reduced, and the iron accumulates in their cytoplasm as ferritin (13). The regulation of hepcidin by inflammation occurs in response to pro-inflammatory cytokines, such as interleukin (IL)-6 (**Figure 1**) (14–16).

In inflammatory processes, soluble transferrin receptor (sTfR) is considered a more reliable and sensitive marker of iron status compared with ferritin, which is also an acute-phase protein (17). Thus, the association of sTfR and hepcidin in the study of iron metabolism probably provides a more valuable information when assessing iron status under inflammatory conditions.

In children with high BMI *z*-score, the supplementation with iron has been proven to be of little efficacy (18), suggesting that iron supplementation is not the best way to improve iron status in these patients. Physical activity and aerobic exercise, and/or adequate changes in nutritional behavior, are considered reliable treatment options for obesity. Indeed, aerobic exercise appears to neutralize the inflammatory state, contributing to

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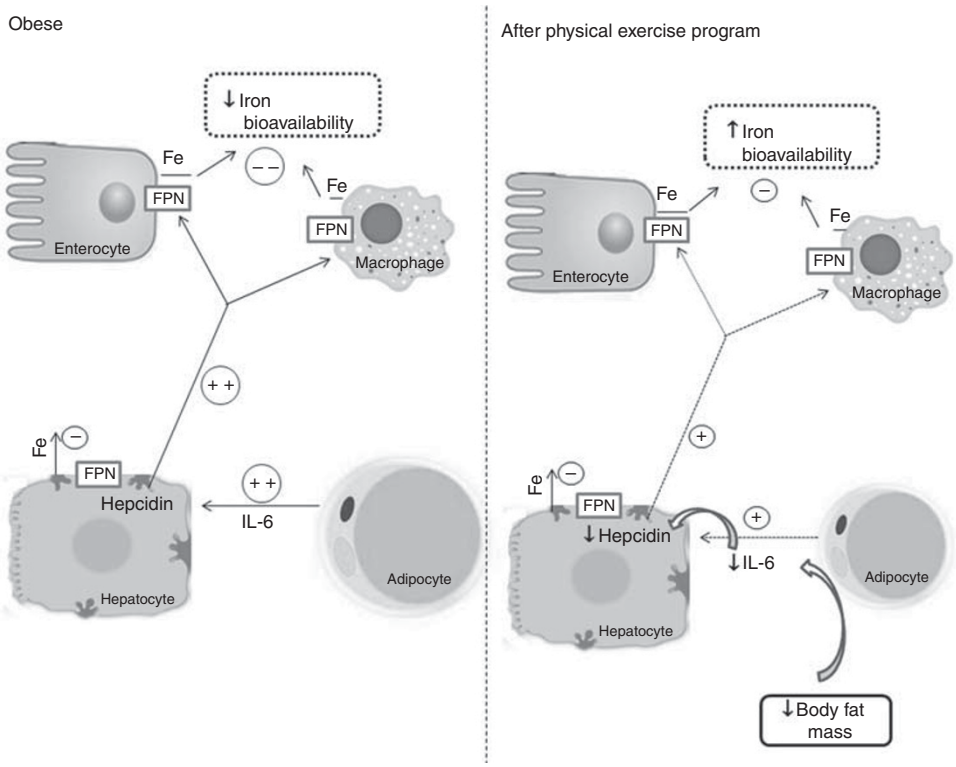

Figure 1. Schematic diagram representative of the interplay between inflammation, hepcidin, and iron bioavailability in obesity and after a physical exercise program. In obese and overweight children and adolescents, the hepatic production of hepcidin is enhanced by inflammation, namely by interleukin (IL)-6, produced by adipocytes; hepcidin induces ferroportin (FPN) degradation at the membrane surface of enterocytes, hepatocytes, and macrophages, inhibiting iron (Fe) absorption and mobilization from iron stores, thus reducing iron bioavailability for erythropoiesis; after an 8-month-long exercise program, a reduction in body fat mass was associated with a decrease in IL-6, leading to a reduction in hepcidin that favors iron absorption and mobilization, increasing iron availability.

improving insulin sensitivity and lipid profile (19). A 6-month-long intervention program including 15 obese children (without a control group) that combined a nutritionally balanced diet with lifestyle modifications induced a BMI decrease that was associated with hepcidin reduction, and, although no alteration was found for iron status markers, iron absorption was improved (20). In agreement, a 1-year-long weight-loss program performed in overweight and obese children reported an improvement in markers of inflammation and iron status; in this study hepcidin levels were not evaluated (21). To better understand the effects of continuous physical exercise (PE) interventions in the relationship between hepcidin, inflammation, and iron availability in obese and overweight children, larger studies evaluating hepcidin and other markers of inflammation, and iron metabolism, are needed.

The aim of this study was to clarify how obesity-associated inflammation can disturb iron metabolism in overweight and obese children and adolescents, and study the effect of an 8-month-long PE intervention program at school on their levels of hepcidin and other markers of inflammation and on iron metabolism.

METHODS

Subjects

This study is included in a larger school-based project that aimed to evaluate the impact of lifestyle intervention on obesity-related comorbidities in children and adolescents (22). Overweight and obese children and adolescents participated in the study, after obtaining informed and written consent from their parents. Two recruitment strategies were used. A part of the population was identified from medical records from two outpatient clinics of pediatric obesity in Oporto and invited to participate during the medical appointments (with the presence of children/adolescents and their parents). A second group of children and adolescents were recruited from public schools from an Oporto suburban setting. In this case, the project was presented to parents and opened to all students meeting the inclusion criteria, explaining that it was directed more towards overweight and obese children and adolescents.

The inclusion criteria were as follows: age 5-17 years, being overweight or obese (BMI > 1 SD or BMI > 2 SDs above age-specific and sex- specific World Health Organization (WHO) reference median values, respectively) (23), having sedentary behavior (absence of regular physical activity—less than 2 h per week), and absence of any medical condition that could affect the study results or limit physical activity. Children and adolescents who were participating in any other PE, nutrition, and/or weight-loss programs were excluded, as well as smokers, subjects with diabetes mellitus, endocrine

disorders, or inflammatory or infectious diseases, and subjects under medication that could interfere with our results.

All eligible participants were invited to participate in a school-based PE promotion program carried out in 5 primary and 2 middle and high public schools from an Oporto suburban setting. The intervention study consisted of an 8-month-long PE program (corresponding to a school-year period) that took place in the aforementioned schools. Only those participants who had a compliance of at least 75% in the PE program, and had undergone blood analytical studies at the beginning and at the end of the study were included in the final analysis.

Forty-four of the seventy-three individuals enrolled in the study agreed to participate in the PE program. Twenty-nine subjects, who accepted to participate in the study, but did not want to participate in the PE program, were included in the control (C) group.

The study protocol was approved by the Committee on Ethics of both Pediatric Clinic and the Review Committee of the Scientific Board of the Faculty of Sport of the University of Porto. The Regional Education Board approved the study protocol, and students, parents, and schools agreed to participate. The nature, benefits, and risks of the study were explained to the volunteers.

Anthropometric Characterization and Clinical Evaluation

Height and weight were measured with participants wearing shorts and t-shirts. Height was measured using a fixed stadiometer (Holtain, Crymych, UK). Weight was measured with the scale Tanita MC 180 MA MA (Tanita, Amsterdam, The Netherlands). BMI was also calculated (kg/m^2). *z*-score values for BMI were obtained with WHO AnthroPlus software, which is available in the WHO website (http://www.who.int/growthref/tools/en/).

Waist circumference was measured with a metallic tape (Holtain) at the superior border of the iliac crest, according to the protocol of the National Health and Nutrition Examination Survey (NHANES), and the ratio between waist circumference and height was calculated.

Body composition was evaluated using dual-energy X-ray absorptiometry (Hologic QDR 4500 A, Hologic, Waltham, MA). This unit was calibrated according to manufacturer's instructions, and a trained technician conducted all examinations. The percentage of body fat mass was measured.

Biological maturation was clinically assessed on the basis of Tanner stages (24).

Physical Activity Intervention Program

Participants were integrated into the "Acorda Project", a school-based intervention program focused on young people with overweight and obesity that aimed to change behaviors by providing easy access to PE associated with food counseling and clinical supervision. The participants were involved in the PE program over a period of 8 months, from September to May. Besides regular classes of PE at school 3 times a week, participants were enrolled in an extra-activity PE program twice a week, resulting in a total of 5 h per week (measured under school/project supervisors) of moderate to vigorous PE, matching the international recommendations (25–27). The PE proposed by the program aimed to increase moderate to vigorous PE intensities. All activities were performed in the schools' indoor sports facilities and were taught by physical education teachers after regular school classes. Exercises and games were progressively intensified as individually tolerated. Training intensity and compliance between individuals was defined to induce heart rate higher than 80% of each child's maximum heart rate. To ensure this, 10 randomly selected participants used a portable heart rate monitor (Polar Team2 Pro, Polar, Finland) and an accelerometer (MTI, model GTX3, as described below) during sessions. The average number of sessions attended (adherence) was >85% for the PE group.

Blood Samples

Blood was collected for laboratory analysis after an overnight fast and after clinical examination at the research centers, before and after the 8-month period. Blood was obtained by venepuncture, collected into EDTA-containing tubes, and processed within 2 h of collection. Aliquots of plasma were prepared and immediately stored at −80°C until assayed.

Assays

Plasma levels of hepcidin, sTfR, and IL-6 were evaluated by enzyme immunoassays (Hepcidin-25, Bachem Group, Peninsula Laboratories, San Carlos, CA; human sTfR immunoassay, R&D Systems, Minneapolis, MN; and Human IL-6 BMS213HS, Bender MedSystems, Vienna, Austria). Iron concentration was determined following a colorimetric method (Iron, Randox Laboratories, North Ireland, UK), whereas ferritin, transferrin, and C-reactive protein (CRP) were measured with immunoturbidimetry (Ferritin, Randox Laboratories; Transferrin, Randox Laboratories ; and CRP (latex) High-Sensitivity, Roche Diagnostics, Basel, Switzerland). Transferrin saturation (TS) was calculated according to the formula TS (%) = 70.9 × serum iron levels (μg/dl)/transferrin (mg/ dl). Red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were evaluated by using an automatic blood cell counter (Sysmex XT-1800i; Sysmex, Hamburg, Germany).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 22.0, Chicago, IL) for Windows. Kolmogorov–Smirnov analysis was used to test whether the results were normally distributed. The parametric variables are presented as mean ± SD and the non-parametric variables are presented as median (interquartile range). Differences between groups at baseline were tested using the chi-squared test and Fisher's exact test for categorical variables; for continuous variables, the unpaired Student's *t*-test or the Mann–Whitney U test was used, according to the distribution of the variable. Within-group changes over the 8-month intervention period were evaluated by Wilcoxon signed ranks test and by the paired Student's *t*-test, in accordance with the Gaussian distribution of the variables. Spearman's rank correlation coefficient was used to evaluate relationships between sets of data. A P value lower than 0.05 was considered statistically significant.

RESULTS

The anthropometric evaluations observed for the PE and the C group are presented in **Table 1**. At baseline, the two groups were matched for BMI, BMI *z*-score, waist-to-height ratio, and percentage of body fat mass, as well as for age, gender, and Tanner stage (76 and 83% with Tanner stage ≥ 2 in PE and control groups, respectively). The PE group showed lower iron and TS values compared with the C group (**Table 1**).

At the end of the study, the C group showed a significant increase in body fat mass percentage (**Table 1**). The overweight and obese children who underwent PE intervention presented a significant decrease in BMI *z*-score and body fat mass (**Table 1**). These alterations were accompanied by a significant decrease in the levels of CRP, IL-6, ferritin, hepcidin, and sTfR, and an increase in iron concentration and TS; a significant increase in mean corpuscular volume was also observed (**Table 1**).

After the 8-month period, the PE group, compared with the C group, showed lower values of waist-to-height ratio (**Table 1**).

At baseline, when considering all the 73 subjects, BMI *z*-score correlated significantly and positively with sTfR ($r = 0.292$, $P = 0.012$) and transferrin ($r = 0.390$, $P = 0.001$); hepcidin correlated positively and significantly with IL-6

Table 1. Anthropometric and analytical data in overweight (OW) and obese (OB) children and adolescents before (T1) and after an 8-month-long exercise program (T2), for the PE and control (C) groups

	C group	(*n* = 29; 26 OB+3 OW)		PE group	(*n* = 44; 35 OB+9 OW)		*P*(C vs. PE)	*P*(C vs. PE)
	T1	T2	*P*(T1vs.T2)	T1	T2	*P*(T1vs.T2)	T1	T2
Anthropometric data								
Age (years)	10.3 (9.1–11.6)	10.7 (9.5–12.1)	**≤ 0.001**	9.1 (7.8–11.7)	9.6 (8.4–12.4)	**≤ 0.001**	0.083	0.092
Gender (%F/%M)	52/48	52/48	—	45/55	45/55	—	0.420	—
Tanner stage (%F/%M)								
Stage 1	3.45/13.79	3.45/6.90	—	9.09/13.64	9.09/6.82	—	0.816[a]	0.512[a]
Stage 2	24.14/13.79	3.45/20.69		18.18/15.91	6.82/18.18			
Stage 3	17.24/13.79	37.93/13.79		11.36/11.36	18.18/13.64			
Stage 4	6.90/3.45	6.90/3.45		6.82/11.36	9.09/9.09			
Stage 5	3.45/0.00	3.45/0.00		0.00/2.27	2.27/6.82			
Height (cm)	144 (138–158)	146 (140–158)	**≤ 0.001**	139 (130–151)	142 (132–155)	**≤ 0.001**	**0.030**	0.106
Weight (kg)	54.0 (41.3–61.7)	55.8 (44.9–66.0)	**0.002**	43.7 (33.6–60.5)	44.6 (36.7–63.3)	**≤ 0.001**	**0.045**	**0.026**
BMI (kg/m^2)	25.4 ± 3.5	25.8 ± 3.7	0.053	24.1 ± 4.8	23.8 ± 4.7	0.152	0.204	**0.040**
BMI *z*-score	2.46 (2.24–2.95)	2.50 (2.15–2.80)	0.210	2.33 (2.03–3.04)	2.10 (1.80–2.90)	**0.003**	0.297	0.070
WC (cm)	82.5 (73.7–93.8)	86.5 (78.7–95.0)	0.216	76.0 (69.6–86.9)	78.6 (70.0–87.2)	0.156	**0.030**	**0.010**
WtH	0.58 ± 0.05	0.58 ± 0.05	0.366	0.56 ± 0.07	0.56 ± 0.06	0.806	0.256	**0.046**
Body fat mass (%)	38.7 (34.2–41.5)	40.4 (36.8–44.7)	**0.009**	40.1 (35.1–43.7)	39.5 (33.8–42.6)	**0.012**	0.250	0.182
Hematologic data								
RBC (×10^{12}/l)	4.96 ± 0.32	4.93 ± 0.36	0.530	4.89 ± 0.29	4.89 ± 0.30	0.939	0.332	0.539
Hb (g/dl)	13.7 ± 0.6	13.6 ± 0.7	0.561	13.5 ± 0.9	13.6 ± 0.8	0.223	0.335	0.941
Hct (%)	41.3 ± 1.89	41.1 ± 2.6	0.671	40.4 ± 2.8	41.0 ± 3.0	0.166	0.115	0.809
MCV (fl)	84.0 (80.5–86.0)	83.0 (82.0–85.5)	0.971	83.0 (80.0–85.6)	84.0 (82.0–86.0)	**0.033**	0.342	0.522
MCH (pg)	27.7 ± 1.4	27.7 ± 1.4	0.947	27.7 ± 1.4	27.9 ± 1.3	0.078	0.991	0.443
MCHC (g/dl)	33.0 (32.6–33.4)	32.8 (32.5–33.9)	0.707	33.5 (32.6–34.2)	33.0 (32.3–33.9)	0.294	0.245	0.866
Inflammatory and iron status parameters								
CRP (mg/l)	0.98 (0.40–2.22)	1.10 (0.28–1.61)	0.888	0.86 (0.21–3.69)	0.61 (0.21–1.70)	**0.002**	0.701	0.596
IL-6 (pg/ml)	0.64 (0.40–0.99)	0.54 (0.42–1.03)	0.837	0.86 (0.53–1.70)	0.72 (0.42–1.06)	**0.048**	0.062	0.765
Hepcidin (ng/ml)	20.4 (14.2–31.3)	20.7 (14.7–29.9)	0.567	22.8 (12.2–34.2)	17.8 (10.8–26.2)	**0.040**	0.978	0.219
Iron (µg/dl)	41.0 (32.5–49.5)	32.0 (25.5–49.5)	0.078	31.5 (22.5–40.8)	40.0 (31.0–56.0)	**0.002**	**0.021**	0.200
sTfR (nmol/l)	24.6 ± 7.5	24.6 ± 6.3	0.977	23.9 ± 4.6	22.3 ± 5.2	**0.010**	0.643	0.107
Transferrin (mg/dl)	279 (258–310)	290 (262–305)	0.682	278 (255–322)	271 (243–301)	0.238	0.782	0.165
TS (%)	9.88(7.74–12.78)	8.89(6.50–12.52)	0.127	7.81(5.85–10.72)	10.06(7.90–14.09)	**0.002**	**0.031**	0.099
Ferritin (ng/ml)	49.1 (39.1–69.7)	48.4 (31.4–66.3)	0.729	49.2 (30.3–79.6)	45.7 (29.9–65.5)	**0.013**	0.752	0.569

BMI, body mass index; CRP, C-reactive protein; F, female; Hb, hemoglobin; Hct, hematocrit; IL, interleukin; M, male; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; PE, physical exercise group; RBC, red blood cell; sTfR, soluble transferrin receptor; TS, transferrin saturation; WC, waist circumference; WtH, waist-to-height ratio. Results are presented as mean ± SD or as median (interquartile range). Statistically significant values are highlighted using bold numbers.
[a]When considering and comparing the two groups - only female gender groups or only male gender groups - the Tanner stage persisted without statistical significance (*P* = 0.795 and *P* = 0.881, at T1, and *P* = 0.539 and *P* = 0.747, at T2, respectively).

(**Figure 2**) and with ferritin ($r = 0.478$, $P < 0.001$); IL-6 correlated negatively with iron ($r = -0.237$, $P = 0.045$) and TS ($r = -0.250$, $P = 0.034$); and CRP correlated positively with IL-6 ($r = 0.308$, $P = 0.006$) and with ferritin ($r = 0.237$, $P = 0.036$).

After the 8-month period, the correlation of hepcidin with IL-6 and that of BMI *z*-score with sTfR were still observed in the C group ($r = 0.416$, $P = 0.025$; $r = 0.445$, $P = 0.016$; respectively) but not in the PE group. Hepcidin correlation with ferritin persisted in the PE and C groups (**Figure 3**).

In the PE group, the percentual change (%Δ), from baseline to 8 months of intervention, of hepcidin correlated significantly and positively with the %Δ of IL-6 (**Figure 4**) and %Δ of ferritin ($r = 0.310$, $P = 0.043$); the %Δ of IL-6 correlated

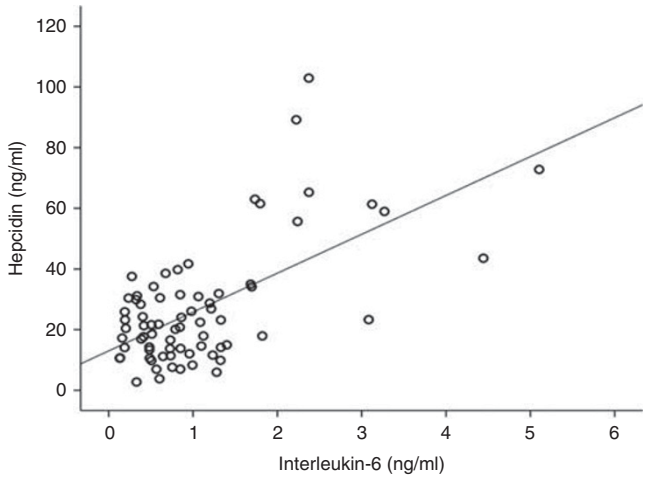

Figure 2. Correlations observed at baseline between circulating levels of hepcidin and interleukin-6 ($n = 73$; $r = 0.398$; $P = 0.001$) in overweight and obese children and adolescents.

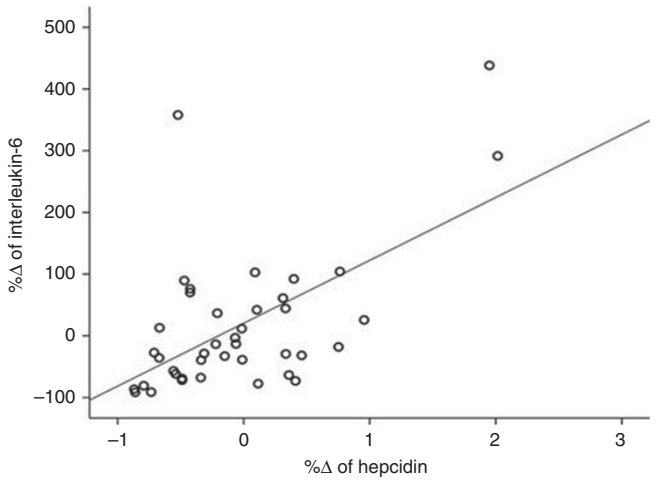

Figure 4. Correlations observed in the physical exercise group of the percentual change (%Δ), from baseline to 8months of intervention, of interleukin-6 with %Δ of hepcidin ($r = 0.465$, $P = 0.002$).

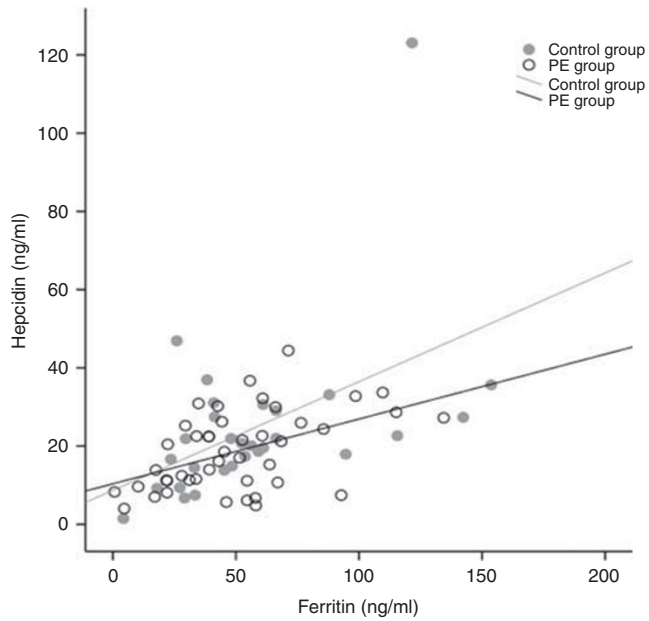

Figure 3. Correlations observed between hepcidin and ferritin after an 8-month-long exercise program in the physical exercise (PE; $n = 44$; $r = 0.459$; $P = 0.002$) and control groups ($n = 29$; $r = 0.525$; $P = 0.003$).

significantly and positively with the %Δ of CRP ($r = 0.583$, $P < 0.001$) and negatively with the %Δ of iron ($r = -0.336$, $P = 0.032$). In the C group, the %Δ of hepcidin correlated significantly and negatively with the %Δ of TS ($r = -0.423$, $P = 0.022$).

DISCUSSION

The improvement in iron status is important for several biological functions, such as hematological and cognitive functions. Indeed, it is widely accepted that iron deficiency has a negative impact on cognition and behavior (28), and thus an adequate iron availability improves these functions. Epidemiological data have shown that disturbances in iron metabolism are common in obese children and adolescents, presenting usually low plasma iron, despite increased ferritin levels (2,5–7,29). Few hypotheses have been proposed to explain this association: namely, an inadequate dietary iron ingestion, an increase in iron requirements, a consequence of a higher body mass and an increase in blood volume, as well as a poor iron absorption (2,5,6). Some studies performed in children and adolescents showed that inadequate iron ingestion *per se* does not explain this relationship (7,30,31). Obesity has also been associated with low-grade inflammation that triggers the production of hepcidin, which is mainly produced by the liver, but it might be also produced by adipose tissue. In fact, few studies have reported that hepcidin levels are higher in obese children as compared with normal weight children (29–31). Hepcidin, by inducing ferroportin degradation, inhibits iron absorption and mobilization from iron stores, reducing circulating iron and increasing iron stores. Hepcidin expression is induced by iron overload and inflammation and is suppressed by hypoxia, iron deficiency, and ineffective erythropoiesis (32,33). During inflammatory conditions, like obesity, the expression of hepcidin is mediated through the IL-6/STAT3 (signal transducer and activator of transcription-3) pathway (15).

To further clarify the disturbances in iron metabolism and its association with inflammation in the pediatric obese population, we conducted an evaluation of inflammatory markers and iron metabolism in a sub-sample of subjects from the Acorda Project, as mentioned earlier. As both transferrin and ferritin levels are known to be altered in inflammation, we also evaluated sTfR, as it is not influenced by inflammation (17). We observed a positive correlation at baseline between sTfR and BMI *z*-score that corroborates the association between obesity and iron deficiency (2–7). In fact, it is well known that the levels of sTfR increase with

erythropoietic activity and with the increase in iron requirements, reflecting an accelerated erythropoiesis and/or an inadequate iron availability for erythropoiesis. We also found a positive correlation between hepcidin and ferritin at baseline and after the intervention in both groups. Hepcidin induces iron accumulation in the cytoplasm of duodenal enterocytes, macrophages, and hepatocytes in the form of ferritin (13), explaining this correlation.

Our results also show a positive correlation between IL-6 and hepcidin and an inverse correlation of IL-6 with iron and TS, showing the role of IL-6 in the modulation of hepcidin and iron metabolism. Indeed, those participants who achieved more substantial reductions in IL-6 also presented more pronounced reductions in hepcidin (**Figure 4**), showing that the increase in the inflammatory mediator IL-6 enhances hepcidin production, favoring a functional iron deficiency in obesity (**Figure 1**).

The adipose tissue is an important source of IL-6 and also expresses hepcidin (34). The adipocytes seem to be able to produce hepcidin in response to an inflammatory stimuli, such as IL-6 (34). This may justify our results, as the correlation of hepcidin with IL-6 and of BMI *z*-score with sTfR remained significant only in the C group, which showed a significant increase in body fat mass and no decrease in BMI *z*-score. It has been hypothesized that hepcidin secreted by increased adipose tissue during obesity may contribute significantly to the circulating levels of hepcidin, disturbing iron metabolism and erythropoiesis(10). This may explain the loss of correlations observed after BMI *z*-score reduction in the PE group, and supports the hypothesis that in the context of obesity IL-6 and hepcidin may exert a major effect in modulating iron metabolism (**Figure 1**). Actually, the lack of correlations in the PE group after intervention may express a more homogenous group of individuals at the end of study.

In the PE group, the physical intervention led to a decrease in BMI *z*-score and body fat mass. The reduction in adipose mass was associated with a decrease in inflammation, as shown by the significant reduction in IL-6, CRP, and ferritin levels. Strengthening the role of inflammation in the disturbances in iron metabolism during obesity, we found that the decrease in IL-6 led to a reduction in hepcidin levels, allowing an improvement in iron availability (**Figure 1**). Indeed, circulating levels of iron and TS increased, showing an improvement in iron absorption and mobilization, and sTfR levels decreased, showing an improvement in iron availability for erythropoiesis. None of these alterations were observed in children from the C group, in whom an enhancement in body fat mass was registered. Our data show that aerobic exercise practice is an efficient way to reduce adiposity, improving iron profile, in obese children.

There are only a few studies addressing the cross-talk between obesity, inflammation, hepcidin, and iron metabolism in children and adolescents, and the results are controversial, probably due to the variety in study designs (e.g. number of obese individuals, type of intervention, and duration of follow-up). In accordance with our data, Amato *et al.* (20) reported a decrease in hepcidin and an increase in iron absorption, following a reduction in BMI. Gong *et al.* (21) also reported that weight loss, achieved by overweight and obese children through a 1-year program, was associated with an improvement in iron status and inflammatory markers. Studies performed in adults also reported an association between fat loss, reduction in inflammation and hepcidin, and improvement in iron profile (35,36).

As the main goal of this work was to evaluate the impact of PE on the several parameters under study, children and their parents chose the group to be included, either the PE or the C group. We found that at baseline the C group presented significantly higher iron and TS. The level of circulating iron increases during childhood, being associated with physiological changes, such as growth and hormonal changes. In the present study, the C and PE groups were equilibrated for age, Tanner stage, and BMI *z*-score, but the C group presented higher values for height and weight at baseline. A more rapid growth of individuals in the C group may justify, at least in part, the higher iron levels and TS values, though a contribution of environmental factors (e.g. diet) cannot be excluded.

At the end of the PE intervention program, when comparing the C and PE groups, in spite of the decrease in BMI in the PE group, we did not find significant differences in the studied analytical markers. A more intense or longer physical intervention program or a program that combines physical activity with nutrition intervention may have a more significant impact on inflammation and iron status. Gong *et al.* (21) reported that those children who underwent a 1-year nutrition-based, comprehensive intervention weight-loss program presented, after this period, lower BMI *z*-scores, and a significant improvement in iron profiles and inflammatory markers IL-6 and CRP, when compared with the control group. As already mentioned, hepcidin levels were not evaluated in this study.

We are aware that our study presents some limitations. Nutritional counseling was provided to all participants; however, we did not administer questionnaires on alimentary habits, which might be a confounding factor in our analyses, even though, and as stated before, inadequate iron ingestion *per se* does not explain the relationship between obesity and iron deficiency (7,30,31). Considering that the groups were self-selected and a dietary analysis was not conducted, the observed changes may not be due to the PE program alone.

The small number of participants in both groups, particularly in the control group, may have diminished the ability to detect significant alterations in other variables, or in their correlations.

We analyzed overweight and obese participants together, and this may have masked some modifications associated with obesity, even though the control and PE groups were equilibrated for body fat mass and BMI *z*-score at baseline (T1), and included a similar number of obese patients (90% and 80% in control and PE groups, respectively; $P = 0.222$). Besides, a control group of lean children and

adolescents was not analyzed, which partially limits the discussion of results found at baseline; nonetheless, CRP values are substantially higher than those reported in non-obese controls in a previous work from our group performed in a Portuguese population (median of CRP = 0.25 mg/l) (37). Furthermore, the observed correlations between markers of inflammation and of iron metabolism strengthen the association of obesity with low-grade inflammation that triggers the production of hepcidin, compromising iron metabolism. Finally, we cannot exclude the existence (and influence) of sporadic physical activity/exercise outside the intervention program (e.g. at home, after school, and during weekends), which was not reported to our team. Actually, the WHO recommends that "children and youth aged 5–17 should accumulate at least 60 min of moderate to vigorous-intensity physical activity daily" (25), but we could control only the 5 school days. Nevertheless, those under other known nutritional or PE (structured) programs were excluded from the present study.

In summary, our data show that increasing adiposity in the pediatric population is associated with increasing IL-6, which stimulates hepcidin synthesis, leading to functional iron deficiency due to inhibition of iron absorption and mobilization from iron stores. Moreover, we showed that an 8-month-long intervention at school allowed a reduction in BMI *z*-score and an improvement in inflammation, namely in IL-6, which causes a reduction in hepcidin levels and in disturbances in iron status.

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