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ORIGINAL ARTICLE

Pro- and anti-inflammatory adipokines are associated with cardiometabolic risk markers in Brazilian schoolchildren

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Abstract

Pro- and anti-inflammatory adipokines have been regarded as potential markers of obesity and cardiometabolic comorbidities. However, few studies have evaluated this association in children. We aimed to evaluate the relationship between adipokine concentrations and cardiometabolic risk markers in Brazilian schoolchildren. This was a cross-sectional study with 378 children aged 8–9 years from Viçosa, Minas Gerais, Brazil. We measured adipokines (leptin, retinol-binding protein 4, adiponectin, and chemerin) and cardiometabolic risk markers (fasting glucose, HOMA-IR, lipid profile, and blood pressure). Cardiometabolic risk markers were compared by quintiles of adipokines with linear regression adjusted for potential confounders. Leptin was positively associated with diastolic blood pressure ($P = 0.03$) and HOMA-IR ($P = 0.01$), and retinol-binding protein 4 was positively associated with total cholesterol ($P = 0.04$). Each standard deviation of leptin and retinol-binding protein 4 was associated to, respectively, a 0.1 (95%CI: 0.1; 0.2), 0.3 (95%CI: 0.1; 0.6), and 2.5 (95%CI: 0.1; 4.9) units increase in diastolic blood pressure, HOMA-IR, and total cholesterol. Adiponectin was negatively associated with diastolic blood pressure ($P = 0.01$) and HOMA-IR ($P = 0.01$), and chemerin was negatively associated with glucose ($P = 0.001$). Each standard deviation of adiponectin and chemerin was associated to, respectively, a −0.1 (95%CI: −0.2; −0.1), −0.2 (95%CI: −0.3; −0.1), and −1.2 (95%CI: −1.9; −0.5) units decrease in diastolic blood pressure, HOMA-IR, and glucose.

Conclusion: Pro- and anti-inflammatory adipokines were positively and negatively associated with cardiometabolic risk markers, respectively, among schoolchildren, indicating this relationship may be identified at earlier ages.

What is Known:
- *Although leptin, retinol-binding protein 4, and adiponectin are well-known adipokines, a consensus regarding their relationship with cardiometabolic risk markers, especially in schoolchildren, has not yet been reached.*
- *Chemerin is an adipokine that has been studied recently. Yet, due to its dependence on the target cell type, its functions are still a controversial topic.*

What is New:
- *Leptin was positively associated with diastolic blood pressure and HOMA-IR, and retinol-binding protein 4 was positively associated with total cholesterol.*
- *Adiponectin was negatively associated with diastolic blood pressure and HOMA-IR, and chemerin was negatively associated with glucose.*

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Introduction

Childhood obesity is a worldwide public health issue, as the excessive accumulation of adipose tissue can negatively impact the children's health [1]. The amount of adipose tissue influences the profiles of adipokines [2], such as leptin, retinol-binding protein 4 (RBP4), adiponectin, and chemerin. Studies with children and adolescents have verified associations of obesity with higher concentrations of leptin [3], RBP4 [4], and chemerin [5], as well as lower adiponectin concentrations [6].

Due to their central role in metabolism and energy expenditure, pro- and anti-inflammatory adipokines might be potential markers of cardiometabolic conditions [7, 8]. Although some studies with children and adolescents indicated that leptin, RBP4, and adiponectin were good markers for insulin resistance [9–11], metabolic syndrome [12], dyslipidemia [9], and hypertension [9, 13, 14], others suggested the opposite [15, 16].

Chemerin is an adipokine that has been studied recently. Yet, its functions still generate controversy, as chemerin's active form depends on the target cell type [17]. Experimental studies with cells and animals demonstrated that chemerin may have pro- and anti-inflammatory effects on muscles [18] and adipocytes [19], respectively. However, research involving children and adolescents remains scarce, and reports positive association of chemerin with markers of inflammation and endothelial activation [5], as well as with insulin concentration [20].

Considering that only a few studies have investigated adipokines in children, the purpose of this study was to evaluate the association between adipokine concentrations and cardiometabolic risk markers in Brazilian schoolchildren. We hypothesized that pro- and anti-inflammatory adipokines are positively and negatively associated with higher cardiometabolic risk, respectively.

Methods

Participants and study design

This was a cross-sectional study conducted with children aged 8 and 9 years from Viçosa, a city within the *Zona da Mata* region in the state of Minas Gerais, Brazil, 227 km distant from Belo Horizonte. According to the 2010 Census, the city had an area of 299 km^2 and 72,244 inhabitants, of whom 93.2% lived in the urban area [21].

The participants were obtained from the Schoolchildren Health Assessment Survey (*Pesquisa de Avaliação da Saúde do Escolar*, PASE, in Portuguese), an investigation that aimed to assess the cardiovascular health of children from Viçosa. In 2015, the city had 17 public and 7 private schools in the urban area that attended children aged 8 and 9 years, with a total of 1464 enrolled children.

Sample calculation, sampling process, and non-inclusion criteria have been previously described [22, 23]. In short, 378 children were selected by stratified random sampling. Children with health conditions that influenced their nutritional status or body composition, with reported chronic use of medicine that affected the glucose and/or lipid metabolism, and/or whose parents/guardians could not be reached after three call attempts, were not included in the study.

This study was conducted according to the guidelines established by the Declaration of Helsinki. All the procedures involving study participants were approved by the Human Research Ethics Committee of the *Universidade Federal de Viçosa* (UFV, in Portuguese) (reference number 663.171/2014), as well as by the Municipal Secretary of Education, the Regional Superintendent of Education and school principals. Written informed consent was obtained from all parents/guardians.

Adipokines

In a 12-h fast, the blood samples were collected by venipuncture in the antecubital region at the UFV Health Center's clinical analysis sector. The serum and plasma were separated and stored in 1.5-ml Eppendorf tubes at −80 °C. We analyzed leptin, RBP4, adiponectin, and chemerin, in duplicate.

Serum leptin was measured by enzyme immunoassay method, with coefficient of variation intra-assay <13.3% and inter-assay <12.7% (KAP2281, DIAsource®, Louvain-la-Neuve, Belgium; standardized protocols from *Diagnósticos do Brasil*).

Plasma RBP4, adiponectin, and chemerin were determined by commercials ELISA sandwich kits, with coefficients of variation intra-assay <10% and inter-assay <12% (human RBP4: SEA929Hu; human adiponectin: SEA605Hu; human chemerin: SEA945Hu, Cloud Clone Corp.®, Houston, TX, USA; standardized protocols from *Laboratório Especializado em Análises Científicas* - LEAC).

Cardiometabolic risk markers

The lipid profile (total cholesterol, high-density lipoprotein—HDL-c, low-density lipoprotein—LDL-c, and triglycerides) and fasting blood glucose were determined by the colorimetric enzymatic method, using the Bioclin® commercial kit (Belo Horizonte, MG, Brazil) and dosed in automatic analyzer equipment (BS-200 Mindray®, Nanshan, China) at the Clinical Analysis Laboratory (LAC) of the Department of Nutrition and Health at UFV. Dyslipidemia was classified in the presence of alteration in at least one of the lipid profile markers [24].

Fasting insulin was determined by the chemiluminescence immunoassay method at the *Diagnósticos do Brasil* laboratory and quantified by the Elecsys Insulin® test. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated according to the equation: fasting insulin (μU/mL) × fasting blood glucose (mmol/L)/22.5 [25]. The HOMA-IR is useful for early identification of children with insulin resistance and exhibited good accuracy for diagnosing metabolic syndrome [26]. According to the cutoff point proposed for children (≥3.02) [27], HOMA-IR was classified to identify insulin resistance.

Blood pressure was measured three times using digital blood pressure monitors (Omron, Vernon Hills, IL, USA), with the child in a sitting position after resting for at least 5 min. The systolic (SBP) and diastolic (DBP) blood pressure's means, in mmHg, were transformed into *Z* score and classified as hypertension, according to sex, age, and height percentile [28].

Sociodemographic, economic, and lifestyle characteristics

A semi-structured questionnaire was applied by researchers and answered by the parents/guardians to assess the child's sociodemographic, economic and lifestyle characteristics, such as sex, age, *per capita* income (US$), maternal education (years), and screen time per day (hours spent watching television, playing video games, and using a cell phone or computer). Screen time > 2 h per day was used to classify sedentary behavior [29]. The household's *per capita* income was categorized into tertiles.

Anthropometry and body composition

Anthropometric measurements were taken by a trained researcher in an appropriate room. At that moment, in the presence of their parents/guardians, the children were barefoot and wore light clothes. Weight and height were measured using, respectively, a digital electronic scale with a capacity of 150 kg and a sensitivity of 100 g (Tanita® Ironman Model BC 553, Tanita Corporation of America Inc, Arlington Heights, USA), and a vertical stadiometer divided in centimeters and subdivided in millimeters (Alturexata®, Belo Horizonte, Brazil).

The *Z* scores for height-for-age and body mass index-for-age (BMI) were obtained from the WHO Anthro Plus software, according to sex- and age-specific references for children [30]. BMI was classified according to the criteria proposed by the International Obesity Task Force (IOTF) [31]. Total body fat (BF) was measured by a specialized technician using the dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems Lunar, Milwaukee, WI, USA) at the UFV Health Center's diagnostic imaging sector. During the examination, children were fasted, wore light clothing, and no metal accessories, remaining in a supine position on a stretcher until the screening was complete. The percentage (%) of BF was categorized into tertiles.

Statistical analyses

Categorical variables were reported as relative frequencies (%), whereas continuous variables were reported as means ± standard deviation (SD). Distribution normality was verified using the Shapiro-Wilk test.

For the analyses, we defined the variables as follows: (1) Exposure: adipokine concentrations (leptin, RBP4, adiponectin, and chemerin) as continuous variables; (2) Outcomes: cardiometabolic risk markers (SBP, DBP, fasting glucose, HOMA-IR, total cholesterol, HDL-c, LDL-c, and triglycerides) as continuous variables.

First, we compared the distribution of outcomes by categories of covariates using mean ± SD. For ordinal exposures, tests for linear trends were derived from linear regression models with cardiometabolic risk markers as continuous outcomes and a variable representing ordinal categories of the covariate as a continuous predictor. For sex and screen time, we used the Wilcoxon rank-sum test.

Next, we compared the adipokine concentrations according to cardiometabolic risk factors (overweight/obesity, dyslipidemias, insulin resistance, and hypertension) using Wilcoxon rank-sum test.

Then, we compared the distributions of outcomes by adipokines categories, using mean differences and 95% confidence intervals (CI) from unadjusted (Model 1) and multiple linear regression models adjusted for age, sex, screen time, and *per capita* income (Model 2). Finally, we added %BF to the multiple model (Model 3). We also estimated unadjusted and adjusted mean differences with 95%CI in cardiometabolic risk markers per 1 SD of adipokine concentrations. The adjustment variables were selected based on the literature, and robust estimates of the variance were specified in all models, which are consistent to heteroskedasticity and non-normality [32].

Analyses were carried out in the Stata® version 14 (StataCorp LP, CollegeStation, TX, USA). The significance level was 5% for all hypothesis tests.

Results

Children's mean age was 8.5 years; 52% were girls, 52% engaged in sedentary behavior, 18% were overweight, and 11% were obese. The means of leptin, RBP4, adiponectin, and chemerin concentrations were 5.7 ng/mL, 4.0 μg/mL, 13.6 μg/mL, and 83.6 ng/mL, respectively. The prevalence of dyslipidemia, insulin resistance, and hypertension were 33%, 2.7%, and 4.8%, respectively.

Girls showed lower glucose and HDL-c concentrations, as well as higher DBP, compared to boys. Height- and BMI-for-age *Z* scores, and %BF were positively associated with SBP, HOMA-IR, and triglyceride. In addition, BMI-for-age and %BF were positively associated with with DBP and LDL-c, and negatively associated with HDL-c. *Per capita* income and maternal education were positively associated with total cholesterol and LDL-c. Furthermore, %BF and *per capita* income were positively and negatively associated with SBP, respectively. Both were positively associated with total cholesterol (Table 1).

Children with overweight/obesity, dyslipidemias, insulin resistance, and hypertension showed a higher leptin concentration. In addition, children with overweight/obesity displayed a higher RBP4 concentration, as well as children with insulin resistance and hypertension showed a lower adiponectin concentration (Table 2).

Leptin was positively associated with DBP and HOMA-IR, and RBP4 was positively associated with total cholesterol. After adjustment, when compared with the first, the fifth quintiles of leptin and RBP4 were related to, respectively, a 0.1 (95%CI: −0.1; 0.3), 0.4 (95%CI: −0.4; 1.1), and 9.2 (95%CI: 1.0; 17.4) units increase in DBP, HOMA-IR, and total cholesterol. Each SD of leptin and RBP4 was associated to, respectively, a 0.1 (95%CI: 0.1; 0.2), 0.3 (95%CI: 0.1; 0.6), and 2.5 (95%CI: 0.1; 4.9) units increase in DBP, HOMA-IR, and total cholesterol (Table 3).

Adiponectin was negatively associated with DBP and HOMA-IR, and chemerin was negatively with glucose. After adjustment, children in the fifth quintiles of adiponectin and chemerin showed, respectively, a −0.3 (95%CI: −0.5; −0.2), −0.6 (95%CI: −1.1; −0.2), and −3.0 (95%CI: −5.4; −0.6) units decrease in DBP, HOMA-IR, and glucose, when compared to children in the first quintiles. Each SD of adiponectin and chemerin was associated to, respectively, a −0.1 (95%CI: −0.2; −0.1), −0.2 (95%CI: −0.3; −0.1), and −1.2 (95%CI: −1.9; −0.5) units decrease in DBP, HOMA-IR, and glucose (Table 3).

Discussion

In our cross-sectional study, we supported the hypothesis that pro- and anti-inflammatory adipokines were positively and negatively associated with cardiometabolic risk markers in children, respectively. These results indicated that, even in childhood, it is already possible to identify relationships between adiposity, inflammation, and cardiometabolic risk. In addition, these findings pointed out the importance of using biomarkers, such as adipokines, for obesity phenotyping beyond BMI and other anthropometric measures, including the total body fat evaluated by DXA (a reference method), something that has already been proposed in the literature [33]. Moreover, adipokines may regulate the myokine expression, which is bioactive factor produced and secreted by the skeletal muscle. Jointly, they may contribute to the development of excess adiposity and, consequently, the onset of insulin resistance [34]. We emphasize that an imbalance of adipokines in childhood may stimulate the early onset of puberty in children with obesity by affecting the hypothalamic-pituitary-gonadal axis [35].

Our findings showed a positive association between leptin and DBP. Leptin may be a potential mediator between body fat and blood pressure in the pediatric population [13, 36]. In a longitudinal study with Chinese children, a higher leptin concentration was associated with a higher risk of hypertension, even after adjustment for BMI [14]. In other study, with young Danish and Norwegian, leptin and DBP remained associated after adjustment for BMI [13]. However, some studies with children and adolescents did not identify this independent association [37, 38]. Leptin is produced and secreted predominantly by adipose tissue and a pro-inflammatory adipokine because it upregulates adhesion molecules production and oxidative stress in endothelial cells [39]. Hyperleptinemia can be caused by changes in the leptin receptor or by deficiencies in its transport system in the blood-brain barrier, characterizing the leptin resistance [40]. The chronic hyperleptinemia increases the sympathetic nervous system activation and oxidative stress, which lead to nitric oxide deficiency, higher renal sodium reabsorption, and, consequently, increased blood pressure [41]. However, further studies are necessary to understand the mechanisms of leptin in the regulation of blood pressure in children.

Moreover, leptin was positively associated with HOMA-IR. Most studies with children and adolescents showed a positive association of leptin with HOMA-IR, insulin, glucose [10, 42, 43], and insulin resistance [11], even after adjustment for BMI or body fat. Insulin stimulates leptin synthesis and secretion, which, in turn, may regulate insulin release through a negative feedback mechanism in β cells [44]. In people with obesity, the leptin resistance activates interleukin-1β (IL-1β) signaling pathways, inducing β cell apoptosis, and triggering insulin resistance and type 2 diabetes mellitus [44].

Table 1 Distribution of cardiometabolic risk markers according to sociodemographic, anthropometric, body composition, and lifestyle characteristics in schoolchildren. Viçosa, Minas Gerais, Brazil, 2015

Characteristics	*n*	SBP (*Z* score) Mean ± SD	DBP (*Z* score) Mean ± SD	Fasting glucose (mg/dL) Mean ± SD	HOMA-IR Mean ± SD	Total cholesterol (mg/dL) Mean ± SD	HDL-c (mg/dL) Mean ± SD	LDL-c (mg/dL) Mean ± SD	Triglyceride (mg/dL) Mean ± SD
Sex[a]									
Female	197	0.26 ± 0.81	0.16 ± 0.62	83.5 ± 7.9	1.3 ± 1.4	152.5 ± 27.3	48.8 ± 9.2	88.5 ± 24.6	82.1 ± 37.4
Male	181	0.24 ± 0.70	0.01 ± 0.53	86.3 ± 7.8	1.1 ± 0.6	152.1 ± 25.5	51.4 ± 10.6	85.3 ± 21.9	75.3 ± 32.8
P		0.91	0.03*	0.001*	0.07	0.99	0.02*	0.31	0.07
Height-for-age (*Z* score)[b]									
−1 to < 0	115	0.13 ± 0.66	0.06 ± 0.54	84.0 ± 8.5	0.9 ± 0.5	152.2 ± 24.4	49.5 ± 10.3	87.8 ± 20.9	72.4 ± 26.4
0 to 1	135	0.17 ± 0.76	0.02 ± 0.60	85.0 ± 7.8	1.0 ± 0.5	152.2 ± 27.8	50.9 ± 9.7	86.2 ± 24.6	78.5 ± 37.6
> 1	128	0.45 ± 0.81	0.19 ± 0.59	85.5 ± 7.5	1.7 ± 1.7	152.5 ± 26.8	49.7 ± 10.0	87.0 ± 24.3	84.9 ± 39.0
P, trend		<0.001*	0.14	0.16	<0.001*	0.84	0.87	0.46	0.03*
BMI-for-age (*Z* score)[b]									
< −2	12	−0.20 ± 0.56	−0.08 ± 0.68	87.3 ± 9.6	0.8 ± 0.4	148.6 ± 21.6	49.8 ± 10.6	85.0 ± 17.1	85.6 ± 52.0
−2 to 1	242	0.08 ± 0.68	−0.02 ± 0.56	84.6 ± 8.1	0.9 ± 0.6	150.9 ± 26.5	51.2 ± 10.2	84.6 ± 23.3	70.9 ± 25.2
> 1 to 2	65	0.46 ± 0.64	0.21 ± 0.46	84.6 ± 7.6	1.6 ± 1.9	155.7 ± 26.2	48.9 ± 9.3	92.4 ± 23.8	90.4 ± 41.4
> 2	59	0.82 ± 0.87	0.43 ± 0.65	85.6 ± 7.4	1.9 ± 1.2	155.0 ± 27.2	47.0 ± 9.2	91.1 ± 23.4	97.2 ± 48.1
P, trend		<0.001*	<0.001*	0.18	<0.001*	0.26	0.005*	0.03*	<0.001*
% body fat (tertiles)[b]									
≤ 18.1	127	0.08 ± 0.68	-0.05 ± 0.57	85.2 ± 8.4	0.8 ± 0.4	148.9 ± 24.3	51.0 ± 10.7	82.2 ± 21.7	73.2 ± 29.2
18.2 to 28.8	124	0.07 ± 0.72	0.02 ± 0.55	84.6 ± 8.1	1.2 ± 1.4	151.8 ± 27.3	51.1 ± 9.4	86.4 ± 24.1	70.4 ± 25.2
> 28.8	127	0.60 ± 0.76	0.30 ± 0.57	84.8 ± 7.4	1.6 ± 1.1	156.2 ± 27.2	48.1 ± 9.6	92.2 ± 23.4	92.6 ± 44.5
P, trend		<0.001*	<0.001*	0.83	<0.001*	0.02*	0.004*	<0.001*	<0.001*
Screen time (h/day)[a]									
≤ 2	198	0.25 ± 0.73	0.11 ± 0.62	84.8 ± 7.4	1.2 ± 1.3	151.3 ± 24.9	50.6 ± 10.1	86.0 ± 24.1	77.3 ± 36.1
> 2	180	0.25 ± 0.80	0.07 ± 0.54	84.9 ± 8.6	1.2 ± 0.9	153.4 ± 28.0	49.5 ± 9.8	88.0 ± 22.6	80.5 ± 34.7
P		0.78	0.61	0.77	0.15	0.56	0.33	0.32	0.38
Per capita income (US$)[bc]									
< 116.46	124	0.29 ± 0.72	0.06 ± 0.56	84.0 ± 8.8	1.3 ± 1.5	151.1 ± 24.1	49.7 ± 9.5	83.9 ± 20.9	78.0 ± 33.1
116.47 to 203.93	128	0.19 ± 0.76	0.12 ± 0.55	85.0 ± 7.7	1.1 ± 0.7	151.0 ± 27.6	49.5 ± 9.7	86.8 ± 24.9	77.3 ± 38.5
> 203.93	126	0.27 ± 0.80	0.08 ± 0.64	85.5 ± 7.4	1.3 ± 1.0	154.8 ± 27.4	51.0 ± 10.7	90.1 ± 23.9	81.2 ± 34.5
P, trend		0.03*	0.05	0.57	0.17	0.04*	0.28	<0.001*	0.23
Maternal education (years)[b]									
≤ 4	53	0.35 ± 0.69	0.05 ± 0.53	85.6 ± 7.4	1.1 ± 0.6	148.7 ± 24.1	49.7 ± 9.0	80.4 ± 19.9	74.6 ± 30.5
5 to 10	109	0.29 ± 0.76	0.17 ± 0.62	84.7 ± 9.1	1.3 ± 1.6	148.1 ± 24.9	49.9 ± 10.1	80.3 ± 21.6	79.3 ± 41.2
11	131	0.26 ± 0.78	0.09 ± 0.56	84.9 ± 7.9	1.2 ± 1.0	151.9 ± 27.5	49.4 ± 10.1	89.2 ± 22.6	76.7 ± 29.7
≥ 12	85	0.14 ± 0.78	0.02 ± 0.61	84.6 ± 6.9	1.1 ± 0.6	160.7 ± 26.4	51.6 ± 10.4	96.4 ± 25.2	84.2 ± 38.1
P, trend		0.06	0.30	0.21	0.39	0.001*	0.26	<0.001*	0.08

SD standard deviation, *95%CI* 95% confidence interval, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HOMA-IR* homeostasis assessment model of insulin resistance, *HDL-c* high-density lipoprotein-cholesterol, *LDL-c* low-density lipoprotein-cholesterol

[a] Wilcoxon rank-sum test

[b] Test for linear trend from linear regression models with cardiometabolic risk markers as continuous outcomes and a variable representing ordinal categories of the characteristic as a continuous predictor. Robust estimates of the variance were specified in all models

[c] Approximate exchange rates of real (R$) to dollar (US$) at the time of this study (US$1.00 = R$ 3.22)

*$P < 0.05$

Table 2 Distribution of adipokine concentrations according to cardiometabolic risk factors in schoolchildren. Viçosa, Minas Gerais, Brazil, 2015

Characteristics	*n*	Leptin (ng/mL) Mean ± SD	RBP4 (μg/mL) Mean ± SD	Adiponectin (μg/mL) Mean ± SD	Chemerin (ng/dL) Mean ± SD
Overweight/obesity					
Yes	109	13.3 ± 13.5	4.1 ± 0.6	12.5 ± 6.9	89.0 ± 63.9
No	269	2.6 ± 4.2	4.0 ± 0.7	14.0 ± 7.6	81.4 ± 65.1
P		<0.001*	0.03*	0.06	0.16
Dyslipidemias					
Yes	113	8.1 ± 11.6	4.1 ± 0.6	13.3 ± 7.5	90.1 ± 67.2
No	265	4.7 ± 8.2	4.0 ± 0.7	13.7 ± 7.4	80.8 ± 63.6
P		0.02*	0.33	0.65	0.07
Insulin resistance					
Yes	10	28.5 ± 25.7	4.0 ± 0.4	9.3 ± 5.4	70.5 ± 36.1
No	364	5.1 ± 7.8	4.0 ± 0.7	13.7 ± 7.4	83.3 ± 64.0
P		0.001*	0.70	0.02*	0.93
Hypertension					
Yes	18	11.9 ± 18.7	4.2 ± 0.7	10.3 ± 4.5	83.0 ± 44.7
No	359	5.4 ± 8.7	4.0 ± 0.7	13.7 ± 7.5	83.7 ± 65.7
P		0.004*	0.21	0.04*	0.34

SD standard deviation, *RBP4* retinol-binding protein 4

*$P < 0.05$; Wilcoxon rank-sum test

Nevertheless, these mechanisms between adiposity, leptin, and insulin resistance in children have not yet been clearly established.

We found a positive association between RBP4 and total cholesterol; however, this relationship has been poorly investigated in childhood [12, 45]. Low-grade inflammation was associated with a decrease in lipogenic factors in omental fat [46], whereas in black adolescents, RBP4 was only associated with triglyceride, after adjustment for adiposity [47]. RBP4 is the main retinol transporting protein, secreted essentially by the liver, and also by adipose tissue [48]. It stimulates the expression of pro-inflammatory molecules involved in leukocyte recruitment and adherence to the endothelium [49]. The retinoids can modulate lipid metabolic processes, including hepatic and intestinal triglyceride production and secretion, β-oxidation of fatty acids, and hepatic production of very-low-density lipoprotein (VLDL-c) [50]. These mechanisms suggested that RBP4 may be positively related to total cholesterol.

The negative association between adiponectin and DBP may be explained by some mechanisms. Adiponectin is an anti-inflammatory adipokine produced exclusively by adipose tissue [51] and has been proposed as an early marker of cardiovascular risk in pediatric subjects [52]. It protects against endothelial dysfunction, by modulating the cross-linking between endothelial cells and platelets, stimulating the production of nitric oxide, and inhibiting vascular endothelial growth factor (VEGF) [53]. In addition, adiponectin has been associated with the renin-angiotensin-aldosterone system [54]. Similarly, other studies have identified a negative association of adiponectin with DBP [38, 42] and hypertension [55], after adjustment for body adiposity.

Besides, this study demonstrated that adiponectin was negatively associated with HOMA-IR in children. Our findings corroborate previous studies that found a negative association with HOMA-IR [56, 57] and insulin resistance [11] in children and adolescents, after adjustment for body adiposity measures. Lower adiponectin concentration was proposed as a marker of β cell dysfunction due to its anti-apoptotic properties, stimulating effects on insulin secretion, and its regulatory role in the gene expression of this hormone [44].

We observed a negative association between chemerin and glucose. This adipokine is mainly produced by adipocytes and hepatocytes, in addition to regulating angiogenesis, adipogenesis, an energy metabolism, as well as acting as a chemoattractant for immune cells [58]. The functions of chemerin are still a controversial issue, due to its active form may have pro- or anti-inflammatory properties, depending on the target cell type [17]. In a study with mice, chemerin increased the insulin-stimulated glucose uptake in 3T3-L1 adipocytes, concurrently with the increase in insulin signaling, thus indicating its importance in regulating insulin sensitivity in adipose tissue [19]. Additionally, another study showed that mice with chemerin deficiency had significantly higher fasting glucose compared to non-chemerin-deficient mice [59]. These researchers indicated that chemerin regulated the function of β cells and played an important role in glucose homeostasis [59]. However, a study that carried out a treatment on primary

Table 3 Cardiometabolic risk markers according to adipokine concentrations in schoolchildren. Viçosa, Minas Gerais, Brazil, 2015

Cardiometabolic risk markers	Adipokine concentrations					Per 1 SD[d]	*P*, trend[e]
	Leptin (quintiles – ng/mL)						
	Q1 (≤ 0.2) (*n* = 72)	Q2 (0.3 to 1.1) (*n* = 79)	Q3 (1.2 to 2.9) (*n* = 74)	Q4 (3.0 to 8.8) (*n* = 74)	Q5 (> 8.8) (*n* = 75)		
DBP (*Z* score)							
Mean ± SD	0.05 ± 0.53	−0.02 ± 0.52	0.06 ± 0.64	−0.02 ± 0.54	0.37 ± 0.58		
Unadjusted difference (95%CI)[a]	Reference	−0.1 (−0.2; 0.1)	0.0 (−0.2; 0.2)	−0.1 (−0.2; 0.1)	0.3 (0.1; 0.5)	0.2 (0.1; 0.2)	<0.001*
Adjusted difference (95%CI)[b]	Reference	−0.1 (−0.2; 0.1)	0.0 (−0.2; 0.2)	−0.1 (−0.2; 0.1)	0.3 (0.1; 0.5)	0.1 (0.1; 0.2)	<0.001*
Adjusted difference (95%CI)[c]	Reference	−0.1 (−0.3; 0.0)	0.0 (−0.2; 0.2)	−0.1 (−0.3; 0.0)	0.1 (−0.1; 0.3)	0.1 (0.1; 0.2)	0.03*
HOMA-IR							
Mean ± SD	1.0 ± 0.5	1.1 ± 0.7	1.0 ± 0.7	1.1 ± 0.6	1.9 ± 2.0		
Unadjusted difference (95%CI)[a]	Reference	0.1 (−0.1; 0.3)	0.0 (−0.2; 0.2)	0.1 (−0.1; 0.3)	0.9 (0.4; 1.4)	0.4 (0.3; 0.6)	<0.001*
Adjusted difference (95%CI)[b]	Reference	0.1 (−0.1; 0.3)	0.0 (−0.2; 0.2)	0.1 (−0.1; 0.3)	0.9 (0.4; 1.3)	0.4 (0.3; 0.6)	<0.001*
Adjusted difference (95%CI)[c]	Reference	0.0 (−0.2; 0.2)	−0.1 (−0.2; 0.1)	−0.1 (−0.3; 0.2)	0.4 (−0.4; 1.1)	0.3 (0.1; 0.6)	0.01*
	RBP4 (quintiles – μg/mL)						
	Q1 (≤ 3.5) (*n* = 75)	Q2 (3.6 to 3.7) (*n* = 76)	Q3 (3.8 to 4.0) (*n* = 75)	Q4 (4.1 to 4.5) (*n* = 77)	Q5 (> 4.5) (*n* = 75)		
Total cholesterol (mg/dL)							
Mean ± SD	145.6 ± 24.1	147.9 ± 19.4	155.6 ± 33.1	155.9 ± 24.8	156.5 ± 27.4		
Unadjusted difference (95%CI)[a]	Reference	2.2 (−4.8; 9.2)	10.0 (0.7; 19.3)	10.3 (2.4; 18.1)	10.8 (2.5; 19.2)	2.9 (0.3; 5.5)	0.03*
Adjusted difference (95%CI)[b]	Reference	1.7 (−5.3; 8.8)	9.1 (−0.1; 18.4)	9.2 (1.2; 17.2)	10.0 (1.7; 18.3)	2.7 (0.2; 5.2)	0.03*
Adjusted difference (95%CI)[c]	Reference	1.5 (−5.5; 8.5)	8.1 (−1.4; 17.5)	8.4 (0.4; 16.4)	9.2 (1.0; 17.4)	2.5 (0.1; 4.9)	0.04*
	Adiponectin (quintiles – μg/mL)						
	Q1 (≤ 7.6) (*n* = 75)	Q2 (7.7 to 10.2) (*n* = 76)	Q3 (10.3 to 13.4) (*n* = 76)	Q4 (13.5 to 18.3) (*n* = 76)	Q5 (> 18.3) (*n* = 75)		
DBP (*Z* score)							
Mean ± SD	0.31 ± 0.64	0.00 ± 0.61	0.11 ± 0.56	0.07 ± 0.58	−0.05 ± 0.46		
Unadjusted difference (95%CI)[a]	Reference	−0.3 (−0.5; −0.1)	−0.2 (−0.4; 0.0)	−0.2 (−0.4; 0.0)	−0.4 (−0.5; −0.2)	−0.1 (−0.2; −0.1)	0.004*
Adjusted difference (95%CI)[b]	Reference	−0.3 (−0.5; −0.1)	−0.2 (−0.4; 0.0)	−0.2 (−0.4; 0.0)	−0.4 (−0.5; −0.2)	−0.1 (−0.2; −0.1)	0.002*
Adjusted difference (95%CI)[c]	Reference	−0.3 (−0.5; −0.1)	−0.2 (−0.4; 0.0)	−0.2 (−0.3; 0.0)	−0.3 (−0.5; −0.2)	−0.1 (−0.2; −0.1)	0.01*
HOMA-IR							
Mean ± SD	1.7 ± 2.0	1.2 ± 0.8	1.1 ± 0.7	1.0 ± 0.6	1.0 ± 0.6		
Unadjusted difference (95%CI)[a]	Reference	−0.5 (−1.0; 0.0)	−0.5 (−1.0; 0.0)	−0.6 (−1.1; −0.1)	−0.7 (−1.2; −0.2)	−0.2 (−0.3; −0.1)	0.002*
Adjusted difference (95%CI)[b]	Reference	−0.4 (−0.9; 0.0)	−0.5 (−0.9; 0.0)	−0.6 (−1.0; −0.1)	−0.7 (−1.2; −0.2)	−0.2 (−0.3; −0.1)	0.002*
Adjusted difference (95%CI)[c]	Reference	−0.5 (−0.9; −0.1)	−0.5 (−0.9; 0.0)	−0.5 (−0.9; 0.0)	−0.6 (−1.1; −0.2)	−0.2 (−0.3; −0.1)	0.01*

Table 3 (continued)

Cardiometabolic risk markers	Adipokine concentrations					Per 1 SD[d]	*P*, trend[e]
	Chemerin (quintiles – ng/mL)						
	Q1 (≤ 35.1) (*n* = 75)	Q2 (35.2 to 52.1) (*n* = 76)	Q3 (52.2 to 74.1) (*n* = 76)	Q4 (74.2 to 117.9) (*n* = 76)	Q5 (> 117.9) (*n* = 75)		
Fasting glucose (mg/dL)							
Mean ± SD	86.1 ± 7.4	84.6 ± 7.8	86.4 ± 7.8	84.1 ± 8.8	83.0 ± 7.7		
Unadjusted difference (95%CI)[a]	Reference	−1.5 (−3.9; 1.0)	0.4 (−2.1; 2.8)	−1.9 (−4.5; 0.6)	−3.1 (−5.5; −0.7)	−1.1 (−1.8; −0.4)	0.002*
Adjusted difference (95%CI)[b]	Reference	−1.0 (−3.4; 1.5)	0.9 (−1.5; 3.4)	−1.6 (−4.2; 1.0)	−2.8 (−5.2; −0.5)	−1.2 (−1.9; −0.5)	0.001*
Adjusted difference (95%CI)[c]	Reference	−1.1 (−3.5; 1.4)	0.9 (−1.6; 3.3)	−1.7 (−4.3; 0.9)	−3.0 (−5.4; -0.6)	−1.2 (−1.9; −0.5)	0.001*

SD standard deviation, *95%CI* 95% confidence interval, *Q* quintile, *DBP* diastolic blood pressure, *HOMA-IR* homeostasis assessment model of insulin resistance, *RBP4* retinol-binding protein 4

[a] From a linear regression model with cardiometabolic risk markers as the continuous outcomes and adipokines as predictors

[b] From linear regression adjusted for sex, age, screen time, and *per capita* income as the continuous outcome

[c] From linear regression adjusted for sex, age, screen time, *per capita* income, and % body fat as the continuous outcome

[d] From a linear regression model with cardiometabolic risk markers as continuous outcomes and adipokine concentrations per 1SD (continuous) as predictors

[e] Test for linear trend when a variable representing ordinal categories of adipokines were introduced into the linear regression as continuous predictors. Robust estimates of the variance were specified in all models

*$P < 0.05$

human skeletal muscle cells with chemerin reported impairments in insulin actions, as well as reduced insulin-stimulated glucose uptake [18]. The relationship between adipose tissue inflammation and glycemic control is complex, as dysfunctional adiposity is characterized by an altered gene expression profile in the context of obesity and type 2 diabetes [60]. Therefore, the mechanisms between chemerin and glucose homeostasis are still unclear, indicating the need for longitudinal studies addressing this relationship, especially in childhood.

Our study has several strengths that should be highlighted. This is, to the best of our knowledge, the first epidemiological study in Brazil that investigated the relationship between chemerin and cardiometabolic risk markers exclusively in children. Second, because adipokines are produced and secreted by adipose tissue, we included body fat, assessed by DXA (reference method), as an adjustment in statistical analysis. Third, we also tested the linear regression model adjusting for the android fat (instead of total body fat) obtained by DXA and results did not change. Finally, this cross-sectional study presented important findings regarding adiposity, inflammation, and cardiometabolic risk in schoolchildren. We emphasize the importance of further longitudinal studies to help understanding the temporal relationship between adiposity-related inflammation and cardiometabolic risk at earlier ages.

As for its limitations, this cross-sectional study did not allow to evaluate the causality between adipokines and cardiometabolic risk. Early puberty is more likely in girls and may play a role in the onset of adiposity, inflammation, and cardiometabolic risk. Although the pubertal stage was not assessed, we selected a representative sample, exclusively composed of children, to minimize potential hormonal influences on body composition and biochemical data. In addition, the lack of consensus on the cut-off points for adipokines precluded the assessment of the prevalence of alterations in these markers in childhood.

We concluded that pro- and anti-inflammatory adipokines were positively and negatively associated with cardiometabolic risk markers, respectively, in Brazilian schoolchildren. These findings demonstrated that the association of low-grade inflammation with cardiometabolic risk can already be observed before age 10 and reinforces that public health actions are a high-priority need to prevent adiposity-related inflammation and co-morbidities in early life stages.

Abbreviations *BF,* Body fat; *BMI,* Body mass index; *CI,* Confidence interval; *DBP,* Diastolic blood pressure; *DXA,* Dual-energy X-ray absorptiometry; *HDL-c,* High-density lipoprotein-cholesterol; *HOMA-IR,* Homeostatic model assessment for insulin resistance; *LDL-c,* Low-den-

sity lipoprotein-cholesterol; *RBP4,* Retinol-binding protein 4; *SBP,* Systolic blood pressure; *SD,* Standard deviation

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Code availability N/A.

Authors' contributions M.S.F.: study conception and design; data collection; analysis; interpretation of data; drafting and writing the manuscript. M.C.P.: study conception and design; revision of the manuscript; co-supervision. J.B.: study conception and design; revision of the manuscript; co-supervision. F.M.A.: data collection; interpretation of data; revision of the manuscript. L.G.S.: interpretation of data; revision of the manuscript. M.A.S.: interpretation of data; revision of the manuscript. J.F.N.: study conception and design; acquisition of funding; writing and revision of the manuscript; supervision. All authors read and approved the final manuscript.

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Data availability N/A.

Declarations

Ethics approval This study was conducted according to the guidelines established by the Declaration of Helsinki. All the procedures involving study participants were approved by the Human Research Ethics Committee of the *Universidade Federal de Viçosa* (UFV) (reference number 663.171/2014), as well as by the Municipal Secretary of Education, the Regional Superintendent of Education and school principals.

Consent to participate Written informed consent was obtained from all parents/guardians.

Consent to publish N/A.

Conflict of interest The authors declare no competing interests.

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