Nutr Hosp. 2016; 33(3):588-594 ISSN 0212-1611 - CODEN NUHOEQ S.V.R. 318

ARÁN

Nutrición Hospitalaria

Trabajo Original

Obesidad y síndrome metabólico

Vaspin association with insulin resistance is related to physical activity and body fat in Brazilian adolescents - A cross-sectional study

La asociación de la vaspina con la resistencia a la insulina está relacionada con la actividad física y la grasa corporal en adolescentes brasileños. Un estudio transversal

Daniela Pala[1], Ana Paula Carlos-Cândido[2], Larissa Leandro-da-Cruz[3], Priscila Oliveira-Barbosa[1], Carla Teixeira-Silva[3], Ana Carolina Pinheiro-Volp[3], George Luiz Lins-Machado-Coelho[1,4] and Renata Nascimento-de-Freitas[1,3]

[1]NUPEB. Federal University of Ouro Preto. Ouro Preto, MG. Brazil. [2]Department of Nutrition. Federal University of Juiz de Fora. Juiz de Fora, MG. Brazil. [3]Department of Clinical and Social Nutrition. School of Nutrition. Federal University of Ouro Preto. Ouro Preto, MG. Brazil. [4]School of Medicine. Federal University of Ouro Preto. Ouro Preto, MG. Brazil

Abstract

Background: Vaspin is a newly-identified adipocytokine associated with insulin resistance (IR).

Objective: The aim of this study was to investigate the correlation between plasma vaspin concentrations and IR and determine whether this association is affected by body composition, physical activity and pubertal stage in adolescents.

Methods: Were studied 484 Brazilian adolescents aged 10–14 years whose anthropometric, clinical, biochemical, and lifestyle measurements were analized. We evaluated the correlation between vaspin and risk factors for IR in adolescents with normal and high body fat percentage (%BF) and did a logistic regression to calculate the odds ratio for IR according to vaspin quartiles sex specific for the sample.

Results: Vaspin was positively correlated with IR in adolescents with high %BF ($r = 0.23$, $p = 0.003$). The logistic regression analysis adjusted for sex, age, BMI, and pubertal stage showed that adolescents in the 2nd (OR = 0.43, 95% CI = 0.23-0.80, p = 0.008) and 3rd (OR = 0.46, 95% CI = 0.25-0.85, p = 0.014) quartile of vaspin concentration had a lower risk for IR. When the model was adjusted for %BF and physical activity, the association remained statically significant only for adolescents in the 2nd quartile.

Conclusion: Vaspin was correlated positively with risk factors associated with insulin metabolism in adolescents with high %BF. Vaspin was associated with a reduced risk of IR independently of BMI and pubertal stage and the association was influenced by body fat and physical activity in these adolescents.

Key words:

Vaspin. Risk factors. Insulin resistance. Physical activity. Body fat.

Resumen

Introducción: la vaspina es una adipocitoquina recientemente identificada que confiere resistencia a la insulina (IR).

Objetivo: el objetivo de este estudio fue investigar la correlación entre las concentraciones plasmáticas de vaspina y la IR para determinar si esta asociación se ve afectada por la composición corporal, la actividad física y la etapa de la pubertad en los adolescentes.

Métodos: fueron analizados las medidas antropométricas, clínicas, bioquímicas y el estilo de vida de 484 adolescentes brasileños de entre 10 y 14 años. Se evaluó la correlación entre los factores de riesgo para vaspina e IR en los adolescentes con porcentaje de grasa corporal normal y alto (%CG) e hicimos una regresión logística para calcular el cociente de probabilidad del IR de acuerdo con los cuartiles de vaspina para la muestra, con respecto al sexo.

Resultados: la vaspina se correlacionó positivamente con IR en adolescentes con alto %CG (r = 0,23; p = 0,003). El análisis de regresión logística ajustada por sexo, edad, índice de masa corporal y etapa puberal mostró que los adolescentes en el segundo (OR = 0,43; IC del 95% = desde 0,23 hasta 0,80, p = 0,008) y tercer (OR = 0,46; IC del 95% = 0,25-0,85, p = 0,014) cuartil de concentración de vaspina tenían un menor riesgo de IR. Cuando el modelo se ajustó para %GC y actividad física, la asociación siguió siendo estadísticamente significativa solo para los adolescentes en el segundo cuartil.

Conclusión: la vaspina se correlacionó positivamente con los factores de riesgo asociados con el metabolismo de la insulina en los adolescentes con alto %GC. Asimismo, se asoció con un menor riesgo de IR independientemente del índice de masa corporal y de etapa de la pubertad, y la asociación estuvo influenciada por la grasa corporal y la actividad física en estos adolescentes.

Palabras clave:

Vaspina. Factores de riesgo. Resistencia a la insulina. Actividad física. Grasa corporal.

Received: 12/09/2015
Accepted: 11/04/2016

Pala D, Carlos-Cândido AP, Leandro-da-Cruz L, Oliveira-Barbosa P, Teixeira-Silva C, Pinheiro-Volp AC, Lins-Machado-Coelho GL, Freitas RN. Vaspin association with insulin resistance is related to physical activity and body fat in Brazilian adolescents - A cross-sectional study. Nutr Hosp 2016;33:588-594

Correspondence:

Renata Nascimento de Freitas. NUPEB and Department of Clinical and Social Nutrition. School of Nutrition. Federal University of Ouro Preto. Campus Universitário. Morro do Cruzeiro.
35400-000 Ouro Preto, MG. Brazil
e-mail: rfreitasmail@gmail.com

INTRODUCTION

Insulin resistance (IR) is characterized by the reduced ability of plasma insulin to promote peripheral cell glucose uptake and has been identified as a public health problem, especially in youth population (1,2). It is well established that IR is present in obese and also in non-obese patients, and thus obese children and adolescents may not be insulin sensitive (3). Several cytokines secreted by the adipose tissue that may be involved in the modulation of insulin metabolism, can influence the risk of insulin resistance and have been studied to understand the association between increased adiposity and IR (4,5).

Vaspin is an adipokine originally isolated from visceral white adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model for abdominal obesity and type 2 diabetes mellitus (T2D), which may play a role on insulin sensitivity (6). Vaspin is a serpin (serine protease inhibitor) that may inhibit proteases that degrade hypoglycemic molecules and anorexigenic factors. Thus, besides being associated with improved blood glucose, vaspin is also associated with lower food intake (7,8). Recently, Kempf et al. (9) observed in the MONICA/KORA study an association between the AA genotype and increased risk of T2D independently of obesity, and they have suggested that the vaspin gene may be responsible for impaired glucose metabolism. Hida et al. (6) detected high vaspin expression in visceral adipose tissue of obese OLETF rats, but vaspin concentrations and expression were reduced after the animals developed T2D. Nevertheless, vaspin concentrations and expression were normalized after rats were treated with insulin and hypoglycemic drugs. Treatment with recombinant vaspin improved glucose tolerance and insulin sensitivity, it altered the expression of genes involved in IR, and acutely reduced food intake (6,10). However, the mechanisms whereby vaspin may act on insulin sensitivity are not fully understood.

Despite the growing number of studies in this area, studies on humans, especially adolescents, are still lacking. Korner et al. (11) observed an inverse association between vaspin and insulin resistance in a case-control study with children and adolescents. In addition, the authors found reduced vaspin concentrations in obese adolescents with IR in an oral glucose load test. Lee et al. (12) reported that a seven-day intensive short-term lifestyle modification including physical activity significantly decreased vaspin levels by 39.3% and improved insulin resistance in overweight and obese adolescents. Adolescence is characterized by significant changes in body composition with pubertal stage, especially body fat percentage (%BF) (13), which may affect vaspin secretion and function. Moreover, physical activity can alter body composition and may also affect vaspin secretion and function. Physical activity has been favorably associated with cardiometabolic risk factors and the risk of developing metabolic syndrome in young adults (14), but the mechanisms by which physical activity acts on these risk factors are not yet fully understood, and available data show that improvement in insulin sensitivity is not fully explained by physical activity (15,16). Because adipose tissue and physical activity may both affect vaspin secretion affecting insulin sensitivity, the objective of the present study was to investigate the association between vaspin concentrations and cardiovascular risk factors in adolescents with normal and high body fat percentage. Furthermore, we also examined the association between vaspin and insulin resistance and the influence of %BF, physical activity and pubertal stage on this association.

METHODS

STUDY POPULATION

This study evaluated data from 484 adolescents (235 boys and 249 girls) aged 10-14 years old (12 ± 1.4 years) who were enrolled in a 2006 cross-sectional population based study in urban area schools in the city of Ouro Preto (17). However 54 samples were not enough to determine vaspin concentration and 180 adolescents did not complete the protocol of physical activity. Informed consent was obtained from all individual participants or responsible for the adolescents included in the study.

VARIABLES MEASURED AND CUT-OFF POINTS

Body weight was measured on calibrated scales (TANITA® BF-542, Tanita Corporation of America, Arlington Heights, IL, USA) with 136 kg maximum capacity and accuracy to 0.2 kg. Adolescents were wearing light clothing, had an empty bladder, and were fasting for 12 hours. The height was evaluated using a WCS® portable stadiometer (Cardiomed, Curitiba, Brazil). The body mass index (BMI) was calculated as weight/height squared (kg/m^2). The waist circumference (WC) was measured at the midpoint between the last rib and the iliac crest, at expiration with the individual standing in the upright position (18). %BF was considered as an indicator of body composition and was estimated from measured tetrapolar bioelectrical impedance (BIA-T, Quantum II, RJL System). The cut-off values for high %BF recommended by William et al. (19) (≥ 30% for girls and ≥ 25% for boys) were adopted to categorize the subjects for comparison with other studies. The systolic and diastolic blood pressure were performed using an OMRON 795CP oscillometric device (Omron Healthcare, Kyoto, Japan) with the subject seated with the left arm at heart level. The extraction of blood samples was performed after 12 hours of fasting by trained pharmaceutical and serum were stored at -80 °C until analysis. The biochemical measurements were performed on stored serum and plasma: total cholesterol and fractions (HDL-c and LDL-c), triacylglycerols, glucose, fasting insulin and vaspin. Vaspin was determined at once in all samples after two years of storage without apparent defrost. According to the manufacturer, the kit is specific for the measurement of natural and recombinant human vaspin and reflects its actual concentration. The rest of analyses were done at once within six months from collection.

Vaspin concentration was measured using commercial kits for human diagnostic (AdipoGen®, Seoul, Korea) by enzyme linked immunosorbent assay (ELISA). Due to the lack of vaspin cut-off

values, the population was categorized into sex-specific vaspin concentration quartiles. Insulin was measured by a chemiluminescence immunoassay using a commercial insulin kit and an ADVIA Centaur® analyzer (Siemens Medical Solutions Diagnostics, New York, USA). Insulin resistance was estimated using the homeostasis model of insulin resistance (HOMA-IR) (20) and considered as high when it was higher than > 3.16, as proposed by Keskin et al. (21) Fasting glucose, triacylglycerols, total cholesterol, and HDL-c were analyzed using an enzymatic colorimetric assay with commercial kits (In Vitro Diagnostics, Itabira, Minas Gerais, Brazil).

Physical activity was assessed based on the Center for Disease Control and Prevention recommendations, which characterize as physically inactive individuals who practice less than 300 min of physical activity per week (22). The stage of sexual maturation was self-reported using Tanner stage ratings (23) to determine the stages for pubic hair, breasts, and genitalia development. The students were classified as prepubertal (Tanner stage PH1), pubertal (Tanner stage PH2-PH3), and post-pubertal (Tanner stage PH4-PH5).

STATISTICAL ANALYSES

As the vaspin concentration is related to body fatness, the clinical and biochemical characteristics of the adolescents are presented for all participants and also after categorization according to %BF. The Shapiro-Wilk test was performed to assess the assumption of normality distribution of all investigated variables. Because none of the variables followed a Gaussian distribution, including vaspin concentration, we used non-parametric tests. The Mann-Whitney U test and the Pearson's Chi-square (χ^2) test were used to compare continuous and categorical risk variables between %BF groups. The adolescents were categorized according to sex specific vaspin concentration quartiles. Logistic regression models were designed to determine the odds ratio (OR) for high HOMA-IR (≥ 3.16) in the different vaspin concentration quartiles, using the 4th quartile as reference, and adjusted for the confounding variables: sex, age, BMI, %BF, pubertal stage, and physical activity. Significant level was set at $p < 0.05$ in all analyses. Analyses were performed using STATA 9.0 (Statistical Software for Professionals) software (Stata Corporation, College Station, USA).

RESULTS

CLINICAL, BIOCHEMICAL AND LIFESTYLE VARIABLES

Selected characteristics of adolescents for the total population and according to %BF are shown in table I. As expected, BMI and WC were higher in adolescents with high %BF ($p < 0.05$). Similarly, cholesterol and LDL-C levels were also higher in the high %BF group ($p < 0.05$). There were no significant differences in vaspin, glucose, insulin, and HOMA-IR concentrations, as well as in pubertal stages and physical activity level between the high and low %BF groups.

VASPIN AND RISK FACTORS

The Spearman correlation showed an association between vaspin and age ($r = 0.10$, $p = 0.03$), insulin ($r = 0.8$, $p \leq 0.001$), and HOMA-IR ($r = 0.16$, $p = 0.002$) in all adolescents (Table II). In the high %BF group, we found associations between vaspin and BMI ($r = 0.17$, $p = 0.018$), triacylglycerols ($r = 0.18$, $p = 0.014$), insulin ($r = 0.25$, $p = 0.001$), and HOMA-IR ($r = 0.23$, $p = 0.003$). We found no association between vaspin and the other variables analyzed.

VASPIN AND INSULIN RESISTANCE

Based on the association between vaspin and IR, we designed logistic regression models to assess the risk for developing IR in adolescents according to vaspin concentration quartiles. Adolescents in the 2nd (OR = 0.48, [95% CI = 0.27-0.88], $p = 0.017$) and 3rd (OR = 0.48, [95% CI = 0.26-0.87], $p = 0.015$) vaspin quartiles had a lower risk for developing IR compared to those in the 4th quartile (Table III).

In addition, when we adjusted the model for BMI (model A) and pubertal stage (model B), the association remained significant, indicating that the relationship between vaspin and the risk for IR is independent of BMI and pubertal stage. However, when we included physical activity in the model, the risk for IR changed and the association was no longer significant for adolescents in the 3rd vaspin quartile (OR = 0.57, [95% CI = 0.30-1.08], $p = 0.083$). A similar result was observed when we adjusted the model for %BF only, indicating that physical activity and %BF affect the association between vaspin concentration and IR in adolescents.

DISCUSSION

In the present study, we observed no differences in plasma vaspin concentrations among adolescents with normal and high %BF. The few studies with adolescents were done with obese subjects who were classified according to BMI, which complicates the comparison of our results with available data. We found no correlation between vaspin and BMI in the total sample of adolescents, unlike some studies conducted with adults (24-26). However, other studies with children, adolescents and adults also found no correlation between vaspin and BMI (11,27,28). On the other hand, we found a positive correlation between vaspin and %BF, indicating that this adipokine has a positive relationship with adiposity. Chang et al. (28) used computed tomography to evaluate the area of visceral adipose tissue in adults and observed a positive association between vaspin and adiposity, but no association between vaspin and BMI in individuals with reduced insulin sensitivity. These results suggest that using body fat measure-

Table I. Clinical and biochemical characteristics of the adolescents and according to body fat percentage

	Total (n = 484)	Normal %BF (n = 267)	High %BF (n = 217)	
	Median IQR[a]			*p value*
Age (years) n = 484	12.0 (11.0-13.0)	13.0 (11.0-14.0)	12.0 (11.0-13.0)	< 0.001[a]
BMI (kg/m²) n = 484	18.19 (16.67-20.47)	17.84 (16.50-19.31)	18.82 (16.99-22.83)	< 0.001
WC (cm) n = 480	64.0 (60.0-69.50)	63.5 (60.0-67.0)	65.0 (61.0-74.0)	< 0.001
SBP (mmHg) n = 473	104.3 (96.0-111.5)	103.0 (95.3-110.7)	106.0 (98.0-113.0)	0.017
DBP (mmHg) n = 473	64.0 (58.8-69.7)	64.0 (58.0-70.0)	64.0 (59.0-69.0)	0.511
%BF n = 453	15.60 (6.50-23.60)	13.55 (5.80-21.90)	18.80 (7.90-28.40)	< 0.001
Cholesterol (mg/dl) n = 479	157.0 (137.5-180.0)	152.0 (131.0- 173.7)	164.0 (140.5-187.0)	< 0.001
HDL-c (mg/dl) n = 479	56.7 (47.1-67.0)	57.0 (48.0-66.2)	55.8 (46.3-67.7)	0.884
LDL-c (mg/dl) n = 478	84.66 (66.30-103.27)	80.38 (62.79-98.05)	91.49 (70.31-110.31)	< 0.001
Triacylglycerols (mg/dl) n = 479	67.60 (51.25-89.70)	64.37 (52.25-84.38)	70.73 (50.62-96.10)	0.156
Glucose (mg/dl) n = 478	83.50 (78.46-89.80)	83.5 5 (78.77-89.25)	83.46 (78.13-89.89)	0.513
Insulin (µUI/ml) n = 403	6.92 (4.89-10.43)	6.90 (4.96-9.97)	6.98 (4.72-10.63)	0.116
HOMA-IR n = 402	1.39 (0.99-2.17)	1.36 (1.04-2.05)	1.40 (0.95-2.18)	0.097
Vaspin (µg/ml) n = 430	0.55 (0.28-1.14)	0.65 (0.31-1.45)	0.48 (0.25-0.95)	0.201
	n (%)			*p value*[b]
Boys	235 (48.60)	105 (44.7)	130 (55.3)	< 0.001
Girls	249 (51.40)	162 (65.1)	87 (34.9)	
Physical activity (n = 304)				0.890
Physically active	102 (33.55)	55 (53.9)	47 (46.1)	
Physically inactive	202 (66.45)	144 (71.3)	58 (28.7)	
Pubertal stage (n = 480)				0.480
Pre-pubertal	224 (46.67)	122 (54.5)	102 (45.5)	
Pubertal	138 (28.75)	74 (53.6)	64 (46.4)	
Pos-pubertal	118 (24.58)	70 (59.3)	48 (40.7)	

%BF: Body fat percentage; BMI: Body mass index; DBP: Diastolic blood pressure; HDL-c: High-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL-c: Low density lipoprotein; SBP: Systolic blood pressure; WC: Waist circumference. [a]Data are given as median and interquartile range for non-parametric variables. Statistical significance was determined by U Man Whitney. [b]For %BF, statistical significance was determined by Chi- square test. Number of subjects varies according to the data availability.

ments instead of BMI is more suitable for evaluating the role of adipokines in obesity and its associated changes.

We observed a positive correlation between vaspin and insulin, HOMA-IR, BMI, and triacylglycerols in adolescents with high %BF. Suleymanoglu et al. (29) also found a positive correlation between vaspin concentrations and the same variables above in obese adolescents, but not in normal ones. We believe that high plasma vaspin concentrations may be an early compensatory response to increased IR with increasing %BF. Li et al. (30) conducted a clinical trial examining short-term continuous subcutaneous insulin infusion and observed a reduction in plasma vaspin in patients with T2D concomitant with reduction in insulin resistance.

The changes in body composition, especially fat distribution, that occur during adolescence render this period critical in initiating or aggravating obesity, because this stage of development is also associated with changes in dietary pattern such as greater intake of high-calorie foods and decreased physical activity (31,32).

The results of the multiple logistic regression analyses showed that the risk for developing IR was approximately 52% lower for individuals in the 2nd and 3rd quartiles compared to those in the 4th quartile. Moreover, adjustment for sex, age, BMI, and pubertal stage did not affect this association (Table III, models A and B). However, when we adjusted the model for physical activity or %BF instead of BMI (Table III, models C and D), the association was no longer significant for individuals in the 3rd quartile, but still significant for those in the 2nd quartile, suggesting a non-linear relationship between vaspin and IR. Thus, the protective role of vaspin for IR would be modulated by physical activity or %BF. Similarly as observed in OLEF rats, vaspin concentration seems

Table II. Correlation of plasma vaspin with risk variables according to body fat percentage

	Total		Normal %BF		High %BF	
Variables	*R*	*p*[a]	*r*	*p*	*r*	*p*
Age (years)	0.10	0.031	0.06	0.336	0.07	0.355
BMI (kg/m²)	0.08	0.087	0.07	0.288	0.17	0.018
WC (cm)	0.05	0.343	0.06	0.374	0.10	0.157
SBP (mmHg)	0.01	0.899	-0.01	0.878	0.06	0.404
DBP (mmHg)	0.06	0.227	0.06	0.485	0.10	0.186
%BF	0.13	0.010	0.15	0.022	0.15	0.041
Cholesterol (mg/dl)	0.01	0.836	-0.01	0.865	0.10	0.178
HDL-c (mg/dl)	-0.02	0.672	0.04	0.590	-0.10	0.169
LDL-c (mg/dl)	0.02	0.702	0.00	0.999	0.11	0.126
Triacylglycerols (mg/dl)	0.06	0.231	-0.03	0.620	0.18	0.014
Glucose (mg/dl)	-0.05	0.347	-0.03	0.687	-0.05	0.456
Insulin (µUI/ml)	0.18	< 0.001	0.14	0.052	0.25	< 0.001
HOMA-IR	0.16	0.002	0.12	0.085	0.23	0.003

%BF: Body fat percentage; BMI: Body mass index; DBP: Diastolic blood pressure; HDL-c: High-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL-c: Low density lipoprotein; SBP: Systolic blood pressure; WC: Waist circumference. [a]*Spearman's correlation analysis for vaspin plasma levels, n = 430.*

Table III. Crude and adjusted odds ratio and 95% CI for insulin resistance according to vaspin quartiles[a]

		Quartiles vaspin (µg/mL)			
		Q1	*Q2*	*Q3*	*Q4*
		(≤ 0.24♂ & 0.35♀)	*(0.25-0.46♂ & 0.36-0.65♀)*	*(0.47-0.86♂ & 0.66-1.49♀)*	*(≥ 0.87♂ & ≥ 1.50♀)*
Crude[b]	OR	0.84	0.48	0.48	1
	95% CI	0.49-1.44	0.27-0.88	0.26-0.87	Ref.
	p	0.520	0.017	0.015	-
Model A	OR	0.84	0.43	0.46	1
	95% CI	0.49-1.46	0.23-0.80	0.25-0.84	Ref.
	p	0.540	0.008	0.012	-
Model B	OR	0.84	0.43	0.46	1
	95%CI	0.48-1.46	0.23-0.80	0.25-0.85	Ref.
	p	0.537	0.008	0.014	-
Model C	OR	1.07	0.49	0.57	1
	95% CI	0.60-1.91	0.26-0.95	0.30-1.08	Ref.
	p	0.814	0.033	0.083	-
Model D	OR	0.95	0.51	0.59	1
	95% CI	0.53-1.72	0.26-0.97	0.31-1.11	Ref.
	p	0.880	0.039	0.102	-

[a]*Insulin resistance considered when HOMA-IR ≥ 3.16.* [b]*Model A: Age, sex e BMI; Model B: Age, sex, BMI and puberty stage; Model C: Age, sex, BMI, puberty stage and physical activity; Model D: Age, sex and %BF.*

to increase in adolescents with %BF until IR onset and after that its concentration decays while insulin concentration increases.

Similar non-linear relationship would occur with the effect of physical activity on the relationship between vaspin and IR.

Lee et al. (12) reported that vaspin concentrations inversely correlated with fasting insulin and HOMA-IR in obese adolescents. After a lifestyle modification program including physical activity, adolescents showed a modification of this correlation with a reduction of approximately 40% in vaspin concentrations with an improvement of HOMA-IR, although Martos-Moreno et al. (33) found no changes in vaspin concentrations in prepubertal children after a physical activity program for BMI reduction. Youn et al. (24) studied adults with normal and impaired glucose tolerance and T2D and found no association between vaspin and anthropometric or metabolic parameters, but after four weeks of physical training, the authors observed that circulating vaspin concentration increased significantly with concomitant reduction in BMI and reduction in insulin resistance. These results and ours suggest a role for physical activity in vaspin secretion and its effects on IR.

However, vaspin behavior after lifestyle modification and vaspin role in insulin sensitivity in combination with physical activity remains unclear. Moreover, it is still unclear whether vaspin modulates improved insulin sensitivity in combination with physical activity or if it is modulated by the altered body fat after a physical activity program. Our study was not designed to clarify this association, and further studies are needed to determine the role of vaspin in this relationship.

Because this is a cross-sectional study, we cannot conclude whether vaspin is acting directly on IR, because the alteration of insulin metabolism may also be the factor affecting vaspin concentrations. Nevertheless, this is an original study that provides evidence that the association between vaspin and IR is mediated by physical activity in adolescents. An additional strength of our study is the fair number of adolescents with several confounds variables available.

Taken together, the results of our study and other studies suggest that vaspin plays an important role in the metabolic changes associated with obesity or that it is, at least, a biomarker for these changes in adolescents. In conclusion, we showed that some risk factors associated with insulin-glucose metabolism are altered in adolescents with high %BF and correlate positively with vaspin concentrations. Even though the association between vaspin and IR remains unknown, increased vaspin concentrations are a protective factor for IR, independently of BMI and pubertal stage, and are highly influenced by body fat and physical activity in these adolescents.

ACKNOWLEDGEMENTS

The study was approved by the Ethics Committee of the Federal University of Ouro Preto, Minas Gerais, Brazil (no. 0017.238.000-05). We thank Professor Roney Luiz de Carvalho Nicolato, Cássio Zumerle Masioli, and Camila Guimarães Santiago for their help with laboratory measurements.

This study was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Federal University of Ouro Preto, Brazil.

COMPLIANCE WITH ETHICAL STANDARDS

In accordance with the principles of the 1964 Helsinki declaration, those responsible for the adolescents provided written informed consent to participate after a clear explanation of the study protocol. The study was approved by the Ethics Committee of the Federal University of Ouro Preto, Minas Gerais, Brazil (no. 0017.238.000-05).

REFERENCES

1. Reaven GM. Role of insulin resistance in human disease (syndrome X): An expanded definition. Ann Rev Med 1993;44:121-31.
2. Ten S, Maclaren N. Insulin resistance syndrome in children. J Clin Endocrinol Metab 2004;89(6):2526-39.
3. Brambilla P, Lissau I, Flodmark CE, Moreno LA, Widhalm K, Wabitsch M, et al. Metabolic risk-factor clustering estimation in children: To draw a line across pediatric metabolic syndrome. Int J Obes 2007;31(4):591-600.
4. Antuna-Puente B, Feve B, Fellahi S, Bastard JP. Adipokines: The missing link between insulin resistance and obesity. Diabetes Metab 2008;34(1):2-11.
5. Wada J. Vaspin: A novel serpin with insulin-sensitizing effects. Expert Opin Investig Drugs 2008;17(13):327-33.
6. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, et al. Visceral adipose tissue-derived serine protease inhibitor: A unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci USA 2005;26;102(30):10610.
7. Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG, et al. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. J Biol Chem 2001;276:33293-6.
8. Blüher M. Vaspin in obesity and diabetes: Pathophysiological and clinical significance. Endocrine 2012;41(2):176-82.
9. Kempf K, Rose B, Illig T, Rathmann W, Strassburger K, Thorand B, et al. (SERPINA12) Genotypes and risk of type 2 diabetes: Results from the MONICA/KORA studies. Exp Clin Endocrinol Diabetes 2010;118(3):184-9.
10. Kloting N, Kovacs P, Kern M, Heiker JT, Fasshauer M, Schon MR, et al. Central vaspin administration acutely reduces food intake and has sustained blood glucose-lowering effects. Diabetologia 2011;54(7):1819-23.
11. Korner A, Neef M, Friebe D, Erbs S, Kratzsch J, Dittrich K, et al. Vaspin is related to gender, puberty and deteriorating insulin sensitivity in children. Int J Obes 2011;35(4):578.
12. Lee MK, Jekal Y, Im JA, Kim E, Lee SH, Park JH, et al. Reduced serum vaspin concentrations in obese children following short-term intensive lifestyle modification. Clin Chim Acta 2010; 411(5-6):381-5.
13. Siervogel RM, Maynard LM, Wisemandle WA, Roche AF, Guo SS, Chumlea WC, et al. Annual changes in total body fat (TBF) and fat free mass (FFM) in children from 8 to 18 years in relation to changes in body mass index (BMI). The Fels Longitudinal Study. Ann NY Acad Sci 2000;904:420-3.
14. Van Mechelen W, Twisk JW, Post GB, Snel J, Kemper H. Physical activity of young people the Amsterdam Longitudinal Growth and Health Study. Med Sci Sports Exerc 2000;32:1610-6.
15. Platat C, Wagner A, Klumpp T, Schweitzer B, Simon C. Relationships of physical activity with metabolic syndrome features and low-grade inflammation in adolescents. Diabetologia 2006;49(9):2078-85
16. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89:2548-56.
17. Candido AP, Benedetto R, Castro AP, Carmo JS, Nicolato RL, Nascimento-Neto RM, et al. Cardiovascular risk factors in children and adolescents living in an urban area of Southeast of Brazil: Ouro Preto Study. Eur J Pediatr 2009;168(11):1373-82.
18. World Health Organization. WHO Expert Committee on Physical Status: The use and interpretation of anthropometry physical status. Geneva: WHO Technical Report Series; 1995.
19. William DP, Going SB, Lohman TG, Harsha DW, Srinivasan SR, Webber LS, et al. Body fatness and risk for elevated blood pressure, total cholesterol and serum lipoprotein ratios in children and adolescents. Am J Public Health 1992;82(3):358-63.
20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function

from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.
21. Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics 2005;115(4):500-3.
22. U.S. Department of Health and Human Services. Physical activity and health: A report of the surgeon general. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion; 1996.
23. Tanner, JM. Growth at adolescence. 2nd ed. Oxford: Blackwell Scientific Publications; 1962. p. 28-39:326.
24. Youn BS, Klöting N, Kratzsch J, Lee N, Park JW, Song ES, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes 2008;57:372-7.
25. Cho JK, Han TK, Kang HS. Combined effects of body mass index and cardio/respiratory fitness on serum vaspin concentrations in Korean young men. Eur J Appl Physiol 2010;108:347-53.
26. Seeger J, Ziegelmeier M, Bachmann A, Lössner U, Kratzsch J, Blüher M, et al. Serum levels of the adipokine vaspin in relation to metabolic and renal parameters. J Clin Endocrinol Metab 2008;93:247-51.
27. Aust G, Richter O, Rohm S, Kerner C, Hauss J, Klöting N, et al. Vaspin serum concentrations in patients with carotid stenosis. Atherosclerosis 2009;204:262-6.
28. Chang HM, Park HS, Park CY, Song YS, Jang YJ. Association between serum vaspin concentrations and visceral adipose tissue in Korean subjects. Metab 2010;59 (9):1276-81.
29. Suleymanoglu S, Tascilar E, Pirgon O, Tapan S, Meral C, Abaci A. Vaspin and its correlation with insulin sensitivity indices in obese children. Diabetes Res Clin Pract 2009;84(3):325-8.
30. Li K, Li L, Yang M, Liu H, Liu D, Yang H, et al. Short-term continuous subcutaneous insulin infusion decreases the plasma vaspin levels in patients with type 2 diabetes mellitus concomitant with improvement in insulin sensitivity. Eur J Endocrinol 2011;164(6):905-10.
31. Bray GA, Popkin BM. Dietary fat intake does affect obesity. Am J Clin Nutr 1998;68:1157-73.
32. Gortmaker SL, Must A, Sobol AM, Peterson K, Golditz GA, Dietz WH. Television viewing as a cause of increasing obesity among children in the United States, 1986-1990. Arch Pediatr Adolesc Med 1996;150:356-62.
33. Martos-Moreno GA, Kratzch J, Korner A, Barrios V, Hawkins F, Kiess W, et al. Serum visfatin and vaspin levels in prepubertal children: Effect of obesity and weight loss after behavior modifications on their secretion and relationship with glucose metabolism. Int J Obes 2011;35(10):1355-62.