



## ORIGINAL ARTICLE

# Adiposity, inflammation and fat-soluble vitamins in adolescents<sup>☆</sup>



Rebecca Peixoto Paes-Silva <sup>a,\*</sup>, Patrícia Calado Ferreira Pinheiro Gadelha<sup>a</sup>,  
Maria da Conceição Chaves de Lemos<sup>a</sup>, Célia Maria Machado Barbosa de Castro<sup>b</sup>,  
Ilma Kruze Grande de Arruda<sup>a</sup>, Alcides da Silva Diniz<sup>a</sup>

<sup>a</sup> Universidade Federal de Pernambuco (UFPE), Departamento de Nutrição, Recife, PE, Brazil

<sup>b</sup> Universidade Federal de Pernambuco (UFPE), Departamento de Medicina Tropical, Recife, PE, Brazil

Received 13 January 2018; accepted 4 May 2018

Available online 29 June 2018

### KEYWORDS

Obesity;  
Inflammation;  
Adolescents;  
Vitamins

### Abstract

**Objective:** Evaluate the association between inflammatory process, adiposity, and vitamins A, D, and E in adolescents, according to gender.

**Methods:** Cross-sectional study with adolescents aged 12–19 years old of both genders attending public schools in Recife. A questionnaire was used to collect data on socioeconomic level, lifestyle, and food intake of adolescents. Then, an anthropometric evaluation and a blood sampling were performed to analyze serum concentrations of  $\alpha$ -1-acid glycoprotein, retinol,  $\beta$ -carotene,  $\alpha$ -tocopherol, and 25-hydroxy-vitamin D.

**Results:** The levels of  $\alpha$ -1-acid glycoprotein were higher for abdominal obesity in both genders. Male adolescents with insufficient serum  $\alpha$ -tocopherol levels had low levels of  $\alpha$ -1-acid glycoprotein ( $p=0.03$ ) and an increased risk of 25-hydroxy-vitamin D and  $\beta$ -carotene deficiency in relation to total and abdominal fat; female adolescents had an increased risk of insufficient  $\beta$ -carotene with abdominal obesity (PR: 1.33; 95% CI: 1.2–1.5).

**Conclusion:** Abdominal adiposity implies a higher risk of inflammation and causes different changes to the levels of fat-soluble vitamins according to gender.

© 2019 Sociedade Brasileira de Pediatria. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

<sup>☆</sup> Please cite this article as: Paes-Silva RP, Gadelha PC, Lemos MC, Castro CM, Arruda IK, Diniz AS. Adiposity, inflammation and fat-soluble vitamins in adolescents. J Pediatr (Rio J). 2019;95:575–83.

\* Corresponding author.

E-mail: [rebecca.peixoto@gmail.com](mailto:rebecca.peixoto@gmail.com) (R.P. Paes-Silva).

**PALAVRAS-CHAVE**

Obesidade;  
Inflamação;  
Adolescentes;  
Vitaminas

**Adiposidade, inflamação e vitaminas lipossolúveis em adolescentes****Resumo**

**Objetivo:** Avaliar a associação entre processo inflamatório, adiposidade e as vitaminas A, D e E em adolescentes, segundo o sexo.

**Métodos:** Estudo transversal com adolescentes de 12 a 19 anos de ambos os sexos de escolas públicas de Recife. Foi utilizado um questionário para coleta de dados socioeconômicos, de estilo de vida e de consumo alimentar dos adolescentes. Em seguida, realizou-se a avaliação antropométrica e coleta de sangue para análise das concentrações séricas de  $\alpha$ -1-glicoproteína ácida, retinol,  $\beta$ -caroteno,  $\alpha$ -tocoferol e 25-hidroxivitamina D.

**Resultados:** Os níveis de  $\alpha$ -1-glicoproteína ácida foram maiores na obesidade abdominal de ambos os sexos. Os meninos com níveis séricos insuficientes de  $\alpha$ -tocoferol expressaram níveis reduzidos de  $\alpha$ -1-glicoproteína ácida ( $p = 0,03$ ) e apresentaram um maior risco de deficiência de 25-hidroxivitamina D e  $\beta$ -caroteno na adiposidade total e abdominal, enquanto as meninas mostraram maior risco de insuficiência de  $\beta$ -caroteno com a obesidade abdominal (RP 1,33; IC 95% 1,2-1,5).

**Conclusão:** A adiposidade abdominal reflete maior risco de inflamação e causa alterações distintas nas concentrações das vitaminas lipossolúveis, de acordo com o sexo.

© 2019 Sociedade Brasileira de Pediatria. Publicado por Elsevier Editora Ltda. Este é um artigo Open Access sob uma licença CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

**Introduction**

Obesity has been considered the most important nutritional disorder<sup>1</sup> and called a worldwide epidemic due to the high occurrence in children, adolescents, and young adults in recent years.<sup>2</sup> Excess weight is implicated in a wide variety of health problems due to the increase in body fat proportion and disorders associated with the increased mechanical load of adipose tissue.<sup>3</sup>

Evidence suggests that, in the presence of obesity, there may be a decrease in the levels of fat-soluble vitamins in plasma, due to a lower intake of these nutrients and/or higher deposition in adipose tissue, which, because they are soluble in fat, are deposited in the adipocytes, thus decreasing their bioavailability in individuals with excess adiposity.<sup>4</sup> In addition, vitamin-carrier proteins would behave as negative proteins in the acute phase of inflammation,<sup>5</sup> thus decreasing their availability in plasma during the chronic inflammatory processes found in obesity.

It has been observed that obese children and adolescents show a higher risk of low levels of fat-soluble vitamins when compared to those with adequate weight.<sup>6,7</sup> Since current nutritional recommendations do not consider the bioavailability of fat-soluble vitamins in the presence of obesity-related metabolic inflammation, a chronic inadequacy state for these micronutrients may place adolescents with excess weight at risk of nutritional disorder. Thus, the present study aimed to evaluate the association between inflammatory process, adiposity, and vitamins A, D, and E in adolescents, according to gender.

**Methods****Study design and sample**

This was a cross-sectional study of adolescents aged between 12 and 19 years, of both genders, carried out from March to April 2013, nested in a prospective cohort performed between 2007 and 2013, with randomly recruited adolescents using a multi-stage sampling process in public schools of Recife, northeastern Brazil. Adolescents receiving vitamin A, D, and E supplements or multivitamins in the last 3 months were excluded.

The sample size was based on an estimated prevalence ( $p$ ) of  $\alpha$ -tocopherol deficiency of 25%, a sampling error ( $d$ ) of 5.5%, a 95% confidence level ( $z$ ), and a design effect ( $c$ ) of 2.1, considering that sampling was carried out by clusters. Using the formula  $n = (z^2 \times p \times q \times c) / d^2$ , corrected for a finite population, that resulted in a minimum sample of 370 individuals. To correct for any losses, a percentage of 11% [ $100 / (100 - 11)$ ] was added. The final sample comprised 411 adolescent students.

**Evaluation methods and techniques**

The application of the food intake questionnaire used in the study, blood collection, and anthropometric measurements were performed by trained technicians who had knowledge of the procedures and study development routine.

**Dietary variables**

Food consumption was assessed by a semiquantitative food frequency questionnaire for adolescents (FFQA), developed

and validated by Slater et al.,<sup>8</sup> which was adapted for foods commonly used in the region. The analysis was performed through the DietSys software version 4.01 (National Cancer Institute – Bethesda, MD, USA). The results of vitamin A, D, and E consumption were compared with the dietary reference intakes (DRI's) proposed by the Institute of Medicine<sup>9,10</sup> and classified according to the estimated average requirement (EAR). An adequate vitamin A intake for boys was considered: >630  $\mu\text{g}/\text{day}$  (9–13 years), >485  $\text{mg}/\text{day}$  (14–18 years); and vitamin A intake for girls: >625  $\text{mg}/\text{day}$  (9–13 years), >500  $\mu\text{g}/\text{day}$  (14–18 years); vitamin E intake: >12  $\text{mg}/\text{day}$ ; and vitamin D >10  $\mu\text{g}/\text{day}$ .

### Anthropometric variables

Weight and height measurements were carried out according to the original technique recommended by Lohman et al.,<sup>11</sup> and waist circumference (WC) was obtained according to the criteria of Taylor et al.<sup>12</sup> The adolescents' nutritional diagnosis was defined according to the World Health Organization (WHO) body mass index (BMI) curves.<sup>13</sup> The indicators BMI for age (BMI/A) and height for age (H/A), expressed in Z-scores, were used to classify the nutritional status of the students. For the H/A indicator, the following were considered: short stature: <score  $-2$ ; adequate height:  $\geq$ score  $-2$  and  $\leq$  score  $+2$  and tall stature: <score  $+2$ . For BMI/A it was considered: low weight: <score  $-2.0$ ; normal weight:  $\geq$ Z-score  $-2$  and  $\leq 1$ ; overweight: >Z-score  $+1$  and  $\leq +2$ ; obesity: >Z-score  $+2$ .<sup>12</sup> Overweight individuals were grouped with their obese peers, being classified in the excess weight category. The diagnosis of excess weight was obtained when it was greater than  $+1$  SD. In the diagnosis of abdominal obesity, the cutoff point for the classification of WC was the one used by Taylor et al.,<sup>12</sup> and the cutoff used for the waist-to-height ratio (WHtR) was that recommended by Li et al.<sup>14</sup>

### Biochemical variables

A 10 mL blood sample was collected for the biochemical analyses after a 10- to 12-hour fast. The tubes were stored and transported for sample processing at laboratory of clinical analyses (LAPAC), where the concentrations of  $\alpha$ -1-acid glycoprotein (AGA) and 25-hydroxyvitamin D (25(OH)D) were analyzed. A serum aliquot (2 mL) was frozen and then sent to the Micronutrient Investigation Center (CIMICRON) of the Universidade Federal da Paraíba (UFPB), to determine the serum levels of retinol,  $\beta$ -carotene, and  $\alpha$ -tocopherol.

#### $\alpha$ -1-Acid glycoprotein

AGA was quantified by immunoturbidimetry using Roche reagents (Roche<sup>TM</sup>, USA), calibrators and controls, in an automated Cobas Mira system (Roche<sup>TM</sup>, USA). The presence of inflammation was defined as a value  $>0.9 \text{ g/L}$ .

#### Retinol, $\beta$ -carotene, and $\alpha$ -tocopherol

The quantification of serum retinol,  $\beta$ -carotene, and  $\alpha$ -tocopherol levels followed the technical procedure

described by Erhardt et al.<sup>15</sup> The cutoff points developed by the WHO for retinol levels were used, considering: retinol deficiency when  $<0.70 \mu\text{mol/L}$ . For  $\alpha$ -tocopherol,  $\geq 12 \mu\text{mol/L}$ <sup>16</sup> was used as reference, while  $\beta$ -carotene values were considered adequate when  $>0.9 \mu\text{mol/L}$ .<sup>17</sup>

### 25-Hydroxy vitamin D

25(OH)D levels were measured using the high-performance liquid chromatography (HPLC) method. 25(OH)D insufficiency was defined as levels  $<72.5 \text{ nmol/L}$ .<sup>18</sup>

### Data analysis

The data were typed in duplicate and verified through VALIDATE, a module of Epi-Info, version 6.0 (WHO/CDC – Atlanta, GE, USA) to verify consistency during the typing process. Statistical analysis of the data was performed with SPSS version 13.0 (SPSS Inc. – Chicago, IL, USA).

Continuous variables were tested for normality using the Kolmogorov-Smirnov test to evaluate the symmetry of the distribution curve of the variables. Data from variables with normal distribution were expressed as mean and standard deviation. The variables with non-Gaussian distribution were presented as median and interquartile range (IQR). In the description of the proportions, the binomial distribution was approximated to the normal distribution by the 95% confidence interval (95% CI).

In the univariate analysis, the strength of the association was assessed by the prevalence ratio and respective confidence interval, and Pearson's chi-squared test. Student's *t*-test or its non-parametric equivalent, Mann Whitney U-test, was used for the comparison between continuous data from independent samples. All statistical tests considered a significance level of 5% to reject the null hypothesis.

### Ethical aspects

The study was approved by the Ethics Committee for Human Research of Lauro Wanderley University Hospital of Universidade Federal da Paraíba (CEP/HULW Registry number 723/10), based on the ethical principles for research involving human beings, as stated, at that time, in Resolution 196/96 of the National Health Council. Adolescents and their parents/guardians were previously informed of the research objectives, as well as the methods to be used. After giving their consent, the parents/guardians signed an informed consent.

### Results

A total of 411 adolescents with a median age of 15 years (IQR: 14–16 years) participated in the study. According to the BMI/age, it was observed that 27.9% had excess weight, with 17.4% ( $n=71$ ) being overweight and 10.5% ( $n=43$ ) obese. When abdominal adiposity was evaluated, 16.8% and 14.4% had elevated WC and WHtR, respectively. Regarding serum levels of fat-soluble vitamins, most had low levels of retinol,  $\alpha$ -tocopherol,  $\beta$ -carotene, and 25(OH)D.

**Table 1** Characteristics of school adolescents in Recife, Northeastern Brazil, 2013.

Variables	<i>n</i>	%	95% CI
<i>Gender</i>			
Male	160	38.9	34.1–43.8
Female	251	61.1	56.1–65.8
<i>BMI/age</i>			
With excess weight	114	27.9	23.6–32.6
Without excess weight	294	72.1	67.4–76.4
<i>WC</i>			
Normal	342	83.2	79.2–86.7
Elevated	69	16.8	13.3–20.8
<i>WhtR</i>			
Normal	352	85.6	81.9–88.9
Elevated	59	14.4	11.1–18.1
<b>Serum levels</b>			
<i>Retinol</i>			
Adequate	265	67.9	63.1–72.6
Insufficient	125	32.1	27.4–36.9
<i>β-carotene</i>			
Adequate	84	25.9	21.2–31.0
Insufficient	240	74.1	68.9–78.7
<i>α-tocopherol</i>			
Adequate	47	11.9	8.9–15.5
Insufficient	347	88.1	84.4–91.1
<i>25(OH)D</i>			
Adequate	202	49.1	44.2–54.1
Insufficient	209	50.9	45.9–55.8
<b>Dietary intake</b>			
<i>Vitamin A</i>			
Adequate	285	71.3	66.5–75.6
Inadequate	115	28.8	24.3–33.4
<i>Vitamin E</i>			
Adequate	14	16.3	2.2–6.6
Inadequate	335	83.8	93.4–97.8
<i>Vitamin D</i>			
Adequate	3	0.7	0.1–2.1
Inadequate	408	99.3	97.9–99.8

BMI, body mass index; WC, waist circumference; WhtR, waist-to-height ratio; CI, confidence interval; 25(OH)D, 25-hydroxy vitamin D, adequate serum retinol: >0.7 μmol/L, adequate β-carotene: >0.9 μmol/L; adequate α-tocopherol: >12 μmol/L; adequate 25 (OH) D: >72.4 nmol/L. Estimated average requirement (EAR) of vitamin A for boys: >630 μg/day (9–13 years), >485 mg/day (14–18 years), vitamin A for girls: >625 mg/day (9–13 years), >500 μg/day (14–18 years); vitamin E >12 mg/day; vitamin D >10 μg/day.

Inadequate values were also observed when evaluating the dietary intake of vitamins A, D, and E, as shown in [Table 1](#).

Regarding the inflammatory process, AGA did not show alterations in serum levels with the increase in total adiposity, but it was increased when male adolescents were classified as having abdominal obesity using the WC ( $p=0.04$ ) and WhtR ( $p=0.03$ ), whereas girls had their levels increased only with elevated WC ( $p<0.01$ ; [Table 2](#)). Regarding vitamin concentrations, AGA showed a reduction in its levels in the presence of insufficient amounts of serum α-tocopherol in male adolescents ( $p=0.03$ ). Regarding dietary intake, AGA did not show a significant difference with variations in the intake of fat-soluble vitamins.

When evaluating the fat-soluble vitamin levels according to the nutritional status, boys showed an increase in retinol and α-tocopherol levels with increased adiposity, as well as a decrease in β-carotene and 25(OH)D levels. As for girls, only α-tocopherol showed significantly higher levels with higher adiposity, as shown in [Table 3](#). When evaluating the association between adiposity type and vitamin levels, boys showed a higher risk of 25(OH)D and β-carotene deficiency in total and abdominal adiposity; however, when adiposity was absent, they showed a risk of retinol and α-tocopherol deficiency ([Table 4](#)). Girls, however, only showed the risk of β-carotene insufficiency when WC (PR = 1.33) and WhtR (PR = 1.33) were elevated.

**Table 2** Serum levels of alpha-1-acid glycoprotein according to nutritional status, serum concentrations and dietary intake of fat-soluble vitamins in adolescent students from Recife, Northeast Brazil, 2013.

	N	alpha-1-acid glycoprotein (g/L)					
		Male gender			Female gender		
		n	Mean ± SD	p <sup>a</sup>	n	Mean ± SD	p <sup>a</sup>
<b>Nutritional status</b>							
<i>BMI/age</i>							
Without excess weight	294	107	0.73 ± 0.19	0.09	187	0.75 ± 0.17	0.33
With excess weight	114	53	0.78 ± 0.17			0.78 ± 0.16	
<i>WC</i>							
Normal	342	121	0.73 ± 0.19	0.04	221	0.75 ± 0.17	<0.01
Elevated	69	39	0.80 ± 0.17		30	0.83 ± 0.16	
<i>WHtR</i>							
Normal	352	130	0.73 ± 0.19	0.03	222	0.75 ± 0.17	0.06
Elevated	59	30	0.81 ± 0.17		29	0.81 ± 0.16	
<b>Serum levels</b>							
<i>Retinol</i>							
Adequate	265	44	0.72 ± 0.20	0.26	81	0.78 ± 0.16	0.10
Insufficient	125	109	0.76 ± 0.19		156	0.75 ± 0.17	
<i>α-tocopherol</i>							
Adequate	47	27	0.82 ± 0.16	0.03	20	0.77 ± 0.13	0.88
Insufficient	347	126	0.73 ± 0.19		221	0.76 ± 0.17	
<i>25(OH)D</i>							
Adequate	202	96	0.73 ± 0.18	0.27	106	0.75 ± 0.16	0.58
Insufficient	209	64	0.77 ± 0.19		145	0.76 ± 0.18	
<i>β-carotene</i>							
Adequate	84	33	0.76 ± 0.23	0.82	51	0.77 ± 0.17	0.75
Insufficient	240	89	0.77 ± 0.17		151	0.76 ± 0.16	
<b>Dietary intake</b>							
<i>Vitamin A</i>							
Adequate	199	09	0.73 ± 0.19	0.68	176	0.77 ± 0.17	0.11
Inadequate	201	46	0.77 ± 0.19		69	0.73 ± 0.15	
<i>Vitamin E</i>							
Adequate	24	24	0.71 ± 0.16	0.31	41	0.73 ± 0.16	0.29
Inadequate	376	131	0.75 ± 0.18		204	0.76 ± 0.17	
<i>Vitamin D</i>							
Adequate	1	2	0.73 ± 0.32	0.93	1	-	0.15
Inadequate	410	158	0.74 ± 0.18		250	0.75 ± 0.17	

<sup>a</sup> Student's *t*-test. BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; adequate serum retinol: >0.7 μmol/L, adequate β-carotene: >0.9 μmol/L; adequate α-tocopherol: >12 μmol/L; adequate 25(OH)D: >72.4 nmol/L. Estimated average requirement (EAR) of vitamin A for boys: >630 μg/day (9–13 years), >485 mg/day (14–18 years), vitamin A for girls: >625 mg/day (9–13 years), >500 μg/day (14–18 years); vitamin E >12 mg/day; vitamin D >10 μg/day.

## Discussion

The high prevalence of excess weight among adolescents reflects the magnitude of the problem in this age group. Although body fat is related to some metabolic complications, several consequences of obesity are more strongly associated with the amount of abdominal fat<sup>19</sup>; this adipose deposition plays a key role and may induce health risks in overweight and obese individuals.<sup>20</sup> It is postulated that obese individuals have persistent inflammation

of the adipose tissue, and the proinflammatory cytokines, associated with the adipose tissue accumulation, would explain the development of obesity-related pathologies.<sup>21</sup> In the evaluation between inflammation and adiposity, it was observed that AGA showed higher concentrations only in the presence of abdominal obesity in both genders, and no difference was observed in total adiposity. There are discordant findings in the literature, since there are reports of elevated AGA in adolescents classified as overweight and obese according to the BMI.<sup>22</sup> Alfadda et al.<sup>23</sup> observed

**Table 3** Serum values of retinol,  $\alpha$ -tocopherol,  $\beta$ -carotene and 25 hydroxy-vitamin D, according to body mass index, waist circumference and waist-to-height ratio in adolescent students from Recife, Northeastern Brazil, 2013.

	Retinol ( $\mu\text{mol/L}$ )			$\alpha$ -tocopherol ( $\mu\text{mol/L}$ )			$\beta$ -carotene ( $\mu\text{mol/L}$ )			25(OH)D (nmol/L)		
	<i>n</i>	Mean $\pm$ SD	<i>p</i> <sup>a</sup>	<i>n</i>	Median (IQR)	<i>p</i> <sup>b</sup>	<i>n</i>	Median (IQR)	<i>p</i> <sup>b</sup>	<i>n</i>	Median (IQR)	<i>p</i> <sup>b</sup>
<b>Male gender</b>												
<i>BMI/age</i>												
With excess weight	53	0.97 $\pm$ 0.35	0.02	51	7.94 (6.33–12.35)	0.02	44	0.8969 (0.8967–0.8975)	<0.001	53	68.25 (58.44–85.41)	0.001
Without excess weight	102	0.84 $\pm$ 0.32		102	6.75 (5.63–8.75)		78	0.8977 (0.8968–0.9249)		107	82.21 (67.42–101.14)	
<i>WC</i>												
Elevated	39	1.02 $\pm$ 0.37	0.03	37	8.56 (6.93–13.83)	0.003	35	0.8969 (0.8967–0.8976)	0.006	69	67.83 (58.34–80.89)	0.002
Normal	116	0.84 $\pm$ 0.31		116	6.59 (5.51–8.64)		87	0.8975 (0.8967–0.9206)		121	81.12 (65.52–101.14)	
<i>WHtR</i>												
Elevated	30	1.05 $\pm$ 0.31	0.02	30	10.67 (7.24–17.12)	<0.001	28	0.8971 (0.8967–0.8978)	0.14	30	66.76 (58.99–75.69)	<0.001
Normal	125	0.85 $\pm$ 0.33		123	6.64 (5.45–8.37)		94	0.8973 (0.8967–0.9159)		130	81.00 (64.97–102.05)	
<b>Female gender</b>												
<i>BMI/age</i>												
With excess weight	60	0.85 $\pm$ 0.40	0.23	60	7.26 (6.34–8.45)	0.03	52	0.8971 (0.8968–0.8985)	0.08	61	66.30 (57.12–78.67)	0.35
Without excess weight	180	0.79 $\pm$ 0.32		179	6.51 (4.65–8.21)		148	0.89780 (0.8970–0.9024)		187	68.72 (59.02–82.21)	
<i>WC</i>												
Elevated	29	0.87 $\pm$ 0.46	0.26	29	7.42 (6.60–8.89)	0.02	24	0.8971 (0.8969–0.8979)	0.09	30	65.41 (55.82–78.57)	0.26
Normal	213	0.80 $\pm$ 0.32		212	6.62 (4.74–8.19)		178	0.8977 (0.8969–0.9022)		221	68.51 (59.12–83.33)	
<i>WHtR</i>												
Elevated	28	0.82 $\pm$ 0.39	0.86	28	7.41 (6.85–8.34)	0.03	24	0.8971 (0.8969–0.8979)	0.10	29	66.53 (56.08–78.67)	0.58
Normal	214	0.81 $\pm$ 0.33		213	6.61 (4.70–8.23)		178	0.8977 (0.8969–0.9022)		222	68.38 (58.49–82.54)	

BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; SD, standard deviation; IQR, interquartile range.

<sup>a</sup> Unpaired Student's *t*-test.

<sup>b</sup> Mann-Whitney test.

**Table 4** Factors associated with inadequate serum levels of retinol,  $\alpha$ -tocopherol,  $\beta$ -carotene, and 25-hydroxy-vitamin D in adolescent students from Recife, Northeastern Brazil, 2013.

	Retinol ( $\mu\text{mol/L}$ )					$\alpha$ -tocopherol ( $\mu\text{mol/L}$ )					$\beta$ -carotene ( $\mu\text{mol/L}$ )					25(OH)D (nmol/L)						
	Insufficient			PR (95% CI)	$p^a$	Insufficient			PR (95% CI)	$p^a$	Insufficient			PR (95% CI)	$p^a$	Insufficient			PR (95% CI)	$p^a$		
	N	n	%			N	n	%			N	n	%			N	n	%			N	n
<b>Male gender</b>																						
<i>BMI/age</i>																						
With excess weight	52	11	21.2	1.00			51	37	72.5	1.00			44	40	90.9	1.46 (1.2–1.8)	<0.01	53	29	54.7	1.67 (1.2–2.41)	0.01
Without excess weight	101	33	32.7	1.54 (0.8–2.8)	0.19		102	89	87.3	1.20 (1.0–1.4)	0.04		78	49	62.8	1.00		107	35	32.7	1.00	
<i>WC</i>																						
Elevated	38	6	15.8	1.00			37	25	67.6	1.00			35	32	91.4	1.40 (1.2–1.7)	<0.01	39	22	56.4	1.63 (1.1–2.3)	0.02
Normal	115	38	33.0	6.33 (2.8–14.4)	0.06		116	101	87.1	1.29 (1.02–1.6)	0.01		87	57	65.5	1.00		121	42	34.7	1.00	
<i>WHtR</i>																						
Elevated	29	3	10.3	1.00			30	18	60.0	1.00			28	24	85.7	1.24 (1.0–1.5)	0.09 <sup>b</sup>	30	19	63.3	1.83 (1.3–2.6)	<0.01
Normal	124	41	33.1	6.47 (2.2–19.9)	0.02		123	108	87.8	1.46 (1.1–1.9)	<0.01		94	65	69.1	1.00		130	45	34.6	1.00	
<b>Female gender</b>																						
<i>BMI/age</i>																						
With excess weight	57	20	35.1	1.64 (0.7–1.6)	0.87		60	52	86.7	1.00			52	44	84.6	1.19 (1.0–1.4)	0.06	61	38	62.3	1.09 (0.9–1.4)	0.55
Without excess weight	178	60	33.7	1.00			179	167	93.3	1.08 (0.97–1.2)	0.11		148	105	70.9	1.00		187	107	57.2	1.00	
<i>WC</i>																						
Elevated	27	9	33.3	1.00			29	24	82.8	1.00			24	23	95.8	1.33 (1.2–1.5)	0.01	30	19	63.3	1.11 (0.8–1.5)	0.56
Normal	210	72	34.3	1.03 (0.6–1.8)	1.00		212	197	92.9	1.12 (0.95–1.3)	0.07 <sup>b</sup>		178	128	71.9	1.00		221	126	57.0	1.00	
<i>WHtR</i>																						
Elevated	25	8	32.0	1.00			28	25	89.3	1.00			24	23	95.8	1.33 (1.2–1.5)	0.01	29	19	65.5	1.15 (0.9–1.5)	0.43
Normal	212	73	34.4	1.08 (0.6–1.9)	1.00		213	196	92.0	1.03 (0.9–1.2)	0.71 <sup>b</sup>		178	128	71.9	1.00		222	126	56.8	1.00	

BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; PR, prevalence ratio; CI, Confidence Interval; Adequate serum retinol >0.7  $\mu\text{mol/L}$ , adequate  $\beta$ -carotene: >0.9  $\mu\text{mol/L}$ ; adequate  $\alpha$ -tocopherol: >12  $\mu\text{mol/L}$ ; adequate 25(OH)D: >72.4 nmol/L.

<sup>a</sup> Chi-squared test.

<sup>b</sup> Fisher's exact test.

that AGA mRNA expression would be correlated with TNF- $\alpha$  and adiponectin expression of subcutaneous adipose tissue, whereas in the visceral adipose tissue this expression is correlated with TNF- $\alpha$ , IL-6, and adiponectin, demonstrating greater AGA expression with abdominal fat deposition. This association could partly justify the present findings, since perhaps the greatest inducer for AGA elevation would be the inflammatory cytokines released by visceral adipose tissue. Additionally, when using only the BMI to classify obesity, a misdiagnosis may occur, because it is not sensitive in predicting the area of fat deposition or if the increase in body mass consists in greater amounts of muscle or fat.

The literature shows that, in the presence of inflammation, the levels of retinol,<sup>24</sup> vitamin E,<sup>25</sup> and vitamin D<sup>6</sup> are significantly lower. Plasma concentrations of micronutrients are at least in part mediated by proinflammatory cytokines that suppress the hepatic production of many carrier proteins, increase capillary permeability, and promote the sequestration of some micronutrients to the liver and other organs.<sup>26</sup>

In the present study, it was not possible to evaluate the vitamins according to the presence of the inflammatory process, since none of the assessed adolescents had inflammation according to the cutoff point established for AGA. Nonetheless, when evaluating the mean concentration of AGA according to vitamin status, it was observed that male adolescents with the highest levels of AGA had adequate  $\alpha$ -tocopherol values. According to previously mentioned reports, it would be expected to find an inverse association between inflammation and  $\alpha$ -tocopherol. However, Ulatowski et al.<sup>27</sup> reported that oxidative stress may increase the expression of the  $\alpha$ -tocopherol transfer protein ( $\alpha$ -TTP) gene, because as this vitamin is the main fat-soluble antioxidant, an increase in the distribution of vitamin E would prevent further oxidative damage.

When assessing vitamin levels in the presence of excess weight according to gender, boys with abdominal obesity showed an increase in retinol and  $\alpha$ -tocopherol, while  $\beta$ -carotene and 25(OH)D were reduced. In girls, only  $\alpha$ -tocopherol showed increased levels in the presence of central adiposity. These results demonstrate that increased abdominal adiposity could have a distinct influence on vitamin levels according to gender, possibly due to variations in hormone action mechanisms on the effects of obesity-mediated inflammation.

In an attempt to elucidate the association between excess adiposity, gender, inflammation, and endocrine signaling, Bloor and Symonds,<sup>28</sup> through a literature review, described an increase in proinflammatory cytokines that occurs in obesity, with a subsequent activation of the hypothalamic-pituitary-adrenal (HPA) axis, which increases cortisol and contributes to increased deposition of visceral adipose tissue in males. Increased central adiposity also occurs in females; however, there is a gradual transition, with a decreased deposition of subcutaneous adipose tissue in the lower and upper limbs to the development of central adiposity, which gives females protection against the inflammatory process, as it prevents the visceral deposit from acting as an increased stress signaling factor in the HPA.

Therefore, boys would be more prone to triglyceride accumulation in adipocytes in the central region, leading to hypertrophy and increased production and secretion of adipokines in circulation, since adipokine gene expression is positively correlated with adipocyte size.<sup>29</sup> As a consequence of the inflammation, there would be initially a greater mobilization of antioxidant vitamins in an attempt to fight oxidative stress, which could justify the increase in retinol and  $\alpha$ -tocopherol levels. As for  $\beta$ -carotene, as in addition to having an antioxidant function, it would also be diverted to conversion into vitamin A in the liver, and it would probably have reduced levels in the presence of the inflammatory process. This increased demand for  $\beta$ -carotene could explain the greater risk of  $\beta$ -carotene insufficiency found in adolescents of both genders with abdominal obesity.

The 25(OH)D, on the other hand, may have its levels reduced by increased storage in adipose tissue.<sup>4</sup> Moreover, plasma levels of 25(OH)D have been reported to be independently associated with CRP (C-reactive protein) and albumin,<sup>30</sup> i.e., in the presence of systemic inflammation, acute-phase negative proteins such as albumin are reduced, which would decrease the transport of this vitamin into the bloodstream.

The approach of the studies carried out in adolescents found in the literature shows the absence of the association between fat-soluble vitamin levels and the inflammatory process. However, Duncan et al.,<sup>31</sup> when evaluating the correlation coefficients of CRP in comparison to several micronutrients (including vitamins A, D, and E) in the plasma of adult individuals, found a poor correlation, indicating that the association is highly variable from patient to patient, suggesting that the interpretation of plasma micronutrient deficiency should only be performed when the presence of inflammation is ruled out.

Thus, the present study demonstrated that excess weight in adolescents may put these individuals at risk of metabolic inflammation when fat accumulation occurs in the abdominal region. Additionally, the increase in abdominal adiposity occurred with distinct alterations in the serum levels of fat-soluble vitamins according to gender.

Although there was no direct association between inflammation and the assessed vitamins, it is suggested that factors such as obesity and inflammation be considered in the biochemical interpretation of fat-soluble vitamins, to prevent the risk of data misinterpretation. However, further studies are required to better understand the mechanisms involved between the chronic inflammatory process present in obesity and the behavior of fat-soluble vitamins, their metabolic pathways, and their action mechanism in this process, considering the difference between genders.

As for the present study limitations, because it was a cross-sectional study, it was not possible to guarantee causal associations, and confounding variables may have affected the reported associations. Additionally, only one biochemical marker was used to monitor the presence of inflammation, i.e., AGA levels, which despite being considered an inflammatory marker of greater clinical validity, may not show greater diagnostic discriminatory power in specific inflammatory situations.



## Funding

This study was funded by Conselho Nacional de Pesquisa (CNPq) (process No. 473387/2010-2) and the Ministry of Science and Technology (IMIP/MCT agreement; process No. 01.0265.00/2005).

## Conflicts of interest

The authors declare no conflicts of interest.

## References

- Pereira PA, Lopes LC. Obesidade infantil: estudo em crianças num ATL. *Millenium*. 2012;42:105–25.
- NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet*. 2017;390:2627–42.
- Wirth MW, Hauner H. The prevention and treatment of obesity. *Dtsch Arztebl Int*. 2014;111:705–13.
- Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr*. 2000;72:690–3.
- Rosales FJ, Ross AC. A Low molar ratio of retinol binding protein to transthyretin indicates vitamin A deficiency during inflammation: studies in rats and a posteriori analysis of vitamin A-supplemented children with measles. *J Nutr*. 1998;128:1681–7.
- Plesner JL, Dahl M, Fonvig CE, Nielsen TR, Kloppenborg JT, Pedersen O, et al. Obesity is associated with vitamin D deficiency in Danish children and adolescents. *J Pediatr Endocrinol Metab*. 2017;31:53–61.
- de Souza LV, da Veiga GV, Ramalho RA. Association of serum concentrations of retinol and carotenoids with overweight in children and adolescents. *Nutrition*. 2007;23:392–7.
- Slater B, Fisberg RM, Philippi ST, Latorre MR. Validation of a semi-quantitative adolescents food frequency questionnaire applies at a public school in São Paulo Brazil. *Eur J Clin Nutr*. 2003;57:629–35.
- Institute of Medicine (IOM). National Research Council. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington DC: National Academy Press; 2002.
- Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academies Press; 2010.
- Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign: Human Kinetics Books; 1988.
- Taylor RW, Jones IE, Williams SM, Goulding A. Evaluation of waist circumference, waist-to-hip ratio, and the conicity index as screening tools for high trunk fat mass, as measured by dual-energy X-ray absorptiometry, in children aged 3–19 y. *Am J Clin Nutr*. 2000;72:490–5.
- de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Sieckmann J, et al. Development of a WHO growth reference for school-aged children and adolescents. *WHO Bull OMS*. 2007;85:660–7.
- Li C, Ford ES, Mokdad AH, Cook S. Recent trends in waist circumference and waist-height ratio among US children and adolescents. *Pediatrics*. 2006;118:8–1390.
- Erhardt JG, Mack H, Sobock U, Biesalski HK.  $\beta$ -Carotene and  $\alpha$ -tocopherol concentration and antioxidant status in buccal mucosal cells and plasma after oral supplementation. *Braz J Nutr*. 2002;87:471–5.
- European Food Safety Authority (EFSA). Scientific opinion on dietary reference values for vitamin E as  $\alpha$ -tocopherol. *EFSA J*. 2015;13:4149.
- Pereira JA, Paiva AA, Bergamaschi DP, Rondó PH, Oliveira GC, Lopes IB, et al. Concentrações de retinol e de beta-caroteno séricos e perfil nutricional de crianças em Teresina, Piauí, Brasil. *Rev Bras Epidemiol*. 2008;11:287–96.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911–30.
- Kelishadi R, Mirmoghtadaee P, Najafi H, Keikha M. Systematic review on the association of abdominal obesity in children and adolescents with cardio-metabolic risk factors. *J Res Med Sci*. 2015;20:294–307.
- Tchernof A, Després JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev*. 2013;93:359–404.
- Pitrez Filho MS, Santana JC, Hauschild JA, Vargas LT, Pinto LA, Spolidoro JV, et al. Fatores de risco cardiovasculares, metabólicos e inflamatórios e suas relações com obesidade em crianças e adolescentes: aspectos clínicos e terapêuticos. *Bol Cient Pediatr*. 2013;2:41–6.
- Murer SB, Aerbeli I, Braegger CP, Gittermann M, Hersberger M, Leonard SW, et al. Antioxidant supplements reduced oxidative stress and stabilized liver function tests but did not reduce inflammation in a randomized controlled trial in obese children and adolescents. *J Nutr*. 2014;144:193–201.
- Alfadda AA, Fatma S, Chishti MA, Al-Naami MY, Elawad R, Mendoza CDO, et al. Orosomucoid serum concentrations and fat depot-specific mRNA and protein expression in humans. *Mol Cells*. 2012;33:35–41.
- Rubin LP, Ross AC, Stephensen CB, Bohn T, Tanumihardjo SA. Metabolic effects of inflammation on vitamin A and carotenoids in human and animal models. *Adv Nutr*. 2017;8:197–212.
- Garcia OP, Ronquillo D, Caamano MC, Martinez G, Camacho M, Lopez V, et al. Zinc, iron and vitamins A, C and E are associated with obesity, inflammation, lipid profile and insulin resistance in Mexican school-aged children. *Nutrients*. 2013;5:5012–30.
- Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448–54.
- Ulatowski L, Dreussi C, Noy N, Barnholtz-Sloan J, Klein E, Manor D. Expression of the alpha-tocopherol transfer protein gene is regulated by oxidative stress and common single-nucleotide polymorphisms. *Free Rad Biol Med*. 2012;53:2318–26.
- Bloor ID, Symonds ME. Sexual dimorphism in white and brown adipose tissue with obesity and inflammation. *Horm Behav*. 2014;66:95–103.
- Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab*. 2007;92:1023–33.
- Ghashut RA, Talwar D, Kinsella J, Duncan A, Mcmillan DC. The effect of the systemic inflammatory response on plasma vitamin 25(OH)D concentrations adjusted for albumin. *PLOS ONE*. 2014;9:e92614.
- Duncan A, Talwar D, Mcmillan DC, Stefanowicz F, O'Reilly DS. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. *Am J Clin Nutr*. 2012;95:64–71.