



ORIGINAL ARTICLE

## Trp64Arg polymorphism of the ADRB3 gene associated with maximal fat oxidation and LDL-C levels in non-obese adolescents<sup>☆,☆☆</sup>



Íncare Correa de Jesus<sup>a,\*</sup>, Lupe Furtado Alle<sup>b</sup>, Eva Cantalejo Munhoz<sup>c</sup>, Larissa Rosa da Silva<sup>a</sup>, Wendell Arthur Lopes<sup>d</sup>, Luciane Viater Tureck<sup>b</sup>, Katia Sheylla Malta Purim<sup>e</sup>, Ana Claudia Kapp Titski<sup>a</sup>, Neiva Leite<sup>a</sup>

<sup>a</sup> Universidade Federal do Paraná (UFPR), Departamento de Educação Física, Curitiba, PR, Brazil

<sup>b</sup> Universidade Federal do Paraná (UFPR), Departamento de Genética, Curitiba, PR, Brazil

<sup>c</sup> Universidade Federal do Rio Grande do Sul (UFRGS), Departamento de Medicina, Porto Alegre, RS, Brazil

<sup>d</sup> Universidade Estadual de Maringá (UEM), Departamento de Educação Física, Maringá, PR, Brazil

<sup>e</sup> Universidade Federal do Paraná (UFPR), Departamento de Medicina, Curitiba, PR, Brazil

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### Abstract

**Objective:** To analyze the association between the Trp64Arg polymorphism of the ADRB3 gene, maximal fat oxidation rates and the lipid profile levels in non-obese adolescents.

**Methods:** 72 schoolchildren, of both genders, aged between 11 and 17 years, participated in the study. The anthropometric and body composition variables, in addition to total cholesterol, HDL-c, LDL-c, triglycerides, insulin, and basal glycemia, were evaluated. The sample was divided into two groups according to the presence or absence of the polymorphism: non-carriers of the Arg64 allele, i.e., homozygous (Trp64Trp: n=54), and carriers of the Arg64 allele (Trp64Arg+Arg64Arg: n=18), in which the frequency of the Arg64 allele was 15.2%. The maximal oxygen uptake and peak of oxygen uptake during exercise were obtained through the symptom-limited, submaximal treadmill test. Maximal fat oxidation was determined according to the ventilatory ratio proposed in Lusk's table.

**Results:** Adolescents carrying the less frequent allele (Trp64Arg and Arg64Arg) had higher LDL-c levels (p=0.031) and lower maximal fat oxidation rates (p=0.038) when compared with non-carriers (Trp64Trp).

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<sup>☆☆</sup> Study carried out at Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil.

\* Corresponding author.

E-mail: [asp.incare@hotmail.com](mailto:asp.incare@hotmail.com) (Í.C. Jesus).

**PALAVRAS-CHAVE**

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**Conclusions:** Although the physiological processes related to lipolysis and lipid metabolism are complex, the presence of the *Arg 64* allele was associated with lower rates of FATMAX during aerobic exercise, as well as with higher levels of LDL-c in adolescents.

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### Polimorfismo *Trp64Arg* do gene *ADRB3* associado à oxidação máxima de gorduras e à concentração de LDL-c em adolescentes não obesos

#### Resumo

**Objetivo:** Analisar a associação entre o polimorfismo *Trp64Arg* do gene *ADRB3*, as taxas de oxidação máxima de gorduras e as concentrações do perfil lipídico em adolescentes não obesos. **Métodos:** Participaram do estudo 72 escolares, de ambos os sexos, com idade entre 11 e 17 anos. Foram avaliadas as variáveis antropométricas e de composição corporal, além do colesterol total, lipoproteína de alta densidade, lipoproteína de baixa densidade, triglicerídeos; insulina e glicemia basal. A amostra foi dividida em dois grupos, segundo a presença ou não do polimorfismo: não portadores do alelo *Arg64*, ou seja, homocigotos (*Trp64Trp*:  $n = 54$ ) e portadores do alelo *Arg64* (*Trp64Arg + Arg64Arg*:  $n = 18$ ), em que a frequência do alelo *Arg64* foi de 15,2%. O consumo máximo de oxigênio e pico de consumo máximo de oxigênio durante o exercício foram obtidos por meio do teste aeróbio submáximo de sintoma limitado em esteira. A oxidação máxima de gorduras foi determinada de acordo com a razão de trocas ventilatórias propostas na Tabela de *Lusk*.

**Resultados:** Os adolescentes portadores do alelo menos frequente (*Trp64Arg* e *Arg64Arg*) apresentaram maiores concentrações de lipoproteína de baixa densidade ( $p = 0,031$ ) e menores taxas de oxidação máxima de gorduras ( $p = 0,038$ ) quando comparados aos não portadores (*Trp64Trp*). **Conclusões:** Embora os processos fisiológicos relacionados à lipólise e ao metabolismo de lipídeos sejam complexos, a presença do alelo *Arg64* associou-se a menores taxas de FATMAX durante exercício aeróbio, bem como maiores níveis de lipoproteína de baixa densidade em adolescentes.

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## Introduction

Aerobic exercise is one of the main strategies in body weight regulation, prevention, and treatment of complex obesity and type 2 diabetes mellitus (DM2), because in addition to potentiating the energy expenditure, it increases glucose absorption of muscle cells and promotes a decrease in insulin resistance.<sup>1</sup> However, the general population does not benefit from the practice of physical exercise, as, in addition to the exercise itself, these effects depend on other environmental factors, such as diet and individual genetic background.<sup>2</sup> The significant role that specific genes play in weight regulation through the action of their products on energy expenditure, substrate oxidation, appetite modulation, lipid metabolism, thermogenesis, and cell differentiation has been well described.<sup>2,3</sup>

In this context, the adrenergic is one of the most important system, acting on the energy balance regulation through brown adipose tissue thermogenesis and white adipose tissue lipolysis, both in humans and in other species.<sup>4,5</sup> Part of this system is found in the  $\beta 3$  receptor, located on chromosome 8p 11.23, expressed mainly in visceral and subcutaneous adipose tissue, acting as lipolysis mediator and, in brown adipose tissue, as thermogenesis regulator,

with both functions performed in response to catecholamine stimulation.<sup>6,7</sup>

Changes in the functionality and amount of the expressed  $\beta 3$  receptors may permeate individual differences in energy expenditure in response to physical activities. Some of its variants associated with metabolic dysfunctions include the single nucleotide polymorphism (SNP) *Trp64Arg* (rs4994), which consists in the substitution of a thymine by a cytosine (T > C), resulting in the exchange of a tryptophan for an arginine at position 64 of the mature protein and corresponding to the first intracellular loop of the  $\beta 3$ <sup>8</sup> receptor. *Arg64* allele carriers have shown to be more resistant to weight loss and decreased visceral fat,<sup>9</sup> in addition to being more likely to have lipid alterations, obesity, DM2,<sup>7</sup> and reduced rates of fat oxidation.<sup>10</sup>

Both the normal metabolic processes that culminate in fat oxidation and the metabolic changes related to the lipid profile are complex systems involving multiple pathways and interactions of exogenous (environmental) and endogenous factors.<sup>11</sup> Despite the underlying complexity of these processes, studies have sought to identify the small portion that can be attributed to allelic variants of genes whose products may influence the abovementioned physiological processes. Therefore, the present study aimed to verify the

association between the *Trp64Arg* polymorphism, the maximal fat oxidation (FATMAX) rates, and the lipid profile levels in non-obese adolescents.

## Methods

### Study design

This was a cross-sectional and observational study, characterized by initial anthropometric screening of all adolescents from public and urban schools in the South of Brazil. A total of 1077 schoolchildren of both genders and aged between 11 and 17 years participated in the study. The prevalence of non-obese adolescents classified between the 5th and 97th percentiles, as proposed by the World Health Organization,<sup>12</sup> was 93%. Subsequently, all students were invited to participate in a physical activity program, in which 165 subjects were volunteers. Participants were evaluated by a single pediatrician through complete physical examination, anthropometric tests, and sexual maturation analysis as proposed by Tanner.<sup>13</sup>

The inclusion criteria were: absence of chronic diseases such as DM2, uncontrolled hypothyroidism, endocrine diseases, infections, and/or use of medications that promote changes in adiposity and metabolic and inflammatory parameters. The exclusion criterion used was the obese nutritional status ( $n=85$ ), considered above the 97th percentile by the World Health Organization.<sup>12</sup> All volunteers and parents signed the informed consent form, according to the research project approved by the Institutional Ethics Committee (protocol number 2460.067/2011-03-UFPR).

After applying the inclusion and exclusion criteria, the selection resulted in 80 non-obese adolescents (38 boys and 42 girls), who did not perform more than 120 min of physical activity a week and did not have any contraindications to the examinations (including absence of heart, pulmonary, and osteo-articular diseases) and were submitted to submaximal treadmill test and blood collection. Of these, five adolescents did not perform the cardiorespiratory evaluation tests and in three it was not possible to perform the genotyping of the *Trp64Arg* polymorphism of the *ADRB3* gene, resulting in 72 non-obese adolescents (33 boys and 39 girls).

### Evaluation of anthropometric and physiological variables

Body mass (BM) (kg) was obtained using a platform scale, with an accuracy of 0.1 kilograms (kg) and a maximum capacity of 150 kg. Height was measured in a stadiometer fixed to the wall, with an accuracy of 0.1 cm and an amplitude of 220 cm. Abdominal circumference (AC) measurement was evaluated following the recommendations of the Centers for Disease Control and Prevention.<sup>14</sup>

Body fat composition assessment was performed with dual energy X-ray absorptiometry (DXA), using a Lunar Prodigy Primo device (General Electric, Healthcare, MA, USA). Lean mass (LM) and fat mass (FM) were determined with an accuracy of 1 g and described in kilograms (kg). To reconstruct the image of the LM and FM, the enCore 2008 software version 12.30 (General Electric, Healthcare, MA, USA) was used.

The maximal oxygen consumption ( $VO_{2max}$ ) and peak oxygen consumption ( $VO_{2peak}$ ) were measured on a treadmill using a K4b2<sup>®</sup> Portable Gas Analyzer (Cosmed<sup>®</sup>, SP, Brazil) with the following protocol: initial velocity of 4 km/h, progressive increase of 0.3 km/h at every 30 seconds and fixed slope of 1% kept constant during the test. Heart rate (HR) was monitored during the test, using a cardiofrequencimeter (Polar<sup>®</sup>, USA), a portable wireless transmission-reception system. The adolescents were advised by a nutritionist to eat a light meal with a predominance of carbohydrates before the test, according to the daily food recommendations.<sup>15</sup> The test was considered maximal when two of the following criteria were observed: A) exhaustion or inability to maintain the required speed; B)  $R \geq 1.09$ ; C) reaching the maximal HR predicted by the formula  $208 - (0.7 \times \text{age})$ , proposed by Tanaka.<sup>16</sup>

To obtain the FATMAX values, the ventilatory exchange ratio ( $R$ ) observed during the symptom-limited submaximal treadmill test were used, according to Lusk's table.<sup>17</sup> This variable was obtained through the product between the caloric equivalent of  $R$  at the FATMAX point and the oxygen consumption observed at the same point.

### Evaluation of biochemical variables

Blood samples were collected in the morning, after a 12-h fast, and stored in appropriate tubes. Plasma levels of total cholesterol, HDL-cholesterol, and triglycerides were determined in mg/dL by enzymatic-colorimetric assay. LDL-cholesterol was calculated by the Friedewald equation in mg/dL.<sup>18</sup> Glucose values were determined by the enzymatic method (Glucose Oxidase - Labtest, SP, Brazil) and insulin was measured by the chemiluminescence technique using an immunometric immunoassay in uU/mL, in an automated equipment.

### Genotyping of the *Trp64Arg* polymorphism of the *ADRB3* gene

DNA extraction from the blood samples was performed using the QIAamp DNA Mini Kit (QIAGEN, MD, USA), according to the Lahiri and Nurnberger<sup>19</sup> method. Genotyping of the *Trp64Arg* polymorphism of the *ADRB3* gene was performed using the TaqMan allelic discrimination assay using the 7500<sup>™</sup> real-time PCR system (Applied Biosystems<sup>®</sup>, CA, USA), the reactions were performed on an Eppendorf Mastercycler Realplex 2 (Biocompare<sup>®</sup>, CA, USA) apparatus, and analyses were performed with the software Eppendorf Realplex v. 1.5 (Biocompare<sup>®</sup>, CA, USA).

DNA extraction was carried out by obtaining the leukocyte layer by centrifugation of the collected whole blood, lysis of erythrocytes, precipitation of proteins, and precipitation of genomic DNA. The PCR technique allowed the detection, cycle by cycle, with high sensitivity and specificity, of the intensity of fluorescence emitted as a result of the amplification of the target DNA sequence. At the end of the reaction, according to the genotype, one or another fluorescence was present in the case of homozygotes and in the case of heterozygotes, both types of fluorescence were present. The genotype distribution among the adolescents enrolled in the study was: *Trp/Trp* ( $n=54$ ; 75%), *Trp/Arg*

( $n = 14$ ; 19.5%), and *Arg/Arg* ( $n = 4$ ; 5.5%). The frequency of the *Arg64* allele was 15.2%, indicating that the data are in Hardy–Weinberg equilibrium, that is, the observed values are similar to those expected ( $p = 0.165$ ). The models of recessive, dominant, and dominance-free allele interaction were tested by adopting the dominant allele interaction model, due to the small number of *Arg64* allele carriers in homozygosis and also due to the observed effect. Thus, individuals were grouped into carriers (*Arg/Arg* + *Trp/Arg*) and non-carriers (*Trp/Trp*) of the *Arg64* allele.

## Statistical analyses

The data were tested for normality and variance homogeneity by the Kolmogorov–Smirnov test and Lilliefors' correction of significance, respectively. The results are shown as means and standard deviation for the variables with normal distribution, and medians and interquartile ranges for those with non-normal distribution. Student's *t*-test was used for variables with normal distribution and Mann–Whitney's *U* test, for variables that did not meet the normality assumption. Fisher's exact test was used for comparisons of proportions between groups regarding the maturation stage. Spearman's correlation was used to analyze the correlation between the biochemical variables and FATMAX. Genotype frequencies were obtained by direct counting, and the comparisons of proportions between the groups regarding gender and Hardy–Weinberg equilibrium groups were verified using the chi-squared test ( $\chi^2$ ). Multiple linear regression was used to verify the degree of association between sexual maturation, gender, fat mass, and the *Trp64Arg* polymorphism of the *ADRB3* gene (independent variables) and the lipid profile and FATMAX (outcome). The power of the study was calculated using the G\*power3 software (G Power, V.3.1.7, Dusseldorf, Germany) with the present sample, and a power ( $1 - \beta$ ) of 0.66 was identified for the comparisons between groups, of 0.84 for the correlations and of 0.82 for regressions. Data were analyzed with

SPSS for Windows (IBM SPSS Statistics for Windows, version 22.0. NY, USA), and the significance level was set at  $p < 0.05$ .

## Results

Table 1 shows the anthropometric, body composition, biochemical, and cardiorespiratory fitness variables of non-carrier adolescents (*Trp64Trp*) and the less frequent allele carriers (*Trp64Arg* + *Arg64Arg*), which were similar. No statistically significant differences were observed between the groups regarding Tanner stage.

Table 2 shows that the group carrying the *Arg64* allele had higher levels of LDL-c when compared with the non-carrier group ( $p = 0.031$ ). Levels of TC, HDL-c, and TG were similar between groups. The group carrying the *Arg64* allele had lower FATMAX rates ( $p = 0.038$ ) when compared with the non-carrier group (*Trp64Trp*).

Table 3 shows Spearman's correlation values, in which levels of HDL-c ( $p = 0.000$ ,  $r = 0.504$ ), LDL-c ( $p = 0.000$ ,  $r = 0.903$ ), and TG ( $p = 0.035$ ) of non-carriers of the *Arg64* allele were directly correlated with total cholesterol (TC). Among the carriers of the *Arg64* alleles, FATMAX showed an indirect correlation with LDL-c ( $p = 0.018$ ,  $r = 0.512$ ) and TC levels ( $p = 0.041$ ,  $r = 0.449$ ). Additionally, serum levels of HDL-c ( $p = 0.030$ ,  $r = 0.474$ ) and LDL-c ( $p = 0.000$ ;  $r = 0.931$ ) were directly correlated with total cholesterol. No significant correlations were observed with the other variables.

The multiple linear regression test (Table 4) showed that the *Trp64Arg* polymorphism of the *ADRB3* gene explained 8% (adjusted  $r^2 = 0.08$ ) of the LDL-c levels ( $\beta = 0.27$ ,  $p < 0.01$ ) and 16% (adjusted  $r^2 = 0.16$ ) of FATMAX ( $\beta = -0.36$ ,  $p < 0.01$ ), whereas FM explained 5% (adjusted  $r^2 = 0.05$ ) of FATMAX ( $\beta = -0.25$ ,  $p < 0.01$ ).

## Discussion

The present study aimed to investigate the association of the *Trp64Arg* polymorphism on FATMAX and biochemical

**Table 1** Comparison of means  $\pm$  standard deviation or medians (confidence intervals) of anthropometric, body composition, biochemical and cardiorespiratory fitness variables among non-carrier adolescents (*Trp64Trp*) and carriers of the less frequent allele (*Trp64Arg* + *Arg64Arg*) of the *ADRB3* gene.

Variable	<i>Trp64Trp</i> ( $n = 54$ )	<i>Trp64Arg</i> + <i>Arg64Arg</i> ( $n = 18$ )	<i>p</i>
Age (years)	14.57 $\pm$ 1.22	14.96 $\pm$ 1.27	0.234
Height (m)	1.65 $\pm$ 0.09	1.65 $\pm$ 0.08	0.786
BM (kg)	59.69 $\pm$ 11.52	62.68 $\pm$ 11.48	0.320
BMI (kg m <sup>-2</sup> ) <sup>a</sup>	20.95 (11.06)	21.77 (12.38)	0.300
BMI-z <sup>a</sup>	0.66 (2.83)	1.12 (3.81)	1.000
AC (cm)	73.58 $\pm$ 8.07	74.39 $\pm$ 8.51	0.705
INS ( $\mu$ UI/mL) <sup>a</sup>	8.90 (17.30)	8.70 (19.30)	0.604
GLU (mg/dL)	87.68 $\pm$ 10.22	87.85 $\pm$ 8.63	0.945
FM (kg) <sup>a</sup>	14.58 (28.10)	17.90 (25.23)	0.300
LM (kg) <sup>a</sup>	40.10 (41.34)	42.40 (27.47)	0.604
VO <sub>2</sub> peak (L min <sup>-1</sup> ) <sup>a</sup>	2.49 (2.98)	2.35 (2.02)	0.300
VO <sub>2</sub> max (L min <sup>-1</sup> kg <sup>-1</sup> )	43.97 $\pm$ 8.24	41.00 $\pm$ 8.07	0.167

BM, body mass; BMI, body mass index; BMI-z, body mass index Z-score; AC, abdominal circumference; INS, basal insulin; GLU, basal glucose; FM, fat mass; LM, lean mass.

<sup>a</sup> Non-parametric variables;  $p < 0.05$ .

**Table 2** Comparison of means  $\pm$  standard deviation or medians (confidence intervals) of the lipid profile and maximal fat oxidation variables in non-carrier adolescents (*Trp64Trp*) and carriers of the less frequent allele (*Trp64Arg + Arg64Arg*) of the *ADRB3* gene.

Variable	<i>Trp64Trp</i>	<i>Trp64Arg Arg64Arg</i>	<i>p</i>
TC (mg/dL)	148.56 $\pm$ 23.73	160.36 $\pm$ 31.65	0.087
HDL-c (mg/dL)	53.68 $\pm$ 9.64	54.91 $\pm$ 10.84	0.635
LDL-c (mg/dL)	76.80 $\pm$ 17.18	90.32 $\pm$ 24.85	0.031 <sup>b</sup>
TG (mg/dL) <sup>a</sup>	77.00 (192.20)	70.00 (122.79)	0.375
FATMAX (kcal/min) <sup>a</sup>	7.78 (8.34)	6.75 (4.21)	0.038 <sup>b</sup>

TC, total cholesterol; HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein; TG, triglycerides; FATMAX, maximal fat oxidation.

<sup>a</sup> Non-parametric variables.

<sup>b</sup>  $p < 0.05$ .

**Table 3** Matrix of Spearman's correlation coefficients between the lipid profile and maximal fat oxidation variables in homozygous adolescents for the most frequent allele (*Trp64Trp*;  $n = 54$ ) and for carriers of the less frequent allele (*Trp64Arg + Arg64Arg*;  $n = 18$ ).

	TC	HDL-c	LDL-c	TG	FATMAX
<i>Trp64Trp</i> ; $n = 54$					
TC (mg/dL)	1.000	0.504 <sup>b</sup>	0.903 <sup>b</sup>	0.296 <sup>a</sup>	-0.047
HDL-c (mg/dL)		1.000	0.233	-0.222	-0.006
LDL-c (mg/dL)			1.000	0.188	-0.102
TG (mg/dL)				1.000	0.062
FATMAX (kcal/min)					1.000
<i>Trp64Arg + Arg64Arg</i> ; $n = 18$					
TC (mg/dL)	1.000	0.474 <sup>a</sup>	0.931 <sup>b</sup>	0.389	-0.449 <sup>b</sup>
HDL-c (mg/dL)		1.000	0.231	-0.269	-0.040
LDL-c (mg/dL)			1.000	0.365	-0.512 <sup>a</sup>
TG (mg/dL)				1.000	-0.337
FATMAX (kcal/min)					1.000

TC, total cholesterol; HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein; TG, triglycerides; FATMAX, maximal fat oxidation.

<sup>a</sup>  $p < 0.05$ .

<sup>b</sup>  $p < 0.01$ .

**Table 4** Linear regression coefficient ( $\beta$ ) and  $r^2$  for gender, sexual maturation, *Trp64Arg* polymorphism of *ADRB3* gene, lipid profile, and maximal fat oxidation variables in adolescents.

		TC	HDL-c	LDL-c	TG	FATMAX
<i>Trp64Arg</i>	$\beta$	0.16	0.01	0.27	-0.18	-0.36
	$r^2$	0.03	-0.01	0.08 <sup>a</sup>	0.24	0.16 <sup>a</sup>
Gender	$\beta$	0.06	-0.06	0.09	-0.01	-0.03
	$r^2$	0.022	0.00	0.02	-0.01	0.020
Tanner	$\beta$	-0.03	-0.03	-0.08	0.18	0.08
	$r^2$	-0.01	-0.01	-0.00	0.01	-0.01
FM	$\beta$	0.18	0.18	0.05	0.05	-0.25
	$r^2$	-0.00	0.00	-0.01	-0.01	0.05 <sup>a</sup>

TC, total cholesterol; HDL-c, high-density lipoprotein; LDL-c, low-density lipoprotein; TG, triglycerides; FATMAX, maximal fat oxidation, FM, fat mass.

<sup>a</sup>  $p < 0.01$ .

variables in non-obese adolescents. These physiological parameters show great variation in the general population, including among adolescents, due to the interaction of multiple determinant genetic factors, and of these with environmental components, such as eating habits and lifestyle.

The results suggest that FATMAX rates were significantly lower and LDL-c levels were higher among adolescents with the *Arg64* allele when compared with those without the allele. Similar results were observed in adults with normal weight, both at rest and during aerobic exercise, in whom the *Trp64Arg* polymorphism was associated with the

reduction of FATMAX.<sup>10</sup> Moreover, in this sense, *in vitro* research with human adipocytes suggested reduced lipolytic activity in homozygous individuals that were carriers of the *Arg64* allele.<sup>20</sup> Although it is a complex physiological process, the genetic variant of the *ADRB3* gene appears to contribute to the variation in FATMAX values in response to physical activity, as well as to a lower response capacity to weight loss.<sup>8</sup>

The prescription of physical activities to the point of FATMAX is an important factor that can contribute to lipid metabolism improvement and weight maintenance,<sup>21</sup> as well as the increase in insulin sensitivity.<sup>22</sup> Training in the FATMAX zone suggests significant increases in this parameter, as observed in obese female adolescents who also showed favorable changes in adipocytokine levels.<sup>23</sup> Thus, understanding the factors that contribute to the individual variation of FATMAX rates may allow the prescription of more individualized training, which will result in more positive outcomes.

Although the present study did not consider functional assays to demonstrate the effect of changes in the functioning of pathways triggered by the  $\beta 3$  receptor activation, previous studies have demonstrated its functionality. A study carried out in rats demonstrated that the *Arg64* allele carrier showed a reduction in the amount of cAMP in response to catecholamines, when compared to the presence of the *64Trp* allele.<sup>24</sup> The same effect was also observed in other cells (ovary cells in hamsters and human embryonic renal cells),<sup>4,5</sup> suggesting that lipolysis may be decreased in the presence of the *Arg64* variant in  $\beta 3$  receptors, when compared with a receptor without the alteration. Still in this sense, such genetic alteration may reflect a less favorable metabolic profile when associated with brown adipose tissue thermogenesis, body weight and early onset of T2DM in some populations.<sup>6,7</sup>

This study indicates that the *Arg64* allele carriers had higher levels of LDL-c, which may represent greater risks for cardiovascular disease development in the future. Likewise, a study carried out with adult Japanese individuals with normal weight showed a significant correlation between LDL-c levels, the *Arg64Arg* genotype, and age, with an annual increase in BMI.<sup>25</sup> However, the literature shows heterogeneity of results. In Italian non-obese adults, no influence of the *Arg64* allele was observed on lipid levels; however, this allele appears to influence abdominal adiposity.<sup>26</sup> A study in Hungarian children with different nutritional states found no influence of the presence of the *Arg64* allele on TG, TC, HDL-c, and glucose levels; however, the authors observed higher values of body mass, fat mass, and fasting insulin, when compared with non-carriers of the allele.<sup>27</sup> In another study, *Arg64Arg* homozygous individuals had significantly lower insulin secretion and higher fasting glucose levels when compared with overweight *Trp64Trp* homozygous individuals.<sup>7</sup>

Furthermore, the results suggest that the higher rates of FATMAX in the non-carrier group of the *Arg64* allele of the *ADRB3* gene showed a negative correlation with TC and HDL-c levels. This relationship is known, since the increase in energy expenditure leads to changes in the lipid profile secondary to physical exercise, through the stimulation of metabolic reactions and the potentiation of energy substrate use by active muscles, which occurs both acutely

and by physiological adaptations that stimulate metabolism throughout the day.<sup>21</sup> Additionally, although it has been observed in the regressions that the *Trp64Arg* polymorphism of the *ADRB3* gene was associated with LDL-c and FATMAX levels, it must be considered the effect of genetic variants on the complex traits in such physiological processes, which can be attributed to the age range and the subjects' nutritional status, as well as the ethnic heterogeneity of the genetic background.

The *Arg64* allele is found at different frequencies in the assessed ethnicities; in the present study the observed frequency was 15.3%. Studies in other populations reported 5.77% in Hungarian obese children,<sup>7</sup> 8% in Euro-North Americans,<sup>25</sup> and 11% in Brazilian adults with different nutritional status.<sup>28</sup>

The present study has some limitations that should be mentioned. First, it had a cross-sectional design; therefore, it was not possible to establish a cause-and-effect association between the presence of polymorphism, FATMAX, and lipid profile, considering that this condition is multifactorial. Second, the small sample size ( $n=72$ ) made it impossible to perform an individualized analysis by gender and, therefore, caution should be adopted when generalizing the results obtained in this study, considering the influence that gender may have in this context. Third, the use of a convenience sample hindered the homogeneity between the selected groups, because differences in gender and Tanner stage may alter FATMAX rates and lipid profile levels. However, no difference was found in the comparisons between genders and Tanner stage in the groups divided by the presence or absence of the *Arg64* allele. Nevertheless, other aspects support the present findings, such as the method used for the analysis of body fat composition (DXA), considered the gold standard; the strict inclusion and exclusion criteria; and the use of the Lusk protocol for assessing FATMAX, which shows good agreement with invasive techniques such as arterial cannulation and muscle biopsies, as well as the easy access and applicability by health professionals.<sup>29</sup>

Identifying the influence of polymorphisms in candidate genes on the maximal rates of fat oxidation and lipid metabolism may contribute to the implementation of more individualized and effective exercise protocols, able to more effectively contribute to health care provision. However, the association between the *Arg64* allele of the *Trp64Arg* polymorphism of *ADRB3* gene, FATMAX, and LDL-cholesterol levels should be interpreted with caution, as it may represent a small portion of complex physiological processes related to lipolysis and metabolism lipids.

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## Conflicts of interest

The authors declare no conflicts of interest.

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