Association of cardiorespiratory fitness, physical activity level, and sedentary behavior with overweight in adolescents

Associação da aptidão cardiorrespiratória, do nível atividade física e do comportamento sedentário com o estado nutricional de adolescentes

Marcelo Jannuzzi Franceschin[1]
https://orcid.org/0000-0002-9992-5921
Gloria Valeria da Veiga[1]
https://orcid.org/0000-0002-7985-0213

Abstract – Physical activity and sedentary behavior promote opposite effects on overweight and obesity, in adolescents; however little is known of their combined impact on these outcomes. We aimed to investigate if there were any associations between overweight, and physical activity level, cardiorespiratory fitness, and sedentary behavior, both as isolated variables and in combination. Data of 1,015 students from two public and four private schools from the metropolitan area of Rio de Janeiro, Brazil were analyzed. Overweight was classified according to body mass index, whereas physical activity level was assessed by the International Physical Activity Questionnaire (IPAQ), cardiorespiratory fitness by a 9-minute test (T9), and sedentary behavior by a self-completed questionnaire, considering the time spent watching television as the main indicator. Associations were examined by binary logistic regression analysis. Odds ratios (ORs) and 95% confidence intervals (95%CIs) were used to measure the magnitude of the associations. IBM SPSS Statistics version 20 was used for all statistical analyses. Adolescents with poor cardiorespiratory fitness (OR=3.71; 95%CI=1.30-10.6) and a high degree of sedentary behavior (OR=1.73; 95%CI=1.24-2.42) had a bigger chance of being overweight. However the combination of these two categories in the same individual was not statistically associated with overweight. The study indicates that both sedentary behavior and poor performance on cardiorespiratory fitness test are significantly independent associated with overweight in adolescents.

Key words: Obesity; Physical fitness; Sedentary behavior.

Resumo – A atividade física e o comportamento sedentário promovem efeitos opostos no sobrepeso e obesidade em adolescentes. Contudo, pouco se sabe sobre seu efeito combinado sobre estes desfechos. Objetivou-se investigar a associação entre sobrepeso e o nível de atividade física, aptidão cardiorrespiratória e comportamento sedentário, isoladamente e de forma combinada. Dados de 1015 estudantes de duas escolas públicas e quatro particulares da região metropolitana do Rio de Janeiro foram analisados. O sobrepeso foi classificado segundo o índice de massa corporal. O nível de atividade física foi avaliado pela forma curta do questionário internacional de atividade física (IPAQ), a aptidão cardiorrespiratória pelo teste de corrida e caminhada de 9 minutos (T9) e o comportamento sedentário pelo tempo do uso de televisão. As associações foram calculadas por regressão logística binária. A magnitude das associações foi avaliada pela odds ratio (OR) e intervalo de confiança de 95% (IC95%). Os cálculos estatísticos foram realizados em IBM SPSS statistical versão 20. Adolescentes com baixa aptidão cardiorrespiratória (OR=3,71; IC95%=1,30-10,6) e com alto nível de comportamento sedentário (OR=1,73; IC95%=1,24-2,42) apresentaram maior chance sobrepeso. Todavia, a coexistência destas duas categorias no mesmo indivíduo não se associou significativamente ao sobrepeso. O estudo indica que tanto o comportamento sedentário quanto o baixo desempenho no teste de aptidão cardiorrespiratória estão associados de forma independente ao sobrepeso em adolescentes.

Palavras-chave: Adolescente; Aptidão física; Comportamento sedentário; Obesidade.

INTRODUCTION

The prevalence of overweight and obesity among adolescents increased significantly, over the past few decades, in Brazil[1] as well as in several other countries[2] leading them to serious health issues. Sedentary lifestyles[3] and inadequate food habits[4] have been considered as the main causes of these conditions[5].

Physical activity not only brings metabolic benefits and bone health, but also cardiorespiratory fitness, and body composition[6]. Besides it improves social skills, self-esteem, cognition and school performance, and prevents from developing depression and anxiety[7]. Sedentary behavior is considered the major cause for the increase in the prevalence of overweight and obesity, among other related chronic diseases[8].

The presence of physical activity or sedentary behavior would suggest the absence of the other[9]. Nevertheless, there are evidences stating that these behaviors coexist regardless of the other[10] and have different effects on an individual's health.

While several studies investigated the isolated associations of physical activity[11] or sedentary behavior[12] with overweight and obesity among adolescents, few studies have took into consideration the influence of both actions on these outcomes. Furthermore, scientific knowledge concerning to the relationship between physical activity and sedentary behavior on individuals' health remains controversial: some studies report that the presence of one behavior would imply the absence of the other[9], while some other studies attest the coexistence of active and sedentary behavior promoting different effects on individuals' health[10,13].

It has been hypothesized that the protective effect offered by physical activity that is performed for a short period, daily, is attenuated by the risk associated with participation in sedentary behaviors for long periods, during the day[9,13].

In this context, the objective of this research is to study the association between physical activity level or cardiorespiratory fitness and sedentary behavior, and a combination of both, with overweight among adolescents. We hope that these findings should clarify how physical activity, cardiorespiratory fitness and sedentary behavior are related to adolescents being overweight, as well as contribute to the formulation of intervention proposals on physical activity practice and sedentary behavior to prevent and treat excessive weight gain.

METHOD

In this cross-sectional study, data from the baseline of the Adolescent Nutritional Assessment Longitudinal Study (ELANA) pertaining to first year high school students from two public and four private schools, located in the metropolitan region of Rio de Janeiro, Brazil, were analyzed. The ELANA's sampling, recruiting methodology, and data collection have been previously described by Moreira et al.[14]. Disabled and pregnant adolescents,

as well as those who were undergoing any obesity drug treatment were excluded from the ELANA sample.

Of the 1131 eligible students, 92 did not took part in this study due to not showing interest, not obtaining parent consent, or not being present on the data collection day. Furthermore, 24 students were excluded due to missing anthropometric data, resulting in a final sample of 1015 students (89.7% of the eligible adolescents).

Data collection was performed between February and August, 2010 by a trained team. Weight was measured using an electronic portable scale (Kratos-Cas Electronic Scales, Brazil) with a 150 kg capacity and 50 g resolution. Height was measured in duplicate, using a portable stadiometer (Alturexata, Brazil) in 0.1 cm graduations. A maximum variation of 0.5 cm was allowed between the two measurements, and the mean value was considered in the analysis. For both, weight and height measurements, adolescents were barefoot, wearing light clothes and no accessories. Body mass index (BMI) was calculated as weight/ (height)^2.

Physical activity level was assessed using the short version of the International Physical Activity Questionnaire (IPAQ), validated for Brazilian adolescents aged from 14 to 18 years or older[15]. This instrument assesses the physical activity performed by adolescents during the week preceding data collection, according to its intensity, weekly frequency and time spent in each activity per day.

It is important to consider the ability of the evaluation method used for physical activity. The IPAQ, the most commonly used instrument to investigate physical activity in epidemiological studies, is based on the memory and auto-perception of individuals and, therefore, is subject to memory bias and subjectivity[16]. It has been showed that cardiorespiratory fitness tests present positive correlation with physical activity tests once people who report higher levels of physical activity have better cardiorespiratory fitness scores[17]. Besides that, it has been already showed that cardiorespiratory fitness is a good indicator of health risk and mortality in adults[18]. Therefore, cardiorespiratory fitness was used as an alternative exposure variable to verify the association with overweight in adolescents as previously studied[19].

Cardiorespiratory fitness was assessed using the 9-minute test (T9) according to the protocol proposed by Gaya and Silva[20]. Adolescents were told to run for as long as possible, until they heard the signal to stop. Walking was allowed when necessary. The distance covered by the adolescent in the test was calculated by multiplying the perimeter of the lane by the number of laps performed plus the distance traveled between the last turn and the location of the student at the moment the test was completed.

Sedentary behavior was assessed using a self-administered questionnaire regarding the frequency and hours spent, daily, watching television (TV) or using a computer or playing video games, based on the following questions: a) Do you watch TV [(1) never or almost never, (2) 1 to 2 times weekly, (3) 3 to 4 times weekly, (4) 5 to 6 times weekly, and (5) daily]? ; b) How many hours a day do you watch TV? The same questions were

proposed regarding the use of computers or videogames. The total daily hours spent on each of these activities was calculated by multiplying the reported number of hours per day and weekly frequency divided by 7 days, as follows: daily hours spent on sedentary behaviors = (number of hours per day) x (weekly frequency) / 7 days.

Data Analysis

BMI, as the dependent variable, was analyzed as a categorical variable through which adolescents were classified in the descriptive analysis as: low weight (Z score<-2), normal weight (Z score -2 ≤ to< 1), overweight (Z score 1≤ to < 2) and obese (Z score ≥ 2) according to the age and sex specific cut off points recommended by the WHO[21]. In the association analysis, adolescents were classified as: overweight (BMI ≥ 1 Z score), and not overweight (BMI <1 Z score).

The independent variables were: a) physical activity level, according to IPAQ data, with the adolescents classified as "physically active" when they participated >=60min of moderate or vigorous physical activity during the day, and "insufficiently active" when they participated <60min/ day of this kind of activity according to the WHO's recommendation[22]; b) cardiorespiratory fitness, as a categorical variable, classified as "risk" and "desired" according to cardiorespiratory fitness health classification proposed by Projeto Esporte Brasil (PROESP)[20]; c) sedentary behavior classified as "absent" (watching <2h of TV/day) and "present" (≥2h of TV/ day), as proposed by the *American Academy of Pediatrics (AAPC)[23.]* The time spent watching TV or using computers and videogames were also analyzed as a continuous variables (hours/day).

The other independent variables were created by the combination of physical activity level and cardiorespiratory fitness with sedentary behavior, based only on the hours spent watching TV because this is the most commonly used variable as a marker of sedentary behavior[8]. These variables were categorized as follows:

Physical activity level + sedentary behavior:
1.'≥60 min/day + <2h of TV/day';
2.'≥60 min/day + ≥2h of TV/ day';
3.'<60min/ day + <2h of TV/ day';
4.'<60min/ day + ≥2h of TV/ day'.

Cardiorespiratory fitness + Sedentary behavior:
1.'desired + <2h of TV/ day';
2.'desired + ≥2h of TV/ day';
3.'risk + <2h of TV/ day';
4.'risk + ≥2h of TV/day'.

The frequencies of the categorical variables were presented according to sex and school type, in the descriptive analysis. The comparison of the frequencies between the groups was performed by a Qui-Square test. Continuous variables 'hours of TV/day' and 'hours of computer and videogames/day' are presented by median and minimum and maximum values, considering their non-normal distributions verified by Kolmogorov

Smirnov test. The comparison of medians between the groups was performed by a Mann-Whitney U test.

The strength of the association between dependent and independent variables was assessed by logistic regression, estimating the odds ratios (ORs) and 95% CIs. Multivariate analyses were performed, estimating the ORs and 95%Cis adjusted by sex (male and female), age (continuous variable), type of school (public or private) and dietary energy intake (continuous variable). The evaluation of dietary energy intake was assessed by a qualitative food consumption frequency questionnaire, validated for teenagers[24].

The Box 1 summarizes all statistical procedures performed in this study.

Box 1. Demonstrative chart of statistical analysis

Objectives	Variables	Typeofvariable	Statisticalanalysis ortest
Descriptive analysis with Comparison between groups (sex and type of school)	Body mass index	Categorical variable	Qui Square test
	Physical activity level (PA)		
	Cardiorespiratory fitness (CF)		
	Sedentary behavior (SB)		
	PA x SB (combined)		
	CF x SB (combined)		
	Daily time watching TV	Continuousvariable	Mann -Whitney U
	Daily time using computer		
Association between overweight (dependent variable) And study variables	Independent variables	Categorical variable	Binary Logistic Regression
	Physical activity level (PA)		
	Cardiorespiratory fitness (CF)		
	Sedentary behavior (SB)		
	PA x SB (combined)		
	CF x SB (combined)		
	Covariables	Categorical variable	
	Sex		
	Age	Continuous variable	
	Energy intake		

All analyses were performed using Statistic Package for Social Sciences Software (SPSS), version 20, and p<0.05 was considered statistically significant.

The ELANA project was approved by the committee of ethics on research of the Social Medicine Institute of the State University of Rio de Janeiro, under the protocol number 0020.0.259.000-9. Only students who were willing to participate, and who provided informed consent forms signed by their parents or guardians took part in the study.

RESULTS

In the whole sample of 1015 adolescents, 53.4% were girls, 50.2% public school students and the mean age 15.3±0.98 years old.

The prevalence of overweight was 18.3% and obesity 8.9%. A higher prevalence of obesity was observed in the boys (11.8%) than the girls (6.3%). Private school students showed a higher prevalence of overweight (22.2%) than those from public schools (14.5%) (Table1).

The recommendation for physical activity practice was not met by 22.5% of the adolescents, which was higher among girls than boys (30% vs. 14.2%, p<0.001), and also higher among public school students than private school students (26.4% vs. 18.8%, p=0.006).

The cardiorespiratory fitness of most adolescents (95%) was in the "risk" category, with higher frequencies observed among public school students than private school students (96.9% vs. 92.5%, p=0.004). Based on the time spent watching TV, as well as using computers and playing videogames, the prevalence rates of sedentary behavior was 69.1% and 71.6%, respectively. The median time spent watching TV and using computers and videogames was 3.0 h (range from 0.0 to 9.0 h). However, private school students spent more hours using computers and videogames than public schools students (3.0 h vs. 2.5 h, p<0.001) (Table1).

Regarding the combination of physical activity and sedentary behavior, it was verified that 12.9% of all the adolescents participated in less than 60 minutes, daily, of physical activity and more than 2 hours, daily, watching TV). A higher number of girls than boys (16.4% *vs.* 9%, p<0.05), and students from private schools than those from public schools (15.8% *vs.* 10.1%, p<0.05) were in this category. Regarding the combination of the cardiorespiratory fitness and sedentary behavior, it was verified that a little more than half of the adolescents (53.3%) were in the worst classification (cardiorespiratory fitness in the "risk" category and watching ≥2h TV/day), with no difference between the sexes and types of school.

A logistic regression model, pertaining the association between overweight and the independent variables, adjusted by sex, age, type of school and dietary energy intake, showed that low levels of physical activity provided a protective effect on overweight (OR=0.67; 95%CI = 0.49-0.98), while cardiorespiratory fitness in the "risk" category increased by almost four times, the chance of having overweight (OR= 3.71; 95%CI=1.30-10.6) compared to that in the "desired" level. Adolescents who watched TV for more than 2 hours a day had almost double the chance (OR= 1.73; 95%CI =1.24-2.42) of being overweight compared to those who spent less than 2 hours daily watching TV (Table 2).

Adolescents who met the WHO's recommendation of participating in moderate or vigorous physical activity for more than 60 minutes per day, but also spent more than 2 hours watching TV, as a marker of sedentary behavior, had a higher chance of being overweight than those who participated in more than 60 minutes per day of moderate or vigorous physical activity as well as spent less than 2 hours daily watching television (OR= 1.51; 95%CI =1.07-2.13) No association was observed between combination of cardiorespiratory fitness and sedentary behavior categories (Table 2).

Table 1 – Characterization of the adolescents with regards to weight status, physical activity level, cardiorespiratory fitness, and sedentary behavior, according to sex and school type – ELANA

		Sex			Type de school		
	Total	Boys	Girls	p value*	Public	Private	p value*
Body Mass Index (kg/m^2)	N=1015	N=473	N=542	0.018	N=510	N=505	0.001
Low Weight	1.5	1.7	1.3	0.598	2.4	0.6	0.02
Normal Weight	71.3	68.9	73.4	0.113	75.1	67.5	0.008
Overweight	18.3	17.5	19.0	0.550	14.5	22.2	0.002
Obesity	8.9	11.8	6.3	0.002	8.0	9.7	0.351
Physical Activity Level (PA)	N=919	N=436	N=483	<0.001	N=468	N=451	0.006
<60min/day	22.5	14.2	30.0		18.8	26.4	
≥60min/day	77.5	85.8	70.0		81.2	73.6	
Cardiorespiratory Fitness (CF)	N=844	N=415	N=429	0,561	N=457	N=387	0.004
Risk	94.9	94.5	95.3		96.9	92.5	
Desired	5.1	5.5	4.7		3.1	7.5	
Sedentary Behavior (SB)							
Television	N=995	N=466	N=529	0,285	N=508	N=486	0.340
>2 h/day	69.1	70.8	67.7		67.8	70.6	
≤2 h/day	30.9	29.2	32.3		32.2	29.4	
Hours/day: Median§	3.0	3.0	3.0	0.995#	3.0	3.0	0.049#
Computer and Videogames	N=995	N=467	N=528	0.008	N=508	N=487	<0.001
>2 h/day	71.6	75.6	68.0		64.4	79.1	
≤2 h/day	28.4	24.4	32.0		35.6	20.9	
Hours/day: Median§	3.0	3.0	3.0	0.108#	2.5	3.0	<0.001#
PA x SB	(N=917)	(N=435)	(N=482)	<0.001	(N=467)	(N=450)	0.032
<60min/day + ≥2h TV/day	12.9	9.0	16.4	**	10.1	15.8	**
<60min/day + <2h TV/day	9.7	5.3	13.7	**	8.8	10.7	
≥60min/day+ ≥2h TV/day	44.5	50.8	38.8	**	47.5	41.3	
≥60min/day + <2h TV/day	32.9	34.9	31.1		33.6	32.2	
CF x SB	(N=842)	(N=414)	(N=428)	0.677	(N=456)	(N=386)	0.023
Risk + ≥2h TV/day	53,6	55,1	52.1		55.3	51.6	
Risk + <2h TV/day	41.3	39.4	43.2		41.7	40.9	
Desired + ≥2h TV/day	3.4	3.9	3.0		1.8	5.4	**
Desired + <2h TV/day	1.7	1.7	1.6		1.3	2.1	

Note. The values express the prevalence percentage; * Chi-Square test; ** p value<0.05; # Mann-Whitney U test; § Minimum value = 0.0; Maximum value = 9.0 for all medians presented

DISCUSSION

This research investigates the association between physical activity level, assessed by the IPAQ, cardiorespiratory fitness, and sedentary behavior (watching TV, and using computers or playing videogames), as well as the combination of these variables with overweight, among adolescents. Our main results suggest that cardiorespiratory fitness in the "risk" category, as well as a high degree of sedentary behavior are significantly independent associated with overweight as expected. On the other hand, physical activity level showed an unexpected direct association with overweight.

Table 2. Logistic regression analysis for the association between overweight and physical activity level, cardiorespiratory fitness, and sedentary behavior, as isolated or grouped variables.

Variables	Overweight N / (%)	Unadjusted OR (95%CI)	Adjusted[1] OR (95%CI)
Physical Activity Level (PA)			
≥60min/day	203 (28.6)	1.00	1.00
<60min/day	47 (22.7)	0.73 (0.51-1.05)	0.67 (0.46-0.98)
Cardiorrespiratory Fitness (CF)			
Desired	4 (9.3)	1.00	1,00
Risk	213 (26.6)	3.53 (1.24-10.0)	3.71 (1.30-10.6)
Sedentary Behavior (SB)			
Television			
<2 h TV/day	47 (22.7)	1.00	1,00
≥2 h TV/day	213 (26.6)	1.74 (1.25-2.40)	1.73 (1.24-2.42)
Computer and Videogames			
<2 h /day	56 (25.9)	1.00	1.00
≥2 h /day	209 (26.8)	1.04 (0.74-1.47)	1.01 (0.71-1.45)
PA x SB			
≥60min/day + <2h TV/ day	72 (23.9)	1.00	1.00
≥60min/ day + ≥2h TV/ day	130 (31.9)	1.49 (1.07-2.09)	1.51(1.07-2.13)
<60min/ day + <2h TV/ day	18 (20.2)	0.81 (0.45-1.44)	0.74 (0.41-1.35)
<60min/ day + ≥2h TV/ day	29 (24.6)	1.04 (0.63-1.70)	0.94(0.57-1.57)
CF x SB			
Desired+ <2h TV/day	1 (7.1)	1.00	1,00
Desired + ≥2h TV/day	3 (10.3)	1.5 (0.14-15.9)	1.40 (0.13-15.0)
Risk + <2h TV/day	76 (21.8)	3.63 (0.47-18.2)	3.63 (0.46-28.5)
Risk + ≥2h TV/day	136 (30.2)	5.61 (0.72-43.4)	(0.72-43.5)

Note. 1. Adjusted by sex, age, type of school attended and dietary energy intake; CI, confidence Interval; OR, odds ratio.

Based on the IPAQ, most of the adolescents met the WHO´s recommendation for physical activity practice (60min/day of moderate or vigorous physical activity); however, only small portion of them had the desired cardiorespiratory fitness level. Due to its correlation with physical activity level[17], it would be expected that the prevalence rates are comparable; however, the magnitude of the prevalence of both variables is considerable. The results of the logistic regression confirm this supposition, as the insufficiently active adolescents showed a lower risk of being overweight than those who were physically active, contradicting the hypothesis that participation on physical activity is a protective factor for overweight. This result must be carefully analyzed because there is a possibility of overestimation of the physical activity level when accessed by IPAQ[25]. However, there are few validated instruments suitable for assessing physical activity level among adolescents, thus the choice of IPAQ is owing to its validation and wide use nationally and internationally[26]. Another explanation for this result could be reverse causality, a limitation of cross-sectional studies, in situations in which the outcome changes the exposure status. Thus, the

adolescents with overweight may have already been engaged in some sort of physical activity as a means of losing weight.

Considering cardiorespiratory fitness, the consistency of the results are verified, as those with cardiorespiratory fitness at the "risk" category have a considerably bigger chance (about 3.5 times) of being overweight than those with a "desired" level of cardiorespiratory fitness. This result is similar to those observed in other studies on Brazilian adolescents[27], in which worse performance on a cardiorespiratory fitness test was associated with obesity and high abdominal obesity. Straatmann and Veiga[19] also observed a negative association between waist circumference and the distance covered in the T9. Therefore, cardiorespiratory fitness could be a more efficient screening option to identify young individuals at a risk of overweight, as it is based on an objective method, free of the inaccuracies associated with subjectivity or memory bias that are inherent to questionnaires that are used to assess the level of physical activity[25].

Sedentary behavior is positively associated with overweight, both as an isolated variable and as well as in combination with physical activity level. Spending excessive time on sedentary behaviors continued to be associated with overweight, even when combined with a high physical activity level; therefore, the prevalence of overweight was higher among those who reported having a high physical activity level but also a high sedentary behavior, compared to those with a high physical activity level but a low degree of sedentary behavior, confirming the results of other studies that describe the negative impacts of sedentary behavior on several health outcomes, even combined with high physical activity level[13].

The association between overweight and cardiorespiratory fitness in the "risk" category combined with a high degree of sedentary behavior was not statistically significant. Only 5% of the adolescents met the desired cardiorespiratory fitness level, in our study, pointing to sample homogeneity, thereby making it hard to identify statistically significant associations in these analyses.

These results point to the independent association of sedentary behavior acting as risk factor[8] and desired cardiorespiratory fitness playing role of protective factor[19] on the development of overweight and obesity, highlighting the importance of multidisciplinary approaches in controlling and preventing overweight and obesity and strategies that not only encourage the practice of physical activity but also promote the reduction in the time spent on sedentary behaviors.

In the context of the effect of sedentary behavior on health-related outcomes, it is important to discuss the sedentary behavior markers. Several studies defined "screen time" as time spent on electronic media such as television, videogames and computers, and its associations with health outcomes, according to different medias, has been investigated separately[8,23] or together[13,28]. In this study, we investigated the difference in the use of these indicators among public and private school students, considering the type of school as a socioeconomic status indicator, as is widely used in Brazilian

studies[29] and the premise that socioeconomic status could be a determinant of the use of different media related to sedentary behavior. Indeed, a larger proportion of private school students spent more than 2 hours, daily, using computers and playing videogames; however, public school students spent more time watching TV. There was no difference between the school types with regards to the prevalence of the overuse of TV. However, only TV exposure was confirmed as a risk factor for overweight. The relationship between the use of TV and overweight, among adolescents, is especially relevant, as it favors low levels of physical activity and the consumption of unhealthy foods, stimulated by television advertising[30], increasing the risk of developing overweight and obesity.

One limitation is the evaluation of sedentary behavior and physical activity through the use of a self-completed questionnaire; therefore, reporting biases can be ruled out. However, this methodology is more feasible for epidemiological studies due to its practicality and low cost[26].

CONCLUSION

The study indicates that both sedentary behavior, expressed by the time spent watching television, and poor performance on the cardiorespiratory fitness test are associated with overweight in adolescents. However, the coexistence of these two factors in the same individual did not increase significantly such association. This reinforces the importance of taking a varied approach, with activities that promote participation in physical activity, consequently, improving cardiorespiratory fitness, and reduction in the time spent on sedentary activities to ensure the greater efficiency of future overweight prevention programs for adolescents.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

Ethical approval
Ethical approval was obtained from the local Human Research Ethics Committee – (Social Medicine Institute of the State University of Rio de Janeiro) and the protocol (no. 0020.0.259.000-9) was written in accordance with the standards set by the Declaration of Helsinki.

Conflict of interest statement
The authors have no conflict of interests to declare.

Author Contributions

Conceived and designed the experiments: GVV. Performed the experiments: MJF, GVV. Analyzed the data: MJF. Contributed reagents/materials/analysis tools: GVV. Wrote the paper: MJF, GVV.

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Corresponding author
Marcelo Jannuzzi Franceschin
Jaime Perdigão street, 135/102
Rio de Janeiro – RJ, Brazil
Zip code: 21920-240
Email: marcelojannuzzi@globo.com