



## Discouraging soft drink consumption reduces blood glucose and cholesterol of Brazilian elementary students: Secondary analysis of a randomized controlled trial☆



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### ABSTRACT

The objective of this study was to evaluate the effect of an educational program aimed at discouraging sugar-sweetened carbonated beverages intake on blood fasting glucose and total cholesterol. Forty-seven fourth grade classes in twenty-two schools have participated in a randomized controlled trial aimed at discouraging soft drink intake in order to prevent excessive weight gain during a school year, in the city of Niterói, Rio de Janeiro. Of 1140 randomized students, 478 (238 in intervention group and 240 in control group) aged 9–12 years old had at least one result on biochemical data and were analyzed to evaluate the effect of the intervention on changes in fasting glucose and total cholesterol at the end of follow-up. Intention-to-treat analysis was performed taking into account the cluster (classes) effect. Statistically significant decrease in fasting glucose ( $-9.12$  mg/dL vs.  $+0.51$  mg/dL,  $p < 0.001$ ) and total cholesterol ( $-10.34$  mg/dL vs.  $+2.14$  mg/dL,  $p < 0.001$ ) were observed among students in the intervention group in comparison with controls. In addition, the prevalence of impaired fasting glucose and hypercholesterolemia decreased in interventions and increased in controls ( $-2.4\%$  vs.  $+8.8\%$ ,  $p = 0.04$  and  $-10.0\%$  vs.  $+2.7\%$ ,  $p = 0.03$ , respectively). Discouraging soft drink consumption among children has led to a reduction in fasting glucose and total cholesterol, suggesting that these beverages may play a role in the development of cardiometabolic risk in childhood.

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### 1. Introduction

Sugar-sweetened beverages (SSB) include all beverages with added sugars, such as soft drinks, fruit juices, sport drinks, energy drinks and also tea-, milk- and soya-based drinks (Centers for Disease Control

and Prevention, 2010). SSB consumption has rose significantly worldwide in the last decades, being higher in Latin America and the Caribbean (Singh et al., 2015b). The widespread consumption of SSB is of great concern since these beverages promote an increase in total daily caloric intake (Houchins et al., 2012; Panahi et al., 2013; Van Wymelbeke et al., 2004), and have already been associated with weight gain in all age groups (Ludwig et al., 2001; Malik et al., 2013). Likewise, replacing SSB by non-caloric alternatives seems to prevent excessive weight gain in children, teenagers and adults (Malik et al., 2013).

Considerable epidemiologic evidences have also shown prospective associations between SSB consumption and metabolic adverse outcomes. Habitual consumption of SSB has been related to greater incidence of type 2 diabetes (Imamura et al., 2015), and a higher consumption of these beverages was also associated with greater risk of developing type 2 diabetes and metabolic syndrome among adults (Malik et al., 2010). Likewise, one-serving per day increase in sugar-sweetened beverage consumption has been associated with greater risks of stroke and myocardial infarction (Narain et al., 2016), as well

Abbreviations: BMI, body mass index; SSB, sugar-sweetened beverages.

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as greater incidence of type 2 diabetes (Imamura et al., 2015) in adults. Similarly, an increase in SSB intake among adolescents have been associated to alterations in glucose homeostasis, blood pressure and serum lipoproteins (Ambrosini et al., 2013), and an increase in plasma HDL-cholesterol over one year was greater among children who decreased their SSB intake in at least one serving per week (Van Rompay et al., 2015). However, there is lack of evidence about how an intervention focused in decreasing SSB consumption could improve metabolic profile in children and adolescents.

In 2005, a randomized controlled trial was conducted with fourth graders of public schools in Niterói, Rio de Janeiro, to evaluate the effect of reducing soft drink consumption on the prevention of excessive weight gain. Intervention group showed significant decrease in daily consumption of sugar-sweetened carbonated beverages compared to control, and overweight children, especially girls, have experienced reduction in body mass index (BMI) (Sichieri et al., 2009). As a secondary aim of the trial, in this study we tested the influence of intervention on blood glucose and total cholesterol among those participants who specifically agreed to provide blood samples.

## 2. Methods

A cluster randomized controlled trial was conducted with fourth graders from twenty-two public schools of Niterói, Rio de Janeiro, Brazil, to evaluate the efficacy of discouraging soft drink consumption on the prevention of overweight over one school year. Because of fasting

blood collection, only morning classes' students were included in the study (Sichieri et al., 2009).

Data collection was performed at baseline, in March 2005, and at the end of follow-up, in December 2005. This study was approved by the Ethics Review Committee of the Institute of Social Medicine, State University of Rio de Janeiro. The participation in the survey was voluntary and written informed consent from parents was obtained.

In the present study, we evaluate the effect of this intervention on both total serum cholesterol and fasting glucose.

### 2.1. Sample size

Sample size was calculated for the original trial (Sichieri et al., 2009), assuming a standard deviation of 1.49 glass of soda, with a power of 80% and a 5% significance level, it would be possible to detect a difference of 0.5 cup in consumption between the two groups and differences of one unit in BMI, with 140 students in each arm of the trial (intervention and control groups). Thus the final sample size with available blood samples of about 480 would be able to detect both weight changes and associated changes in metabolic markers such as glucose and cholesterol (Fig. 1).

To prevent contamination of intervention, schools were randomized instead of classes. After ranking schools based on their prevalence of overweight, randomization was computer generated by researchers using blocks of four schools, following the ranking, balancing intervention and control groups by BMI. The last two on the list were randomly assigned to the intervention and control groups.

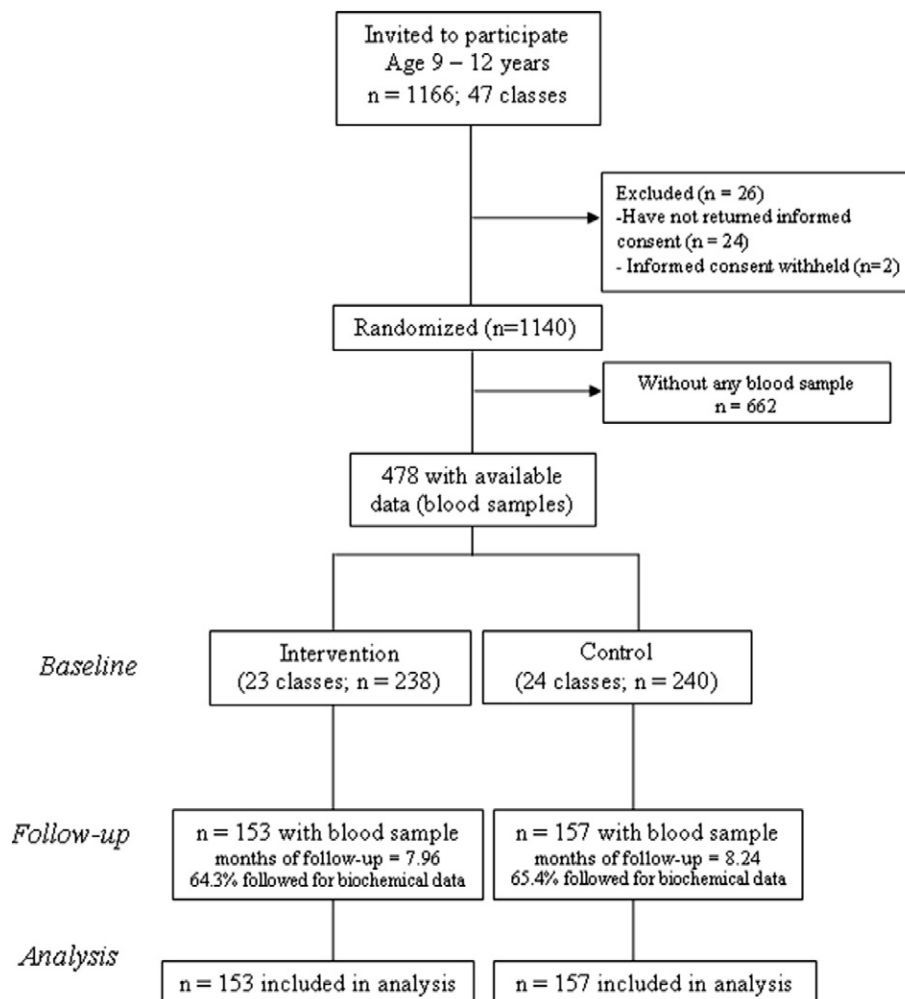


Fig. 1. Flowchart of the study (Niterói/Brazil, 2005).

## 2.2. Intervention

Considering that schools play an important role in obesity prevention (Story et al., 2006), our intervention consisted of a one-year school time healthy lifestyle educational program, delivered via classroom activities distributed in ten one-hour sessions, conducted by four trained research assistants. Full description of the program can be found elsewhere (Sichieri et al., 2009). In short, banners promoting water consumption were hung in intervention schools, and children were taught the importance of drinking water through classroom activities using simple messages that encouraged the consumption of water instead of sugar-sweetened carbonated beverages, such as games, and quizzes, as well as drawing and songs competition, using “water v. sugar-sweetened carbonated beverages” as theme. Teachers were encouraged to reaffirm the message during their classes. In the control group, only two one-hour sessions were developed, with approach on general health issues, and students received printed guidelines on healthy eating.

## 2.3. Main outcome

The main outcome in this analysis is the change in fasting glucose and total cholesterol from baseline to end of follow-up. Blood samples were collected in schools by trained laboratory technician. Children were asked to be fasting 10–12 h as previously scheduled, and adherence of fasting was asked before collection. After collection, blood samples were kept in thermal bags with ice gel packs for transportation to the laboratory of Physiology, Nutrition and Development of the State University of Rio de Janeiro, where samples were centrifuged and serum was immediately stored at  $-70^{\circ}\text{C}$ . Serum cholesterol and plasma glucose were obtained by enzymatic-colorimetric method (GoldAnalisa Kit) using the Konelab 6.0.1 device with automated reading. All biochemical analyzes were performed within one month after finishing all blood collection.

The prevalence of impaired fasting glucose ( $\geq 100$  mg/dL) (American Diabetes Association, 2016), moderately high total cholesterol ( $\geq 150$  mg/dL) (Xavier et al., 2013) and hypercholesterolemia ( $\geq 170$  mg/dL) (Xavier et al., 2013) were also determined.

## 2.4. Compliance with the reduction of sodas

Dietary intake was assessed by two 24-hour recall, at baseline and the end of follow-up. Total energy and macronutrients intake were estimated by the Nutrition Support Programme (Nutwin, version 2.0), a Brazilian software that contains usual portion sizes (Departamento de Informática em Saúde, Universidade Federal de São Paulo, Brazil).

Soft drink intake was also estimated using a self-administered frequency questionnaire on consumption of beverages, drawn from the food frequency questionnaire validated for adolescents (Araujo et al., 2010), consisting of 14 questions regarding the type and frequency of drinks usually consumed, except alcoholic. A usual serving of drink was standardized in a 250 ml glass for all drinks, which were fruit juice, milk, yogurt, soft drink and guaraná drink. The frequency of consumption included eight possible answers: never or less than once per month; 1–3 times per month; one time per week; 2–4 times a week; 5–6 times per week; one time per day; 2 to 3 times a day and  $>3$  times a day. For analyses, we considered only the frequency of soft drink grouped into three categories: never to 4 times per month, 2 to 6 times per week and once a day or more. Questionnaires were checked at the time of delivery, making it possible to correct errors in filling in.

## 2.5. Anthropometric evaluation

Body weight and stature were measured using standardized procedures (Gordon et al., 1988). With both measures, we calculated BMI and analyzed it as a continuous variable but also as categorical. BMI was classified according to the World Health Organization references

for children and adolescents (de Onis, 2007) and grouped into two categories: Overweight (overweight + obesity) and non-overweight (thinness + normal weight).

## 2.6. Data analysis

Continuous variables were tested for normality distribution. BMI was log-transformed due to skewed distribution. The mean, standard deviation and frequency of the variables of interest were calculated.

In order to address differences between intervention and control groups at the end of follow-up, the effect size of intervention on each outcome was obtained by calculating Cohen's *d* (Cohen, 1992), by the formula:  $(M_1 - M_2)/SD_p$ , where  $M_1$  was the mean of control group,  $M_2$  was the mean of intervention group and  $SD_p$  is the pooled standard deviation for both groups at the end of follow-up.

Temporal changes for continuous variables (BMI, fasting glucose, total cholesterol, soft drink consumption, energy and macronutrients intake) were evaluated using linear mixed models (Singer and Willett, 2003) (PROC MIXED command in SAS 9.1), whereas changes on categorical outcomes (impaired fasting glucose, moderately high total cholesterol and hypercholesterolemia) were evaluated through generalized linear models (PROC GENMOD procedure in SAS 9.1). Both analyses were intention-to-treat and included all observations available of each one of the subjects regardless of loss to follow-up or compliance. Analysis of changes from baseline for fasting glucose and total cholesterol included its baseline values as a covariate. All analysis was performed taking into account cluster effect (classes). The term of interest was treatment  $\times$  time interaction, which estimates the rate of changes in the outcomes over time. Statistical significance was set at  $p \leq 0.05$  for all analyses.

## 3. Results

Among the 1166 eligible students, twenty-six refused to participate in the research or did not return the informed consent. Four hundred and seventy-eight children (238 in intervention group and 240 in control group) had blood samples collected at either baseline or follow-up and were included in our analysis. On the intervention and control groups, respectively, 64.3% and 65.4% of the children had biochemical data for both baseline and the end of follow-up (Fig. 1).

As the study had many losses on biochemical data due to refusal to take part in blood collection or absence on collection days, baseline characteristics were compared between students who had at least one blood sample during the study and those who did not participate in any blood collection. Frequency of adolescents who consumed soft drink at least once a day was higher among those with no blood samples (22.9% vs. 16.5%) compared to those with at least one blood sample (Table 1).

**Table 1**

Baseline characteristics of all randomized students, according to their availability of blood samples (Niterói/Brazil, 2005).

Characteristics	No blood samples (n = 662)	At least one blood sample (n = 478)	p-Value <sup>a</sup>
Female - % (n)	50.8 (336)	55.6 (266)	0.10
Non-white skin - % (n)	55.2 (282)	60.8 (282)	0.08
Soft drink intake - % (n)			
Never to once/week	29.1 (149)	30.5 (141)	0.04
2 to 6 times/week	48.0 (246)	53.0 (245)	
At least once/day	<b>22.9 (117)</b>	<b>16.5 (76)</b>	
Overweight - % (n)	20.4 (134)	19.1 (91)	0.58
Age (years) - mean (SD)	10.9 (0.78)	10.8 (0.78)	0.05
Body mass index (kg/m <sup>2</sup> ) - mean (SD)	18.2 (3.33)	18.2 (3.55)	0.76

Data are expressed as mean (standard deviation) or frequency (number of observations).

<sup>a</sup> Student's or chi-square test. In bold -  $p < 0.05$  on chi-square partition.

Baseline characteristics were also compared between intervention and control groups, among the 478 adolescents included in our study. Intervention and control groups were similar at baseline for almost all variables analyzed, except for soft drink intake, mean of fasting glucose, mean of total cholesterol and the frequency of high cholesterol levels, that were all greater in the intervention group (Table 2).

Mean changes from baseline for anthropometric, dietary and biochemical data, as well as effect size of intervention on each outcome, are shown in Table 3. At the end of follow-up, we observed statistically significant differences in mean changes between intervention and control groups for both fasting glucose ( $-9.12$  mg/dL vs.  $+0.51$  mg/dL;  $p < 0.001$ ) and total cholesterol ( $-10.34$  mg/dL vs.  $+2.14$  mg/dL;  $p < 0.001$ ). Intervention group had also a greater reduction in total energy ( $-384.0$  kcal vs.  $-193.7$  kcal;  $p = 0.03$ ), carbohydrate ( $-59.3$  g vs.  $-31.1$  g;  $p = 0.04$ ), lipids ( $-11.6$  g vs.  $-3.4$  g;  $p = 0.01$ ) and soft drink intake ( $-142.3$  g/day vs.  $-25.5$  g/day;  $p = 0.003$ ) from baseline to the end of follow-up when compared with the control group. On the other hand, fruit juices consumption has rose in both intervention ( $+12.4$  g/day) and control ( $+29.1$  g/day) groups, with no statistically significant difference between them. The effect sizes of intervention on each outcome variables were small, being the effect on serum glucose (0.24) and lipids intake (0.25) the highest ones.

Additionally, we have also evaluated changes in the prevalence of impaired blood glucose and cholesterol levels by groups. A decrease in the prevalence of high glucose levels among interventions with a concomitant increase among controls (12.9% to 10.5% and 11.0% to 19.8%, respectively;  $p = 0.04$ ) was observed. The same pattern was perceived for the prevalence of hypercholesterolemia, in which intervention group showed a decrease with increase in the control group (39.1% to 29.1% and 26.0% to 28.7%, respectively;  $p = 0.03$ ) (Table 4).

#### 4. Discussion

In the present study, a reduction of blood fasting glucose and total cholesterol among adolescents in the intervention group compared with control was observed. The intervention group had statistically

**Table 2**  
Baseline characteristics of participants (Niterói/Brazil, 2005).

Variable	Intervention (n = 238)	Control (n = 240)	p-Value <sup>a</sup>
Boys - % (n)	55.0 (131)	56.2 (135)	0.79
Non-white skin - % (n)	62.4 (146)	59.1 (136)	0.47
Age (years) - mean (SD)	10.9 (0.83)	10.8 (0.73)	0.58
Body mass index (kg/m <sup>2</sup> ) - mean (SD)	18.0 (3.46)	18.4 (3.64)	0.26
Overweight - % (n)	18.9 (45)	19.2 (46)	0.92
Frequency of soft drinks - % (n)			
Never to 4 times/month	31.7 (73)	29.3 (68)	0.51
2 to 6 times/week	50.4 (116)	55.6 (129)	
Once/day or more	17.8 (41)	15.1 (35)	
Dietary intake - mean (SD)			
Total energy (kcal/day)	2339.0 (930.9)	2255.5 (917.6)	0.33
Carbohydrate (g/day)	354.4 (145.1)	338.3 (143.8)	0.23
Protein (g/day)	88.4 (35.5)	86.1 (35.6)	0.48
Lipids (g/day)	63.6 (31.5)	61.8 (33.9)	0.56
Soft drinks (g/day)	350.1 (423.8)	274.4 (374.7)	0.04
Fruit juices (g/day)	104.7 (207.8)	84.8 (187.3)	0.28
Glucose (mg/dL) - mean (SD)	85.12 (13.2)	81.58 (14.5)	0.006
≥ 100 mg/dL - % (n)	12.87 (30)	10.97 (26)	0.52
Total cholesterol (mg/dL) - mean (SD)	162.39 (24.9)	153.59 (26.3)	<0.001
≥ 150 mg/dL - % (n)	70.67 (159)	53.19 (125)	<0.001
≥ 170 mg/dL - % (n)	39.11 (88)	25.96 (61)	0.003

Data are expressed as mean (standard deviation) or frequency (number of observations).  
<sup>a</sup> Student's *t* or chi-square test.

**Table 3**

Crude mean (standard deviation) in the follow-up, changes from baseline ( $\Delta$ ) and effect sizes for biochemical and dietary parameters after one school year by groups (Niterói/Brazil, 2005).

Variables	Mean (SD)	$\Delta^a$	p-Value <sup>a</sup>	Effect size <sup>b</sup>
Glucose (mg/dL)				
Intervention	76.57 (15.76)	-9.12	<0.001	0.24
Control	81.22 (22.22)	+0.51		
Total cholesterol (mg/dL)				
Intervention	154.21 (29.67)	-10.34	<0.001	0.06
Control	156.18 (31.24)	+2.14		
Total energy (kcal/day)				
Intervention	1952.5 (719.0)	-384.0	0.03	0.15
Control	2068.5 (796.0)	-193.7		
Carbohydrate (g/day)				
Intervention	294.8 (113.2)	-59.3	0.04	0.11
Control	308.1 (126.2)	-31.1		
Protein (g/day)				
Intervention	78.2 (33.4)	-10.0	0.50	0.01
Control	78.5 (30.2)	-7.6		
Lipids (g/day)				
Intervention	51.8 (24.4)	-11.6	0.01	0.25
Control	58.6 (30.2)	-3.4		
Soft drinks (g/day)				
Intervention	206.1 (286.2)	-142.3	0.003	0.16
Control	264.0 (431.3)	-25.5		
Fruit juices (g/day)				
Intervention	119.1 (225.2)	+12.4	0.48	-0.03
Control	112.5 (217.0)	+29.1		

<sup>a</sup> Model include time, treatment and time  $\times$  treatment interaction adjusted for baseline values and cluster effect.

<sup>b</sup> Cohen's *d*.

significant reduction of carbonated sugar-sweetened beverages compared to the control, as well as reduction in total calories, carbohydrate and lipids intake.

These findings are in line with results from a systematic review about the effects of soft drink consumption on total caloric intake (Vartanian et al., 2007) and also with studies which have investigated the effect of increasing SSB intake on cardiometabolic risk profile. Overweight adults participating in an intervention study had an increase of 11.4 mg/dL in serum cholesterol and 3.4 mg/dL in fasting glucose after six months consuming 1 L/day of cola soft drink (Maersk et al., 2012). In a cohort of Australian adolescents, increasing the consumption of SSB between 14 and 17 years old resulted in decreased HDL-cholesterol and increased cardiovascular risk and serum concentrations of triglycerides at the age of 17, independent of BMI (Ambrosini et al., 2013). In a Canadian sample of 8 to 10 years old children, a 100 mL higher SSB consumption has been associated with a 0.1 unit higher HOMA-IR and a 1.1 mm Hg higher systolic blood pressure (Wang et al., 2013). Among European children and adolescents of multicenter cross-sectional studies, consuming SSB more than five times per week was independently associated with increased insulin resistance, indicated by the HOMA-IR index (Kondaki et al., 2013), and the highest consumption of these beverages was related to greater odds of having cardiovascular risk (Bel-Serrat et al., 2013). Besides that, it has been shown that the total intake of fructose (either from fruits and vegetables or sweets and SSB) among overweight adolescents was predictive of smaller-sized LDL cholesterol particles (Aeberli et al., 2007), which are potentially more atherogenic (Berneis, 2002).

Although significant, intervention had a small effect in reducing serum glucose (0.24) and cholesterol (0.06), as well as the intake of total energy, carbohydrates, protein and lipids, and soft drink consumption in our sample. In a meta-analysis that addressed strategies for the promotion of healthy eating in primary school children, Dudley and colleagues reported curriculum approaches for reducing sugar consumption had also a small ( $d = 0.28$ ) mean effect size, but cross-curricular approaches seemed to be more effective ( $d = 0.42$ ) for reducing sugar consumption, although only two interventions had this kind of strategy (Dudley et al., 2015).

**Table 4**

Frequencies of impaired fasting glucose ( $\geq 100$  mg/dL), moderately high total cholesterol ( $\geq 150$  mg/dL) and hypercholesterolemia ( $\geq 170$  mg/dL) on baseline and at the end of follow-up, by groups.

	Frequencies on baseline		Frequencies on follow-up		p-Value
	% (n)		% (n)		
	Intervention <sup>a</sup>	Control <sup>b</sup>	Intervention <sup>c</sup>	Control <sup>d</sup>	
Glucose $\geq 100$ mg/dL	12.9 (30)	11.0 (26)	10.5 (16)	19.8 (31)	0.04
Cholesterol $\geq 150$ mg/dL	70.7 (159)	53.2 (125)	55.6 (84)	56.7 (89)	0.002
Cholesterol $\geq 170$ mg/dL	39.1 (88)	26.0 (61)	29.1 (44)	28.7 (45)	0.03

<sup>a</sup>  $n = 233$  for glucose;  $n = 225$  for cholesterol.

<sup>b</sup>  $n = 237$  for glucose;  $n = 235$  for cholesterol.

<sup>c</sup>  $n = 153$  for glucose;  $n = 151$  for cholesterol.

<sup>d</sup>  $n = 157$  for glucose and cholesterol analyses.

A limitation of our study is that only 43% of the randomized sample accepted to participate in blood collection on either baseline or follow-up. We compared students who had no blood samples with those who had at least one blood sample to identify possible bias due to refusals. The only difference observed was a higher frequency of adolescents consuming soft drinks at least once a day among those with no blood samples, what could lead our results towards the null hypothesis, since greater consumers of soft drinks could have better adherence to intervention. Even though, we observed improvement in metabolic profile for those who provided blood samples.

Another limitation was that intervention and control groups had differences on baseline for soft drink intake, mean of fasting glucose, mean of total cholesterol and the frequency of high cholesterol levels. Nevertheless, all analyses were adjusted by baseline values, in order to work around this question. The use of only one dietary 24-hour recall in each phase of the trial was also a limitation of our study, since it could be not representative of the usual intake of respondents. We have tried to avoid this limitation by using a 3-day diary during the pre-test, but most children did not return them or brought them with incomplete data. Even so, one 24-hour dietary recall provides a good estimation of group intake (classes) (Buzzard, 1998) and was also consistent to data from food frequency questionnaire on baseline in our sample (Sichieri et al., 2009).

In the present study, discouraging soft drink consumption among children has led to a decrease in both fasting glucose and total cholesterol at the end of one school year, suggesting that these beverages may play a role in the development of cardiometabolic risk in childhood. The importance of our results, which corroborate many observational studies, is due to the experimental design. In most of the observational studies, it is difficult to disentangle the effects of beverages on metabolic indicators since changes in adiposity are also related to beverage drinking. Besides that, this was the first study in Brazil assessing the effect of an intervention focused on reducing SSB consumption in metabolic profile of children and adolescents.

Given the potential harm that SSB consumption may offer, public health strategies have been discussed in an attempt to change the current scenario of excessive consumption of these beverages, such as increasing tax rates for soft drinks (Blecher, 2015; Brownell and Frieden, 2009). Especially among children and adolescents, changing school environment could be a good alternative to reduce the intake of SSB, since it has been shown that students who usually buy SSB from the school canteen are more likely to be high consumers of these beverages (Hebden et al., 2013). Those initiatives, along with health campaigns and nutrition education, especially when facilitated by school staff, parents and families (Meiklejohn et al., 2016), can help change the scenario of excessive consumption of SSB. This reduction could contribute to the potential decrease in the burden of non-communicable diseases (Singh et al., 2015a), preventing its deleterious effects on the entire population.

#### Authorship

M.M.M. participated in the data analysis and manuscript conception, writing and final revision; M.F.F.M. participated in the data analysis and

collaborated in the manuscript conception and revision; R.A.G.S. participated in all phases of the study and collaborated in the manuscript conception and revision; A.S.M. participated in the biochemical analyses and manuscript final revision; G.V.V. was a co-investigator of the project and participated in conceptualization of the intervention and manuscript conception, writing and final revision; R.S. was the principal investigator, led the research project and participated in all phases of the study.

#### Ethical standards disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Review Committee of the Institute of Social Medicine, State University of Rio de Janeiro. Written informed consent was obtained from all subjects' parents.

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#### Transparency document

The Transparency document associated with this article can be found, in the online version.

#### Conflict of interest

None.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jpmed.2017.04.035>.

#### References

- Aeberli, I., Zimmermann, M.B., Molinari, L., Lehmann, R., L'Allemand, D., Spinaz, G.A., Berneis, K., 2007. Fructose intake is a predictor of LDL particle size in overweight schoolchildren. *Am. J. Clin. Nutr.* 86, 1174–1178.
- Ambrosini, G.L., Oddy, W.H., Huang, R.C., Mori, T.A., Beilin, L.J., Jebb, S.A., 2013. Prospective associations between sugar-sweetened beverage intakes and cardiometabolic risk factors in adolescents. *Am. J. Clin. Nutr.* 98:327–334. <http://dx.doi.org/10.3945/ajcn.112.051383>.
- American Diabetes Association, 2016. Classification and diagnosis of diabetes. *Diabetes Care* 39:S13–S22. <http://dx.doi.org/10.2337/dc16-S005>.
- Araujo, M.C., Yokoo, E.M., Pereira, R.A., 2010. Validation and calibration of a semiquantitative food frequency questionnaire designed for adolescents. *J. Am. Diet. Assoc.* 110: 1170–1177. <http://dx.doi.org/10.1016/j.jada.2010.05.008>.
- Bel-Serrat, S., Mouratidou, T., Börnhorst, C., Peplies, J., De Henauw, S., Marild, S., Molnár, D., Siani, A., Tornaritis, M., Veidebaum, T., Krogh, V., Moreno, L.A., 2013. Food consumption and cardiovascular risk factors in European children: the IDEFICS study. *Pediatr. Obes.* 8:225–236. <http://dx.doi.org/10.1111/j.2047-6310.2012.00107.x>.
- Berneis, K.K., 2002. Metabolic origins and clinical significance of LDL heterogeneity. *J. Lipid Res.* 43:1363–1379. <http://dx.doi.org/10.1194/jlr.R200004-JLR200>.

- Blecher, E., 2015. Taxes on tobacco, alcohol and sugar sweetened beverages: linkages and lessons learned. *Soc. Sci. Med.* 136–137C:175–179. <http://dx.doi.org/10.1016/j.socscimed.2015.05.022>.
- Brownell, K.D., Frieden, T.R., 2009. Ounces of prevention—the public policy case for taxes on sugared beverages. *N. Engl. J. Med.* 360:1805–1808. <http://dx.doi.org/10.1056/NEJMp0902392>.
- Buzzard, M., 1998. 24 hour dietary recall and food record methods. In: Willett, W.C. (Ed.), *Nutritional Epidemiology*. Oxford University Press, New York, NY, pp. 50–73.
- Centers for Disease Control and Prevention, 2010. *The CDC Guide to Strategies for Reducing the Consumption of Sugar-sweetened Beverages*.
- Cohen, J., 1992. A power primer. *Psychol. Bull.* 112:155–159. <http://dx.doi.org/10.1037/0033-2909.112.1.155>.
- de Onis, M., 2007. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* 85:660–667. <http://dx.doi.org/10.2471/BLT.07.043497>.
- Dudley, D.A., Cotton, W.G., Peralta, L.R., 2015. Teaching approaches and strategies that promote healthy eating in primary school children: a systematic review and meta-analysis. *Int. J. Behav. Nutr. Phys. Act.* 12:28. <http://dx.doi.org/10.1186/s12966-015-0182-8>.
- Gordon, C.C., Chumlea, W.C., Roche, A.F., 1988. Stature, recumbent length, and weight. In: Lohman, T.G., Roche, A.F., Martorell, R. (Eds.), *Anthropometric Standardization Reference Manual*. Human Kinetics Books, Champaign, pp. 3–8.
- Hebden, L., Hector, D., Hardy, L.L., King, L., 2013. A fizzy environment: availability and consumption of sugar-sweetened beverages among school students. *Prev. Med.* 56, 416–418.
- Houchins, J.A., Burgess, J.R., Campbell, W.W., Daniel, J.R., Ferruzzi, M.G., McCabe, G.P., Mattes, R.D., 2012. Beverage vs. solid fruits and vegetables: effects on energy intake and body weight. *Obesity (Silver Spring)* 20:1844–1850. <http://dx.doi.org/10.1038/oby.2011.192>.
- Imamura, F., O'Connor, L., Ye, Z., Mursu, J., Hayashino, Y., Bhupathiraju, S.N., Forouhi, N.G., 2015. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. *BMJ* 351:h3576. <http://dx.doi.org/10.1136/bmj.h3576>.
- Kondaki, K., Grammatikaki, E., Jiménez-Pavón, D., De Henauw, S., González-Gross, M., Sjöström, M., Gottrand, F., Molnar, D., Moreno, L.A., Kafatos, A., Gilbert, C., Kersting, M., Manios, Y., 2013. Daily sugar-sweetened beverage consumption and insulin resistance in European adolescents: the HELENA (healthy lifestyle in Europe by nutrition in adolescence) study. *Public Health Nutr.* 16, 479–486.
- Ludwig, D.S., Peterson, K.E., Gortmaker, S.L., 2001. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. *Lancet* 357:505–508. [http://dx.doi.org/10.1016/S0140-6736\(00\)04041-1](http://dx.doi.org/10.1016/S0140-6736(00)04041-1).
- Maersk, M., Belza, A., Stødkilde-Jørgensen, H., Ringgaard, S., Chabanova, E., Thomsen, H., Pedersen, S.B., Astrup, A., Richelsen, B., 2012. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. *Am. J. Clin. Nutr.* 95:283–289. <http://dx.doi.org/10.3945/ajcn.111.022533>.
- Malik, V.S., Pan, A., Willett, W.C., Hu, F.B., 2013. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *Am. J. Clin. Nutr.* 98:1084–1102. <http://dx.doi.org/10.3945/ajcn.113.058362>.
- Malik, V.S., Popkin, B.M., Bray, G.A., Després, J.-P., Willett, W.C., Hu, F.B., 2010. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care* 33:2477–2483. <http://dx.doi.org/10.2337/dc10-1079>.
- Meiklejohn, S., Ryan, L., Palermo, C., 2016. A systematic review of the impact of multi-strategy nutrition education programs on health and nutrition of adolescents. *J. Nutr. Educ. Behav.* 48, 631–646.e1. <http://dx.doi.org/10.1016/j.jneb.2016.07.015>.
- Narain, A., Kwok, C.S., Mamas, M.A., 2016. Soft drinks and sweetened beverages and the risk of cardiovascular disease and mortality: a systematic review and meta-analysis. *Int. J. Clin. Pract.* <http://dx.doi.org/10.1111/ijcp.12841>.
- Panahi, S., El Khoury, D., Luhovyy, B.L., Goff, H.D., Anderson, G.H., 2013. Caloric beverages consumed freely at meal-time add calories to an ad libitum meal. *Appetite* 65:75–82. <http://dx.doi.org/10.1016/j.appet.2013.01.023>.
- Sichieri, R., Paula Trotte, A., de Souza, R.A., Veiga, G.V., 2009. School randomised trial on prevention of excessive weight gain by discouraging students from drinking sodas. *Public Health Nutr.* 12:197–202. <http://dx.doi.org/10.1017/S1368980008002644>.
- Singer, J.D., Willett, J.B., 2003. *Applied Longitudinal Data Analysis – Modeling Change and Event Occurrence*. Oxford University Press, New York.
- Singh, G.M., Micha, R., Khatibzadeh, S., Lim, S., Ezzati, M., Mozaffarian, D., 2015a. Estimated global, regional, and national disease burdens related to sugar-sweetened beverage consumption in 2010. *Circulation* 132:639–666. <http://dx.doi.org/10.1161/CIRCULATIONAHA.114.010636>.
- Singh, G.M., Micha, R., Khatibzadeh, S., Shi, P., Lim, S., Andrews, K.G., Engell, R.E., Ezzati, M., Mozaffarian, D., 2015b. Global, regional, and national consumption of sugar-sweetened beverages, fruit juices, and milk: a systematic assessment of beverage intake in 187 countries. *PLoS One* 10, e0124845. <http://dx.doi.org/10.1371/journal.pone.0124845>.
- Story, M., Kaphingst, K.M., French, S., 2006. The role of schools in obesity prevention. *Futur. Child.* 16, 109–142.
- Van Rompay, M.L., McKeown, N.M., Goodman, E., Eliasziw, M., Chomitz, V.R., Gordon, C.M., Economos, C.D., Satchell, J.M., 2015. Sugar-sweetened beverage intake is positively associated with baseline triglyceride concentrations, and changes in intake are inversely associated with changes in HDL Cholesterol over 12 months in a multi-ethnic sample of children. *J. Nutr.* 145:2389–2395. <http://dx.doi.org/10.3945/jn.115.212662>.
- Van Wymelbeke, V., Bérudot-Thérond, M.-E., de La Guéronnière, V., Fantino, M., 2004. Influence of repeated consumption of beverages containing sucrose or intense sweeteners on food intake. *Eur. J. Clin. Nutr.* 58:154–161. <http://dx.doi.org/10.1038/sj.ejcn.1601762>.
- Vartanian, L.R., Schwartz, M.B., Brownell, K.D., 2007. Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. *Am. J. Public Health* 97:667–675. <http://dx.doi.org/10.2105/AJPH.2005.083782>.
- Wang, J.W., Mark, S., Henderson, M., O'Loughlin, J., Tremblay, A., Wortman, J., Paradis, G., Gray-Donald, K., 2013. Adiposity and glucose intolerance exacerbate components of metabolic syndrome in children consuming sugar-sweetened beverages: QUALITY cohort study. *Pediatr. Obes.* 8:284–293. <http://dx.doi.org/10.1111/j.2047-6310.2012.00108.x>.
- Xavier, H.T., Izar, M.C., Faria Neto, J.R., Assad, M.H., Rocha, V.Z., Sposito, A.C., Fonseca, F.A., dos Santos, J.E., Santos, R.D., Bertolami, M.C., Faludi, A.A., Martinez, T.L.R., Diamant, J., Guimarães, A., Forti, N.A., Moriguchi, E., Chagas, A.C.P., Coelho, O.R., Ramires, J.A.F., 2013. V Diretriz Brasileira de Dislipidemias e Prevenção da Aterosclerose. *Arq. Bras. Cardiol.* 101:01–22. <http://dx.doi.org/10.5935/abc.2013S010>.