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ORIGINAL ARTICLE

Multicomponent intervention effect on cardiometabolic risk factors among overweight/obese Brazilian children: a mediation analysis

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Abstract

Purpose To verify whether percentage of body fat, physical fitness, physical activity and calorie intake mediates the multicomponent intervention effect on cardiometabolic risk factors in overweight/obese children, and present the relative contribution of each mediator.

Methods This is an intervention study, developed with 35 overweight/obese school-aged children (control group = 17 and intervention group = 18), aged between 7 and 13 years (9.05 ± 1.90). A 12-week multicomponent intervention was performed, consisting of physical exercise, nutritional education sessions and parental support. The following variables were evaluated at baseline and post-intervention: anthropometric measures and percentage of body fat, physical fitness, physical activity assessed by accelerometer, total calorie intake and biochemical assays. For statistical analysis, generalized linear models were used.

Results The intervention effect on glucose was mediated by percentage of body fat (24%), muscular fitness (22%) and total calorie intake (40%). The same was observed for alanine aminotransferase (ALT), with a mediation proportion of 26, 31 and 35%, respectively, as well as for HDL-C (percentage of body fat − 30%, muscular fitness − 30% and total calorie intake − 33%); while vigorous physical activity mediated the intervention effect on glucose (40%), HDL-C (39%) LDL-C (43%) and total cholesterol (37%).

Conclusion Interventions strategies should focus on reducing percentage of body fat and calorie intake, and enhancing muscular fitness and vigorous physical activity to achieve effective changes on cardiometabolic risk factors.

Keywords Obesity · Physical exercise · Cardiometabolic health

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Abbreviations

NAFLD	Nonalcoholic fatty liver disease
ULS	Upper-limb strength
LLS	Lower-limb strength
TC	Total cholesterol
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
TG	Triglycerides
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
HOMA-IR	Homeostasis model assessment of insulin resistance

Introduction

Cardiometabolic dysfunction and childhood obesity have been identified as important determinants of health, in early ages and adulthood. Research has indicated several related risk factors, such as dyslipidemia, insulin resistance, and nonalcoholic fatty liver disease (NAFLD) [1]. Moreover, in youth population, physical fitness and physical activity exert an important role in this context, as studies have shown that high levels of cardiorespiratory, muscular fitness, and physical activity [2, 3], along with dietary intake, may prevent the development of metabolic diseases and obesity [4].

However, a large proportion of children and adolescents are not compliant with healthy eating and physical activity recommendations [5, 6]. Furthermore, a recent study with Brazilian children and adolescents indicated an increased occurrence of clustered cardiometabolic risk factors (evaluated through body mass index and cardiorespiratory fitness) and musculoskeletal risk (flexibility and abdominal strength/resistance) over the years [7].

Physical activity intervention programs have been shown to provide cardiorespiratory, muscular fitness, and body composition improvements among pediatric populations [8, 9]. Similarly, there is substantial evidence showing the positive impact of exercise programs on risks for cardiometabolic dysfunctions, such as insulin resistance, low-grade inflammation, dyslipidemia, and NAFLD in children and adolescents with normal weight or obese [9–11]. In addition, multidisciplinary approaches that combine parental support and nutrition education, besides physical exercise, seems to be appropriate strategies to promote health in this population [10, 12].

It is important to consider that the relationship between changes in cardiometabolic risk factors after an intervention program may be mediated by indirect mechanisms. Álvarez et al. [13] recently suggested that improvements in lower body strength and decreases in waist circumference of school-aged children may explain the observed improvements in glucose control of insulin resistance after six weeks of resistance or high-intensity interval training. Therefore, we hypothesized that changes in physical fitness, physical activity, percentage of body fat, and calorie intake are important mediators to consider along the causal pathway between the intervention program and its positive effects in cardiometabolic risk factors in overweight/obese youths. According to our knowledge, few studies have addressed the potential role of different modifiable risk factors in cardiometabolic risk factors after a multicomponent intervention. The knowledge of mediating mechanisms allows the determination of the main aspects the intervention should focus. Thus, the aim of the present study was to verify whether percentage of body fat, physical fitness, physical activity, and calorie intake mediates the multicomponent intervention effect on cardiometabolic risk factors in overweight/obese children, and present the relative contribution of each mediator.

Methods

This is an intervention study, which is part of the "Action for Health" (Adolescents and Children in a Training Intervention for Health) project, developed with overweight/obese low-income children of both genders, aged between 7 and 13 years. This multicomponent intervention program aims to promote physical activity after-school classes, nutritional education sessions, and parental support (Fig. 1).

The evaluation methods and procedures were approved by the Scientific Board of the Research Unit that leads the project and is registered at https://www.clinicaltrials.gov (Number: NCT02929472). Also, all the Helsinki Declarations' ethical aspects were followed. More detailed information about study design and intervention was published before [14].

Participants and procedures

The program was developed by four physical education teachers, one nutritionist, and one pediatrician who previously participated in weekly meetings (over 6 months), where methodologies and intervention sessions were prepared. The project was publicized in two educational poles (six public schools) from João Pessoa-PB (Brazil), which were located in regions nearby the intervention site. All schools were located in a deprived area, with low socioeconomic status. Parents of potential participants ($n = 276$) were invited to participate in early evening information meetings with the multidisciplinary staff. From those, ninety-six parents attended the meetings, where all the potential participants were screened for inclusion criteria, defined as being overweight or obese, according to Cole et al. criteria [15], and being not involved in any other physical exercise or diet

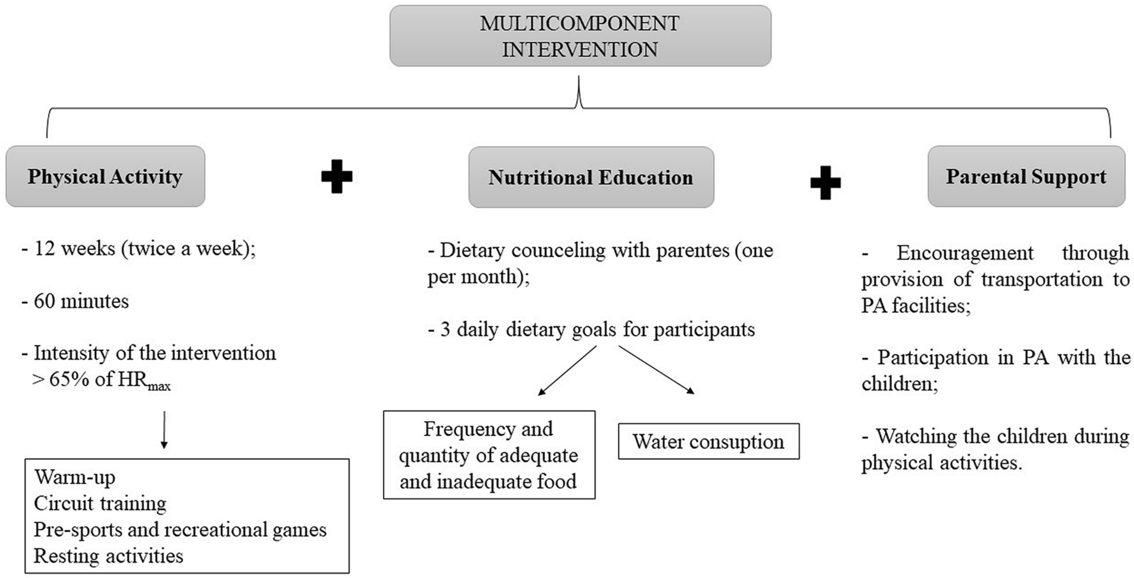

Fig. 1 Organization of the multicomponent intervention. *PA* physical activity, HR_{max} maximum heart rate

program. From those attending the first screening, fifty-three were excluded, leaving forty-three in the study, which were allocated by convenience in the control group (CG, $n=20$) and intervention group (IG, $n=23$). However, two participants did not complete baseline evaluation in CG, and six in IG, leaving a total of 17 participants in CG and 18 in IG. Children and parents of the CG were advised to not change their lifestyle during the period between the evaluations (12 weeks). The parents from those who agreed to participate signed the consent form, as well as the assent form was signed for children and adolescents.

Multicomponent intervention program

Physical exercise intervention

The exercise sessions aimed to develop general motor fitness performance, and took 60 min, twice a week, during 12 weeks. Each session included 10 min of warm-up; 30 min of circuit training; 15 minutes of pre-sports and recreational games; and 5 min of resting activities. Warm-up included aerobic/anaerobic and recreational activities. The circuit, divided into six stations, included activities that prioritized conditional and physical fitness components (muscular strength, cardiorespiratory fitness, balance, motor coordination, and agility) [16]. Exercises were structured so that participants could maintain a high intensity throughout the circuit. Every week, time in each station was increased, or exercise complexity was changed (i.e. balance with opened eyes and both feet on the floor, balance in an unstable surface, balance with closed eyes), to guarantee load´s increments.

Considering physical exercise protocol aimed to improve general motor fitness performance, balance and coordination exercises, for example, in its nature, demand lower heart rate. Therefore, training intensity and compliance between individuals were defined to induce heart rate (HR) higher than 65% of HR_{max} of each participant [17].

To monitor the activity, ten randomly selected participants wore a portable HR monitor (Polar Team^2 Pro, Polar, Finland) during sessions. The attendance average in physical exercise intervention was 85% in the IG.

Nutritional education intervention

The nutritional session was designed by the nutritionist staff and consisted of two actions. The first one named dietary counseling. Each month, all participants and their parents were invited for a nutritional appointment in which was provided information about the food pyramid, weight loss importance, fat-free and low-energy foods, food calories, good nutritional choices on preparing low-cost meals, and decisions on food choices. The three sessions (1/month) were based on the information recorded through the participant's 24 h-recorded-dietary evaluation, which was done by the nutritional staff at baseline. The attendance to appointments was 100%. The second action consisted of the attempt to accomplish three daily dietary goals, during all the intervention development. These goals were focused on three main points: (a) increase frequency and quantity intake of adequate food; (b) decrease of inadequate food intake; and (c) increase water consumption. At the beginning of the intervention, participants received a worksheet to record day by day whether they met the daily goals.

Parental support intervention

Simultaneously, exercise sessions were offered to all parents to encourage family support. Adults' participation was voluntary and the parental support intervention was focused on improvement of three aspects: (a) encouragement through the provision of transportation to physical activity facilities (100% attendance); (b) participation in physical activity with the children, when applicable (82% attendance); (c) watching the children during physical activities (95% attendance). The activities were carried out by a trained physical education teacher in the center of sports of Federal University of Paraíba, João Pessoa (Brazil), during children's same schedule. Parent's attendance average for the three different actions was 88%.

Measures

Baseline measurements were conducted during a 14-day period, for both groups. On the first day, it was performed the blood collection and anthropometric measures, on the second day, children did the body scan and participants and parents were interviewed about children's 3-day food intake record. The third and fourth days were used to administer physical fitness tests. On the fourth day, participants received their accelerometer to be used during the last 10 days of evaluation (Fig. 2). All physical tests were carried out at the Physical Education Department of the Federal University of Paraiba. The blood analysis was carried out at a convened laboratory in João Pessoa/Brazil. For post-intervention evaluation, the same procedures were followed.

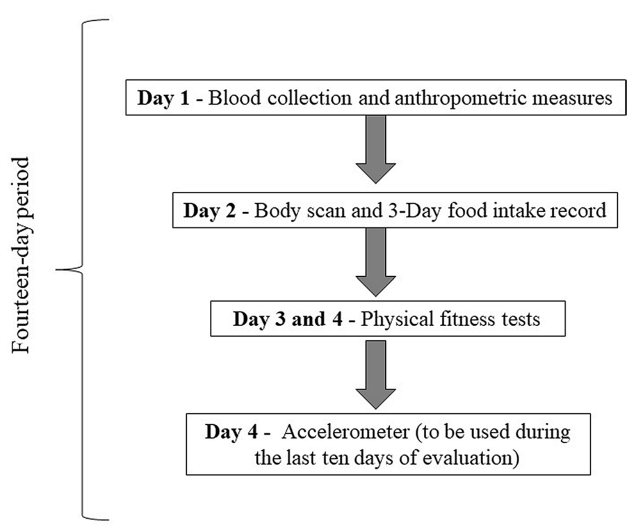

Fig. 2 Sequence of evaluations for intervention and control groups

Anthropometric measures and body composition

Height and weight were determined by a "Holtain" stadiometer, for this evaluation children should be barefoot and light clothing. Then, body mass index was calculated, by dividing body mass (in kilograms) by height (in square meters). Percentage of body fat was measured after 4-h-fasting and low water intake with a bioimpedance scale (Inbody 720, Biospace Co. Ltd.). These measures were taken following standardized procedures [18].

Physical fitness

Cardiorespiratory fitness was measured using the 20 m shuttle-run test. Participants completed 20 m shuttle runs keeping in time with an audible "beep" signal. The frequency of the sound signals were increased every minute, by 0.5 km/h, increasing the intensity of the test, and youth were encouraged to run to exhaustion [19]. The number of completed shuttles was considered for each participant.

Upper limb strength (ULS) was measured through a handgrip dynamometer. The participant squeezed gradually and continuously for at least two seconds, performing the test with the right and left hands in turn, and with the elbow in full extension. The test was performed twice and the maximum score for each hand was recorded in kilograms. The sum of the scores achieved by left and right hands was used in the analysis. Lower limb strength (LLS) test was assessed by the standing-long jump. From a parallel standing position and with arms hanging loose to the side, participants were instructed to jump twice as far as possible in horizontal direction and to land on both feet. The test score (best of two trials) was the distance in centimeters, measured from the starting line to the point where the back of the heel landed on the floor. Abdominal strength was assessed by the cadence-based curl up test (Cooper Institute for Aerobics Research 1999) [20], the test was finished when the subject was unable to maintain the required cadence or unable to maintain the proper curl up technique for 2 consecutive repetitions. Each subject completed as many repetitions as possible. The results of the ULS, LLS and abdominal strength test were transformed to standardized values (z-scores). Then the sum of z-scores was performed to create a muscular fitness score.

Physical activity

Physical activity was measured by an accelerometer (Actigraph, GT3X model, Florida). Participants used an accelerometer for ten consecutive days, and were provided with a diary of activities in which they were required to record use and non-use time, and also habitual physical activities. Data reduction was performed by Actlife software, version 6.11.7. Criteria for a successful recording were a minimum of 4 days of the week and 1 day of the weekend and more than 480 min per day, and the time of non-use was estimated based on periods of more than 20 consecutive minutes of zero [21]. Of these 4 days, 2 days should correspond to intervention days and 2 days without intervention (one weekend day). The epoch period was set at 15 s, based on recommendations to a similar sample [22], and the output was expressed as counts per minute (counts/min). Activity counts were summed for each hour that the accelerometer was worn. Specific cut points proposed by Evenson et al. [23] were used to determine vigorous physical activity (counts/15 s $\geq$ 1003).

Total calorie intake

Food intake was carried out by a 24-h recall [24]. Participants, along with their parents, informed food intake during the past two weekdays and one weekend day before evaluation. Data from these 3 days were tabulated in the software "Virtual Nutri" to obtain the total energy intake values for each of the 3 days. For analytical procedures, the mean value of caloric intake among the 3 days was used and data were recorded in kcal.

Biochemical assays

The circulating levels of plasma insulin, glucose, cholesterols [total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)], triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), were measured through peripheral puncture in the cubital vein after a nocturnal 12 h-fasting, by laboratory specialists, using standard techniques. The analysis of TC, HDL-C, TG, and glucose was carried out by spectrophotometry (Cobas Integra 400 Plus) with Roche® kits. The LDL-C fraction was indirectly calculated using the Friedewald formula [25]. AST and ALT levels were determined by enzyme kinetic assay for spectrophotometrically. Insulin was determined by Luminex-100 IS (Integrated System: Luminex Corporation, Austin, TX, USA), using the Linco Human Gut Hormone panel kit (Linco Research Inc., MO, USA). As a proxy measure of insulin resistance, Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the product of basal glucose (mmol/L) and insulin (μIU/mL) levels divided by 22.5 [26]. All samples were run in duplicates and the means were calculated.

Statistical analyses

Descriptive data are presented as means and standard errors. All variables were checked for normality. Independent *t* tests were conducted to test for differences between the IG and CG in cardiometabolic risk factors at baseline.

Generalized linear models were used to analyze the mediated effect. To assess mediating effects, the product-of-coefficient test was used. This test consist of: (1) estimating the main effect of the intervention on changes in the dependent variables, where in the dependent variables (glucose, AST, ALT, HDL-C, LDL-C, TC, triglycerides, insulin and HOMA-IR) at post-intervention was regressed on the intervention condition and dependent variables at baseline (c-coefficient); (2) estimating the effect of the intervention on changes in the potential mediators by regressing the PI-values of the mediators (percentage of body fat, cardiorespiratory fitness, muscular fitness, vigorous physical activity and total calorie intake) onto the intervention condition, adjusted for baseline values of the mediators (a-coefficient); (3) estimating the independent effect of changes in the potential mediators on changes in the dependent variables, adjusted for the intervention condition, by regressing the post-intervention values of dependent variables onto the intervention condition, baseline values of dependent variables and the post-intervention and baseline values of the potential mediators (*b*-coefficient); and (4) computing the product of the two coefficients ($a*b$), representing the mediated effect. The statistical significance of the mediated effect was estimated by the Sobel test. All analyses were adjusted for sexual maturation. Whether there was no effect of the intervention on cardiometabolic risk factors mediation analyses was not conducted.

Furthermore, the proportion mediated was calculated by dividing the product-of-coefficient ($a*b$) by the total main effect of the intervention condition on the dependent variables (*c*-coefficient).

All analyses were carried out using the IBM SPSS 21 (SPSS, Inc., Chicago, Illinois/USA). The sample size was calculated a posteriori in the software G*power, considering that participants were volunteered selected. Thus, for linear multiple regression, a medium effect of the intervention and a medium effect of mediation on dependent variables ($F^2 = 0.15$–0.35) were considered and the value of the statistical power was between 0.60 and 0.80, as well as the level of statistical significance was established as $p < 0.05$.

Results

The sample was composed of 35 overweight/obese children (22 girls and 13 boys), aged between 7 and 13 years. There was no difference in the potential mediators between CG and IG group at baseline: muscular fitness (CG: -0.01 ± 0.23, IG: 0.01 ± 0.12, $p = 0.91$); percentage of body fat (CG: 14.13 ± 1.62, IG: 13.60 ± 2.44, $p = 0.85$), vigorous physical activity (CG: 11.76 ± 2.02, IG: 8.04 ± 1.61,

$p = 0.16$) and total calorie intake (CG: 1706.17 ± 72.12, IG: 1807.62 ± 100.17, $p = 0.41$). Cardiorespiratory fitness was the only potential mediator that showed difference at baseline (CG: 7.24 ± 0.57, IG: 10.82 ± 1.05, $p = 0.005$) (Table 1).

Table 2 shows the mean values of the cardiometabolic risk factors in CG and IG, as well as the intervention effect. Insulin was the only variable that presented difference at baseline between groups (CG: 6.30 ± 0.95, IG: 9.09 ± 0.98, $p = 0.05$). Furthermore, there was a significant intervention effect on changes in fasting glucose, ALT, TC, HDL-C, LDL-C, insulin, and HOMA-IR.

Table 3 shows the effect of the intervention on the mediator (*a*-coefficient), the effect of the mediator on dependent variables (*b*-coefficient) and the mediated effects for the intervention effect on the dependent variables ($a*b$). Percentage of body fat mediated the intervention effect on ALT (26%), TC (21%), HDL-C (30%) and glucose (24%). Cardiorespiratory fitness did not mediate the intervention effect on any of the cardiometabolic risk factors, while muscular fitness mediates the intervention effect on ALT (31%), TC (22%), HDL-C (30%), and glucose (22%). Regarding vigorous physical activity, it was observed a mediation effect on TC (37%), HDL-C (39%), LDL-C (43%), and glucose (40%). Finally, total calorie intake mediate the intervention effect on TC (32%), ALT (35%), HDL-C (33%) and glucose (36%).

Discussion

Considering that the direct effect of the intervention on cardiometabolic risk factors is already known, our study aimed to add new information regarding the role of potential mediators in this relationship. Thus, our findings revealed that there was a significant intervention effect on changes in glucose, ALT, TC, HDL-C, LDL-C, insulin, and HOMA-IR in overweight/obese children. Besides, the percentage of body fat, muscular fitness, total calorie intake, and vigorous physical activity mediated the intervention effect on the above-mentioned cardiometabolic risk factors.

Our findings indicated that the multicomponent intervention was effective to reduce ALT levels, while for AST there were no changes. In line with the present results, a systematic review investigated the effect of lifestyle interventions on NAFLD and found a decrease in ALT levels in children [11]. More recently, Gonzales-Ruiz et al. [27] indicated that exercise was associated with a reduction

Table 1 Potential mediators in control group and intervention group at baseline

Potential mediators	CG Mean (SD)	IG Mean (SD)	*p*
Muscular fitness (*z*-score)	−0.01 (0.23)	0.01 (1.12)	0.91
Cardiorespiratory fitness (laps)	7.24 (0.57)	10.82 (1.05)	0.005
Percentage of body fat (%)	14.13 (1.62)	13.60 (2.44)	0.85
Vigorous physical activity (min/day)	11.76 (2.02)	8.04 (1.61)	0.16
Total calorie intake (kcal)	1706.17 (72.12)	1807.62 (100.17)	0.41

CG control group, *IG* intervention group, *SD* standard deviation

Table 2 Cardiometabolic risk factors in control and intervention group at baseline and post-intervention and intervention effect on ardiometabolic risk factors.

Cardiometabolic risk factors	Baseline			Post-intervention		Intervention effect	
	Control ($n = 18$)	Intervention ($n = 17$)	*P*	Control ($n = 18$)	Intervention ($n = 17$)		
	Mean (SE)	Mean (SE)		Mean (SE)	Mean (SE)	*c*-coefficient (95%)	*p*
ALT (U/L)	18.95 (0.99)	19.53 (1.02)	0.68	23.77 (3.01)	16.69 (3.10)	−0.82 (−1.28, −0.35)	**< 0.001**
AST (U/L)	28.50 (3.05)	28.65 (1.73)	0.96	27.93 (1.13)	26.19 (1.48)	−0.02 (−0.51, 0.46)	0.92
TC (mg/dL)	158.11 (6.23)	171.64 (6.41)	0.14	169.00 (6.36)	156.22 (6.55)	−0.88 (−1.28, −0.49)	**< 0.001**
HDL-C (mg/dL)	50.61 (2.49)	46.00 (2.57)	0.20	44.87 (2.37)	54.89 (2.44)	1.20 (0.82, 1.59)	**< 0.001**
LDL-C (mg/dL)	94.49 (5.96)	97.93 (4.14)	0.65	100.81 (6.24)	94.79 (4.62)	−0.58 (−0.97, −0.20)	**0.003**
TG (mg/dL)	80.63 (6.67)	76.05 (6.82)	0.63	100.83 (8.17)	95.58 (5.82)	−0.10 (−0.80, 0.59)	0.77
Glucose (mg/dL)	86.27 (1.48)	83.12 (1.53)	0.15	90.27 (1.38)	82.96 (1.42)	−0.91 (−0.42, 0.73)	**< 0.001**
Insulin (UI/mL)	6.30 (0.95)	9.09 (0.98)	**0.05**	7.66 (0.90)	7.10 (0.92)	−0.66 (−1.30, −0.03)	**0.04**
HOMA-IR	1.36 (0.14)	1.88 (0.25)	0.09	1.71 (0.17)	1.46 (0.20)	−0.79 (−1.38, −0.19)	**0.009**

SE standard error, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance

Table 3 Potential mediators of the intervention effect on cardiometabolic risk factors

	Intervention effect on mediator *a* (95% CI)	*p*	Effect mediator on dependent variable *b* (95% CI)	*p*	Mediated effect *a***b* (95% CI)	*p*	Proportion mediated (%)
Percentage of body fat	−0.25 (−0.35, −0.15)	<0.001					
ALT (U/L)	–	–	−0.88 (−1.48, −0.27)	0.004	0.22 (0.06, 0.37)	**0.007**	26.0
TC (mg/dL)	–	–	−0.76 (−1.31, −0.20)	0.007	0.19 (0.06, 0.32)	**0.01**	21.0
HDL-C (mg/dL)	–	–	1.50 (0.98, 2.01)	<0.001	0.37 (0.22, 0.52)	**< 0.001**	30.0
LDL-C (mg/dL)	–	–	−0.21 (−0.72, 0.30)	0.42	0.05 (−0.06, 0.16)	0.42	–
Glucose (mg/dL)	–	–	−0.90 (−1.57, −0.23)	0.008	0.22 (0.04, 0.39)	**0.01**	24.0
Insulin (UI/mL)	–	–	−0.21 (−0.77, 0.53)	0.71	0.05 (−0.10, 0.20)	0.52	–
HOMA-IR	–	–	−0.29 (−0.90, 0.32)	0.35	0.07 (−0.06, 0.20)	0.35	–
Cardiorespiratory fitness	0.35 (−0.25, 0.95)	0.25					
ALT (U/L)	–	–	−0.79 (−1.31, −0.27)	0.003	0.27 (−0.22, 0.76)	0.27	–
TC (mg/dL)	–	–	−0.84 (−1.29, −0.38)	< 0.001	0.29 (−0.24, 0.76)	0.26	–
HDL-C (mg/dL)	–	–	1.14 (0.70, 1.58)	< 0.001	0.39 (−0.79, 1.57)	0.25	–
LDL-C (mg/dL)	–	–	−0.57 (−1.01, −0.12)	0.01	0.19 (−0.16, 0.54)	0.28	–
Glucose (mg/dL)	–	–	−0.82 (−1.32, −0.32)	< 0.001	0.28 (−0.22, 0.78)	0.27	–
Insulin (UI/mL)	–	–	−0.41 (−1.12, 0.29)	0.25	0.14 (−0.19, 0.47)	0.41	–
HOMA-IR	–	–	−0.53 (−1.18, 0.12)	0.11	0.18 (−0.19, 0.55)	0.34	–
Muscular fitness	0.27(0.02, 1.52)	0.02					
ALT (U/L)	–	–	−1.06 (−1.54, −0.58)	< 0.001	0.28 (0.01, 0.55)	**0.04**	31.0
TC (mg/dL)	–	–	−0.77 (−1.18, −0.36)	< 0.001	0.20 (0.01, 0.39)	**0.05**	22.0
HDL-C (mg/dL)	–	–	1.03 (0.62, 1.44)	< 0.001	0.27 (0.02, 0.25)	**0.03**	30.0
LDL-C (mg/dL)	–	–	−0.69 (−1.10, −0.28)	< 0.001	0.18 (−0.01, 0.35)	0.06	–
Glucose (mg/dL)	–	–	−0.95 (−1.48, −0.42)	< 0.001	0.25 (0.001, 0.50)	**0.05**	22.0
Insulin (UI/mL)	–	–	−0.94 (−1.56, −0.32)	0.003	0.25 (−0.02, 0.52)	0.07	–
HOMA-IR	–	–	1.05 (−1.64, −0.46)	< 0.001	0.28 (−0.09, 0.45)	0.06	–
Vigorous physical activity	0.40 (0.10, 0.71)	0.009					
ALT (U/L)	–	–	−0.56 (−1.02, −0.10)	0.01	0.22 (−0.01, 0.45)	0.07	–
TC (mg/dL)	–	–	−0.84 (−1.29, −0.38)	< 0.001	0.33 (0.04, 0.62)	**0.03**	37.0
HDL-C (mg/dL)	–	–	1.17 (0.74, 1.60)	< 0.001	0.46 (0.09, 0.83)	**0.01**	39.0
LDL-C (mg/dL)	–	–	−0.63 (−1.06, −0.20)	0.004	0.25 (0.02, 0.48)	**0.04**	43.0
Glucose (mg/dL)	–	–	0.92 (−1.46, −0.38)	< 0.001	0.36 (0.03, 0.69)	**0.03**	40.0
Insulin (UI/mL)	–	–	−0.54 (−1.17, 0.09)	0.09	0.21 (−0.08, 0.50)	0.15	–
HOMA-IR	–	–	−0.67 (−1.26, −0.09)	0.02	0.26 (−0.03, 0.55)	0.08	–
Total calorie intake	−0.34 (−0.64, −0.04)	0.02					
ALT (U/L)	–	–	−0.87 (−1.38, −0.36)	< 0.001	0.29 (0.001, 0.58)	**0.05**	35.0
TC (mg/dL)	–	–	−0.88 (−1.31, −0.46)	< 0.001	0.29 (0.001, 0.58)	**0.04**	32.0
HDL-C (mg/dL)	–	–	1.20(0.78, 1.61)	< 0.001	0.40 (0.03, 0.77)	**0.03**	33.0
LDL-C (mg/dL)	–	–	−0.64 (−1.02, −0.27)	< 0.001	0.21 (−0.001, 0.42)	0.06	–
Glucose (mg/dL)	–	–	0.92 (−1.46, −0.38)	< 0.001	0.36 (0.03, 0.69)	**0.03**	40.0
Insulin (UI/ml)	–	–	−0.50 (−1.15, 0.15)	0.13	0.17 (−0.08, 0.42)	0.20	–
HOMA-IR	–	–	−0.62 (−1.23, −0.01)	0.04	0.21 (−0.06, 0.48)	0.13	–

ALT alanine aminotransferase, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance

in visceral, subcutaneous, and intrahepatic fat, but did not alter AST and ALT among overweight/obese youths. Given the direct effect of the intervention on ALT, we also showed that decreases in the percentage of body fat and total calorie intake, as well as increasing muscular fitness, mediate this effect in 26, 35, and 31%, respectively.

We are not aware of studies investigating the role of potential mediators of the intervention effect on these variables.

Thus, we hypothesized that these parameters mediate the intervention effect, considering that they are closely associated with changes in NAFLD parameters. Adiposity is a key factor for NAFLD [28], as well as dietary habits [29], thus reducing the percentage of body fat and total calorie intake may mediate the effect of a multicomponent intervention on ALT. More recently, it has been suggested that increasing muscular fitness presented benefits on hepatic fat content reduction in youth [30]. The mechanisms through which muscular fitness might influence NAFLD have not been clarified, but it has been suggested that increased muscular fitness might improve lipid profile, glucose metabolism, as well as increased secretion of myokines, leading to the development of NAFLD at early ages [31].

The positive effect of the multicomponent intervention on lipid profile parameters is in accordance with previous studies developed with obese children youth [11, 32]. In addition, our findings indicated that the change in percentage of body fat, muscular fitness, and total calorie intake mediate the intervention effect on TC and HDL-C. Moreover, vigorous physical activity mediates the intervention effect in the same variables, additionally LDL-C. This means that change in the percentage of body fat, muscular fitness, total calorie intake, and vigorous physical activity might be a biologically plausible mechanism explaining the intervention effects on lipid profile parameters. Some cross-sectional studies have already explored the role of some of the potential mediators investigated in our study on different cardiometabolic risk factors in children [33, 34]. For example, increased vigorous physical activity was associated with reduced cardiometabolic risk factors, including HDL-C and LDL-C in children [35]. However, previous intervention research has not specifically examined the mediating role of change in lipid profile.

Regarding glucose metabolism, the intervention also showed a positive effect, with reductions in fasting insulin, glucose, and HOMA-IR. These findings are in agreement with previous systematic review and meta-analysis in the young population with an excess of adiposity [11]. Mediation analysis showed that the percentage of body fat, muscular fitness, total calorie intake, and vigorous physical activity mediated the intervention effect only for fasting glucose. Although the intervention was effective in reducing HOMA-IR and insulin, surprisingly none of the potential mediators mediate the intervention effect on these variables. Probably the amount of changes in the mediators was not enough to promote changes in the intervention effect of these variables. A recent study in Chilean children showed that waist circumference changes directly contribute to explaining the variance in HOMA-IR changes (38%), whereas the lower body strength might directly contribute to explaining the variance (27%) in glucose control and indirectly contribute to the improvement in body composition [13].

It is also important to consider that for the mediation of total calorie intake on ALT and total cholesterol, as well as the mediation of muscular strength on glucose, there was a small effect size as the inferior 95% CI was almost near zero. Nevertheless, we highlight the clinical relevance of this finding considering the short period duration of the intervention, and also approaching a specific population of overweight/obese children.

Some limitations of this study should be acknowledged. The intervention covered a limited time period (12 weeks) and, therefore, it would be interesting knowing whether a long-term program would result in continued improvement. Also, not randomization and the small sample size requires precautious generalization regarding extrapolation of these results to the wider population of Brazilian overweight/obese children. The main strength is that as far as we know this is the first study using mediation analysis in the intervention effect on several cardiometabolic risk factors. Besides, we considered the role of different mediators. Also, we have evaluated a specific population of overweight/obese youth, and although there are different criteria to classify this condition, such as percentage of body fat, waist circumference, and waist-to-height ratio [36], the use of body mass index has been widely accepted [37, 38]. Finally, the multicomponent design of the intervention that included besides physical exercise, nutritional education, and parent intervention is another important issue.

In conclusion, the percentage of body fat, muscular fitness, vigorous physical activity, and calorie intake mediates the multicomponent intervention effect on cardiometabolic risk factors in overweight/obese children. Thus, the results of this study provide new evidence, indicating that some intermediate variables should be considered when analyzing the relationship between the intervention effects on cardiometabolic risk factors. We also emphasize that to achieve this goal, clinical practice should recommend lifestyle changes, not only resulting in a combination of decreasing percentage of body fat and total calorie intake, but also increasing muscular fitness and vigorous physical activity, for a better cardiometabolic health profile among children.

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Compliance with ethical standards

Conflict of interest There are no potential conflict of interest.

Ethical approval The evaluation methods and procedures were approved by the Scientific Board of the Research Unit that leads the project and is registered at https://www.clinicaltrials.gov (Number:

NCT02929472). Also, all the Helsinki Declarations' ethical aspects were followed.

Informed consent The parents from those who agreed to participate signed the consent form, as well as the assent form was signed for children and adolescents.

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