Effects of Resistance Training on Obese Adolescents

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ABSTRACT

DIAS, I., P. FARINATTI, M. D. G. C. DE SOUZA, D. P. MANHANINI, E. BALTHAZAR, D. L. S. DANTAS, E. H. DE ANDRADE PINTO, E. BOUSKELA, AND L. G. KRAEMER-AGUIAR. Effects of Resistance Training on Obese Adolescents. *Med. Sci. Sports Exerc.*, Vol. 47, No. 12, pp. 2636–2644, 2015. **Purpose**: The effects of resistance training (RT) alone upon endothelial function, metabolic and hemodynamic profiles, physical fitness, body composition, and inflammatory biomarkers in nondiabetic obese adolescents were investigated. **Methods**: Adolescents were assigned into nonobese control (CG, $n = 20$; 14.7 ± 1.4 yr) and obese (OB, $n = 24$; 14.1 ± 1.0 yr) groups. Muscle and skin endothelial reactivity, body composition, at-office and 24-h ambulatory blood pressure, metabolic profile, adipocytokines, aerobic and strength fitness were assessed before and after 12 wk of RT (CG, only at admission). **Results**: After RT, body mass did not change in OB, but significant reductions in body fat (1.6%; $P = 0.01$), waist circumference (2.9%; $P < 0.001$), waist-to-hip ratio (3.3%; $P < 0.001$), homeostasis model assessment for insulin resistance (15.4%; $P = 0.02$), endothelin-1 (14.2%; $P = 0.04$), and fibrinogen (6.9%; $P = 0.03$) were found. Both at-office and ambulatory blood pressure decreased, whereas skin endothelium-dependent vasodilation (32%; $P = 0.02$), $\dot{V}O_2$ (14.3%; $P = 0.04$), and HR (5.3%; $P = 0.04$) during submaximal exercise and isokinetic strength (extension, 21.3%; flexion, 29.9%; $P < 0.0001$) increased. Forearm vascular conductance increased at rest (28.1%; $P = 0.03$) and during postocclusive reactive hyperemia (25.2%; $P = 0.02$). After RT differences between CG and OB at admission were no longer detected for most outcomes. **Conclusions**: RT alone improved endothelial function, hemodynamic and metabolic profiles, body composition, and physical fitness in nondiabetic obese adolescents regardless of changes in body mass. **Key Words**: RESISTANCE EXERCISE, VASCULAR FUNCTION, LOW-GRADE INFLAMMATION, OBESITY

Childhood obesity has increased dramatically in the last decades, being associated with metabolic disorders and increasing risk of cardiovascular disease (CVD) in adulthood, which has been attributed to a premature atherosclerotic process (24). Endothelial dysfunction is an early marker of atherosclerosis, being acknowledged to be associated with risk factors such as type 2 diabetes mellitus (T2DM), hypercholesterolemia, and hypertension (40). Strategies to reduce risk exposure include early detection and improvement of endothelial dysfunction, especially at younger ages.

Regular physical exercise improves endothelial function by increasing the bioavailability of nitric oxide (NO) (8), and aerobic training has been recommended to reduce cardiovascular risk in adults (26) and children (38). However, evidence suggests that muscular fitness would be an independent marker of prevalence and mortality because of CVD and metabolic syndrome (MS) (18). Hence, specific strategies to increase strength and muscle mass, such as dynamic resistance training (RT), might help in decreasing cardiovascular risk and have been recommended as complementary to aerobic exercise in obese adults (11).

There is evidence that in distinct adiposity states, RT may induce long-term anti-inflammatory effects (31). Notwithstanding, there is a lack of research about isolated effects of RT on endothelial function and inflammatory biomarkers in obese adolescents. In most cases, the few available studies investigated the combined effects of resistance and aerobic trainings (e.g., concurrent training) (19,42). It is unclear whether isolated RT might improve endothelial and inflammation risk markers for CVD. Given the limited success of obese subjects in other kinds of activity as endurance and team sports (23), RT could be an alternative strategy to increase adherence to physical activity (39).

APPLIED SCIENCES

In brief, RT programs should be further investigated with regard to different outcomes related to cardiovascular risk. The aim of this study was to investigate the effects of a supervised RT program on endothelial function, metabolic and hemodynamic profiles, inflammatory biomarkers, body composition, and physical fitness in nondiabetic obese adolescents. We hypothesized that RT alone would improve these markers, irrespective of changes in body weight.

SUBJECTS AND METHODS

Subjects. Forty-four adolescents (24 girls; body mass index (BMI), 20.6 ± 2.4 kg·m^{-2}) participated in the study and were classified according to SD scores for BMI (z-BMI). Local ethics committee approved the study (CAE: 0165.0.228.000-09), and a written informed consent was provided by subjects' parents or legal guardians. Inclusion criteria were as follows: age between 13 and 17 yr and pubertal maturation level from 3 to 5 according to the Tanner scale. Exclusion criteria were as follows: 1) z-BMI from 1 to 2 (overweight but not obese), 2) T2DM, nephropathy, or hypertension, 3) smoking or use of any medication for weight, glucose, or blood pressure control, and 4) self-reported regular physical activity >45 min·wk^{-1}, besides curricular physical education.

Study design. Initially, subjects completed a screening for physical activity and clinical status. Arterial hypertension, MS, and T2DM were diagnosed according to the Eighth Joint National Committee (17), International Diabetes Federation (46), and American Diabetes Association (1) criteria, respectively. After laboratory tests for eligibility, subjects were assigned to groups according to z-BMI: obese (OB, z-BMI from 2 to 3) and controls (CG, z-BMI −2 to 1).

Baseline assessment included the following variables: anthropometric—BMI, z-BMI, waist circumference (WC), and body composition; hemodynamic—at-office systolic and diastolic blood pressures (SBP/DBP) and 24-h ambulatory BP measurement (ABPM-24); laboratorial—lipids, glucose metabolism, and adipocytokines; endothelial function at skin and muscle sites; physical fitness—strength and aerobic submaximal capacity. Subsequently, only OB underwent a 12-wk RT program and performed postintervention measurements. All tests (before and after intervention) were applied in the same period of the day. Subjects were instructed to keep their regular activities, avoiding major lifestyle changes. At the end of the experiment, volunteers were referred for treatment at the outpatients care unit.

RT program. The RT protocol included one to three sets of exercises for all major muscle groups, performed in the following order: chest and leg press, low row, leg extension, seated bilateral cable row, leg and arm curls, leg adduction, triceps extension, leg abduction, plantarflexion, and push-ups. RT was performed on a circuit training format without rest interval between sets and exercises. The first 2 wk was dedicated to adaptation to training and learning of movements.

The 12-wk program was performed three times per week at nonconsecutive days, totaling 36 sessions with duration of 30–40 min each.

Ten-repetition maximum tests (10RM) were applied to determine the training workloads for all exercises. The loads corresponding to 10RM were determined 48–72 h before the first training session and reassessed at the end of the second and sixth weeks of intervention to ensure an accurate exercise prescription. RT progression was based on available recommendations for strength training in adolescents (13), as follows: one set of 10–15 repetitions with 50%–70% 10RM in the first 2 wk, two sets of eight to 12 repetitions with 60%–80% 10RM from weeks 3 to 6, and three sets of six to 10 reps with 70%–85% 10RM from weeks 7 to 12. Subjects attending less than 90% of RT sessions were considered as noncompleters. All exercise sessions were supervised by the same trained physical education professional.

Anthropometry. The same trained examiner performed the following measures: body mass (kg), height (m), and waist and hip circumferences (cm). Waist-to-hip ratio (WHR) and BMI were calculated. SD scores for BMI were obtained from growth charts of the National Center for Health Statistics/World Health Organization (Anthroplus 1.0.3, Geneva, Switzerland).

At-office and 24-h ABPM and HR. At-office SBP and DBP were assessed following available recommendations (32) by means of a semiautomatic device (G·Tech; Onbo Eletronics™, Shenzhen, Guangdong, China). The 24-h ABPM and HR were measured each 30 min by automatic noninvasive ambulatory monitor (Spacelabs™ Medical, Redmond, WA). Recordings were analyzed for daytime (0600 to 2200), night (2200 to 0600), and 24 h, using the ABP Report Management System Software (version 2.00.09).

Body composition. The following body fractions were determined by dual energy x-ray absorptiometry (Hologic 4500A; Hologic™, Bedford, MA): lean body mass and fat content (9). The amount of fat was expressed in absolute (kg) and relative (%) terms. Variables analyzed in this study were total body fat (%), central fat (%), android fat distribution (%), and lean/fat mass (kg).

Muscle microvascular reactivity. Forearm blood flow (FBF) was noninvasively assessed at the left arm using venous occlusion plethysmography (EC-6; Hokanson™, Bellevue, WA) with the subject in supine position (20). A mercury-filled strain-gauge was placed on the maximal diameter of the upper third of the forearm with two inflatable cuffs on the arm and wrist. The average of four cycles was used to determine resting FBF (mL·min^{-1} per 100-mL tissue). FBF during postocclusive reactive hyperemia (PORH) was used to assess endothelial-dependent vasodilation after 3-min arterial occlusion and normalized as flow per unit of BP to estimate forearm vascular conductance (FVC).

Skin microvascular reactivity. The skin microcirculation is an accessible vascular bed for which dysfunction correlates with markers of CVD (34). Net red blood cell flux (in arbitrary units (PU)) was measured after acclimatization

in a quiet, temperature-controlled room, with subjects in sitting position using laser-Doppler flowmetry (Perimed™ AB, Stockholm, Sweden). Endothelium-dependent and -independent vasodilation were evaluated after iontophoretic release of acetylcholine (ACh) and sodium nitroprusside, respectively (9). Recordings were performed with the probe positioned on the ventral side of the forearm.

Cardiorespiratory fitness. Because of poor reproducibility and precision of maximal exercise testing in very sedentary and unfit adolescents, cardiorespiratory responses to submaximal workload were adopted as markers of aerobic fitness. Although maximal exercise testing is considered the gold standard for assessing aerobic fitness, its application is questionable in very sedentary and unfit adolescents whose performance may be limited because of pain or fatigue rather than exertion as well as in cases where maximal exercise testing is contraindicated (29). In this context, cardiorespiratory responses to submaximal exercise are acknowledged to overcome many of the limitations of maximal exercise testing. By any means, if a similar work is performed with lower HR and oxygen uptake ($\dot{V}O_2$), this fact reflects greater aerobic and cardiovascular efficiency during submaximal effort. This strategy has evident relation with daily tasks and increased safety during the evaluation of cardiorespiratory fitness in our sample of extremely sedentary adolescents.

The $\dot{V}O_2$ was measured using a $\dot{V}O2000$ analyzer (Medical Graphics™, Saint Louis, MO) as described elsewhere (6). After familiarization, tests were performed on a cycle ergometer (Cateye EC-1600; Cateye™, Tokyo, Japan) using an intermittent protocol, which included the following: 1) 3-min warm-up without load and 2) three consecutive stages of 6 min at 30 W interspersed with 3-min intervals. Pedaling rate was fixed at 50–60 rpm. Gas exchange variables were 20-s stationary time-averaged, which provided good compromise between removing data noise while maintaining underlying trend. The means of two higher $\dot{V}O_2$ and HR values obtained in the three 6-min stages were recorded as markers of cardiorespiratory efficiency during submaximal aerobic exercise.

Isokinetic strength. The isokinetic strength of the lower limbs was measured before and after intervention (Biodex™ System 4 PRO, Biodex Medical Systems, Shirley, NY). Immediately before the test, a specific warm-up consisting of 15 repetitions with angular velocity fixed at $120°·s^{-1}$ was performed. Maximal isokinetic strength of knee extensors and flexors was measured in concentric–eccentric muscle action with the dominant limb. Motion range varied between 0° and 90°, with execution speed fixed in $60°·s^{-1}$. Subjects performed three sets of 10 repetitions with 120-s intervals. The peak torque was defined as the highest value obtained in the three sets, whereas values obtained in each set for total work and work fatigue were averaged and adopted as final results. Peak torque and total work were used as indicators of force production, and work fatigue percentage, as reflecting fatigue resistance.

Laboratory analysis. Biochemical measurements were performed twice after 10–12 h of fasting with automated methods (Modular Analytics E 170 and P; Roche™, Basel, Switzerland). Fasting plasma glucose, total cholesterol, insulin, triglycerides, and HDL-cholesterol (HDL-c) were measured as described elsewhere (9), and plasma LDL-cholesterol (LDL-c) and homeostasis model assessment for insulin resistance (HOMA-IR) were calculated. Adiponectin, interleukin 6 (IL-6), leptin, tumor necrosis factor α (TNF-α), resistin, and endothelin 1 (ET-1) were measured by enzyme-linked immunosorbent assay (R&D Systems™, Minneapolis, MN), nonesterified fatty acids (NEFA), by enzymatic colorimetric method assay (Wako Chemicals™, Richmond, VI), and high-sensitivity C-reactive protein was measured by turbidimetry using the latex method. Intra- and interassay coefficients and sensitivity of measurements for adiponectin, IL-6, leptin, TNF-α, resistin, ET-1, and NEFA were, respectively, 5.8%, 2.5%, and 0.246 $\mu g·mL^{-1}$; 2.4%, 3.1%, and 0.039 $pg·mL^{-1}$; 2.3%, 3.1%, and 7.8 $ng·mL^{-1}$; 6.2%, 7.8%, and 0.106 $pg·mL^{-1}$; 5.35%, 0.18%, and 0.026 $ng·mL^{-1}$; 5.44%, 3.07%, and 0.087 $pg·mL^{-1}$; and 4.7%, 3.7%, and 0.0014 mM.

Statistical analysis. Statistical calculations were performed using GraphPad Prism 5.0 (GraphPad™ Software, San Diego, CA). Data are expressed as mean ± SD or median (first to third tertiles). Data normality was tested by D'Agostino and Pearson Omnibus test, and unpaired Student's t-test or Mann–Whitney test was used to compare groups (CG vs OB at baseline, and CG vs OB at postintervention). Within-group differences (OB group at baseline vs postintervention) were determined by paired Student t-test or Wilcoxon test. Changes in clinical/laboratorial/hemodynamic variables in OB were expressed as differences between postintervention and baseline values. Correlations between changes in outcomes were tested by Pearson or Spearman tests only in OB. Significance level was set at $P < 0.05$.

RESULTS

Adherence. Initially, 83 adolescents were recruited for the study. Of these, 23 nonobese subjects missed the scheduled day for the exams (dropouts) and seven obese adolescents were excluded because of T2DM. A total of 53 volunteers was therefore assigned to either nonobese control group (CG, $n = 24$) or obese group (OB, $n = 29$). During the training period, four controls and five obese adolescents dropped out. The final sample consisted of 20 controls (CG, seven girls) and 24 nondiabetic obese adolescents (OB, 17 girls).

Clinical, laboratorial, and body composition variables. Table 1 exhibits data from clinical, laboratory, and body composition assessments. At admission, differences between groups with regard to some clinical (body mass, WC, hip circumference, WHR, BMI, z-BMI, SBP, and DBP), laboratorial (insulin, HOMA-IR, HDL-c, triglycerides, fibrinogen, and MS diagnosis), and body composition (total,

TABLE 1. Clinical, laboratorial, and body composition characteristics in normal weight (CG) and obese adolescents (OB), at baseline and postintervention.

		OB ($n = 24$)	
	CG ($n = 20$)	Baseline	Postintervention
Clinical characteristics			
Age (yr)	14.7 ± 1.4	14.1 ± 1.0	—
Height (m)	1.65 ± 0.1	1.66 ± 0.1	—
Tanner stage	—	—	—
Female (PH;B)	4.0 (4.0–5.0); 4.0 (3.0–4.0)	4.0 (3.2–5.0); 4.0 (4.5–5.0)	
Male (PH;G)	4.0 (3.0–4.0); 4.0 (3.0–5.0)	4.0 (3.0–4.0); 4.0 (4.0–4.0)	
Body mass (kg)	56.1 ± 10.0	87.8 ± 11.3*	87.2 ± 11.5*
WC (cm)	72.4 ± 7.9	103.9 ± 8.9*	100.9 ± 9.4*,**
Hip circumference (cm)	87.0 ± 7.7	111.9 ± 7.9*	111.8 ± 8.6*
WHR	0.83 ± 0.07	0.93 ± 0.06*	0.90 ± 0.07*,**
BMI (kg·m^{-2})	20.6 ± 2.4	32.1 ± 3.6*	31.7 ± 3.7*
z-BMI	0.5 ± 0.2	2.6 ± 0.3*	2.6 ± 0.4*
SBP (mm Hg)	109.7 ± 11.5	122.4 ± 9.1*	110.1 ± 8.3**
DBP (mm Hg)	65.3 ± 5.9	76.1 ± 7.1*	69.0 ± 6.4**
Laboratorial characteristics			
FPG (mg·dL^{-1})	84.3 ± 5.7	84.7 ± 4.1	85.4 ± 4.1
Insulin (mU·mL^{-1})	7.0 (5.2–11.5)	25.5 (17.2–36.5)*	21.5 (16.7–33.2)*,***
HOMA-IR	1.4 (1.0–2.3)	5.2 (3.7–7.4)*	4.4 (3.6–6.9)*,***
Postload PG	95.6 ± 21.1	105.8 ± 25.3	98.1 ± 25.3
Total cholesterol (mg·dL^{-1})	138.5 (124.5–178.8)	159.5 (139.3–181.3)	152.0 (136.8–185.5)
HDL-c (mg·dL^{-1})	49.0 (45.2–57.0)	41.5 (35.2–50.2)****	42.0 (32.7–52.7)
LDL-c (mg·dL^{-1})	85.8 ± 26.1	95.2 ± 22.0	94.5 ± 25.7
Triglycerides (mg·dL^{-1})	66.6 ± 28.2	93.0 ± 47.8****	81.7 ± 29.2
Fibrinogen (mg·dL^{-1})	276.0 (255.0–315.3)	421.0 (387.3–447.5)*	392.0 (354.8–444.8)*****,******
MS diagnosis (%)	0	16.6****	0******
Body composition			
Total fat (%)	24.3 ± 7.6	44.6 ± 4.3*	43.9 ± 4.1****,******
Central fat (%)	22.1 ± 9.2	47.8 ± 4.2*	46.8 ± 4.5****,******
Android fat distribution (%)	24.8 ± 12.4	56.4 ± 4.5*	55.1 ± 5.2*****,******
Lean mass (kg)	40.5 ± 7.4	46.9 ± 7.3*	46.6 ± 7.3*
Fat mass (kg)	13.2 ± 5.2	37.4 ± 6.4*	36.9 ± 6.7*

Data are expressed as means ± SD or medians (first to third).
*Significant difference compared with CG group, $P < 0.001$.
**Significant difference compared with OB group (baseline), $P < 0.001$.
***Significant difference compared with OB group (baseline), $P < 0.01$.
****Significant difference compared with CG group, $P < 0.05$.
*****Significant difference compared with CG group, $P < 0.01$.
******Significant difference compared with OB group (baseline), $P < 0.05$.
B, breast; BMI; body mass index; DBP, at-office diastolic blood pressure; FPG, fasting plasma glucose; G, genitalia (categorical data; expressed as medians and first and third quartiles); HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; MS, metabolic syndrome; PH, pubic hair; SBP, at-office systolic blood pressure; WHR, waist-to-hip ratio; z-BMI, standard deviation score for BMI.

central and android fats, and lean and fat masses) characteristics were detected.

After the intervention, improvements were observed in OB for WC ($P < 0.0001$) and WHR ($P < 0.0001$), without any change in absolute lean ($P = 0.89$) and fat mass ($P = 0.55$). In addition, the percent body fat (total, $P = 0.01$; central, $P = 0.006$; and android, $P = 0.008$) were reduced after training, albeit remaining higher in OB versus CG ($P < 0.05$). At-office BP decreased after intervention in OB ($P < 0.05$), becoming similar to CG. Percent subjects with diagnosis of MS significantly reduced (16.6% vs 0%) after RT ($P = 0.03$). Accordingly, insulin levels ($P = 0.008$), HOMA-IR ($P = 0.02$), and fibrinogen ($P = 0.03$) also decreased after training.

Physical fitness and ABPM-24 h variables. Table 2 depicts the results obtained for isokinetic and submaximal aerobic tests as well as for ABPM-24 h. At admission, work fatigue during knee extension and flexion as well as $\dot{V}O_2$ and HR at 30 W were higher in OB versus those in CG. Differences of SBP or DBP during ABPM-24 h were not detected, but HR measured at day and night was higher in OB versus those in CG. The RT induced favorable adaptations in peak torque (extension, $P = 0.004$; flexion, $P = 0.006$), total work (extension, $P = 0.02$; flexion, $P = 0.045$); $\dot{V}O_2$ at 30 W ($P = 0.04$), and HR at 30 W ($P = 0.04$) in OB. Exercise intervention significantly decreased SBP and DBP measured by ABPM-24 h in OB (except during sleep).

Muscle/skin microvascular reactivity and inflammatory biomarkers. Table 3 presents data for microvascular reactivity and inflammatory biomarkers. At admission, CG showed higher FVC at rest ($P = 0.03$) and during PORH ($P = 0.02$) versus OB. On the other hand, OB exhibited higher leptin ($P < 0.0001$), IL-6 ($P < 0.0001$), and NEFA ($P = 0.02$) but lower adiponectin levels ($P = 0.006$). After RT, an increase of endothelial-dependent vasodilation ($P = 0.02$) was observed in OB whereas endothelial-independent vasodilation remained unaltered ($P = 0.79$). With regard to microvascular reactivity, the FVC at rest ($P = 0.03$) and during PORH ($P = 0.02$) were improved in OB after RT. Consequently, FVC at rest ($P = 0.74$) and during PORH ($P = 0.66$) became similar in OB and CG. A significant change in NEFA was not detected in OB ($P = 0.63$), but values in OB and CG became similar after training ($P = 0.12$). Only ET-1 significantly decreased after RT in OB ($P = 0.04$).

APPLIED SCIENCES

TABLE 2. Physical fitness characteristics and ABPM-24 h in normal weight (CG) and obese adolescents (OB) at baseline and postintervention.

| | CG (n = 20) | OB (n = 24) | |
		Baseline	Postintervention
Physical fitness characteristics			
Peak torque extension (N·m)	151.1 ± 44.9	145.8 ± 23.5	185.2 ± 32.0*,**
Peak torque flexion (N·m)	85.6 ± 28.6	91.3 ± 19.8	130.3 ± 44.3*,**
Total work extension (J)	1376.4.0 ± 377.5	1363.5 ± 297.1	1405.4 ± 275.5*,***
Total work flexion (J)	697.12 ± 267.7	714.7 ± 234.4	737.6 ± 210.2*,***
Work fatigue extension (%)	11.2 ± 13.1	14.9 ± 9.9*	16.5 ± 9.5*
Work fatigue flexion (%)	20.6 ± 13.7	25.9 ± 14.9*	29.5 ± 8.1*
$\dot{V}O_2$ at 30 W (L·min^{-1})	0.5 ± 0.2	0.8 ± 0.3*	0.7 ± 0.2*,***
HR at 30 W (bpm)	121 ± 10	129 ± 11*	123 ± 14*,***
ABPM-24 h (mm Hg)			
SBP all time	118.3 ± 9.2	121.7 ± 8.1	118.9 ± 8.3***
SBP daytime	120.8 ± 10.2	125.0 ± 8.9	121.8 ± 9.2***
SBP nighttime	112.7 ± 9.3	114.5 ± 7.5	112.9 ± 7.8
DBP all time	69.1 ± 7.2	69.6 ± 5.1	67.2 ± 5.2***
DBP daytime	72.2 ± 8.6	73.9 ± 6.5	70.2 ± 5.8***
DBP nighttime	62.8 ± 6.8	60.4 ± 4.2	60.9 ± 6.2
MAP all time	87.3 ± 6.4	88.0 ± 5.9	85.8 ± 5.7***
MAP daytime	89.8 ± 7.8	91.7 ± 6.9	88.6 ± 6.0***
MAP nighttime	81.6 ± 7.0	82.8 ± 6.0	76.2 ± 10.1
HR all time (bpm)	77.7 ± 6.8	85.6 ± 8.2****	80.1 ± 7.3*****
HR daytime (bpm)	81.5 ± 7.4	89.8 ± 9.2****	84.5 ± 6.0***
HR nighttime (bpm)	68.8 ± 7.5	77.2 ± 7.5******	72.0 ± 8.2***

Data are expressed as means ± SD.
*Significant difference compared with CG, $P < 0.05$.
**Significant difference compared with OB (baseline), $P < 0.001$.
***Significant difference compared with OB (baseline), $P < 0.05$.
****Significant difference compared with CG, $P < 0.01$.
*****Significant difference compared with OB (baseline), $P < 0.01$.
******Significant difference compared with CG, $P < 0.001$.
ABPM, ambulatory blood pressure measurement; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure; $\dot{V}O_2$, oxygen uptake.

Correlation analysis. The reduction in percent total body fat significantly correlated with the decrease in WC ($r = 0.42$; $P = 0.04$) and fibrinogen levels ($r = 0.52$; $P = 0.01$). WC was the only anthropometric index that correlated with at-office SBP ($r = 0.46$; $P = 0.01$). The variation in insulin was related to changes in percent central fat ($r = 0.47$; $P = 0.03$), at-office DBP ($r = 0.57$; $P = 0.01$), and mean arterial pressure (MAP) ($r = 0.50$; $P = 0.02$) during daytime measured by ABPM-24. Changes in HOMA-IR showed significant association with variations in DBP ($r = 0.60$; $P < 0.01$) and MAP ($r = 0.58$; $P < 0.01$) during daytime and with DBP ($r = 0.47$; $P = 0.03$) and MAP ($r = 0.46$; $P = 0.03$) during all day. No significant association was found between changes in endothelium-dependent vasodilation and SBP ($r = -0.26$; $P = 0.20$) or DBP ($r = -0.25$; $P = 0.22$). In addition, significant correlations between changes in cardiorespiratory fitness ($\dot{V}O_2$ at 30 W) or isokinetic strength (peak torque at extension and flexion) versus

TABLE 3. Muscle and skin microvascular blood flow and inflammatory biomarkers in normal weight (CG) and obese adolescents (OB) at baseline and postintervention.

| | CG (n = 20) | OB (n = 24) | |
		Baseline	Postintervention
Skin microvascular blood flow			
Iontophoresis (%)			
ACh	1050 (667–1595)	1234 (707–1649)	1805 (1078–2206)*
SNP	1598 (1136–1959)	1735 (1322–2015)	1529 (1086–2884)
Muscle microvascular blood flow			
FVC at resting (FBF per mm Hg)	24.8 (18.4–40.0)	18.2 (13.0–24.8)**	25.2 (16.3–37.3)*
FVC during PORH (FBF per mm Hg)	79.7 (60.8–97.7)	63.7 (54.6–66.9)**	85.0 (50.3–111.1)*
Inflammatory biomarkers			
Leptin (pg·mL^{-1})	3.858 (1.297–9.239)	39.010 (22.710–50.060)***	35.400 (18.780–52.320)***
IL-6 (pg·mL^{-1})	0.6 (0.5–1.0)	1.4 (0.9–2.3)***	1.6 (1.2–2.4)***
Resistin (ng·mL^{-1})	5.6 (4.5–8.2)	7.3 (5.6–8.6)	7.1 (6.0–9.3)
TNF-α (pg·mL^{-1})	1.8 (1.2–2.7)	1.8 (1.2–2.7)	1.8 (1.2–2.7)
Adiponectin (ng·mL^{-1})	10.150 (4.848–13.840)	5.119 (3.110–7.763)***	5.125 (3.025–7.167)***
ET-1 (pg·mL^{-1})	1.6 (1.2–2.2)	1.3 (1.0–1.5)	1.2 (1.0–1.4)*
NEFA (mM)	0.40 (0.26–0.54)	0.48 (0.35–0.70)**	0.40 (0.26–0.54)
C-reactive protein (mg·dL^{-1})	276.0 (255.0–315.3)	421.0 (387.3–447.5)***	392.0 (354.8–444.8)*,****

Data are expressed as medians (first to third).
*Significant difference compared with OB group (baseline), $P < 0.05$.
**Significant difference compared with CG group, $P < 0.05$.
***Significant difference compared with CG group, $P < 0.001$.
****Significant difference compared with CG group, $P < 0.01$.
Ach, acetylcholine; ET-1, endothelin-1; fbf, forearm blood flow; FVC, forearm vascular conductance; IL-6, interleukin-6; NEFA, nonesterified fatty acids; TNF-α, tumoral necrosis factor-α; SNP, sodium nitroprusside.

microvascular reactivity or hemodynamic data (reflected by FVC and BP) could not be detected ($P > 0.05$).

DISCUSSION

The present investigation is the first to demonstrate that an isolated RT program can improve endothelial-dependent microvascular reactivity at muscle and skin sites in nondiabetic obese adolescents, with potential favorable effects on systemic endothelial function (35). Further positive results were as follows: 1) decreased BP, ET-1, and fibrinogen levels, 2) increased cardiorespiratory efficiency during submaximal exercise and muscular strength, and 3) improved body composition and distribution. It is also important to mention that after chronic RT, the body mass in OB did not change whereas the percent of subjects diagnosed with MS significantly reduced probably because of improved insulin sensitivity. Altogether, our findings have originally shown that RT alone improved the physical fitness and reduced cardiovascular and metabolic risk profiles in a group of nondiabetic obese adolescents.

The $\dot{V}O_2$ and mean HR during submaximal exercise decreased in OB after training, suggesting an improvement in cardiovascular efficiency (6). With regard to the isokinetic test, the peak torque represents muscle's maximum strength capability. On the other hand, the total work and work fatigue are related to the muscle's capability to maintain torque throughout the whole test. The peak torque was similar between controls and obese adolescents and increased in OB after training.

Because the total work is in great extent affected by the peak torque, as anticipated, no differences were found between CG and OB at baseline, whereas an increase in overall muscle work capacity was detected in the obese adolescents after RT. The work fatigue is calculated by the ratio between work produced in the first and last third of a given bout of repetitions. In our study, this marker was somewhat higher in OB versus that in CG and remained relatively stable after RT. In theory, as subjects improve their muscle function, the work in the first and last thirds of a set should become more equal (13,33). However, this can be influenced by the maximal strength and consistency of muscle contraction during the test (7,33). For instance, when the peak torque and total work increase, there is more room for differences between the first and last thirds of sets and the average work fatigue might stabilize or even increase. Furthermore, if the subject does not perform a consistent effort, the work completed in the first third may be less than the work in the last third—this is not unusual in subjects not used to maximal effort or discomfort, which was perhaps the case of our sample. Anyway, our findings suggest that RT was able to improve the muscle strength and work capacity of obese adolescents but not necessarily their capacity to maintain the total work across successive sets.

Obesity is related to the atherosclerotic process, and endothelial dysfunction is a premature and surrogate marker of this process. Previous research showed that aerobic and concurrent training (aerobic plus RT) may improve endothelial function and blood flow in overweight and obese adolescents, irrespective of changes in lipid profile (44), body composition (42), and percentage body fat (27). Possibly, these findings are secondary to increased shear stress during exercise, which upregulates endothelial NO synthase expression (22). However, the specific effects of isolated RT in respect of endothelial function remain unclear, particularly in nondiabetic obese adolescents. The present study provides essential information on this issue.

The effects of regular exercise on reducing obesity-related vascular dysfunction may involve several pathways. It is likely that chronic exercise increases not only shear stress but also angiogenesis and blood flow, thereby reducing hypoxia and associated inflammation even at the adipose tissue (45). In this scenario, there is evidence suggesting that RT can be effective to improve vascular function in healthy adult patients, adult patients with hypercholesterolemia, and adult patients with heart failure (2,30,36). Our results suggest that beneficial vascular adaptations due to RT, expressed by increased FVC at rest and during PORH, also occur in nondiabetic obese adolescents.

The cutaneous microcirculation might be viewed as a model to easily and noninvasively assess overall microvascular function. In our study, isolated RT potentiated endothelium-dependent but not -independent vasodilation in OB. Previous studies about the effects of RT on skin microvascular reactivity have mostly investigated adults with T2DM, but the number of trials is limited and their results are mixed. Actually, available reports claimed that either an improvement (3) or no change (4) of endothelium-dependent vasodilation would be induced by RT.

The absence of a nonexercise control group might limit speculation and inferences related to the effect of RT upon the endothelial-dependent vasodilation. However, previous research has indicated that vasodilation capacity may be improved by aerobic exercise (15,25), particularly endothelial-dependent vasodilation (25). It is therefore acknowledged that chronic exercise would have beneficial effects on vasodilation capacity, at least in obese adults (25) and adults with hypertension (15). However, no previous study had until now demonstrated that the endothelial-dependent vasodilation capacity might also respond favorably to isolated RT. Furthermore, this is the first trial showing that this important marker of cardiovascular risk may be improved even in adolescents, which is evidently useful information in the context of early prevention of CVD in obese young populations.

In young individuals, it could be expected that endothelium-independent vasodilation was not impaired at baseline. Hence, there would be limited room for improvement due to chronic exercise because structural damage at the muscle layer of vessels would not be present yet (28). Most likely, damage caused by metabolic disturbances as a result of obesity at this age would occur at a functional level, which would be reflected by changes in endothelium-dependent vasodilation. Considering

that beneficial adaptations to vascular health due to a relatively short-term RT intervention would be probably induced by increased shear stress (41), this might help explain why improvement was detected only in endothelium-dependent reactivity. Furthermore, it is worth mentioning that endothelium-independent vasodilation was similar in OB and CG groups at baseline. These data reinforce the premise that in our sample of obese adolescents, changes due to training would be more likely to occur in endothelium-dependent than endothelium-independent vasodilation.

At microcirculatory level, pathways involved on vessel relaxation are influenced not only by NO but also by prostaglandins and endothelium-derived hyperpolarizing factor (34). The exact pathway underlying the endothelial response to RT remains unknown and warrants further investigations. At skin level, the exact action of iontophoretic ACh also remains controversial (34). Although ACh effects on NO synthase are well defined, at skin, the ciclooxigenase-dependent pathway may have also influenced our findings (4). On the other hand, FVC during PORH tested at muscle site is probably NO-dependent, which reinforces that RT was indeed capable to induce beneficial effects on endothelium-dependent pathway.

In the current investigation, decreases in SBP and DBP were found in assessments performed during consultation (at-office BP) and during 24 h (AMBP 24 h). Few studies investigated the chronic effects of isolated RT on BP, at least one of them suggesting that moderate RT would be able to reduce BP in sedentary adults with normal BP or hypertension (5). A possible reason for the benefits of RT on BP would be an increase in endothelium-dependent vasodilatation combined with reduced chronic inflammation (2,30). Our data partially confirmed this possibility, as along with the improvement in endothelium-dependent vasodilation, there was a decrease in ET-1 levels.

Data about the effects of RT on ET-1 and on fibrinogen are still limited. In obese and insulin-resistant subjects, endothelium-derived peptide ET-1 acts as vasoconstrictor. Insulin resistance is involved in the physiopathology of endothelial dysfunction and also hypertension (40). Previous research has shown that ET-1 regulates glucose metabolism via receptor-dependent mechanisms in insulin-resistant subjects (37). Therefore, it could be speculated that the presently observed effects of RT on insulin sensitivity might have had a role not only in reducing ET-1 but also in lowering BP. Furthermore, a reduction of fibrinogen levels after RT was detected, which correlated with changes in percent body fat. The fibrinogen is acknowledged to affect blood coagulation, platelet aggregation, and endothelial function (12). For that reason, it is feasible to think that the reduction of fibrinogen levels induced by RT could have provoked some beneficial effect on endothelial function. NEFA levels did not change after RT but became similar in CG and OB after training, which might also reflect improved insulin sensitivity.

Chronic low-grade inflammation associated with central obesity may increase insulin resistance (14,16). There is evidence showing that RT can be effective in reducing central obesity in adults (18). With regard to children, a recent review suggested that RT of moderate intensity can induce favorable effects on body composition (10). Van Der Heijden et al. (43) showed improvements in strength, lean body mass, and hepatic insulin sensitivity after 3 months of RT in obese adolescents. More recently, Lee et al. (21) showed that RT was capable to prevent weight gain and reduce abdominal fat, intrahepatic lipid content, and insulin sensitivity in obese male adolescents. Our results concur with some of these findings, including weight gain prevention, increased insulin sensitivity, and improved fat distribution, expressed by reduced WC, WHR, and percent central fat.

The main limitation of this study is the lack of a non-exercise obese control group. It must be acknowledged that some of testing procedures applied in OB may have led to overestimation of the training impact because aspects related to learning effects could not be controlled. The imbalance of females and males in CG and OB might also be considered as a limitation of the study. If both groups were tested before versus after intervention, this difference could result in important bias. However, because we tested only OB at postintervention with special emphasis on intragroup comparison, the potential bias due to such imbalance was probably minimized.

Other potential sources of bias are the absence of objective data about the diet and leisure time spent with physical activities. However, it must be noted that specific recommendations have been given in the sense that habitual activities and diet should be kept and that any kind of additional physical training was forbidden during the experiment. Furthermore, indirect measures of insulin resistance, such as HOMA-IR and fasting insulin, also have moderate correlation with clamp techniques, limiting inferences about insulin sensitivity. Maximal exercise testing to determine the actual aerobic capacity would also be desirable. However, the present sample was composed by extremely sedentary adolescents and the choice of evaluating the submaximal instead of maximal exercise capacity was justified to avoid a potential "ceiling effect" when assessing $\dot{V}O_{2max}$ because of early peripheral fatigue. Finally, mechanistic insights into NO-dependent pathway for endothelial responses to RT could be more specifically addressed if nitrite levels at baseline and postintervention have been assessed.

In conclusion, a 12-wk RT program alone was capable to improve the endothelial function, hemodynamic and metabolic profiles, body composition, and physical fitness in a cohort of nondiabetic obese adolescents. No change in body mass was found, showing that weight gain was at least prevented. These findings suggest that the effects induced by RT were independent of changes in body mass. These results warrant further investigation about the potential effects of RT on risk factors for cardiovascular and metabolic disease in obese children and reinforce the idea that this type of exercise can be an alternative for exercise programs designed to prevent and treat obesity in this population.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes–2014. *Diabetes Care*. 2014;37(1 Suppl):S14–80.

2. Brooks NJ, Layne JE, Gordon PL, Roubenoff R, Nelson ME, Castaneda-Sceppa C. Strength training improves muscle quality and insulin sensitivity in Hispanic older adults with type 2 diabetes. *Int J Med Sci*. 2006;4(1):19–27.

3. Cohen ND, Dustan DW, Robinson C, Vulikh E, Zimmet PZ, Shaw JE. Improved endothelial function following a 14-month resistance exercise training program in adults with type 2 diabetes. *Diabetes Res Clin Pract*. 2008;79(3):405–11.

4. Colberg SR, Parson HK, Nunnold T, Herriot MT, Vinik AI. Effect of an 8-week resistance training program on cutaneous perfusion in type 2 diabetes. *Microvasc Res*. 2006;71(2):121–7.

5. Cornelissen VA, Fagard RH. Effect of resistance training on resting blood pressure: a meta-analysis of randomized controlled trials. *J Hypertens*. 2005;23(2):251–9.

6. Cunha FA, Midgley AW, Monteiro WD, Farinatti PT. Influence of Cardiopulmonary exercise testing protocol and resting VO(2) assessment on %HR(max), %HRR, %VO(2max) and %VO(2)R relationships. *Int J Sports Med*. 2010;31(5):319–26.

7. Davies GI, Heiderscheit B, Brinks K. Test interpretation. In: Brown LE, editor. *Isokinetics in Human Performance*. Champaign (IL): Human Kinetics 2000:3–24.

8. Delp MD, Laughlin MH. Regulation of skeletal muscle perfusion during exercise. *Acta Physiol Scand*. 1998;162(3):411–9.

9. Dias I, Panazzolo DG, Marques MF, et al. Relationships between emerging cardiovascular risk factors, z-BMI, waist circumference and body adiposity index (BAI) on adolescents. *Clin Endocrinol (Oxf)*. 2013;79(5):667–74.

10. Dietz PS, Hoffmann S, Lachtermann E, Simon P. Influence of exclusive resistance training on body composition and cardiovascular risk factors in overweight or obese children: a systematic review. *Obes Facts*. 2012;5(4):546–60.

11. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK, American College of Sports Medicine. Position Stand: appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc*. 2009;41(7):459–71.

12. Ernst E, Koenig W. Fibrinogen and cardiovascular risk. *Vasc Med*. 1997;2(3):115–25.

13. Faigenbaum AD, Kraemer WJ, Blimkie CJ, et al. Youth resistance training: updated position statement paper from the national strength and conditioning association. *J Strength Cond Res*. 2009;(5 Suppl):S60–79.

14. Ferroni P, Basili S, Falco A, Davi G. Inflammation, insulin resistance, and obesity. *Curr Atheroscler Rep*. 2004;6(6):424–31.

15. Higashi Y, Sasaki S, Kurisu S, et al. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation*. 1999;100(11):1194–202.

16. Hunter GR, Bryan DR, Wetzstein CJ, Zuckerman PA, Bamman MM. Resistance training and intra-abdominal adipose tissue in older men and women. *Med Sci Sports Exerc*. 2002;34(6):1023–8.

17. James PA, Oparil S, Carter BL, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA*. 2014;311(5):507–20.

18. Katzmarzyk PT, Craig CL. Musculoskeletal fitness and risk of mortality. *Med Sci Sports Exerc*. 2002;34(5):740–4.

19. Kim ES, Im JA, Kim KC, et al. Improved insulin sensitivity and adiponectin level after exercise training in obese Korean youth. *Obesity (Silver Spring)*. 2007;15(12):3023–30.

20. Kraemer-Aguiar LG, Bahia LR, Villela N. Metformin improves endothelial vascular reactivity in first-degree relatives of type 2 diabetic patients with metabolic syndrome and normal glucose tolerance. *Diabetes Care*. 2006;29(5):1083–9.

21. Lee S, Bacha F, Hannon T, Kuk JL, Boesch C, Arslanian S. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys: a randomized, controlled trial. *Diabetes*. 2012; 61(11):2787–95.

22. Leung FP, Yung LM, Laher I, Yao X, Chen ZY, Huang Y. Exercise, vascular wall and cardiovascular diseases: an update (part 1). *Sports Med*. 2008;38(12):1009–24.

23. Lloyd RS, Faigenbaum AD, Stone MH, et al. Position statement on youth resistance training: the 2014 International Consensus. *Br J Sports Med*. 2014;48(7):498–505.

24. McGill HC, McMahan CA, Herderick EE, et al. Obesity accelerates the progression of coronary atherosclerosis in young men. *Circulation*. 2002;105(23):2712–8.

25. Mestek ML, Westby CM, Van Guilder GP, Greiner JJ, Stauffer BL, DeSouza CA. Regular aerobic exercise, without weight loss, improves endothelium-dependent vasodilation in overweight and obese adults. *Obesity (Silver Spring)*. 2010;18(8):1667–9.

26. Metkus TS, Baughman KL, Thompson PD. Exercise prescription and primary prevention of cardiovascular disease. *Circulation*. 2010;121(23):2601–4.

27. Meyer AA, Kundt G, Lenschow U, Schuff-Werner P, Kienast W. Improvement of early vascular changes and cardiovascular risk factors in obese children after a six-month exercise program. *J Am Coll Cardiol*. 2006;48(9):1865–70.

28. Montero D, Walther G, Perez-Martin A, et al. Effects of a lifestyle program on vascular reactivity in macro- and microcirculation in severely obese adolescents. *J Clin Endocrinol Metab*. 2014;99(3): 1019–26.

29. Noonan V, Dean E. Submaximal exercise testing: clinical application and interpretation. *Phys Ther*. 2000;80(8):782–807.

30. Olson TP, Dengel DR, Leon AS, Schmitz KH. Changes in inflammatory biomarkers following one-year of moderate resistance training in overweight women. *Int J Obes (Lond)*. 2007;31(6): 996–1003.

31. Phillips MD, Patrizi RM, Cheek DJ, Wooten JS, Barbee JJ, Mitchell JB. Resistance training reduces subclinical inflammation in obese, postmenopausal women. *Med Sci Sports Exerc*. 2012; 44(11):2099–110.

32. Pickering TG, Hall JE, Appel JL, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation*. 2005;111(5):697–716.

33. Pincivero DM, Lephart SM, Karunakara RA. Reliability and precision of isokinetic strength and muscular endurance for the quadriceps and hamstrings. *Int J Sports Med*. 1997;18(2):113–7.

34. Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation*. 2012;19(1):47–64.

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35. Sax FL, Cannon RO, Hanson C, Epstein SE. Impaired forearm vasodilator reserve in patients with microvascular angina. Evidence of a generalized disorder of vascular function? *N Engl J Med*. 1987;317(22):1366–70.

36. Selig SE, Carey MF, Menzies DG, et al. Moderate-intensity resistance exercise training in patients with chronic heart failure improves strength, endurance, heart rate variability, and forearm blood flow. *J Card Fail*. 2004;10(1):21–30.

37. Shemyakin A, Salehzadeh F, Bohm F, et al. Regulation of glucose uptake by endothelin-1 in human skeletal muscle in vivo and in vitro. *J Clin Endocrinol Metab*. 2010;95(5):2359–66.

38. Shih KC, Janckila AK, Kwok CF, Ho LT, Chou YC, Chao TY. Effects of exercise on insulin sensitivity, inflammatory cytokines, and serum tartrate resistant acid phosphatase 5a in obese Chinese male adolescents. *Metabolism*. 2010;59(1):144–51.

39. Sothern MS, Loftin JM, Udall JN, et al. Safety, feasibility, and efficacy of a resistance training program in preadolescent obese children. *Am J Med Sci*. 2000;319(6):370–5.

40. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest*. 1996;97(11):2601–10.

41. Tinken TM, Thijssen DH, Hopkins N, et al. Impact of shear rate modulation on vascular function in humans. *Hypertension*. 2009; 54(2):278–85.

42. Tjonna AE, Stolen TO, Bye A, et al. Aerobic interval training reduces cardiovascular risk factors more than a multitreatment approach in overweight adolescents. *Clin Sci (Lond)*. 2009;116(4): 317–26.

43. Van Der Heijden GJ, Wang ZJ, Chu Z, et al. Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. *Med Sci Sports Exerc*. 2010;42(11):1973–80.

44. Watts K, Beye P, Siafarikas A, et al. Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents. *J Am Coll Cardiol*. 2004;43(10):1823–7.

45. You T, Arsenis NC, Disanzo BL, Lamonte MJ. Effects of exercise training on chronic inflammation in obesity: current evidence and potential mechanisms. *Sports Med*. 2013;43(4):243–56.

46. Zimmet P, Alberti G, Kaufman F, et al. The metabolic syndrome in children and adolescents. *Lancet*. 2007;369(9579):2059–61.

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