Effects of a lifestyle program on vascular reactivity in macro and microcirculation in severely obese adolescents

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ABSTRACT

Context and Objective: This study aimed to comprehensively assess the macro- and microcirculation of severely obese adolescents (SOA) and normal-weight counterparts, and determine the longitudinal effects of weight loss on vascular function in SOA.

Design, Setting, Participants, and Outcome Measures: Seventeen SOA (BMI z-score = 4.22 ± 0.73) before and after a 4-month weight loss program, and nineteen puberty-matched normal-weight counterparts (BMI z-score = -0.02 ± 1.04) were studied. Brachial artery flow-mediated dilation (FMD) and response to sublingual nitrate (NMD) were assessed by high-resolution ultrasound. Microvascular reactivity was evaluated by laser Doppler flowmetry in response to NMD, iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP), and local hyperthermia. Plasma insulin, leptin, resistin, C-reactive protein, myeloperoxidase (MPO), and tissue plasminogen activator (tPA) were measured.

Results: At baseline, SOA had similar FMD and impaired NMD in the brachial artery compared to normal-weight adolescents. Similarly, peak responses to ACh and SNP iontophoresis and to local hyperthermia were unaltered whereas cutaneous blood flow following NMD was lower in the forearm microcirculation of SOA. All plasma measurements were significantly higher in SOA. After the program, SOA presented a weight reduction of 7.4 ± 3.1 %, but neither brachial artery nor microvascular reactivity variables were improved. Significant decreases were detected in plasma leptin, MPO and tPA.

Conclusions: Macro- and microvascular endothelial function are preserved in adolescents with severe obesity. Conversely, a 7 % weight loss does not improve their impaired smooth muscle response to exogenous organic nitrate in both vascular beds, despite reducing plasma markers adversely related to vascular homeostasis.
Severe obesity in pediatric population is a major public health concern, as well as one of the fastest growing obesity categories in childhood obesity (1), which is associated with vascular risk factors and disease in adulthood (2). Moreover, the progressive exacerbation of preclinical signs of vascular disease might be particularly accelerated in obese adolescents on account of the pro-inflammatory and pro-oxidative changes that occur during puberty, plausibly hampering vascular function (3). The early detection of vascular alterations is thus a major clinical goal to identify subjects at risk for cardiovascular morbidity, and to initiate strategies to reduce risk exposure.

Endothelial dysfunction is a primary sign of the early stage of atherosclerotic disease, appearing long before the symptoms (4). This alteration has been observed at the macrovascular level in severely obese children and adolescents (5). However, data remain scarce concerning the endothelial function at the microcirculation level in severe childhood obesity (6). The microcirculation, in turn, is increasingly recognized to be independently involved in vascular diseases previously thought to be primarily a macrocirculation matter (7). Furthermore, the specific vascular profile of severely obese pubertal adolescents (SOA) is unknown, since the aforementioned studies included pre-pubertal children (5; 6).

Although lifestyle interventions, including physical activity and/or diet, result in well-known vascular benefits associated with a decrease in markers of inflammation and oxidative stress in moderately obese children and adolescents (3), their effects remain unknown in severe childhood obesity. Therefore, the aims of the present study were 1) to investigate, in a comprehensive manner, the vascular function in the macro- and microcirculation of severely obese and normal weight adolescents, and 2) to determine the longitudinal effects of a weight loss program on both vascular beds in SOA.

MATERIALS AND METHODS

Subjects
Seventeen adolescents with severe obesity were recruited from a pediatric weight management center. Obesity was defined according to the age and sex-specific cut-off points of childhood obesity as indicated by the International Obesity Task Force (8). BMI z-scores were calculated and values greater than 3 defined severe obesity (9). Nineteen healthy normal-weight pubertal stage-matched adolescents were recruited from the community to serve as controls (Table 1). All subjects were normotensive (defined as a pressure < 95th sex-, age-, and height-specific percentiles), non-diabetic, and free from further known obesity-related comorbidities. Exclusion criteria for all subjects included a family history of premature cardiovascular disease, intake of any medication, pubertal status assessed by Tanner stage < 2, weight loss larger than 5% of their total weight during the previous 3 months, and non-sedentary status (> 3 h of exercise per week) to minimize training effects. Informed consent was obtained from the parents and adolescents. The study protocol was approved by the local Ethics Committee and performed in accordance with the principles outlined in the Declaration of Helsinki.

**Weight loss program**

The SOA group underwent a weight loss program consisting in diet and exercise managed by the pediatric weight center. Clinical and vascular assessment of SOA was performed within the first week, and four months later after inclusion in the center. A 4-month period between assessments was considered sufficient time to detect successful weight loss (estimated at 7% of the initial weight) and potential vascular improvement in obese subjects (10). SOA received a moderately hypocaloric diet (reduction of ~300 to 500 calories/day) based on a balanced distribution of carbohydrates (55%), proteins (15%), and lipids (30% total, with less than 10% saturated fat), while performing a physical activity program consisting of four 90-minute supervised sessions per week. Each session mostly involved aerobic exercise, including dancing, tennis, and recreational games, intended to encourage physical activity in the subjects.

**Vascular measurements**
All vascular measurements were performed after fasting overnight in a quiet room with a controlled temperature between 22 and 24 °C. All subjects had abstained from strenuous exercise for 48 h before the test. Measurements commenced after 20 minutes of acclimatization in supine position, and blood pressures were measured on the left arm by an automated system (Dinamap, GE Medical Systems, Milwaukee, USA).

Macrovascular assessment of the brachial conduit artery was performed by the same investigator (A.V.), according to the International Brachial Reactivity Task Force Guidelines (11). Brachial measurements were achieved using high-resolution vascular ultrasonography (MyLab30, Esaote SpA, Firenze, Italy), with a 10-MHz multi-frequency linear probe. B-mode images and Doppler signals were continuously and simultaneously recorded for off-line analysis. All results were calculated as the average of 5 consecutive measurements. Flow mediated dilation (FMD), a well-established noninvasive method to estimate endothelial function in conduit artery, was performed. Briefly, a pneumatic cuff was put on the right forearm near the elbow. The ultrasound probe was placed approximately midway between the antecubital and axillary regions, and baseline brachial artery diastolic lumen diameter was measured. The cuff was then inflated to 250 Hg mm for 5 minutes before sudden cuff deflation induced post-ischemic hyperemia. Fifteen minutes later, baseline measurements were repeated before 0.4 mg of isosorbide dinitrate (Isocard, Schwarz Pharma, Monheim, Germany), an endothelium-independent vasodilator, was given sublingually to assess endothelium-independent vasodilation (nitrate-mediated dilation, NMD). This procedure is described in detail elsewhere (12). FMD and NMD were expressed as the percentage change of peak diastolic brachial diameter after reactive hyperemia and exogenous organic nitrate administration, respectively, relative to the baseline diastolic diameter. Time-averaged mean blood flow velocity and blood flow were determined, as previously described (13). Shear rate (s⁻¹) was calculated as 4 × time-averaged mean blood flow velocity/mean brachial diameter, to estimate resting and peak shear stress (14). FMD was normalized by the net shear rate stimulus (peak minus resting shear rate, ∆shear rate). Although the validity of shear normalization is controversial, we added this measurement as it is commonly used by
other researchers. Within-subject coefficient of variations in our laboratory at rest were 1.8% for arterial
diameters, 13.2% for time averaged mean velocity and 12.7% for blood flow (13).

Microvascular assessment of cutaneous blood flow (CBF) was performed by one investigator (D.M.) by
means of the laser Doppler flowmetry (LDF) technique. LDF continuously monitors perfusion by
measuring microvascular red blood flow using the Doppler principle. The LDF technique has been
previously described in detail (15). Cutaneous blood flow (CBF) was measured in conventional perfusion
units (PU) using a LDF system (Periflux PF 5000, Perimed, Stockholm, Sweden), equipped with a
thermostatic LDF probe with an effective surface of 0.95 cm$^2$ (PF 481, Perimed, Stockholm, Sweden), on
the volar surface of the left forearm. Before commencing the iontophoresis protocol, resting forearm CBF
was calculated by averaging a 3-minute steady recording using a non-drug-containing LDF probe. A
direct current for drug iontophoresis was provided by a battery-powered current stimulator (Perilont,
Perimed, Stockholm, Sweden). Iontophoresis allows non-invasive drug delivery to the skin without
systemic effects and perturbation of the skin. Microvascular responses to iontophoresis of acetylcholine
(ACh) and sodium nitroprusside (SNP) were assessed. SNP 1% and ACh 1% solutions, adjusted to a
physiological ionic strength (0.154 M) by adding saline solution, were administered via two drug delivery
electrodes, each inserted within an LDF probe, positioned 10 cm apart avoiding superficial veins and
broken epidermal areas. In order to minimize non-specific vasodilatory effects, the iontophoresis protocol
consisted in a single anodal (ACh) or cathodal (SNP) pulse of 0.021 mA/cm$^2$ for 370 s, yielding a total
charge of 7.8 mC/cm$^2$ (16). In addition, a non-drug containing LDF probe determined the CBF response
to sublingual administration of organic nitrate (NMD). LDF probes were maintained at a constant
temperature of 33ºC throughout the whole measuring process. To assess the local hyperthermia response,
a non-drug containing LDF probe was heated to 42ºC for 5 minutes. Only the plateau phase
(endothelium-dependent) of the hyperthermia response was analyzed. Peak CBF responses to ACh, SNP,
NMD and local hyperthermia were determined as the maximum average value over a 10 s period within
their respective procedures. Concerning the spatial variability of LDF measurements, the specific volar
forearm location of each LDF probe and electrode was approximately maintained in all subjects, especially for SOA before and after the weight loss program in which locations were noted in relation to anatomical landmarks.

Blood analysis

Blood samples were collected after fasting overnight. Biochemical markers related to vascular function such as leptin, resistin, C-reactive protein (CRP), myeloperoxidase (MPO), and tissue plasminogen activator (tPA) were determined in plasma by bead-based multiplex immunoassays (FlowCytomix, eBioscience, San Diego, CA, USA). Plasma insulin was measured using the radioimmunoassay method (coat-a-count radioimmunoassay kit TKIN2, Siemens, Berlin, Germany).

Statistical analysis

All normally distributed variables were expressed as mean ± SD. Data that were not normally distributed (CRP, tPA, and microvascular variables) were log-transformed to approximate normality before parametric testing, and were expressed as median (interquartile range) in Tables 1 and 3. SOA versus normal-weight subjects were compared by independent t-tests and analysis of covariance (ANCOVA), including gender as a covariate. Paired student t-tests were used to assess SOA before and after the weight loss program. Bivariate associations between vascular and study variables were determined by calculating Pearson’s correlation coefficients. A two-tailed p-value less than 0.05 was considered significant. All statistical analyses were performed using MedCalc software (bvba, Mariakerke, Belgium).

RESULTS

The major clinical characteristics of the subjects are shown in Table 1. At baseline, SOA presented higher BMI, BMI z-score, and waist circumference than normal-weight adolescents. Likewise, SOA showed higher insulin, leptin, resistin, CRP, MPO, and tPA plasma levels (Table 1).
With respect to the vascular assessment in the brachial artery, similar FMD values were found between groups, whereas resting blood flow, resting shear rate, and peak shear rate were higher in SOA (Table 2). Normalization of FMD by Δshear rate yielded similar values between groups. Conversely, SOA presented lower NMD of the brachial artery than normal-weight adolescents (Table 2).

In the microcirculation, peak CBF during NMD was also reduced in SOA (Table 3). Resting CBF was lower in SOA, but peak CBF after ACh, SNP iontophoresis and local hyperthermia were unaltered between groups (Table 3). All previous results did not differ when adjusted for gender.

Following correlation analysis (Table 4), Δshear rate was positively associated with BMI ($r = .372$, $P = .036$), waist circumference ($r = .366$, $P = .043$), and tPA ($r = .363$, $P = .045$). Significant inverse associations were detected between NMD and adiposity measurements (weight, BMI, BMI z-score, and waist circumference), the strongest being for NMD and waist circumference ($r = -.473$, $P = .006$).

Similarly, forearm resting CBF was inversely associated with adiposity measurements, and leptin ($r = -.447$, $P = .009$). Negative associations were also detected for forearm peak CBF during NMD with resistin ($r = -.528$, $P = .002$), and MPO ($r = -.381$, $P = .031$) (Table 4).

After the weight loss program, SOA exhibited a mean weight reduction of 7.4 ± 3.1 %, as well as decreased BMI, BMI z-score, and waist circumference (Table 1). Plasma leptin, MPO and tPA measurements were also significantly reduced in SOA (Table 1). As regards brachial artery variables, resting diameter was increased, while resting shear rate, peak shear rate and Δshear rate were diminished (Table 2). No significant changes were observed in FMD or NMD (Table 2). Moreover, NMD remained impaired in SOA when adjusted for gender compared to normal-weight adolescents ($P = .038$). In the microcirculation, peak CBF during NMD was further reduced in SOA after the weight loss program, whereas no significant changes were noted in other microvascular variables (Table 3).

DISCUSSION
The most important findings in this study are: 1) SOA exhibit preserved endothelial function but impaired
smooth muscle response to exogenous organic nitrate in both macro- and microcirculation, and 2) a 4-
month weight-loss program does not improve NMD in either of the two vascular beds assessed in SOA,
despite of a significant weight loss and decreased plasma levels of selective markers adversely related to
vascular function. These findings add new evidence for endothelial function preservation in childhood
obesity, which was proposed to be a transitory adaptation to chronic hyperemia. Nevertheless, the absence
of improvement in NMD after a 7% weight loss suggests the necessity of longer and/or more intense
weight loss programs in SOA.

Although our finding of preserved FMD in SOA is not universal (5), the present study is in accordance
with recent and larger published works comparing obese children and adolescents to their normal-weight
counterparts (17-19). Since brachial blood flow at rest and during hyperemia was higher in obese
children, Charakida et al. (19) hypothesized that the endothelial function of conduit arteries may be
temporarily adapted to the hemodynamic consequences of adiposity, thus partially counteracting the well-
known adverse effects of obesity on vascular function. Likewise, we observed that SOA had increased
both resting and hyperemic shear rate, which is the primary hemodynamic stimulus to induce endothelial
nitric oxide synthase (eNOS) expression (20), probably increasing the endothelium-mediated vasodilatory
capacity of SOA. Preserved endothelial function was also observed in the microcirculation via two
different stimuli. Peak responses to iontophoresis of ACh and local hyperthermia in the forearm
microcirculation were not attenuated in SOA. To our knowledge, no other studies have assessed the
microvascular reactivity to ACh and/or local hyperthermia in obese adolescents without co-morbidities.
Altogether, these findings support the hypothesis that during childhood there may be intervals in which
obese children present a preserved endothelial function in both the macro- and microcirculation. Further
research is needed to evaluate whether such intervals of adaptive compensatory endothelial function
might imply a progressive or even more accelerated deterioration of vascular function during adulthood
(21).
Unfortunately, previous reports indicating preserved endothelial function in obese children and adolescents (17-19) did not assess smooth muscle function, which is, apart from its intrinsic interest, recommended in order to confirm endothelial function results (11). Our results indicate that the smooth muscle response to exogenous organic nitrate (NMD) is impaired in the brachial artery and the microvasculature of SOA. This NMD impairment in conduit arteries was already described in obese children (22) and adolescents (5; 23; 24), but always in the presence of endothelial dysfunction (impaired FMD). Given that the FMD response includes at least in part the function of the smooth muscle, the unaltered FMD in SOA suggests that there is either normal smooth muscle function at submaximal vasodilation or impaired smooth muscle function at submaximal vasodilation counterbalanced by increased endothelial function, in line with the aforementioned hypothesis (19). Similarly, at microvascular level, endothelial responses (peak CBF after ACh iontophoresis and hyperthermia) were normal, however peak CBF following NMD was decreased in the forearm microcirculation of SOA. In contrast, a similar peak perfusion following SNP iontophoresis was detected in both groups. The reasons explaining the different results of these two nitrodiators are unclear. At first sight, considering that there is an increase of oxidative stress markers in the plasma of SOA, we might speculate that the orally administered organic nitrate (isorbide dinitrate, NMD) underwent a more prolonged exposure to oxidative stress-mediated inactivation than SNP, which was transdermally delivered. However, the fact that the dilator activity of organic nitrates depends on its conversion to nitric oxide (NO) inside the smooth muscle cells (25) weakens this hypothesis. Another potential explanation may be related to the vasodilatory effects inherently associated with the iontophoresis procedure (26). The electric current of iontophoresis could stimulate, to some extent, underlying mechanisms of vasodilation related to the so-called axon reflex (27). Even though we tried to avoid non-specific effects of iontophoresis by reducing the current intensity, we cannot discard the possibility of some effects of SNP iontophoresis being attributed to the axon reflex response. Nevertheless, overall, these findings demonstrated for the first time
the proof of a widespread decreased smooth muscle function in SOA compared to normal-weight
adolescents.

Obesity and puberty, characterized by dynamic hormonal and physiological changes in boys and girls,
may alter metabolic and vascular homeostasis by promoting a pro-inflammatory and pro-oxidant state (3).
Effectively, plasma markers related to adiposity, inflammation, oxidative stress and endothelial activation
were altered in SOA. At baseline, SOA presented elevated levels of insulin and leptin, two related
hormones with overlapping effects on the hypothalamic control of energy homeostasis (28). Leptin is also
associated with increased sympathetic activity (29), which was suggested to be compatible with the lower
resting blood flow noticed in obese adults (30). Identically, SOA presented lower CBF at rest than
normal-weight counterparts in relation to their higher leptin level (Table 4).
In addition, SOA presented higher values of CRP and MPO, suggesting the presence of systemic
inflammation (31) and neutrophil-mediated oxidative stress (32), respectively. In correlation analysis,
MPO was inversely associated with NMD response in the microcirculation, but not in the brachial artery
(Table 4), suggesting a higher vulnerability to oxidative stress-mediated inactivation of NO in the smaller
vessels. Moreover, tPA, considered to reflect endothelial activation (33), was augmented in SOA and
positively associated to Δshear rate. Increased vascular shear stress was shown to stimulate tPA
expression and secretion by endothelial cells (34), which may explain to some extent the higher plasma
levels of tPA found in SOA. Regarding the prognostic significance of these markers, increases in plasma
CRP and MPO were recently associated with cardiovascular risk in obese children (35), while high levels
of tPA were found to precede the development of type 2 diabetes in a large longitudinal study (36).
Furthermore, resistin was increased in SOA as well as negatively associated with peak NMD response in
the microcirculation (Table 4). The latter finding could give new clues concerning the controversy on the
role of resistin in the pathogenesis of obesity-related comorbidities (37). Otherwise, a 7 % weight loss did
not modify the plasma levels of insulin, resistin and CRP in SOA. Nonetheless, the significant reductions
in leptin, MPO and tPA after weight loss reinforce the therapeutic effects of hypocaloric diets and physical activity programs in obese subjects.

The success of the weight loss program in reducing adiposity and plasma markers potentially altering vascular homeostasis in SOA, did not involve beneficial effects on macro- and microvascular function. The impaired NMD in the brachial artery was not significantly enhanced in SOA. To our knowledge, only one prior study evaluated the effect of a non-pharmacological program in obese children presenting impaired NMD (38). Similarly, they reported no significant change in brachial NMD in a randomized controlled trial assessing the effects of 3-month of exercise training in obese children (38). However, that exercise strategy without dietary intervention did not result in any appreciable weight loss, whereas in our study SOA presented a 7% weight loss, which was previously associated to vascular function improvement in obese subjects (10). One explanation for the lack of enhancement of brachial NMD in our study may be related to the elevated resting brachial shear rate, even after weight loss, observed in SOA. It is conceivable that chronic shear rate-stimulated overexpression of eNOS, thus increasing nitric oxide (NO) release, might lead to some degree of smooth muscle tolerance to NO-mediated vasodilation (39) that was not reversed by the 7% weight loss in SOA. Furthermore, the NMD response was further reduced in the microcirculation of SOA after weight loss. Currently, reasons for such decreased microvascular smooth muscle function are unclear and requires further investigation. Taken together, the outcomes of this study suggest that longer and/or more intense weight loss programs might be needed to restore smooth muscle function in the macro- and microcirculation of SOA.

There are several limitations to the present study. This is a single-center study with limited sample size and conclusions must be taken with caution. Due to the nature of human research, there are likely to be unrecognized variables leading to residual confounding. For instance, despite the baseline assessment of SOA was performed within the first week after being admitted in the center, we cannot discard the possibility that some SOA anticipated a somewhat control of nutrient intake before their entrance in the
center, which might have positively influenced their baseline vascular responses. Furthermore, the cutaneous microcirculation, which we investigated, may raise doubts about its overall significance for vascular risk assessment. However, there is substantial evidence that cutaneous microcirculation is representative of the microcirculation in general. This is underscored by several reports indicating that cutaneous microvascular function mirrors generalized systemic microvascular function (40).

In summary, we have shown that endothelial function is preserved in the macro- and microcirculation of adolescents with severe obesity. Nevertheless, a 7% weight loss does not induce an improvement of their impaired smooth muscle response to organic nitrate in whichever vascular bed assessed. Further studies are required to determine whether longer and/or more intense weight loss programs can enhance arterial smooth muscle function in this population.

REFERENCES


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Table 1 Clinical characteristics and biochemical measurements of normal-weight and severely obese adolescents before and after a 4-month weight loss program

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls/boys</td>
<td>11/8</td>
<td>12/5</td>
<td>12/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>15.09 ± 1.32</td>
<td>13.45 ± 1.18*</td>
<td>13.79 ± 1.18†‡</td>
</tr>
<tr>
<td>Pubertal stage (Tanner)</td>
<td>3.50 ± 0.73</td>
<td>3.33 ± 1.13</td>
<td>—</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.32 ± 9.05</td>
<td>162.71 ± 5.95*</td>
<td>163.88 ± 6.18†</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.14 ± 9.56</td>
<td>88.65 ± 15.62*</td>
<td>81.91 ± 13.69†‡</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.32 ± 2.32</td>
<td>33.36 ± 4.86*</td>
<td>30.43 ± 4.42†‡</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-0.02 ± 1.04</td>
<td>4.22 ± 0.73*</td>
<td>3.46 ± 0.68†‡</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>68.05 ± 7.32</td>
<td>109.50 ± 14.32*</td>
<td>101.25 ± 13.83†‡</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>65.52 ± 10.75</td>
<td>65.50 ± 9.80</td>
<td>63.41 ± 9.74</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>102.84 ± 8.69</td>
<td>98.41 ± 9.44</td>
<td>101.53 ± 7.84</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>60.05 ± 6.72</td>
<td>57.06 ± 6.47</td>
<td>56.24 ± 8.45</td>
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<tr>
<td>Insulin (µU/mL)</td>
<td>3.32 ± 1.47</td>
<td>6.15 ± 4.88*</td>
<td>5.04 ± 2.86‡</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>17.29 ± 15.41</td>
<td>47.56 ± 27.18*</td>
<td>38.38 ± 21.08†‡</td>
</tr>
<tr>
<td>Resistin (ng/mL)</td>
<td>8.47 ± 1.95</td>
<td>10.69 ± 2.11*</td>
<td>10.63 ± 2.15‡</td>
</tr>
<tr>
<td>CRP (µg/mL)</td>
<td>0.10 (0.05-0.37)</td>
<td>1.15 (0.49-4.51)*</td>
<td>0.55 (0.35-2.35)‡</td>
</tr>
<tr>
<td>MPO (ng/mL)</td>
<td>27.31 ± 10.69</td>
<td>45.76 ± 19.20*</td>
<td>35.14 ± 11.08†‡</td>
</tr>
<tr>
<td>tPA (ng/mL)</td>
<td>2.25 (1.99-2.73)</td>
<td>3.17 (2.49-4.10)*</td>
<td>2.74 (2.24-3.32)†‡</td>
</tr>
</tbody>
</table>

Values are mean ± SD, except for CRP and tPA which are median (IQR); CRP = C-reactive protein; DBP = diastolic blood pressure; IQR = interquartile range; MPO = myeloperoxidase; SBP = systolic blood pressure.
pressure; SD = standard deviation; SOA = severely obese adolescents; tPA = tissue plasminogen activator.

* Severely obese adolescents before weight loss versus normal-weight: $P < .001$ for age, weight, BMI, BMI z-score, waist circumference, leptin, and CRP; $P = .002$ for MPO; $P = .003$ for resistin; $P = .039$ for insulin; $P = .044$ for tPA.

† Severely obese adolescents before versus after weight loss: $P < .001$ for age, height, weight, BMI, BMI z-score, and waist circumference; $P = .014$ for tPA; $P = .016$ for leptin; $P = .020$ for MPO.

‡ Severely obese adolescents after weight loss versus normal-weight: $P < .001$ for weight, BMI, BMI z-score, and waist circumference; $P = .002$ for leptin; $P = .004$ for age, resistin, and CRP; $P = .041$ for insulin; $P = .044$ for MPO, and tPA.
**Table 2** Macrocirculation in normal-weight and severely obese adolescents before and after a 4-month weight loss program

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting brachial artery diameter (mm)</td>
<td>3.13 ± 0.50</td>
<td>2.88 ± 0.55</td>
<td>3.05 ± 0.49†</td>
</tr>
<tr>
<td>Resting brachial blood flow (ml min⁻¹)</td>
<td>42.27 ± 25.65</td>
<td>65.30 ± 28.51*</td>
<td>62.63 ± 32.24‡</td>
</tr>
<tr>
<td>Resting brachial shear rate (s⁻¹)</td>
<td>109.31 ± 47.11</td>
<td>232.05 ± 68.01*</td>
<td>193.49 ± 96.25† ‡</td>
</tr>
<tr>
<td>Peak brachial blood flow (ml min⁻¹)</td>
<td>219.78 ± 80.22</td>
<td>278.17 ± 133.60</td>
<td>251.95 ± 98.27</td>
</tr>
<tr>
<td>Peak brachial shear rate (s⁻¹)</td>
<td>490.06 ± 122.00</td>
<td>744.32 ± 245.00*</td>
<td>570.98 ± 99.32†</td>
</tr>
<tr>
<td>∆shear rate (s⁻¹)</td>
<td>386.63 ± 139.59</td>
<td>511.24 ± 227.54</td>
<td>386.06 ± 87.72†</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>8.27 ± 3.27</td>
<td>7.53 ± 2.57</td>
<td>7.92 ± 3.30</td>
</tr>
<tr>
<td>FMD/∆shear rate</td>
<td>0.024 ± 0.010</td>
<td>0.018 ± 0.008</td>
<td>0.021 ± 0.013</td>
</tr>
<tr>
<td>NMD (%)</td>
<td>24.55 ± 8.04</td>
<td>18.21 ± 5.26*</td>
<td>20.42 ± 5.54</td>
</tr>
</tbody>
</table>

Values are mean ± SD. FMD = flow-mediated dilation; NMD = nitrate-mediated dilation; SOA = severely obese adolescents. ∆shear rate means peak brachial shear rate minus resting brachial shear rate.

* Severely obese adolescents before weight loss versus normal-weight: $P < .001$ for resting brachial shear rate; $P = .005$ for peak brachial shear rate; $P = .011$ for NMD; $P = .017$ for resting brachial blood flow.

† Severely obese adolescents before versus after weight loss: $P = .011$ for resting brachial artery diameter, and peak brachial shear rate; $P = .035$ for resting brachial shear rate; $P = .047$ for ∆shear rate.

‡ Severely obese adolescents after weight loss versus normal-weight: $P = .004$ for resting brachial shear rate; $P = .046$ for resting brachial blood flow.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>SOA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm resting CBF (PU)</td>
<td>8.69 (6.38-14.20)</td>
<td>6.66 (5.45-7.66)*</td>
</tr>
<tr>
<td>Forearm peak ACh iontophoresis CBF (PU)</td>
<td>63.68 (52.43-84.40)</td>
<td>70.96 (40.67-104.28)</td>
</tr>
<tr>
<td>Forearm peak hyperthermia CBF (PU)</td>
<td>41.15 (35.40-70.51)</td>
<td>51.45 (33.89-74.01)</td>
</tr>
<tr>
<td>Forearm peak SNP iontophoresis CBF (PU)</td>
<td>18.96 (10.98-27.04)</td>
<td>20.41 (9.11-38.29)</td>
</tr>
<tr>
<td>Forearm peak CBF during NMD (PU)</td>
<td>21.54 (15.83-30.67)</td>
<td>13.29 (9.73-17.42)*</td>
</tr>
</tbody>
</table>

Values are median (IQR). ACh = acetylcholine; CBF = cutaneous blood flow; IQR = interquartile range; PU = perfusion units; SNP = sodium nitroprusside; SOA = severely obese adolescents.

* Severely obese adolescents before weight loss versus normal-weight: \( P = .014 \) for forearm peak CBF during NMD; \( P = .019 \) for forearm resting CBF.

† Severely obese adolescents before versus after weight loss: \( P = .015 \) for forearm peak CBF during NMD.

‡ Severely obese adolescents after weight loss versus normal-weight: \( P < .001 \) for forearm peak CBF during NMD; \( P = .008 \) for forearm resting CBF.
Table 4 Associations of adiposity and biological measurements with vascular measures in normal-weight and severely obese adolescents before a 4-month weight loss program

<table>
<thead>
<tr>
<th>Variable</th>
<th>∆shear rate</th>
<th>Brachial NMD</th>
<th>Forearm resting CBF</th>
<th>Forearm peak CBF during NMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Adiposity measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>.343</td>
<td>NS</td>
<td>-.458</td>
<td>.006</td>
</tr>
<tr>
<td>BMI</td>
<td>.372</td>
<td>.036</td>
<td>-.400</td>
<td>.019</td>
</tr>
<tr>
<td>BMI Z-score</td>
<td>.337</td>
<td>NS</td>
<td>-.428</td>
<td>.012</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>.366</td>
<td>.043</td>
<td>-.473</td>
<td>.006</td>
</tr>
<tr>
<td>Biological measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>.014</td>
<td>NS</td>
<td>-.189</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin</td>
<td>.343</td>
<td>NS</td>
<td>-.146</td>
<td>NS</td>
</tr>
<tr>
<td>Resistin</td>
<td>.150</td>
<td>NS</td>
<td>-.110</td>
<td>NS</td>
</tr>
<tr>
<td>CRP</td>
<td>.261</td>
<td>NS</td>
<td>-.162</td>
<td>NS</td>
</tr>
<tr>
<td>MPO</td>
<td>.048</td>
<td>NS</td>
<td>-.132</td>
<td>NS</td>
</tr>
<tr>
<td>tPA</td>
<td>.363</td>
<td>.045</td>
<td>-.286</td>
<td>NS</td>
</tr>
</tbody>
</table>

CBF = cutaneous blood flow; CRP = C-reactive protein; DBP = diastolic blood pressure; MPO = myeloperoxidase; NMD = nitrate-mediated dilation; tPA = tissue plasminogen activator. ∆shear rate means peak brachial shear rate minus resting brachial shear rate.