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 ºC. 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, Δshear 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 nitrodilators 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

255 the proof of a widespread decreased smooth muscle function in SOA compared to normal-weight
256 adolescents.
257
258 Obesity and puberty, characterized by dynamic hormonal and physiological changes in boys and girls,
259 may alter metabolic and vascular homeostasis by promoting a pro-inflammatory and pro-oxidant state (3).
260 Effectively, plasma markers related to adiposity, inflammation, oxidative stress and endothelial activation
261 were altered in SOA. At baseline, SOA presented elevated levels of insulin and leptin, two related
262 hormones with overlapping effects on the hypothalamic control of energy homeostasis (28). Leptin is also
263 associated with increased sympathetic activity (29), which was suggested to be compatible with the lower
264 resting blood flow noticed in obese adults (30). Identically, SOA presented lower CBF at rest than
265 normal-weight counterparts in relation to their higher leptin level (Table 4).
266 In addition, SOA presented higher values of CRP and MPO, suggesting the presence of systemic
267 inflammation (31) and neutrophil-mediated oxidative stress (32), respectively. In correlation analysis,
268 MPO was inversely associated with NMD response in the microcirculation, but not in the brachial artery
269 (Table 4), suggesting a higher vulnerability to oxidative stress-mediated inactivation of NO in the smaller
270 vessels. Moreover, tPA, considered to reflect endothelial activation (33), was augmented in SOA and
271 positively associated to Δshear rate. Increased vascular shear stress was shown to stimulate tPA
272 expression and secretion by endothelial cells (34), which may explain to some extent the higher plasma
273 levels of tPA found in SOA. Regarding the prognostic significance of these markers, increases in plasma
274 CRP and MPO were recently associated with cardiovascular risk in obese children (35), while high levels
275 of tPA were found to precede the development of type 2 diabetes in a large longitudinal study (36).
276 Furthermore, resistin was increased in SOA as well as negatively associated with peak NMD response in
277 the microcirculation (Table 4). The latter finding could give new clues concerning the controversy on the
278 role of resistin in the pathogenesis of obesity-related comorbidities (37). Otherwise, a 7 % weight loss did
279 not modify the plasma levels of insulin, resistin and CRP in SOA. Nonetheless, the significant reductions

280 in leptin, MPO and tPA after weight loss reinforce the therapeutic effects of hypocaloric diets and
281 physical activity programs in obese subjects.
282
283 The success of the weight loss program in reducing adiposity and plasma markers potentially altering
284 vascular homeostatis in SOA, did not involve beneficial effects on macro- and microvascular function.
285 The impaired NMD in the brachial artery was not significantly enhanced in SOA. To our knowledge, only
286 one prior study evaluated the effect of a non-pharmacological program in obese children presenting
287 impaired NMD (38). Similarly, they reported no significant change in brachial NMD in a randomized
288 controlled trial assessing the effects of 3-month of exercise training in obese children (38). However, that
289 exercise strategy without dietary intervention did not result in any appreciable weight loss, whereas in our
290 study SOA presented a 7 % weight loss, which was previously associated to vascular function
291 improvement in obese subjects (10). One explanation for the lack of enhancement of brachial NMD in our
292 study may be related to the elevated resting brachial shear rate, even after weight loss, observed in SOA.
293 It is conceivable that chronic shear rate-stimulated overexpression of eNOS, thus increasing nitric oxide
294 (NO) release, might lead to some degree of smooth muscle tolerance to NO-mediated vasodilation (39)
295 that was not reversed by the 7% weight loss in SOA. Furthermore, the NMD response was further
296 reduced in the microcirculation of SOA after weight loss. Currently, reasons for such decreased
297 microvascular smooth muscle function are unclear and requires further investigation. Taken together, the
298 outcomes of this study suggest that longer and/or more intense weight loss programs might be needed to
299 restore smooth muscle function in the macro- and microcirculation of SOA.
300
301 There are several limitations to the present study. This is a single-center study with limited sample size
302 and conclusions must be taken with caution. Due to the nature of human research, there are likely to be
303 unrecognized variables leading to residual confounding. For instance, despite the baseline assessment of
304 SOA was performed within the first week after being admitted in the center, we cannot discard the
305 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 assessement. 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

1. **Skelton J, Cook S, Auinger P, Klein J, Barlow S** 2009 Prevalence and trends of severe obesity among US children and adolescents. Acad Pediatr 9:322-329

2. **Must A, Jacques P, Dallal G, Bajema C, Dietz W** 1992 Long-term morbidity and mortality of overweight adolescents. A follow-up of the Harvard Growth Study of 1922 to 1935. N Engl J Med 327:1350-1355

3. **Montero D, Walther G, Perez-Martin A, Roche E, Vinet A** 2012 Endothelial dysfunction, inflammation, and oxidative stress in obese children and adolescents: markers and effect of lifestyle intervention. Obes Rev 13:441-455

4. **Raitakari OT, Celermajer DS** 2000 Flow-mediated dilatation. Br J Clin Pharmacol 50:397-404

5. **Tounian P, Aggoun Y, Dubern B, Varille V, Guy-Grand B, Sidi D, Girardet JP, Bonner D** 2001 Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. Lancet 358:1400-1404

6. **Schlager O, Willfort-Ehringer A, Hammer A, Steiner S, Fritsch M, Giurgea A, Margeta C, Lilaj I, Zehetmayer S, Widhalm K, Koppensteiner R, Gschwandtner ME** 2011 Microvascular function is impaired in children with morbid obesity. Vasc Med 16:97-102

7. **Wiernsperger N, Rapin JR** 2012Microvascular diseases: is a new era coming? *Cardiovasc Hematol Agents* Med Chem 10:167-183

8. **Cole T, Bellizzi M, Flegal K, Dietz W** 2000 Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 320:1240-1243

9. **Rolland-Cachera M, Cole T, Sempe M, Tichet J, Rossignol C, Charraud A** 1991 Body Mass Index variations: centiles from birth to 87 years. Eur J Clin Nutr 45**:**13-21

10. **Hamdy O, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, Moussa A, Caselli A, Caballero AE, Economides PA, Veves A, Horton ES** 2003 Lifestyle modification improves endothelial function in obese subjects with the insulin resistance syndrome. Diabetes Care 26:2119-2125

11. **Corretti M, Anderson T, Benjamin E, Celermajer D, Charbonneau F, Creager M, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force** 2002 Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 39:257-265

12. **Karpoff L, Vinet A, Schuster I, Oudot C, Goret L, Dauzat M, Obert P, Perez-Martin A** 2009 Abnormal vascular reactivity at rest and exercise in obese boys. Eur J Clin Invest 39:94-102

13. **Walther G, Nottin S, Dauzat M, Obert P** 2006 Femoral and axillary ultrasound blood flow during exercise: a methodological study. Med Sci Sports Exerc 38:1353-1361

14. **Betik AC, Luckham VB, Hughson RL** 2004 Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. Am J Physiol Heart Circ Physiol 286:H442-448

15. **Leahy MJ, de Mul FF, Nilsson GE, Maniewski R** 1999 Principles and practice of the laser-Doppler perfusion technique. Technol Health Care **7**:143-162

16. **Droog EJ, Henricson J, Nilsson GE, Sjoberg F** 2004 A protocol for iontophoresis of acetylcholine and sodium nitroprusside that minimises nonspecific vasodilatory effects. Microvasc Res 67:197-202

17. **Koopman LP, McCrindle BW, Slorach C, Chahal N, Hui W, Sarkola T, Manlhiot C, Jaeggi ET, Bradley TJ, Mertens L** 2012 Interaction between myocardial and vascular changes in obese children: a pilot study. J Am Soc Echocardiogr 25**:**401-410 e401

18. **Naylor LH, Green DJ, Jones TW, Kalic RJ, Suriano KL, Shah M, Hopkins N, Davis EA** 2011 Endothelial function and carotid intima-medial thickness in adolescents with type 2 diabetes mellitus. J Pediatr 159:971-974

19. **Charakida M, Jones A, Falaschetti E, Khan T, Finer N, Sattar N, Hingorani A, Lawlor DA, Smith GD, Deanfield JE** 2012 Childhood Obesity and Vascular Phenotypes: A Population Study. J Am Coll Cardiol; 60(25):2643-2650

20. **Davis ME, Cai H, Drummond GR, Harrison DG** 2001 Shear stress regulates endothelial nitric oxide synthase expression through c-Src by divergent signaling pathways. Circ Res 89:1073-1080

21. **Zalesin KC, Franklin BA, Miller WM, Peterson ED, McCullough PA** 2008 Impact of obesity on cardiovascular disease. Endocrinol Metab Clin North Am 37:663-684

22. **Aggoun Y, Farpour-Lambert NJ, Marchand LM, Golay E, Maggio AB, Beghetti M** 2008 Impaired endothelial and smooth muscle functions and arterial stiffness appear before puberty in obese children and are associated with elevated ambulatory blood pressure. Eur Heart J 29:792-799

23. **Pena AS, Belobrajdic DP, Wiltshire E, Gent R, Hirte C, Couper J** 2010 Adiponectin relates to smooth muscle function and folate in obese children. Int J Pediatr Obes 5:185-191

24. **Pena AS, Wiltshire E, MacKenzie K, Gent R, Piotto L, Hirte C, Couper J** 2006 Vascular endothelial and smooth muscle function relates to body mass index and glucose in obese and nonobese children. J Clin Endocrinol Metab 91:4467-4471

25. **Mehta JL** 1995 Endothelium, coronary vasodilation, and organic nitrates. Am Heart J 129:382-391

26. **Roustit M, Cracowski JL** 2012 Non-invasive assessment of skin microvascular function in humans: an insight into methods. Microcirculation 19:47-64

27. **Durand S, Fromy B, Bouye P, Saumet JL, Abraham P** 2002 Current-induced vasodilation during water iontophoresis (5 min, 0.10 mA) is delayed from current onset and involves aspirin sensitive mechanisms. J Vasc Res 39:59-71

28. **Niswender KD, Schwartz MW** 2003 Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. Front Neuroendocrinol 24:1-10

29. **Monroe MB, Van Pelt RE, Schiller BC, Seals DR, Jones PP** 2000 Relation of leptin and insulin to adiposity-associated elevations in sympathetic activity with age in humans. Int J Obes Relat Metab Disord 24:1183-1187

30. **Doupis J, Rahangdale S, Gnardellis C, Pena SE, Malhotra A, Veves A**.2011 Effects of Diabetes and Obesity on Vascular Reactivity, Inflammatory Cytokines, and Growth Factors. Obesity 19(4):729-735

31. **Rocha VZ, Folco EJ** 2011 Inflammatory concepts of obesity. Int J Inflam:529061

32. **Loria V, Dato I, Graziani F, Biasucci LM** 2008 Myeloperoxidase: a new biomarker of inflammation in ischemic heart disease and acute coronary syndromes. Mediators Inflamm: 135625

33. **Bagg W, Ferri C, Desideri G, Gamble G, Ockelford P, Braatvedt GD** 2001 The influences of obesity and glycemic control on endothelial activation in patients with type 2 diabetes. J Clin Endocrinol Metab 86:5491-5497

34. **Diamond SL, Sharefkin JB, Dieffenbach C, Frasier-Scott K, McIntire LV, Eskin SG** 1990 Tissue plasminogen activator messenger RNA levels increase in cultured human endothelial cells exposed to laminar shear stress. J Cell Physiol 143:364-371

35. **Olza J, Aguilera CM, Gil-Campos M, Leis R, Bueno G, Martinez-Jimenez MD, Valle M, Cañete R, Tojo R, Moreno LA, Gil A** 2012 Myeloperoxidase is an early biomarker of inflammation and cardiovascular risk in prepubertal obese children. Diabetes Care 35:2373-2376

36. **Eliasson MC, Jansson JH, Lindahl B, Stegmayr B** 2003 High levels of tissue plasminogen activator (tPA) antigen precede the development of type 2 diabetes in a longitudinal population study. The Northern Sweden MONICA study. Cardiovasc Diabetol 2:19

37. **Kusminski CM, McTernan PG, Kumar S** 2005 Role of resistin in obesity, insulin resistance and Type II diabetes. Clin Sci 109:243-256

38. **Farpour-Lambert NJ, Aggoun Y, Marchand LM, Martin XE, Herrmann FR, Beghetti M** 2009 Physical activity reduces systemic blood pressure and improves early markers of atherosclerosis in pre-pubertal obese children. J Am Coll Cardiol 54:2396-2406

39. **Yamashita T, Kawashima S, Ohashi Y, Ozaki M, Rikitake Y, Inoue N, Hirata K, Akita H, Yokoyama M** 2000 Mechanisms of reduced nitric oxide/cGMP-mediated vasorelaxation in transgenic mice overexpressing endothelial nitric oxide synthase. Hypertension 36**:**97-102

40. **Holowatz LA, Thompson-Torgerson CS, Kenney WL** 2008 The human cutaneous circulation as a model of generalized microvascular function. J Appl Physiol 105:370-372

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

Characteristics	Controls	SOA	
		Before	After
Girls/boys	11/8	12/5	12/5
Age (years)	15.09 ± 1.32	13.45 ± 1.18*	13.79 ± 1.18† ‡
Pubertal stage (Tanner)	3.50 ± 0.73	3.33 ± 1.13	—
Height (cm)	165.32 ± 9.05	162.71 ± 5.95	163.88 ± 6.18†
Weight (kg)	53.14 ± 9.56	88.65 ± 15.62*	81.91 ± 13.69† ‡
BMI (kg/m^2)	19.32 ± 2.32	33.36 ± 4.86*	30.43 ± 4.42† ‡
BMI z-score	-0.02 ± 1.04	4.22 ± 0.73*	3.46 ± 0.68† ‡
Waist circumference (cm)	68.05 ± 7.32	109.50 ± 14.32*	101.25 ± 13.83† ‡
Heart rate (beats/min)	65.52 ± 10.75	65.50 ± 9.80	63.41 ± 9.74
SBP (mm Hg)	102.84 ± 8.69	98.41 ± 9.44	101.53 ± 7.84
DBP (mm Hg)	60.05 ± 6.72	57.06 ± 6.47	56.24 ± 8.45
Insulin (µU/mL)	3.32 ± 1.47	6.15 ± 4.88*	5.04 ± 2.86‡
Leptin (ng/mL)	17.29 ± 15.41	47.56 ± 27.18*	38.38 ± 21.08† ‡
Resistin (ng/mL)	8.47 ± 1.95	10.69 ± 2.11*	10.63 ± 2.15‡
CRP (µg/mL)	0.10 (0.05-0.37)	1.15 (0.49-4.51)*	0.55 (0.35-2.35)‡
MPO (ng/mL)	27.31 ± 10.69	45.76 ± 19.20*	35.14 ± 11.08† ‡
tPA (ng/mL)	2.25 (1.99-2.73)	3.17 (2.49-4.10)*	2.74 (2.24-3.32)† ‡

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

425 pressure; SD = standard deviation; SOA = severely obese adolescents; tPA = tissue plasminogen
426 activator.
427
428 * Severely obese adolescents before weight loss versus normal-weight: $P < .001$ for age, weight, BMI,
429 BMI z-score, waist circumference, leptin, and CRP; $P = .002$ for MPO; $P = .003$ for resistin; $P = .039$ for
430 insulin; $P = .044$ for tPA.
431 † Severely obese adolescents before versus after weight loss: $P < .001$ for age, height, weight,
432 BMI, BMI z-score, and waist circumference; $P = .014$ for tPA; $P = .016$ for leptin; $P = .020$ for
433 MPO.
434 ‡ Severely obese adolescents after weight loss versus normal-weight: $P < .001$ for weight, BMI,
435 BMI z-score, and waist circumference; $P = .002$ for leptin; $P = .004$ for age, resistin, and CRP; $P =$
436 .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

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

Values are mean ± SD. FMD = flow-mediated dilation; NMD = nitrate-mediated dilation; SOA = severely obese adolescents. Δshear 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 Δshear 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 3 Microcirculation in normal-weight and severely obese adolescents before and after a 4-month weight loss program

Variable	Controls	SOA	
		Before	After
Forearm resting CBF (PU)	8.69 (6.38-14.20)	6.66 (5.45-7.66)*	6.61 (4.87-7.55)‡
Forearm peak ACh iontophoresis CBF (PU)	63.68 (52.43-84.40)	70.96 (40.67-104.28)	60.37 (51.48-88.49)
Forearm peak hyperthermia CBF (PU)	41.15 (35.40-70.51)	51.45 (33.89-74.01)	43.04 (35.36-51.81)
Forearm peak SNP iontophoresis CBF (PU)	18.96 (10.98-27.04)	20.41 (9.11-38.29)	9.85 (7.52-21.08)
Forearm peak CBF during NMD (PU)	21.54 (15.83-30.67)	13.29 (9.73-17.42)*	9.31 (7.21-12.79)† ‡

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

	Δshear rate		Brachial NMD		Forearm resting CBF		Forearm peak CBF during NMD	
Variable	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Adiposity measurements								
Weight	.343	NS	-.458	.006	-.348	.047	-.280	NS
BMI	.372	.036	-.400	.019	-.393	.024	-.342	NS
BMI Z-score	.337	NS	-.428	.012	-.365	.037	-.319	NS
Waist circumference	.366	.043	-.473	.006	-.392	.026	-.266	NS
Biological measurements								
Insulin	.014	NS	-.189	NS	-.171	NS	.203	NS
Leptin	.343	NS	-.146	NS	-.447	.009	-.195	NS
Resistin	.150	NS	-.110	NS	-.254	NS	-.528	.002
CRP	.261	NS	-.162	NS	-.276	NS	-.329	NS
MPO	.048	NS	-.132	NS	-.229	NS	-.381	.031
tPA	.363	.045	-.286	NS	-.142	NS	-.079	NS

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