

Diet, Exercise, and Endothelial Function in Obese Adolescents

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abstract

BACKGROUND AND OBJECTIVES: Endothelial dysfunction is the first, although reversible, sign of atherosclerosis and is present in obese adolescents. The primary end point of this study was to investigate the influence of a multicomponent treatment on microvascular function. Additional objectives and end points were a reduced BMI SD score, improvements in body composition, exercise capacity, and cardiovascular risk factors, an increase in endothelial progenitor cells (EPCs), and a decrease in endothelial microparticles (EMPs).

METHODS: We used a quasi-randomized study with 2 cohorts of obese adolescents: an intervention group ($n = 33$; 15.4 ± 1.5 years, 24 girls and 9 boys) treated residentially with supervised diet and exercise and a usual care group ($n = 28$; 15.1 ± 1.2 years, 22 girls and 6 boys), treated ambulant. Changes in body mass, body composition, cardiorespiratory fitness, microvascular endothelial function, and circulating EPCs and EMPs were evaluated after 5 months and at the end of the 10-month program.

RESULTS: Residential intervention decreased BMI and body fat percentage, whereas it increased exercise capacity ($P < .001$ after 5 and 10 months). Microvascular endothelial function also improved in the intervention group ($P = .04$ at 10 months; $+ 0.59 \pm 0.20$ compared with $+ 0.01 \pm 0.12$ arbitrary units). Furthermore, intervention produced a significant reduction in traditional cardiovascular risk factors, including high-sensitivity C-reactive protein ($P = .012$ at 10 months). EPCs were increased after 5 months ($P = .01$), and EMPs decreased after 10 months ($P = .004$).

CONCLUSIONS: A treatment regimen consisting of supervised diet and exercise training was effective in improving multiple adolescent obesity-related end points.

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WHAT'S KNOWN ON THIS SUBJECT: Adolescent obesity is characterized by endothelial dysfunction at the macrovascular and microvascular level; high endothelial microparticle (EMP) and low endothelial progenitor cell (EPC) counts contribute to these processes. Although reversal of macrovascular endothelial dysfunction is feasible, clinical evidence regarding microvascular endothelial dysfunction is scarce.

WHAT THIS STUDY ADDS: Ten months of diet and exercise training improves microvascular endothelial function (peak response) in obese adolescents. EPC and EMP displayed a biphasic response, with an increase in EPC at 5 months and a decrease in EMP at the end of the treatment.

The rapidly rising prevalence of childhood obesity poses a threat to future health management because of the increased risk of death from cardiovascular disease in adulthood.¹ Impaired endothelial function is a key step in the pathogenesis of atherosclerosis. Under physiologic conditions, the endothelium creates an antiatherosclerotic milieu, thereby preventing leukocyte adhesion and vascular smooth muscle proliferation. Its main role, however, is to regulate vascular smooth muscle tone, allowing the adaptation of the arterial diameter to suit blood flow demands. Using flow-mediated dilation, brachial artery diameter responses can be objectified, thereby evaluating macrovascular endothelial function. Recently, the Endo-PAT device was developed to assess microvascular endothelial dysfunction. The Endo-PAT uses pneumatic probes, placed on the index fingers, and measures changes in finger arterial tone. By applying these techniques, it was demonstrated that the flow-associated dilatory capacity of the endothelium in obese adolescents is reduced both at the macrovascular² and microvascular level.³ Moreover, microvascular endothelial dysfunction appears to precede macrovascular endothelial dysfunction,⁴ indicating that these manifestations reflect various early stages of vascular disease. Endothelial function is negatively affected by both traditional cardiovascular risk factors, such as hypertension and sedentary lifestyle, and novel cardiovascular risk factors, including inflammatory (eg, high C-reactive protein) and antiinflammatory markers (eg, low adiponectin).⁵

Macrovascular endothelial function in obese adolescents, assessed using flow-mediated dilation, can be improved by exercise training⁶ and diet,⁷ and combining both induces the greatest improvements.⁸ However, whether lifestyle interventions are also capable of reversing endothelial dysfunction at the microvascular level is currently not clear.⁹

In recent decades, novel insights have been obtained into the mechanistic basis for endothelial homeostasis. First, endothelial progenitor cells (EPCs) were recognized as important contributors of endothelial repair¹⁰ because they are able to replace lost endothelial cells. Second, endothelial microparticles (EMPs), shed from activated and apoptotic endothelial cells, were identified as potent (dys) regulators of endothelial function.¹¹ In our previous work, we observed an imbalance between endothelial damage and repair in obese adolescents.¹² Obese adolescents had higher EMP counts and lower EPC numbers in their circulation compared with age- and gender-matched normal-weight adolescents. In multiple linear regression analysis, systolic blood pressure and blood counts of EMPs and EPCs were identified as independent determinants of microvascular endothelial function.

The primary end point of this study was the effect of a residential treatment program combining diet and exercise training on microvascular function in obese adolescents (peak response as measured with Endo-PAT). BMI and exercise capacity were used to evaluate the effectiveness of therapy (secondary end points), and numbers of circulating EMPs and EPCs were reported to evaluate the balance between endothelial damage and repair.

METHODS

Study Population

Sixty-one obese adolescents aged between 12 and 18 years were recruited from the revalidation center Zeepreventorium (De Haan, Belgium). This is a secondary center specialized in inpatient treatment of obese children and adolescents. Inclusion criteria were a BMI \geq 97th age- and gender-specific percentile for adolescents younger than 16 years and BMI \geq 35 for adolescents older than 16 years.

Exclusion criteria were an acute or chronic inflammatory process, use of nonsteroidal antiinflammatory or immunosuppressive drugs, structural heart disease or other cardiovascular diseases, and active malignant hematologic disease.

Ethical Approval

The protocol complied with the Declaration of Helsinki, was approved by the ethics committee of the Antwerp University Hospital, and was registered at ClinicalTrials.gov (NCT01461226). All participants and their parents gave their written informed consent.

Study Design

Participants were recruited for this quasi-randomized trial at the beginning of a 10-month residential treatment program (intervention group) and from the waiting list of the institution (usual care group) (Fig 1). Participants were evaluated at baseline and after 5 and 10 months.

Inpatient treatment included dietary restriction (1500–1800 kcal/day), physical activity, and psychological support under medical supervision.¹³ Besides 2 hours of supervised play and lifestyle activities each day, adolescents engaged in 2 hours of physical education per week at school and 3 supervised training sessions every week, with an effective training time of 40 minutes each. One session a week was spent in a fitness area focusing on cardio training (cycling or running) and resistance training. In addition, a swimming session was organized focusing on swimming laps and improving swimming technique. The third session was preserved for running, setting continuous running for 30 minutes as the final aim. Qualified physiotherapists supervised the participants during all training sessions.

Participants on the waiting list were treated by their general practitioner or pediatrician, focusing on caloric

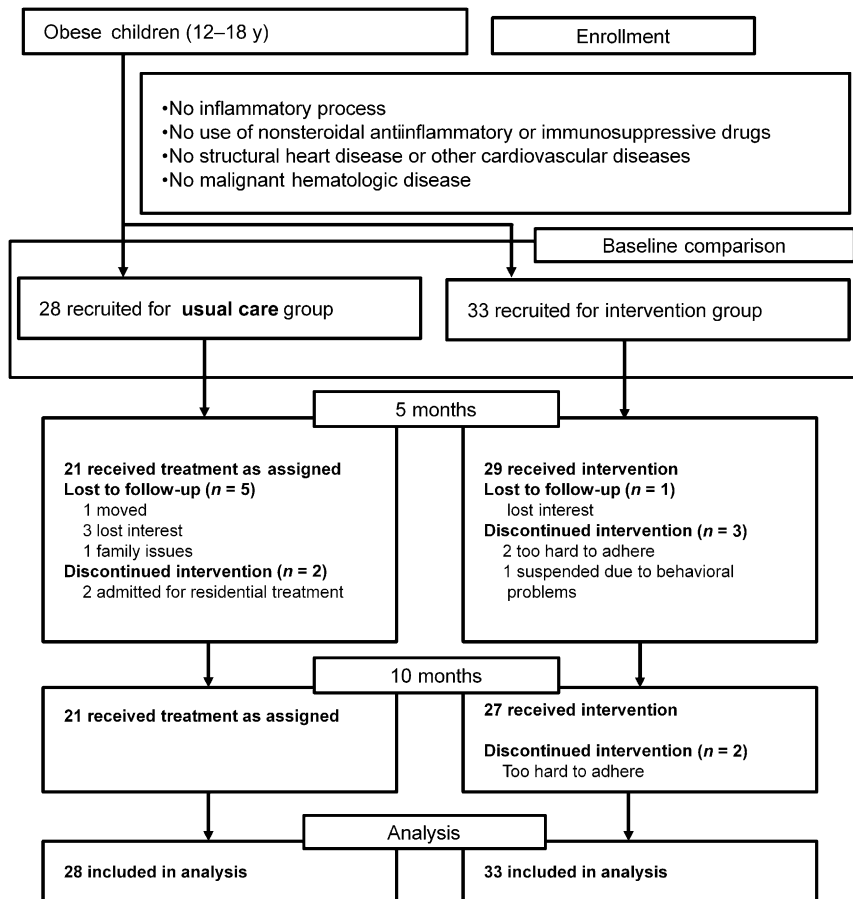


FIGURE 1
Flow diagram of study design.

restriction and encouragement to participate in sports activities.

Blood Sampling

After an overnight fast, serum samples were taken for biochemical analyses, and whole blood was collected in an acid citrate dextrose (ACD) tube (BD Biosciences, Erembodegem, Belgium) for quantification of EMPs and EPCs.

Biochemical Analyses

Routine parameters were determined at the Zeepreventorium medical laboratory (Olympus AU 400, Beckman Coulter) with the exception of low-density lipoprotein (LDL) cholesterol (Dimensions Vista, Siemens Healthcare, Erlangen, Germany) and insulin (Modular, Roche, Basel, Switzerland), which were measured at the department of

clinical chemistry, Antwerp University Hospital. The homeostatic model assessment of insulin resistance was calculated by using glucose concentration and insulin levels.¹⁴ The total adiponectin level in serum was objectified using the Human Total Adiponectin/Acrp30 Quantikine ELISA Kit (R&D systems, Abingdon, England) according to the manufacturer's instructions.

Anthropometry and Body Composition

Weight was measured to the nearest 0.1 kg using a digital-balanced scale, and height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Age- and gender-specific SD scores (SDS) were calculated.¹⁵ Body composition was determined using a Lunar Prodigy Advance Full Size (GE Healthcare, Diegem,

Belgium) dual-energy radiograph absorptiometry scanner, and SDS values were computed.¹⁶

Cardiopulmonary Exercise Testing

Symptom-limited cardiopulmonary exercise testing was performed on a bicycle, and registration was done with an Ergocard (Medisoft Belgium, Sorinnes, Belgium). Peak oxygen uptake (V_{O_2} peak) was determined as the mean value of V_{O_2} measurements during the final 50 seconds of exercise and expressed in milliliter/minute/kilogram fat-free mass. Starting workload (30 or 50 watts) was increased with 20 or 25 watts, respectively, every 2 minutes, and the same protocol was used for repeat testing.

Arterial Stiffness and Endothelial Function

Blood pressure and markers of arterial stiffness were registered by using the Arteriograph (Tensiomed, Budapest, Hungary) according to recent guidelines.¹⁷ The mean of 3 measurements was calculated for systolic and diastolic blood pressure, augmentation index, and pulse wave velocity. Gender-, age-, and height-specific percentiles for systolic and diastolic blood pressures were calculated.¹⁸

As previously described, pulse amplitude tonometry (Endo-pat, Itamar Medical, Caesarea, Israel) was applied to assess microvascular endothelial function.¹⁹ This apparatus uses pneumatic probes that are placed on both index fingers and measures the diameter of finger arterioles. After a 5-minute baseline assessment, a blood pressure cuff around the upper arm is insufflated, and the resulting dilation of finger arterioles is measured for 5 minutes. The Reactive Hyperemia Index (RHI) is automatically calculated, which was initially chosen as the primary end point of the study. However, emerging evidence demonstrated that the fixed time period of RHI generally misses the maximal dilation (peak response)

in adolescents.¹⁹ For that reason, peak response, and not RHI, is correlated with clinical parameters in obese adolescents.¹² Therefore, in May 2013, before ending of the study and data analysis, we decided to set forth peak response as the primary end point.

Flow Cytometry

For quantification of EMPs, defined as CD31+/CD42b- particles <1 μ m, ACD tubes were centrifuged for 20 minutes at 1 525 *g* twice, to obtain platelet poor plasma (PPP). Fifty microliters of PPP was incubated with 3 μ L of CD31- phycoerythrin (cloneWM59) and 4 μ L of CD42b- FITC (clone HIP1; BD Biosciences). Samples were measured using a BD FACSCanto II flow cytometer as previously published,²⁰ allowing the calculation of circulating EMPs per microliter PPP.

For quantification of EPCs, defined as CD34+/KDR+/CD45dim/- cells, blood collected in an ACD tube was fixated by using TransFix (Caltag Medsystems, Buckingham, United Kingdom) and processed 2 to 3 days after sampling.²¹ Fixated whole blood was lysed using NH₄Cl, centrifuged and supernatant was decanted. FcR blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) was added to the pellet as well as the following antibodies: 17.5 μ L anti-CD3 PerCP, 10 μ L anti-KDR APC, 7.5 μ L anti-CD34 phycoerythrin Cy7 and 5 μ L anti-CD45 APC H7 (BD Biosciences, except anti-KDR APC, which was from R&D Systems). We used Syto 13 (Invitrogen, Paisley, United Kingdom) to gate on nucleated cells. Measurements were performed as recommended²² and analyzed by using BD FACSDIVA software version 6.1.2 (BD Biosciences). EPCs were expressed as cells per million mononuclear cells.

Statistical Methods

Continuous data are expressed as mean \pm SD or median and interquartile range (25th and 75th percentile). Continuous data of groups were compared using the independent

samples *t* test or Mann-Whitney *U* test, and the χ^2 test was used for categorical data. Effects of the intervention at 5 months and at completion of the entire program were compared between groups by using linear mixed models analysis. A model was set up with an autoregressive type of repeated covariance, and independent variables were the group indicator variable and the visit number. Reported *P* values for this model are derived from the interaction of group and visit number. Simple regression analysis was performed to identify baseline characteristics that could affect primary outcome. All analyses were performed by using SPSS Statistics version 22.0 (SPSS Inc, Chicago, IL).

RESULTS

Baseline Characteristics

See Table 1. Adolescents in the intervention and usual care group were of similar age (15.4 \pm 1.5 vs 15.1 \pm 1.2 years; *P* = .420) and gender (24 girls and 9 boys vs 22 girls and 6 boys; *P* = .60). Both groups were comparable for the majority of baseline characteristics. However, the homeostatic model assessment of insulin resistance (*P* = .006) and systolic and diastolic blood pressure were significantly higher in the usual care group (*P* = .04 and *P* = .004, respectively), and number of circulating EMPs was lower as well (*P* = .02). However, in simple linear regression analysis, the baseline values of these parameters did not explain the significant effects on peak response, and therefore no post hoc correction was applied to mixed models analysis.

Effects of the Intervention

As shown in Table 1, the number of dropouts was similar in both groups (*P* = .78); 27 intervention group participants (82%) and 21 adolescents from the usual care group (75%) could be followed up until the end of the program. Reasons for dropout are summarized in Fig 1.

Five months of treatment led to a mean weight loss of 18.9 \pm 6.9 kg in the intervention group, reducing BMI with 6.8 \pm 2.2 and 0.76 \pm 0.21 SDS (Fig 2), whereas a weight gain of 3.4 \pm 5.0 kg was observed in the usual care group, raising the BMI with 1.0 \pm 1.6 and 0.04 \pm 0.15 SDS (mixed models analysis for all parameters, *P* < .001). Lipid profile changed with a significant decrease in LDL cholesterol of 7.5 \pm 15.3 mg/dL in the intervention group versus + 17.0 \pm 24.2 mg/dL in the usual care group (*P* < .001). Compared with this group, adolescents in the intervention group demonstrated improved exercise capacity, as maximal workload increased with 16.7 \pm 25.7 watts and V_{O₂peak} with 3.5 \pm 6.0 mL/min/kg fat free mass (vs - 5.7 \pm 19.1 watts and - 2.6 \pm 6.2 mL/min/kg fat-free mass; *P* < .001 for both). Despite no clear improvements in microvascular endothelial function + 0.40 \pm 0.16 vs 0.37 \pm 0.16 arbitrary units (*P* = .60), a marked increase in circulating EPC was noted (+ 9.3 \pm 3.2 vs - 6.8 \pm 3.5 per million mononuclear cells *P* = .01; Fig 2).

At completion of the 10-month program, adolescents in the intervention group achieved a total weight loss of 26.9 \pm 11.9 kg compared with a weight gain of 7.4 \pm 7.5 kg for adolescents in the usual care group. Accordingly, BMI was reduced with 9.9 \pm 3.8 and 1.27 \pm 0.44 SDS in the intervention group and increased with 1.7 \pm 2.3 and 0.05 \pm 0.19 SDS in the usual care group (all *P*s < .001). Body fat percentage was reduced with 13.9% \pm 8.4% in the intervention group, compared with 0.6% \pm 1.8% increase in the usual care group (*P* < .001). LDL cholesterol was lowered by 18.9 \pm 15.2 in the intervention group versus 8.9 \pm 20.7 mg/dL in the usual care group, and high-density lipoprotein cholesterol increased to 5.0 \pm 9.0 in the intervention group versus - 4.3 \pm 6.8 mg/dL in the usual care group (*P* = .04 and .002, respectively).

TABLE 1 Baseline Characteristics and Effects of the Interventional Program Among Participants

	Usual Care Group			Intervention Group			Baseline Characteristics P	Effects After 5 Mo Treatment P	Effects of Entire Intervention P
	Baseline	5 mo	10 mo	Baseline	5 mo	10 mo			
	28	21	21	33	29	27			
Participants, n									
Anthropometry									
Wt, kg	103.7 ± 19.7	110.6 ± 20.0	113.6 ± 20.2	102.7 ± 18.2	84.4 ± 14.2	78.3 ± 12.6	.84	<.001	
Wt, SDS	2.63 ± 0.72	2.83 ± 0.70	2.84 ± 0.70	2.58 ± 0.65	1.73 ± 0.71	1.17 ± 0.77	.76	<.001	
BMI	36.72 ± 5.83	38.40 ± 5.62	39.10 ± 5.26	36.44 ± 4.82	30.07 ± 3.85	27.39 ± 3.57	.84	<.001	
BMI, SDS	2.77 ± 0.51	2.89 ± 0.46	2.90 ± 0.42	2.76 ± 0.36	2.04 ± 0.45	1.55 ± 0.53	.90	<.001	
Body fat, %	51 (48–53)	50 (49–54)	52 (49–54)	51 (49–54)	43 (39–47)	39 (31–43)	.83	<.001	
Fat %, SDS	2.86 (2.75–3.01)	2.85 (2.66–3.07)	2.86 (2.71–3.01)	2.91 (2.71–3.05)	2.42 (2.01–2.57)	1.93 (1.50–2.23)	.94	<.001	
Laboratory parameters									
Cholesterol, mg/dL									
Total	153.3 ± 25.0	159.0 ± 21.0	153.9 ± 19.5	150.5 ± 21.8	133.5 ± 31.0	140.4 ± 19.9	.65	.12	
LDL	91.6 ± 23.6	114.1 ± 29.1	86.8 ± 14.1	88.3 ± 19.0	80.9 ± 15.8	71.1 ± 12.9	.55	.04	
HDL	45.5 ± 9.4	44.1 ± 7.3	41.9 ± 10.4	42.5 ± 10.1	41.7 ± 10.2	48.3 ± 10.1	.24	.002	
HOMA-IR	5.91 (2.79–7.25) ^a	5.88 (4.28–9.84)	3.90 (3.05–5.02)	3.32 (2.32–4.05)	2.60 (1.80–3.55)	2.46 (1.70–3.04)	.006	.15	
hsCRP, mg/dL	0.25 (0.15–0.45)	0.30 (0.12–0.64)	0.37 (0.22–0.64)	0.27 (0.15–0.60)	0.18 (0.06–0.26)	0.13 (0.03–0.36)	.94	.01	
Adiponectin, ng/mL	9.8 ± 4.4	—	7.6 ± 3.7	7.9 ± 4.9	—	14.6 ± 6.4	.29	<.001	
Blood pressure									
Systolic, mm Hg	126 ± 12 ^a	119 ± 11	120 ± 11	120 ± 9	108 ± 9	108 ± 6	.04	.04	
Systolic, percentile	87 (70–98)	73 (51–84)	69 (52–92)	75 (66–91)	26 (13–56)	26 (18–43)	.08	.002	
Diastolic, mm Hg	68 ± 7 ^a	63 ± 7	63 ± 7	63 ± 6	59 ± 4	60 ± 4	.004	.29	
Diastolic, percentile	55 ± 24 ^a	40 ± 23	39 ± 23	40 ± 21	27 ± 13	29 ± 14	.01	.45	
Exercise capacity									
Maximal load, W	150 (130–170)	150 (130–170)	130 (130–170)	150 (130–170)	170 (130–210)	170 (150–210)	.56	<.001	
V _O 2-peak/FFM, mL/min/kg	41.4 ± 6.8	38.3 ± 7.5	34.4 ± 3.9	39.0 ± 6.0	43.0 ± 6.7	44.4 ± 7.2	.150	<.001	
Endothelial function									
AIX, %	24.5 (4.9–32.7)	29.2 (3.4–46.2)	13.8 (4.6–47.3)	13.3 (7.4–43.1)	16.4 (4.1–31.2)	10.4 (1.1–13.1)	.77	.04	
PWV, m/s	6.1 (5.4–7.2)	6.5 (6.0–8.1)	6.2 (5.7–6.9)	5.9 (5.2–6.6)	5.4 (5.0–5.7)	5.2 (5.0–5.7)	.17	.08	
Baseline PWA, AU	372.46 ± 187.49	298.97 ± 188.83	427.20 ± 191.86	375.07 ± 195.12	267.08 ± 166.97	277.25 ± 227.96	.96	.05	
RHI, AU	2.05 ± 0.59	2.26 ± 0.70	2.30 ± 0.58	2.24 ± 0.74	2.15 ± 0.54	2.45 ± 0.85	.28	.73	
Peak response, AU	1.42 (1.24–1.84)	1.55 (1.40–2.19)	1.47 (1.25–1.70)	1.48 (1.26–2.01)	2.01 (1.58–2.50)	1.94 (1.53–3.00)	.64	.04	
EPC, #/10e6 MNC	22.9 (8.7–33.0)	18.0 (10.0–26.9)	16.9 (12.2–23.1)	15.6 (13.0–19.7)	25.4 (18.0–38.7)	17.4 (14.2–26.1)	.09	.12	
EMP, #/μL	199.0 (170.7–268.9) ^a	266.6 (173.0–343.5)	281.9 (205.6–362.2)	304.3 (214.3–374.9)	291.5 (207.6–372.5)	229.0 (191.1–280.2)	.006	.004	

Results are shown as mean ± SD or median (interquartile range). AU, arbitrary units; AIX, augmentation index; FFM, fat-free mass; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; MNCs, mononuclear cells; PWV, pulse wave velocity.

^a Significant baseline difference for usual care versus intervention group.

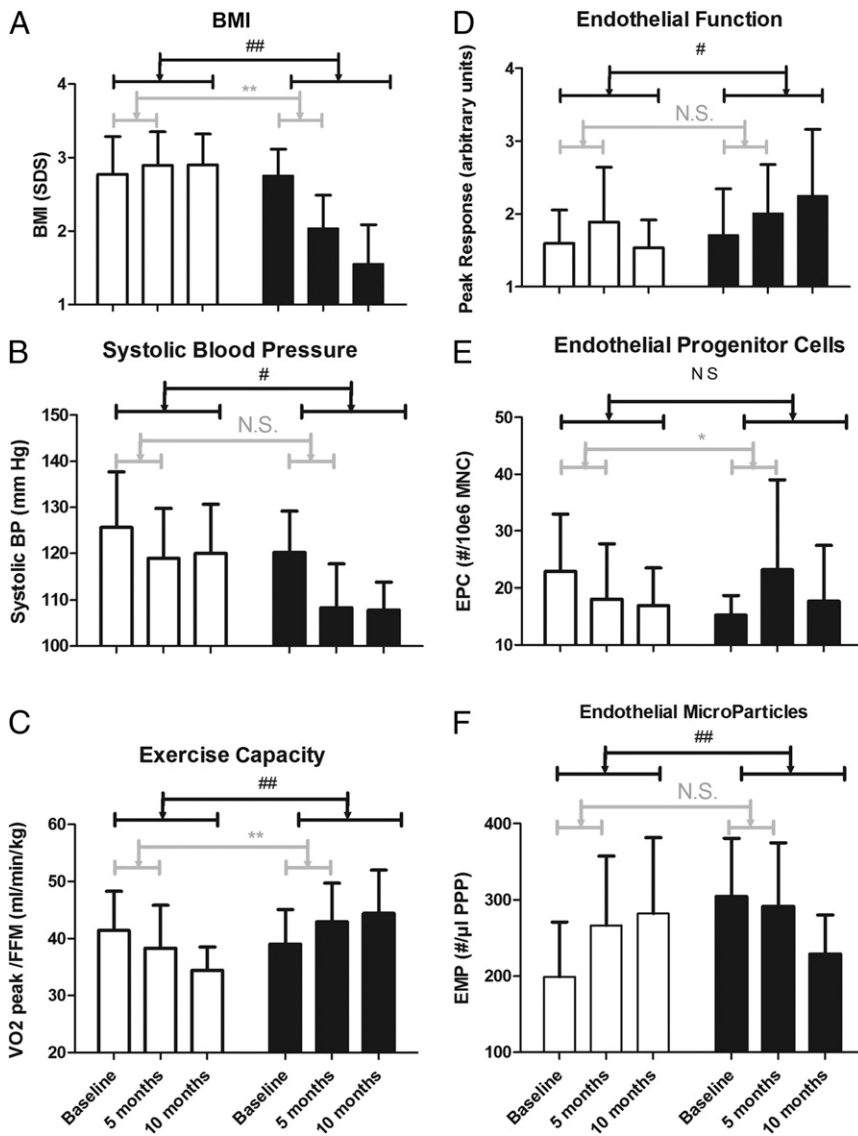


FIGURE 2 Changes in BMI, blood pressure, exercise capacity, and markers of endothelial function after 5 and 10 months in children: obese usual care group (white bars) and obese intervention group (black bars). A, BMI was expressed as SDS values; B, systolic blood pressure is expressed as mm Hg; and C, maximal load as watts. Data are shown as mean \pm SD. D, Microvascular endothelial function was measured as the peak response in arbitrary units; E, EPCs are expressed as cells per million mononuclear cells; and F, EMPs as particles per microliter of PPP. Data are shown as median and interquartile range. *P* values are given for mixed models analyses at 5 months (in gray, *) and for the entire intervention period (in black, #). * or # *P* < .05; ** or ## *P* < .01. NS, no statistical significance.

Residential treatment significantly improved low-grade inflammation, reflected in a decline of high-sensitivity C-reactive protein (*P* = .01) and a rise in adiponectin (*P* < .001) in the intervention compared with the usual care group. Exercise capacity was better in the intervention compared with the usual care group in that we observed an increase in $VO_{2peak}/\text{fat free mass}$ of

5.7 ± 6.5 mL/min/kg fat free mass (*P* < .001) and $+ 25.0 \pm 22.3$ watts (*P* < .001) maximal workload. Systolic blood pressure dropped with a mean of 11 ± 2 versus 7 ± 2 mm Hg (*P* = .04), and the augmentation index was significantly reduced to $12.3\% \pm 3.7\%$ in the intervention group compared with $-1.6 \pm 3.7\%$ in the usual care group (*P* = .04).

At the end of the treatment program, adolescents in intervention group achieved a significant amelioration of peak response of $+ 0.59 \pm 0.20$ arbitrary units compared with $+ 0.01 \pm 0.12$ arbitrary units in the ambulatory treated group (*P* = .04). There was a significant reduction in the number of circulating EMPs of 49.3 ± 122.5 in the intervention group versus a rise of 61.64 ± 114.54 EMPs per microliter in usual care adolescents (*P* = .004).

DISCUSSION

In this report, the effects of a residential treatment program compared with those of an ambulant usual care treatment in obese adolescents are described. The main inferences of the current study are the following:

1. A 10-month residential multidisciplinary therapeutic program, consisting of a supervised diet and exercise training, restored impaired microvascular endothelial function (ie, peak response) through significant reductions in BMI, improved exercise capacity, and reduction in multiple cardiovascular risk factors such as hypertension, adverse lipid profile, low-grade inflammation, and low adiponectin values.
2. This process occurred along with a biphasic response in circulating EPCs and EMPs.

Endothelial dysfunction is considered as the primum movens of atherosclerosis and is present in obese adolescents.^{2,23} However, endothelial dysfunction occurs differently in macro- versus microvascular beds.²³ Our results demonstrate for the first time that microvascular endothelial dysfunction in obese adolescents can be improved to values comparable in normal-weight adolescents¹² by participation in a combined diet plus exercise regimen. This improvement in microvascular endothelial function could only be achieved after

successful completion of the entire 10-month residential program, supporting the need for sustained therapy as an approach to improve microvascular dysfunction in obese adolescents. The cost of such programs may imply that such treatment modality is to be reserved for a minority of motivated severely obese children and adolescents.

Circulating numbers of EPCs were significantly increased after 5 months of therapy. A significant rise in EPCs had been observed previously in obese adolescents after 12 weeks of exercise training.²⁴ The EPC phenotype, however, differed from what is currently accepted.²² In accordance with the latest recommendations, Walther et al demonstrated that 1 year of additional exercise during school time is an effective means to markedly increase the number of circulating CD45low/CD34+/KDR+ EPC.²⁵ However, only 13% of the adolescents in that study were overweight or obese.

Intriguingly, our residential treatment program induced a biphasic response in EPC with an increase at 5 months and a decrease at completion of the 10-month program. Initially, elevated numbers of EPCs might have been released from bone marrow into the circulation to contribute to vasculoprotection. We postulate that these EPCs were incorporated in the endothelium, based on previous findings in young healthy men in which an increase in EPCs was noted up to 24 hours after maximal bicycle

ergometry.²⁶ Later, due to long-term healing of endothelium by exercise, the amount of EPCs may decrease again.

The number of EMPs declined significantly after 10 months of residential treatment. To our knowledge, the effect of a diet plus exercise treatment on the number of circulating EMPs in obese adolescents has not been described. Exercise-induced increases in blood flow across the endothelium²⁷ may well explain lowered EMP levels because in vitro findings proved that high shear stress conditions limit EMP release.²⁸ In future studies, quantifying numbers of EMPs and EPCs, in addition to endothelial function assessment, could be useful for further improvement of exercise training protocols.

A limitation of this study is that we did not use a randomized controlled trial design because we considered it unethical to deny adolescents a treatment based on a randomization process. To solve this problem, we recruited patients from the institution's waiting list as a control group. The treatment in a residential setting is a clear benefit of this study, allowing intensive follow-up of participants and leading to overall better results than ambulatory follow-up. As is often inherent with clinical studies, not all baseline parameters were comparable between the 2 obese groups. However, the primary purpose of this study was to consider how (endothelial) parameters changed

over time in response to treatment, for which the mixed-models analysis was highly appropriate. Finally, the number of participants was limited, although comparable to landmark papers on this matter.^{29,30} The results from the current study are to be confirmed in larger cohort studies.

CONCLUSIONS

A 10-month residential treatment program for obese adolescents, consisting of moderate diet and exercise training, significantly improved microvascular endothelial function, and known and novel cardiovascular risk factors were managed concomitantly. In addition, EPCs and EMPs displayed a biphasic response with an initial significant increase in circulating EPCs at 5 months followed by a marked reduction in EMP numbers after completion of the treatment program.

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This trial has been registered at www.clinicaltrials.gov (identifier NCT01461226).

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