Endothelial Progenitor Cells and Endothelial Microparticles Are Independent Predictors of Endothelial Function

Luc Bruyndonckx, MD[1,2,3], Vicky Y. Hoymans, MSc, PhD[1,2], Geert Frederix, MSc[1], Ann De Guchtenaere, MD, PhD[4], Hilde Franckx, MD[4], Dirk K. Vissers, PhD[5], Christiaan J. Vrints, MD, PhD[1,2,6], José Ramet, MD, PhD[3], and Viviane M. Conraads, MD, PhD[1,2,6,*]

Objective To examine the degree of microvascular endothelial dysfunction in relation to classical cardiovascular risk factors, arterial stiffness, and numbers of circulating endothelial progenitor cells (EPCs) and endothelial microparticles (EMPs), in obese and normal-weight children.

Study design Cross-sectional study with 57 obese (15.2 ± 1.4 years) and 30 normal-weight children (15.4 ± 1.5 years). The principal outcome was microvascular endothelial function measured with peripheral arterial tonometry. Fasting blood samples were taken for biochemical analysis and EMPs ($CD31^+/CD42b^-$ particles) and EPCs ($CD34^+/KDR^+/CD45dim/^-$ cells) flow cytometry. Characteristics between groups were compared by use of the appropriate independent samples test; a stepwise multiple regression analysis was used to determine independent predictors of microvascular endothelial function.

Results Microvascular endothelial function was significantly impaired in obese children and inversely correlated with body mass index Z scores ($r = -0.249$; $P = .021$) and systolic blood pressure ($r = -0.307$; $P = .004$). The number of EPCs was significantly lower in obese children and correlated with endothelial function ($r = 0.250$; $P = .022$), and the number of EMPs was significantly greater in obese children and correlated inversely with endothelial function ($r = -0.255$; $P = .021$). Multivariate analysis revealed that systolic blood pressure and numbers of circulating EPCs and EMPs are important determinants of endothelial function.

Conclusion Obese children demonstrate impaired endothelial microvascular function, increased arterial stiffness, fewer EPCs, and more EMPs. Besides systolic blood pressure, EPC and EMP counts independently predict the presence of microvascular endothelial dysfunction. *(J Pediatr 2014;165:300-5)*.

Obese children are more prone to develop early cardiovascular morbidity and are at increased risk for cardiovascular mortality in their adult life.[1] Severe obesity in children is associated with both arterial wall stiffness and endothelial dysfunction.[2,3] These manifestations represent different aspects of vascular disease, yet both are adversely affected by the accumulation of multiple cardiovascular risk factors, such as hypertension, dyslipidemia, inflammation, and oxidative stress, present in obese children.[4]

A strong inverse correlation between cardiovascular risk factors and the number of circulating endothelial progenitor cells (EPCs) has been reported in obese adults.[5] These bone marrow-derived EPCs have gained considerable attention as they play a pivotal role in the repair of damaged endothelium. Obese adults were reported to have a reduced number of circulating EPCs, and this was inversely associated with an increased intima media thickness.[6] Obesity was a more prominent predictor of the number of EPC than other cardiovascular risk factors, and weight loss was associated with an increased EPC count and an improved brachial artery flow-mediated dilation. Little is known about the number of EPCs and the correlation with endothelial function in children. In the study of Jung et al,[7] overweight children surprisingly had more EPCs than thinner teenagers. However, functional or structural damage to arteries was not investigated.

Circulating endothelial microparticles (EMPs) are released from endothelial cells into the circulation upon activation or apoptosis.[8] In obese adults, the EMP count is increased and correlates inversely with endothelial function.[9] Similarly, in overweight and obese children, an increased number of circulating EMPs is detected, but currently there are no data available linking EMP counts with

ACD	Acid citrate dextrose	hsCRP	High-sensitivity C-reactive protein
Aix	Augmentation index		
AU	Arbitrary units	PPP	Platelet-poor plasma
BMI	Body mass index	PWA	Pulse-wave amplitude
EMP	Endothelial microparticle	PWV	Pulse-wave velocity
EPC	Endothelial progenitor cell	RHI	Reactive hyperemia index
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance		

From the [1]Laboratory of Cellular and Molecular Cardiology, Department of Cardiology, University Hospital Antwerp; [2]Cardiovascular Diseases, Department of Translational Pathophysiological Research, University of Antwerp; [3]Department of Pediatrics, University Hospital Antwerp, Antwerp, Belgium; [4]Zeepreventorium, De Haan, Belgium; [5]Faculty of Medicine and Health Sciences, University of Antwerp; and [6]Department of Cardiology, University Hospital Antwerp, Antwerp, Belgium

*Deceased.

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endothelial function and vascular structure.[10] Therefore, the aim of this study was to examine the relationship between circulating EPCs and EMPs and microvascular endothelial function and arterial stiffness in obese and normal weight children.

Methods

Fifty-seven obese children and 30 normal-weight controls (12-18 years of age) were enrolled. Obesity was defined for children younger than 16 years, as a body mass index (BMI) ≥97th age- and sex-specific percentile,[11] whereas for those older than 16 years, a BMI ≥35 kg/m^2 was set as cut-off. Obese children with an acute or a chronic inflammatory process, a structural or other cardiac disease, or with active malignant hematologic processes were excluded from the study. Participants who were taking nonsteroidal anti-inflammatory or immunosuppressive drugs or were active smokers also were excluded. Obese patients were recruited from the Zeepreventorium (De Haan, Belgium) and from the obesity clinic of the Department of Pediatrics of the Antwerp University Hospital (Edegem, Belgium). Healthy, normal-weight children were recruited from high schools surrounding Antwerp University Hospital. The exclusion criteria were similar to those for obese children. The use of medications (except for oral contraception) also disqualified normal-weight children from inclusion in the study. All children were recruited between April and August to avoid confounding caused by seasonal variation of endothelial function.[12]

The protocol complied with the Declaration of Helsinki and was approved by the ethics committee of the Antwerp University Hospital (B300201110926). All participants and their parents provided their written informed consent.

Overnight fasting serum and acid citrate dextrose (ACD) anticoagulated blood (Vacutainer tube; BD Biosciences, Erembodegem, Belgium) were obtained from an antecubital vein via the use of a 21-gauge butterfly needle. Serum was centrifuged 30 minutes after blood collection, and aliquots were stored at −80°C until analysis. Routine blood tests were analyzed at a certified laboratory (Zeepreventorium, De Haan, Belgium or Department of Clinical Chemistry, Antwerp University Hospital, Edegem, Belgium). The ACD Vacutainer tube was used for flow cytometric enumeration of EPC and EMP.

Body weight and stature were measured to the nearest 0.1 kg and 0.1 cm, respectively, by using a digital-balanced scale and a wall-mounted stadiometer, and anthropometric z-scores were calculated.[11] In normal-weight children, the percentage of total body fat was estimated by bioelectrical impedance analysis (Body Logic Pro Body Fat Analyzer; Omron, Kyoto, Japan); in obese children, body fat determination was performed with dual-energy X-ray absorptiometry (Lunar Prodigy Advance Full Size; GE Healthcare, Waukesha, Wisconsin). Pubertal staging (pubic hair status) was assessed by clinical examination according to the method of Tanner.

After the patient rested for 10 minutes in a recumbent position, peripheral blood pressure and arterial stiffness were assessed via use of the TensioMed Arteriograph (TensioMed Kft, Budapest, Hungary). Measurements were performed at a similar time of day (8:00-10:00 a.m.) for all patients in a temperature-controlled (21-24°C), dimly lit room.[13] The instrument enables oscillometric measurement of systolic and diastolic blood pressure. Then the cuff is inflated to suprastystolic pressures to record the time interval between the direct and reflected systolic pressure wave (return time). In the condition of brachial artery occlusion, the influence of the brachial arterial wall is practically eliminated, and the recorded curves reflect central pressure waves.

By subtracting the difference between the amplitude of the direct and the reflected wave by the pulse pressure, we are able to compute the augmentation index (Aix). Aortic pulse-wave velocity (PWV) is then calculated by dividing the straight-line distance from sternal notch to the pubic bone (as a marker of aortic length) by the return time.[14] Measurements with an SD larger than 1.1 m/s were rejected. We used the average of 3 accepted recordings for the calculation of systolic and diastolic blood pressure, Aix, and PWV. Age-, sex-, and height-specific systolic and diastolic blood pressure percentiles were determined.[15]

Peripheral microvascular endothelial dysfunction was measured noninvasively at the distal phalanx of the index fingers with Endo-PAT (Itamar Medical Ltd, Caesarea, Israel). The time point at which peak dilation occurs is much more variable in children and adolescents than in adults,[16] meaning that the automatically calculated reactive hyperemia index (RHI) might not always reflect maximal dilation. We calculated a ratio of postocclusion pulse-wave amplitude (PWA) to baseline amplitude every 30 seconds after occlusion. By doing so, we could identify peak response as well as the time to peak response by using the midpoint of the corresponding 30 seconds average amplitude interval. An area under the curve was calculated with PWA data for every 30-second interval until 4.5 minutes after occlusion via use of the trapezoidal rule.

To quantify the number of circulating CD31$^+$/CD42b$^-$ EMP, ACD anticoagulated whole blood was centrifuged within 30 minutes at 1525g without acceleration or brake for 20 minutes. The top 1.5 mL of plasma was carefully aspirated and centrifuged again at 1525g for 20 minutes to obtain platelet-poor plasma (PPP). Fifty microliters of PPP was incubated with specific antibodies for 30 minutes at 4°C in the dark: 3 μL of anti CD31-PE and anti 4 μL of CD42b-FITC (BD Biosciences) were added per test. Samples were diluted with 0.22 μm of filtered phosphate-buffered saline and measured on a BD FACSCanto II flow cytometer (BD Biosciences) as previously described.[17] Samples were acquired on a low flow rate during 180 seconds and run in duplicate; the coefficient of variation limit between duplicates was set at <15%. The flow rate was objectified with Trucount Beads (BD Biosciences), allowing calculation of circulating EMP number per microliter of PPP.

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