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High levels of soluble intercellular adhesion molecule-1, insulin resistance and saturated fatty acids are associated with endothelial dysfunction in healthy adolescents

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ABSTRACT

Objective: Atherosclerosis begins and progresses during childhood and adolescence. Endothelial dysfunction is one of the earliest abnormalities that can be detected in the development of atherosclerosis. As the determinants of endothelial function in childhood are unknown, we investigated the influence of cardiovascular risk factors on endothelial function in a cohort of healthy adolescents.
Methods: A total of 257 adolescents (age: 14.5 ± 1.0 years, 138 girls) participated in this study. Endothelial function was measured as reactive hyperemic index (RHI) using a fingertip peripheral arterial tonometry device. Blood samples were collected for analysis of lipids, insulin, glucose, fatty acid composition of plasma phospholipids, and markers of inflammation and endothelial function.
Results: There was no gender difference in RHI. Boys had higher plasma level of vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin and monocyte chemoattractant protein-1, and lower level of insulin, total cholesterol, high-density lipoprotein–cholesterol (HDL), low-density lipoprotein–cholesterol (LDL), ApoA1, ApoB, and docosahexaenoic acid of plasma phospholipids than girls. There was no gender difference regarding triacylglycerol, triacylglycerol/HDL, LDL/HDL and ApoB/ApoA. The RHI was inversely associated with plasma ICAM-1 ($p = 0.0003$), HOMA index for insulin resistance (HOMA-IR, $p = 0.001$) and saturated fatty acids of plasma phospholipids (SFA, $p = 0.001$). The associations remained significant after adjusting for age, height, BMI-*z*-score, sex, blood pressure, HDL and smoking.
Conclusion: In healthy adolescents impaired endothelial function is significantly associated with high level of soluble ICAM-1, HOMA-IR and SFA.

1. Introduction

Endothelial function reflects the integrated effects of risk factors on the vasculature. Given that endothelial dysfunction is one of the earliest abnormalities that can be detected in the development of atherosclerosis [1], study of endothelial dysfunction has utility for the identification of novel risk factors for cardiovascular disease (CVD) and for the evaluation of interventions to reduce CVD risks [2]. In adults, the endothelial function is known to correlate to various classic cardiovascular risk factors such as smoking, hypertension, dyslipidemia, obesity and chronic systemic inflammation [1]. Recent studies show that the endothelial function can predict the progression of carotid intima–media thickness and incident cardiovascular events in adults free of clinical CVD at baseline [3,4]. There is evidence that atherosclerosis begins in childhood [5], and high-risk children, such as those with obesity and familial hypercholesterolemia, have impaired endothelial function compared with healthy controls [6,7]. However, we know very little about the factors that influence the endothelial function in healthy children and adolescents. The aim of this study was to evaluate the relations of classic CVD risk factors, insulin resistance, plasma fatty acid composition, and inflammatory and endothelial markers to endothelial function in a cohort of healthy adolescents. Given that gender plays an important role on cardiovascular physiology and pathology via the endothelial system [8], and gender difference in the artery intima–media thickness occurs early in childhood [9], we also investigated gender differences in endothelial function, plasma concentration of

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Table 1
Anthropometrical and biochemical variables.

Variables	Girls	*n*	Boys	*n*	*p*
Age (y)	14.5 ± 1.0 (12.5–16.4)	138	14.5 ± 1.0 (12.3–16.7)	119	*n.s.*
Weight (kg)	57.0 ± 10.0	137	61.9 ± 15.0	116	0.003
Height (m)	1.64 ± 0.06	137	1.71 ± 0.09	116	0.000
BMI	21.2 ± 3.3	137	21.0 ± 3.6	116	*n.s.*
BMI-*z*-score	0.47 ± 1.08	137	0.47 ± 1.11	116	*n.s.*
RHI	1.7 (1.4–2.0)	134	1.7 (1.4–2.0)	116	*n.s.*
SBP (mm Hg)	107.7 ± 8.4	137	110.3 ± 8.8	117	*n.s.*
DBP (mm Hg)	60.2 ± 5.4	137	57.5 ± 5.7	117	0.000
Glucose (mmol/L)	4.6 (4.5–5.0)	114	4.8 (4.6–5.0)	105	*n.s.*
Insulin (mU/L)	6.5 (4.5–9.3)	116	4.5 (2.8–6.6)	107	0.000
HOMA-IR	1.3 (0.9–2.0)	113	0.9 (0.6–1.4)	104	0.000
TG (mmol/L)	0.7 (0.5–1.0)	115	0.6 (0.4–0.9)	107	*n.s.*
Total cholesterol (mmol/L)	4.2 (3.7–4.9)	115	3.9 (3.3–4.2)	107	0.000
HDL (mmol/L)	1.3 (1.2–1.5)	115	1.2 (1.0–1.4)	107	0.000
LDL (mmol/L)	2.6 (2.2–3.0)	115	2.3 (1.9–2.6)	107	0.002
TG/HDL	0.53 (0.42–0.75)	115	0.54 (0.36–0.8)	107	*n.s.*
LDL/HDL	1.96 (1.53–2.23)	115	1.93 (1.59–2.3)	107	*n.s.*
ApoA (g/L)	1.26 ± 0.18	115	1.14 ± 0.18	108	0.000
ApoB (g/L)	0.67 ± 0.15	115	0.61 ± 0.13	108	0.004
ApoB/ApoA	0.54 (0.45–0.64)	115	0.54 (0.45–0.63)	108	*n.s.*

Data are presented as means ± SD or median (interquartile range). For age, the minimum and maximum values are presented in brackets. BMI, body mass index; RHI, reactive hyperemia index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment index for insulin resistance; TG, triacylglycerol; HDL, high-density lipoprotein–cholesterol; LDL, low-density lipoprotein–cholesterol; ApoA, apolipoprotein-A; ApoB, apolipoprotein-B.

inflammatory and endothelial markers, and plasma fatty acid composition.

2. Materials and methods

2.1. Study population

A total of 554 healthy adolescents in three schools in the Gothenburg region were invited to participate in the study (age 14.2 ± 0.9, 289 girls). Informed consent and written protocols, approved by the Ethics Committee at the Sahlgrenska Academy at the University of Gothenburg, were presented to the adolescents and their parents. Written consents by both the adolescents and their parents were obtained from 257 (138 girls) adolescents. Of these, we obtained blood samples from 227 (117 girls) subjects and data about endothelial function measurement from 250 (134 girls) subjects. Weight and height were measured. BMI-*z*-scores were calculated according to large databases presented by Cole et al. [10]. Resting blood pressure levels were measured indirectly in the right arm with an electronically sphygmomanometer (Welch Allyn Inc., New York). Three separate readings were taken 2 min apart, and the average of the second and third readings was calculated.

2.2. Blood sample analyses

Fasting blood samples were collected and kept at −70 °C. Fasting total cholesterol, high-density lipoprotein–cholesterol (HDL) and triacylglycerol (TG) were analyzed using enzymatic methods, and apolipoproteins A and B by immunoturbidimetric assays (Roche Diagnostics, Germany). Low-density lipoprotein–cholesterol (LDL) concentrations were calculated by using Friedewald's equation. Plasma levels of vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin, L-selectin, interleukin (IL)1α, IL1β, IL2, IL4, IL6, IL8, IL10, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) were measured by Evidence® array biochip analyzer (Randox Laboratories Ltd., United Kingdom). The functional sensitivity and inter-assay coefficient of variation (CV) for these biomarkers were: VCAM-1: 7.3 ng/mL, 6.5%; ICAM-1: 7.8 ng/mL, 10.7%; E-selectin: 1.6 ng/mL, 7.7%; P-selectin: 4.1 ng/mL, 7.1%; L-selectin: 15.5 ng/mL, 6.1%; IL1α: 0.5 pg/mL, 8%; IL1β: 0.8 pg/mL, 7.5%; IL2: 4.1 pg/mL, 8.9%; IL4: 2.8 pg/mL, 11.6%; IL6: 0.3 pg/mL, 12.7%; IL8: 1.5 pg/mL, 9%; IL10: 1.0 pg/mL, 7.5%; TNF-α: 1.8 pg/mL, 9.2%; MCP-1: 19.4 pg/mL, 9.2%; VEGF: 25.3 pg/mL, 9%; EGF: 2.6 pg/mL, 7%. Serum insulin was analyzed by enzyme-linked immunosorbent assay (Mercodia, Sweden) and glucose was determined using an enzymatic method (hexokinase). Homeostatic model assessment index for insulin resistance (HOMA-IR) was calculated with formula: HOMA-IR = insulin (μU/ml) × glucose (mmol/l)/22.5. Fatty acid composition of plasma phospholipids was analyzed as described previously [11].

2.3. Endothelial function measurements

Endothelial function was assessed using reactive hyperemia peripheral arterial tonometry (RH-PAT) method with Endo-PAT® device (Itamar Medical Ltd., Israel). Briefly, pulse wave amplitude was recorded for 5 min before and after 5-min arterial flow occlusion. The reactive hyperemic index (RHI) was calculated as the ratio of the average pulse wave amplitude measured over 60 s starting 1 min after cuff deflation to the average pulse wave amplitude measured at baseline, and then divided by the same ratio in the contra-lateral finger to correct for changes in systemic vascular tone. The reproducibility of RH-PAT measurements was investigated in a separate group of healthy children ($n = 15$). Each subject was studied twice with a 10-week interval. The mean RHI was 1.862 (range 1.233–3.060) for the first measurement and 1.818 (1.238–2.762) for the second. The mean intra-subject standard deviation of the RHI was 0.26 and the CV was 14.9%.

2.4. Statistics

Statistical analyses were performed with SPSS15.0. All continuous variables were tested for normal distribution by Kolmogorov–Smirnov test. Normally distributed variables are presented as means ± SD, while variables with skewed distribution are presented as medians and interquartile ranges. Gender differences were tested by Student's *t*-test for normally distributed variables or by Mann–Whitney's *U*-test for variables with skewed distribu-