Atherosclerosis 220 (2012) 215–218

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Trunk weighted obesity, cholesterol levels and low grade inflammation are main determinants for enhanced thrombin generation

Florian Prüller[a], Reinhard B. Raggam[a], Verena Posch[a], Gunter Almer[a], Martie Truschnig-Wilders[a], Renate Horejsi[b], Reinhard Möller[b], Daniel Weghuber[c], Rottraut Ille[d], Wolfgang Schnedl[e], Harald Mangge[a,*]

[a] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria
[b] Institute of Physiological Chemistry, Center of Physiological Medicine, Medical University of Graz, Austria
[c] Department of Pediatrics, Paracelsus Private Medical School Salzburg, Austria
[d] Institute for Psychology, Karl-Franzens University Graz, Austria
[e] General Practice for Internal Medicine, Bruck an der Mur, Austria

ARTICLE INFO

Article history:
Received 15 June 2011
Received in revised form 20 August 2011
Accepted 25 September 2011
Available online 14 October 2011

Keywords:
Endogenous thrombin potential (ETP)
Trunk obesity
Low grade inflammation
Thrombosis risk

ABSTRACT

Objective: Endogenous thrombin generation (ETP) may be critically involved in obesity associated thromboembolism.
Methods: Three hundred and one participants of the STyrian Juvenile OBesity (STYJOBS)/Early DEteCTion of Atherosclerosis (EDECTA) study cohort (age, 16–58 years) were analysed. ETP was measured by the new CE-IVD marked Siemens-Innovance® ETP test on a BCS-XP analyser, and correlated to clinical findings and extended lipometry-based anthropometric data, biomarkers, and coagulation parameters.
Results: In the overweight/obese study group, ETP and fibrinogen levels were significantly higher compared to controls ($p < 0.001$). In a multiple stepwise regression including all subjects, subcutaneous adipose tissue thickness of upper back, cholesterol and ultrasensitive C-reactive protein were the best predictors for ETP.
Conclusion: Trunk weighted obesity together with low grade inflammation and hypercholesterolemia enhance thrombin generation.

1. Introduction

Obesity represents a major public health problem. Recently reported associations between increased BMI, impaired fibrinolysis and vascular burden imply that a prothrombotic tendency is also accounting for the health threatening risk of obesity [1,2]. On the other hand, physical training was observed to improve negative effects especially in obese juveniles [3–7]. However, these investigations were based on a limited number of investigated subjects [3–5] and did not specifically look at different depots of fat distribution [2]. The endogenous thrombin potential (ETP) defines the pro- and anticoagulant balance as a global function test of clotting [8].

Increased ETP levels were seen in obesity associated myocardial infarction [9], stroke [10], and pulmonary embolism [11]. However, ETP in asymptomatic young and middle-aged subjects with increased risk for these endpoints remains to be clarified [7]. Our rationale is to investigate this preclinical phase as basis for an improved prevention and intervention. To the best of our knowledge, we are the first to analyse ETP in conjunction with extended lipometry-based anthropometric data and metabolic/inflammatory biomarkers in a well defined controlled large cohort of young and middle aged overweight/obese subjects.

2. Methods and procedures

2.1. Subjects

Participants were derived from the prospective, observational STYJOBS/EDECTA study (ClinicalTrials.gov Identifier NCT00482924) which is designed to improve the understanding of the development of atherosclerosis, and obesity linked metabolic risk patterns by investigation of the "non-biased" early stage. The inclusion criterion for the overweight (obese) subjects was BMI > 90th ≤ 97th (>97th) percentile if below 18 years of age, and BMI > 25 ≤ 29.9 kg/m^2 (>30 kg/m^2) if above 18 years of age. Participants were 107 obese (female 53/male 47), 55 overweight

* Corresponding author at: Clinical Institute of Medical and, Chemical Laboratory Diagnosis, Medical University of Graz, Auenbruggerplatz 30, A-8036 Graz, Austria. Tel.: +43 316 385 83340, fax: +43 316 385 14024.
E-mail address: harald.mangge@klinikum-graz.at (H. Mangge).

0021-9150/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2011.09.035

Table 1a
Baseline characteristics of the study subjects.

Variable	Controls	Overweight	Obese
Individuals	$n = 139$	$n = 55$	$n = 107$
Female/male	93/46	29/26	53/47
Age (years)	32.9 ± 9.7	37.9 ± 11.9	33.6 ± 12.3
Body height (m)	1.7 ± 0.09	1.7 ± 0.1	1.7 ± 0.1
Body weight (kg)	64.2 ± 9.8	82.1 ± 10.9***	107.8 ± 21.7*** §§§
BMI (kg/m^2)	22 (20–23)	27 (26–29)$^{+++}$	35 (32–39)$^{+++}$ ◊◊◊
Metabolic syndrome (IDF criteria)	0/139	12/55	58/107
SBP (mmHg)	117 (110–125)	130 (110–135)$^{++}$	130 (120–140)$^{+++}$ ◊
DBP (mmHg)	75 (70–80)	80 (70–85)$^{+}$	80 (75–90)$^{+++}$ ◊
Triglycerides (mmol/l)	0.83 (0.6–1.1)	1.0 (0.8–1.5)$^{+++}$	1.5 (1.1–2.2)$^{+++}$ ◊◊◊
Cholesterol (mmol/l)	4.8 ± 0.9	5.1 ± 1.0	5.2 ± 1.0*
HDL-cholesterol (mmol/l)	1.8 (1.5–2.1)	1.6 (1.2–1.9)$^{+++}$	1.2 (1.0–1.5)$^{+++}$ ◊◊◊
LDL-cholesterol (mmol/l)	2.6 ± 0.9	3.1 ± 0.9**	3.2 ± 0.7***
Oxidized LDL (mmol/l)	1.0 (0.8–1.4)	1.5 (1.1–1.7)$^{+++}$	1.6 (1.2–2.1)$^{+++}$
Glucose (mmol/l)	3.2 (4.3–4.8)	4.9 (4.6–5.4)$^{+++}$	4.8 (4.4–5.3)$^{++}$
Insulin (μE/ml)	6.7 (4.2–10.4)	10.2 (6.1–13.9)$^{++}$	16.2 (10.8–22.2)$^{+++}$ ◊◊◊
US-CRP (mg/l)	0.8 (0.5–2.0)	2.0 (0.8–3.7)$^{+++}$	3.3 (2.0–5.7)$^{+++}$ ◊◊◊
Interleukin-6 (pg/ml)	1.6 (1.5–2.2)	2.0 (1.5–2.7)$^{+}$	3.1 (1.9–4.1)$^{+++}$ ◊◊◊
Creatinine (μmol/l)	76.0 ± 11.5	81.3 ± 15.0*	75.1 ± 14.7§
Uric acid (μmol/l)	267.7 ± 71.4	321.2 ± 71.4***	354.6 ± 84.5*** §
ALT/GPT (U/l)	18 (15–24)	31 (20–40)$^{+++}$	30 (20–49)$^{+++}$
AST/GOT (U/l)	25.5 (22–30)	27 (24–35)$^{+}$	29 (23–35)$^{++}$
Gamma-GT (U/l)	17 (13–23)	27 (17–48)$^{+++}$	28 (20–42)$^{+++}$
Prothrombin time (INR)	1.02 (1.08–0.96)	0.97 (1.06–0.92)$^{+}$	0.97 (1.06–0.92)
APTT (s)	32 (30–34)	31 (30–33)	33 (31–34)$^{◊}$
Fibrinogen (mg/dl)	282.8 ± 62.1	325 ± 70.2***	351 ± 83***
ETP (extinction)	383.8 ± 53.9	413.2 ± 58.2**	422.8 ± 51.9***
ETP (%)	96.5 ± 14.3	104.6 ± 15.4**	107.0 ± 13.6***
Antithrombin (%)	109 ± 17	115 ± 24	108 ± 22
D-dimer (μg/ml)	0.2 (0.15–0.33)	0.20 (0.15–0.35)	0.24 (0.18–0.37)

BMI, body mass index; IDF, International Diabetes Federation; SBP, systolic blood pressure; DBP, diastolic blood pressure; US-CRP, ultra-sensitive C-reactive protein; APTT, activated partial thromboplastin time; ETP, endogenous thrombin potential.
Results are expressed as mean ± SD, analysed by Student's *t*-test ($^{*§}p < 0.05$, $^{**§§}p < 0.01$, $^{***§§§}p < 0.001$), or as median (25th–75th percentile) and analysed by Mann–Whitney *U* test ($^{+◊}p < 0.05$, $^{++◊◊}p < 0.01$, $^{+++◊◊◊}p < 0.001$) depending on the distribution of data. The symbols ($^{*+}$) are indicators of significance between controls and overweight subjects, and between controls and obese subjects; ($^{§◊}$) indicate the significance between overweight and obese subjects. Variables outlined as median (25th–75th percentile) are not normally distributed and logarithmically transformed for statistical calculations.

(29/26), and 139 normal-weight (93/46) subjects aged from 16 to 58 years (Table 1a). All subjects were free of acute or chronic disease and none had a family history of haemorrhagic diathesis. The adolescents were postpubertal and nine women studied were postmenopausal, but none received HRT. Standard anthropometric data (height, weight, waist-, hip-circumference, waist to hip-, waist to height-ratio) were obtained from each subject. Subjects wore light clothing (e.g. shorts and a light top) and no shoes during the measurements. Standing height was measured to the nearest 0.01 cm using a portable calibrated stadiometer (SECA®-220, Hamburg, Germany). Body mass was measured to the nearest 0.01 kg using calibrated electronic scales (Soehnle® 7700, Murrhardt, Germany). The BMI was calculated as the weight in kilograms divided by the square of height in meters. Waist-circumference was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks. Resting blood pressure (BP) was measured on the right arm, at the end of the physical examination with the participant sitting. Physical activity and smoking were evaluated by a structured interview. Before starting with the clinical examination, written informed consent of all participants was obtained.

2.2. Extended anthropometry (Lipometer®)

Measurements of SAT thickness were performed by means of a patented optical device (EU Pat.Nr. 0516251) on 15 anatomically well-defined body sites [12] distributed from neck to calf on the right and left side of all study participants, and then averaged for both body sides. The sensor head of the lipometer that is held perpendicular to the measurement site, consists of a light source of light emitting diodes (λ = 660 nm, light intensity 3.000 mcd) and a photodetector, that measures the corresponding light intensities that are back scattered in the SAT. Calibration and evaluation were done using computed tomography (CT) as the reference method [12].

2.3. Sample material and laboratory methods

Fasting blood samples, collected from 08:00 to 10:30 h by a standard procedure (cubital vein puncture with butterfly), were immediately centrifuged at 3500 rpm at ambient temperature and stored at −80 °C until analysis. Deep-frozen (−80 °C) aliquots of platelet free plasma, derived from citrated blood samples were used for ETP analysis, which was performed by the new CE-IVD labeled Innovance® ETP test kit on a BCS-XP analyser (Siemens Healthcare Diagnostics, Marburg, Germany). Standard clotting parameters including prothrombin time (PT), activated thromboplastin time (APTT), fibrinogen, antithrombin and D-dimer, were measured with LIATEST reagents using a STAGO STA-R Evolution coagulation analyzer (Roche Diagnostics). Cholesterol, HDL-, LDL-cholesterol and triglycerides were measured by means of ECLIA (ElectroChemiLuminiscenceAssay) on an Elecsys™ 2010 analyzer (Roche Diagnostics Mannheim, Germany), oxidized low dense lipoprotein (oxLDL) by a commercially available ELISA (Mercodia oxidized LDL Competitive ELISA, SE-754 50 Uppsala, Sweden). Ultrasensitive-CRP (US-CRP) was analysed with a particle-enhanced immunoturbidimetric assay [Tina-quant® C-reactive protein latex ultrasensitive assay (Roche Diagnostics)], plasma insulin by ELISA (Mercodia, Uppsala, Sweden), and plasma glucose by the glucose hexokinase method on a Hitachi 917 chemical analyzer. HOMA-IR