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Haemostatic alterations in overweight children: Associations between metabolic syndrome, thrombin generation, and fibrinogen levels☆

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ABSTRACT

Background: The metabolic syndrome (MetS) is associated with central obesity and leads to increased morbidity and mortality due to cardiovascular disease (CVD). Since obesity is associated with a hypercoagulable state, it has been speculated that hypercoagulation is linking MetS to CVD.
Methods: We prospectively examined 81 overweight children and 32 normal-weight children aged 10–16 years. We analyzed blood pressure, fasting lipids, glucose, insulin, fibrinogen, and thrombin generating test determining time to peak (TTPeak), peak, time preceding the thrombin burst (lag-time), and 'endogenous' thrombin potential (ETP).
Results: Overweight children demonstrated significantly higher fibrinogen levels ($p < 0.001$), shorter lag-time ($p < 0.001$), and TTPeak ($p = 0.038$) compared to normal-weight children. Furthermore, ETP ($p < 0.001$) and peak ($p < 0.001$) were significantly higher in overweight than in normal-weight children. Fibrinogen and all parameters of the clotting test correlated significantly (p always <0.05) to body mass index (BMI) but not significantly to insulin resistance index HOMA-IR or occurrence of MetS in multiple linear backward regression analyses adjusted for age and gender.
Conclusions: The increased fibrinogen levels and the changes in the thrombin generation test points towards a haemostatic alteration in overweight children. The parameters of the clotting test were related to the degree of overweight but not to insulin resistance or occurrence of MetS questioning a direct association between MetS and the coagulation system. Longitudinal studies are needed to confirm these findings.

1. Introduction

A large body of epidemiological data indicates that people with abdominal adiposity, glucose intolerance, elevated blood pressure and dyslipidemia levels have an increased risk of cardiovascular disease (CVD) [1,2]. The common clustering of these factors in a single individual is summarized in the definition of the metabolic syndrome, which is associated with high insulin levels due to insulin resistance [1,2]. However, the known parameters of the metabolic syndrome do not adequately explain the excess cardiovascular disease morbidity and mortality attributed to this syndrome [3]. In the past decade, explanations for the increased CVD suffered by this group include their accelerated development of atherosclerosis and their increased propensity to develop thrombosis [3–6].

Indeed, obesity has been shown to be a modest risk factor for arterial and venous thromboembolic events [3,7]. Obesity is associated with a hypercoagulable state consisting of increased levels of clotting factors (for example fibrinogen) as well as inhibition of the fibrinolytic pathway (for example increased plasminogen activator inhibitor-1 (PAI-1) and decreased tissue plasminogen (tP) activator activity) [7]. Increased fibrinogen levels have been reported in obese children [8–11], which normalized in physical training or weight loss due to lifestyle intervention [10,11].

Since hyperinsulinemia has been suggested to influence coagulation [3], it is discussed controversially whether obesity per

Abbreviations: BMI, body mass index; SDS, standard deviation score; SB, Psystolic blood pressure; DBP, diastolic blood pressure; CVD, cardio vascular disease; TG, thrombin generation; tP, tissue plasminogen; PAI-1, plasminogen activator inhibitor-1; TTPeak, time to peak; ETP, 'endogenous' thrombin potential; Lag, time-time preceding the thrombin burst; HOMA, homeostatic model assessment.

☆ This study is registered at http://www.clinicaltrials.gov/ (NCT00435734).

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se or the insulin resistance in the metabolic syndrome is the main influence factor of the changes in the coagulation system [3–5,7–12].

To shed some light into this controversy, we analyzed thrombin generation (TG) and fibrinogen levels in overweight children with and without metabolic syndrome to determine the relationships between coagulation, obesity, and factors of the metabolic syndrome. Coagulation analyses in overweight children are rare [13,14] and especially studies in overweight children with metabolic syndrome are still lacking. Studying children has some potential advantages that there is likely no additional confusion with other diseases, medications, or active tobacco smoking probably influencing the coagulation. Furthermore, the metabolic syndrome begins in childhood [15] and is associated with early atherosclerotic vessel injury as demonstrated by measurements of increased intima-media thickness [16,17]. We decided to perform thrombin generation tests for clotting analyses. Since in contrast to various conventional clotting tests, which only reflect a small part of the complex haemostatic/thrombotic system, measurements of thrombin generation represent an overall function test, detecting the entire process of the overlapping steps of initiation, amplification, propagation and the termination phase of coagulation [14].

We hypothesized that the changes in the coagulation systems were stronger related to the occurrence of metabolic syndrome than overweight. Therefore, we compared clotting test of normal-weight children, overweight children without metabolic syndrome, and overweight children with metabolic syndrome.

2. Patients and methods

The local ethics committee of the University of Witten/Herdecke approved this study. Written informed consent according to the Declaration of Helsinki was obtained from all subjects and their parents.

We prospectively examined 81 overweight white children aged 10–16 years and 32 normal-weight children of similar age and gender recruited from the STYrian Juvenile OBesity Study [18]. None of the children suffered from endocrine or syndromal disorders, were smokers, or were on any medication.

All children were screened for factors of the metabolic syndrome (MetS) as described below. Additionally, thrombin generation test were performed and fibrinogen concentrations were measured. Furthermore, in the overweight children, pubertal stage was determined based on Tanner stages [19,20].

Height was measured to the nearest centimetre. Weight was measured in underwear to the nearest 0.1 kg using a calibrated balance scale. We used box-cox transformation to calculate SDS–BMI as a measure for degree of overweight due to the skewness of the BMI distribution [21]. Overweight was defined by a BMI above the 90th percentile for German children [22].

Blood pressure was measured using a validated protocol [23]. Systolic (SBP) and diastolic (DBP) blood pressure were measured at the right arm twice after a 10-min rest in the supine position by using a calibrated sphygmomanometer and averaged.

Blood sampling was performed in the fasting status after an overnight fasting between 8:00 and 9:00. Venous blood was drawn using precitrated tubes. Immediately after venipuncture, plasma was separated by centrifugation at $8000 \times g$ for 10 min at room temperature and stored at −82 °C. All analyses were performed in triplicate in one specialised laboratory [14]. Plasma fibrinogen was determined by the Clauss method using the reagent kit of Technoclone GmbH™, Vienna, Austria. Triglyceride, HDL-cholesterol, insulin, and glucose concentrations were measured using commercially available test kits (HDL-C-Plus™ Roche Diagnostics, Mannheim, Germany; Vitros™ analyzer Ortho Clinical Diagnostics, Neckarge-muend, Germany; MEIA™, Abbott, Wiesbaden, Germany). Homeostasis model assessment (HOMA) was calculated as follows [24]: HOMA-IR = (insulin [mU/l] × glucose [mmol/l])/22.5. The oGTT was performed in overweight children between 08:00 and 09:00 according to guidelines [25] after an overnight fast of at least 8 h. Impaired fasting glucose was defined by glucose values >5.5 mmol/l (100 mg/dl) [25]. Impaired glucose tolerance was defined by 2-h serum glucose >140 mg/dl in the oGTT.

We defined the MetS following the recent IDF definition [26] (waist circumference >90th percentile [27] + ≥2 of the following criteria: impaired fasting glucose or impaired glucose tolerance, systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg, triglycerides >150 mg/dl, and HDL-cholesterol <40 mg/dl).

We performed thrombin generation test (TG) by means of calibrated automated thrombography, developed by Hemker et al. [28]. This continuous measuring method can be applied using platelet-poor plasma containing fibrinogen and is based on the conversion of a thrombin-specific fluorogenic substrate, Z-Gly-Gly-Arg-AMC (amino-methyl-coumarin), purchased from Bachem, Bubendorf, Switzerland [14]. Thrombin activity was calculated as a function of time by comparing the fluorescent signal from the thrombin-generating sample with that from a known and stable sample, in parallel measured standard activity, using the thrombinoscope software [28]. Plasma was activated with low amounts of 5 pM tissue factor (TF), 4 μM phospholipids and calcium. The measurement process was performed using a fluorometer (Fluoroscan Ascent, Thermolabsystems OY, Helsinki, Finland) with an excitation filter at 390 nm and an emission filter at 460 nm.

In this TG test the 'endogenous' thrombin potential (ETP), the time preceding the thrombin burst (lag-time), the peak and the time to peak (TTPeak) are measured (Supplementary Fig. 1). The area under the curve of generated thrombin represents the ETP and has been shown to correlate with plasma-based hypercoagulable states [14,28]. Antithrombin, protein S, protein C deficiency and the plasmatic changes as a result of prothrombin hyperexpression and activated protein C resistance are associated with increased ETP levels. The lag-time is the time from the coagulation activation until the thrombin burst starts, shown as the first raise of the curve. The lag-time is strongly dependent on the concentration of coagulation activators and inhibitors as factors VII, X, and Protein C and S. After a fast rise the curve reaches its peak, showing the maximum concentration of thrombin generated. The time to the reversal of the curve shows the TTPeak. A recent study showed that the peak measured after weak activation is a very sensitive parameter for all coagulation factors [29].

Statistical analyses were carried out with Winstat for Exel™. Normal distribution was confirmed by the Kolmogorov–Smirnov test for all variables. Comparisons were performed by ANOVA, student *t*-test, and Chi-square test as appropriate. All parameters of the TG and fibrinogen were correlated to anthropometrics and parameters of the MetS by Pearson correlation. To differentiate whether degree of overweight, insulin resistance, or occurrence of MetS determinate the disturbances of coagulation, multiple backward linear regression analyses were calculated with parameters of the thrombin generation test and fibrinogen as dependent variables, and age, gender, BMI, and the insulin resistance index HOMA-IR as independent variables. Each variable of the TG was included as independent variable in a separate model. Additionally, occurrence of MetS instead of insulin resistance index HOMA-IR was included in these models. Gender (0 = male; 1 = female) and occurrence of MetS (0 = no; 1 = yes) were used as classified variables in these models. A p-value < 0.05 was considered as significant. Data are presented as mean ± standard deviation and as percentage.