Thrombin Generation in Severely Obese Children

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Introduction

In recent years the prevalence of obesity has raised to an alarming level. Especially the increasing rate of obese children across all pediatric ages is a cause of concern. Several studies made likely a close connection between obesity and alterations in hemostasis by findings that obese subjects tend to have higher values of fibrinogen, prothrombin fragment 1 and 2 (F1 + 2), factor VII, factor VIII, von Willebrand factor (vWF), and plasminogen activator inhibitor (PAI) compared to non-obese subjects [1, 2]. These hemostatic alterations in obese patients may contribute to the development of cardiovascular disease.

Despite its undisputed importance, data on single factors or components involved in the coagulation cascade reflect only a small part of the complex hemostatic-thrombotic system and cannot replace an overall function test [3]. Therefore, we investigated the general influence of obesity in childhood on thrombin generation (TG) performing the Calibrated Automated Thrombography (CAT) representing a global test of the blood clotting system. In contrast to various conventional clotting tests, such as the partial thromboplastin time (PTT) or the prothrombin time (PT), the TG – assay is sensitive to hypercoagulable changes in the plasma [4]. Measurements of the TG represent a method to estimate the individual thrombotic risk by assessing the coagulability of blood, and – determined with platelet poor plasma – reflect the function of all plasmatic pro- and anticoagulant factors [5]. The area under the curve of generated thrombin represents the so-called »endogenous thrombin potential (ETP)« and has been shown to correlate with plasma-based hypercoagulable states [4].

In this study we compared the TG of obese children with that of healthy, normal weight, and age matched controls by means of the CAT.

Materials and Methods

Collection and Preparation of Blood

Patients were recruited from the STYrian Juvenile OBesity Study (STYJOBS) representing an attempt to focus on health related problems of obese juveniles. For this purpose children, each with a BMI greater than the 97th percentile, were examined
to assess the risk of preatherosclerotic lesions and metabolic disorders (such as insulin resistance, diabetes mellitus type 2, dyslipidemia, hypertension, and others) associated with juvenile obesity [6].

We examined plasma samples of the 13 most obese children from this cohort. The study group consisted of 9 male and 4 female, aged 4.02 to 13.60 years (mean 9.62), the BMI ranged between 27.3 and 46.8 (mean 33.24). The BMI SDS ranged from 8.02 to 15.1 (mean 10.27). The control group consisted of 13 healthy, age and sex matched children. Venous blood was drawn into precitrated tubes. Plasma was separated by centrifugation at 4000 rpm for 10 minutes at room temperature and stored at −70 °.

Automated Fluorogenic Measurement of TG under Standard Conditions

TG was measured in platelet-poor plasma using a slow fluorogenic substrate (Z-Gly-Gly-Arg-AMC), purchased from Bachem, Bubendorf, Switzerland, using the Fluoroscan Ascent (Thermolabsystems OY, Helsinki, Finland) with an excitation filter at 390 nm and an emission filter at 460 nm after addition of tissue factor, phospholipids, and calcium performing the CAT, developed by Hemker’s group in Maastricht [4, 5]. The measurement process lasted 40 minutes. Thrombin activity was calculated as a function of time by comparing the fluorescent signal from the thrombin-generating sample to that from a known and stable, in parallel measured standard activity.

Results

The characteristics of a typical resultant curve are shown in Figure 1 comparing an obese child with an age matched child of the control group: During the lag time no thrombin formation is detectable and the curve remains flat. After this period a steep increase up to a peak is seen. The inclination of the curve is described as time to peak. The thrombin concentration goes down again and the start tail marks the time, when the curve reaches the abscissa. The area under the curve (ETP) represents the amount of thrombin built.

Table 1. Results of TG measurement in obese children and controls

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Controls</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ETP</td>
<td>1948.92 ± 264.72</td>
<td>1421.33 ± 170.34</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mean Lag time (min.)</td>
<td>2.26 ± 0.21</td>
<td>1.64 ± 0.18</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mean Peak (nM )</td>
<td>509.42 ± 55.20</td>
<td>436.38 ± 48.51</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean TTP (min.)</td>
<td>4.05 ± 0.30</td>
<td>3.30 ± 0.21</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mean Start tail (min.)</td>
<td>16.33 ± 1.99</td>
<td>13.0 ± 1.07</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

ETP = endogenous thrombin potential; TTP = time to peak
Fig. 1. TG - curves of an obese and a normal weight child.
TG in a 13.1 year old girl with a BMI of 43.10 and a BMI<sub>SDS</sub> of 10.3 (▲) compared to a 13.05 year old, healthy, and normal weight girl with a BMI of 22.4 and a BMI<sub>SDS</sub> of 1.14 (■).
ETP was significantly higher in obese children compared to controls. The lag time was significantly longer, and the time until the thrombin peak was reached (TTP) was prolonged in obese patients compared to controls. The thrombin peak (maximum concentration) was significantly higher in comparison to controls. The mean start tail in the obesity group was significantly higher compared to controls (Table 1).

Discussion

In this study we investigated whether differences in TG exist between obese and normal weight children. Our data show that overweight children after a significantly prolonged lag phase generate significantly higher amounts of thrombin, and that the time until the thrombin concentration approaches zero is significantly elongated in comparison to age matched, normal weight, healthy controls. Studying the metabolic and hemostatic effects of obesity in a grown-up population is often fraught with problems concerning additional risk factors like smoking, stress, and alcohol consumption. Additionally, these patients often have other co-morbidities, which manipulate the outcome directly or by their therapy. Many of these problems can be overcome by studying children who have little co-morbidities besides their obesity.

Our findings confirm the close relationship between obesity and alterations in the coagulation system. The amount of thrombin generated, the pivotal enzyme in the thrombotic-hemostatic system, represented as area under the curve (ETP), displays the individual risk of possibly being affected by thrombosis [5]. Our results are in accordance with previous papers showing that obesity is often associated with alterations in the coagulation and fibrinolytic system representing a risk factor for the development of cardiovascular disease. The fact, that elevated levels of coagulation factors i.e. FVII, FVIII, and fibrinogen are frequently detected in obese patients, could be a plausible explanation for the heightening of the ETP [1, 2, 5]. Some prospective studies have shown that increased plasma levels of fibrinogen, as well as of factors VII, and VIII indicate a risk of cardiac (i.e. ischemic heart disease, myocardial infarction) respectively vascular (i.e. arterial thrombosis) events [7, 8]. The fact that metabolic parameters exert influence on the coagulation system is confirmed by recent studies suggesting that the adipocyte itself, as a metabolically active secretory cell, is able to produce proteins like TF, contributing to atherogenesis in atherosclerotic lesions, or PAI-1, possibly explaining the high levels found in obesity [1, 8]. The association between PAI-1 antigen (PAI-1-Ag) and adiposity exists already in childhood [9]. As main physiological inhibitor of fibrinolysis in blood, elevated PAI-1 levels stand for a decreased fibrinolytic capacity and represent a risk factor for future development of atherothrombosis [10, 11, 12].

Considering all known alterations of the coagulation system in an obese population, the fact that we found a significantly prolonged lag time is surprising. This is in contrast to the observed high peaks and high ETP in obese children. We hypothesize that – using small amounts of lipidated TF as in our experiments – this prolongation of clotting time in obese patients represented as lag time in the thrombo-
gram assay may possibly be caused by an elevation of inhibitors of coagulation such as tissue factor pathway inhibitor (TFPI). This theory is corroborated by several studies showing a correlation between TFPI levels and BMI, demonstrating that elevated TFPI levels highly depend on obesity index [13].

Our results confirm the assumption that a hypercoagulable state exists even in children with severe obesity and make likely that the observed changes in hemostatic parameters may be part of the pathomechanisms of atherosclerosis in obese patients. However, our findings with a prolonged lag phase and then increased TG show that the mechanisms of the altered TG in obese children may be more complex to explain than simply by the elevation of some clotting factors, and suggest that more work has to be done to explain the details of the observed altered TG.

References