Circulating procoagulant microparticles in obesity

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SUMMARY
Aim: Obesity is a risk factor for cardiovascular diseases and venous thromboembolism. Circulating procoagulant microparticles (MP) have been described in various clinical situations associated with thrombosis and in diabetic patients. The aim of this preliminary study was to evaluate the presence of MP in obese patients without any other vascular risk factor in particular diabetes.

Methods: Fifty-eight obese women < 50 year-old without other cardiovascular risk factors were recruited from a single out-patient nutrition clinic. They were compared to 45 age-matched healthy normal weight controls. Main outcome was MP levels in patients and controls. Relationships between MP concentrations and parameters reflecting insulin resistance in patients were also studied.

Results: Obese patients were 33.3 ± 1.2 years old and had a mean BMI of 42.4 ± 0.9 kg/m². There was a greater proportion of smokers in the obese group (34.5 vs 15.6%). Mean MP levels were markedly higher in obese patients compared to controls (10.6 ± 0.5 vs 3.2 ± 0.3 nMSeq, P < 0.001). There was no difference in MP concentrations between smokers and non smokers. In the obese group, there was a negative correlation between MP and BMI (r = -0.265, P < 0.05) but no relationship could be established between MP concentrations and markers of insulin resistance.

Conclusion: This increase in circulating MP levels reflects cell activation and could account for the increased risk of thrombotic complications in obesity. Further studies are ongoing to explore the relationships between MP levels and coagulation markers and to assess the effect of weight reduction.

Key-words: Microparticles · Microvesicles · Obesity · Thrombosis.

RÉSUMÉ
Objectif : L’obésité est un facteur de risque de maladies cardiovasculaires et de thromboses veineuses. Les microparticules procoagulantes (MP) ont été décrites dans différentes situations cliniques associées à des thromboses et chez des diabétiques. Le but de cette étude préliminaire était d’évaluer la présence de MP chez des patientes obèses n’ayant pas d’autre facteur de risque vasculaire en particulier pas de diabète.

Méthodes : Cinquante-huit femmes obèses âgées de moins de 50 ans, sans autre facteur de risque cardiovasculaire recrutées à partir de la structure ambulatoire d’un service de Nutrition ont été comparées à 45 femmes de poids normal apparées sur l’âge. Le critère principal était la concentration de MP. Les relations entre MP et les marqueurs d’insulinorésistance ont également été étudiées chez les patientes.

Résultats : Les patientes obèses étaient âgées de 33,3 ± 1,2 ans et avaient un IMC de 42,4 ± 0,9 kg/m². Il y avait une plus grande proportion de fumeuses chez les obèses par rapport aux témoins (34,5 vs 15,6 %). La concentration de MP était plus élevée chez les patientes obèses que chez les témoins (10,6 ± 0,5 vs 3,2 ± 0,3 nMSeq, P < 0,001). Il n’y avait pas de différence de concentrations des MP entre les fumeuses et les non fumeuses. Chez les obèses, il y avait une corrélation négative entre les MP et l’IMC mais aucune relation n’a pu être établie entre les taux de MP et les marqueurs d’insulinorésistance.

Conclusion : L’élévation des MP témoigne d’une activation cellulaire et pourrait expliquer en partie l’augmentation du risque de thrombose dans l’obésité. Des études sont en cours pour explorer les relations entre les MP et des marqueurs de la coagulation et mesurer l’effet d’une réduction pondérale.

Mots-clés : Microparticules · Obésité · Thrombose.
Obesity is a risk factor of cardiovascular diseases and venous thromboembolism [1]. Various hemostasis abnormalities have been described in obesity, mainly concerning fibrinolysis and in particular increased PAI-1 [2], but other anomalies of coagulation factors and platelets have been reported [3]. Microparticles (MP) are fragments shed from the plasma membrane after challenge of cells by a variety of stimuli (procoagulant, proinflammatory or apoptogenic) [4]. Some of them bear active tissue factor and all of them expose procoagulant aminophospholipids, phosphatidylserine and phosphatidylethanolamine, which confer a procoagulant phenotype. High levels of circulating MP have been associated with various disorders associated with thrombosis such as heparin-induced thrombocytopenia, thrombocytopenic purpura, hemolytic uremic syndrome, paroxysmal nocturnal hemoglobinuria and acute coronary syndromes [4,5]. Elevated MP have also been reported in diabetes mellitus and have been proposed as a marker of microangiopathic complications [6-8]. Hence, MP can actually be viewed as markers of cell activation but probably also as true actors at the onset of vascular disorders [5].

The aim of this study was to assess the level of circulating MP in otherwise healthy obese women without clinically apparent vascular complication and without diabetes mellitus.

Patients and methods

Fifty-eight obese women (mean age: 33.3 ± 1.2 years, mean BMI: 42.4 ± 0.9 kg/m², mean waist circumference: 117.1 ± 2.2 cm) consecutively explored in the nutrition outpatient clinic of our institution were enrolled in the study after giving informed consent. Inclusion criteria were: age < 50 years, absence of any symptom of cardiac, cerebral, or peripheral vascular disease, absence of cardiovascular risk factor other than obesity in particular diabetes, hypertension or hyperlipemia. Patients with fasting plasma glucose ≥ 1.26 g/l, total cholesterol ≥ 2.20 g/l, triglycerides ≥ 2 g/l, systolic blood pressure ≥ 140 mmHg or diastolic pressure 90 mmHg were not included. Every patient had a standard exploration including a dietary intake study, fasting glucose, insulin, cholesterol, triglycerides, HDL-cholesterol and leptin determinations.

The controls were 45 healthy non obese women under 50 year-old (mean age: 34.4 ± 1.2, mean BMI: 20.9 ± 1.6 kg/m²) working in clinical department or in the haematology laboratory. There were slightly more smokers in the obese group than in the control group (20/58 vs 7/45, χ² = 4.7, P = 0.04). Blood was immediately mixed with one tenth volume of 0.129M trisodium citrate and platelet depleted plasma was obtained after 2 centrifugations steps, one at 1,500 g for 15 min, the second at 12,000 g for 2 min. Plasma was stored at -80°C until assay. Microparticles were captured on to immobilized annexin V. In brief, annexin V was biotinylated and then insolubilized onto streptavidin-coated microtitration plates, and the anionic phospholipid content was determined by a prothrombinase assay. In the assay, the blood clotting factor and calcium concentrations (factor Xa, factor Va, prothrombin, and CaCl₂) were determined to ensure that phosphatidylserine was the rate limiting parameter of the reaction. Results were expressed as nanomolar phosphatidylserine equivalent (nMPSeq) by reference to a standard curve constructed by the use of liposomes of defined composition.

The HOMA-R was calculated to evaluate insulin resistance, using the following formula [9]: (plasma glucose [mmol/l]x insulinemia [µU/ml])/22.5.

Mean results between obese subjects and controls were compared using Student t-test and multiple regression analysis (stepwise linear regression). In the obese group, as biological data were available only for the patients, comparisons between terciles of HOMA values were conducted by ANOVA. Relationships between MP and the other parameters were studied by multiple regression analysis. Results are given as mean ± SEM. P < 0.05 was considered significant.

Results

MP were markedly elevated in obese patients compared to controls (10.6 ± 0.5 vs 3.2 ± 0.3 nMPSeq, P < 0.001) (figure 1). Forty four obese patients (75.9%) had MP concentrations above 7.7 nMPSeq, a threshold which can be considered as the upper normal range (mean + twice the standard deviation of the normal population).

There was no difference for MP concentrations between smokers and non smokers neither in the control group (3.3 ± 0.3 vs 3.0 ± 0.4 nMPSeq, P = 0.694) nor in the obese group (11.0 ± 0.5 vs 10.4 ± 0.7 nMPSeq, P = 0.498).

Multiple correlations were calculated for patients and controls, using MP as dependant variable and age, BMI and

![Figure 1](image-url)
HOMA-R in obese subjects was 3.23 were excluded from the study of insulin resistance. Mean confirmed in multivariate analysis (P = 0.024).

Discussion

This preliminary study demonstrates that MP are increased in obese women without other cardiovascular risk factors compared to normal age-matched controls but this increase does not seem to be related to insulin resistance. Circulating MP have been described in numerous clinical situations, in particular those associated with increased thrombotic risk, but their role in health and disease may be dual [5]. In a general manner, their presence reflects cell activation and our study provides evidence for in vivo cell activation in healthy obese subjects. MP have also been recognized as vectors in the transcellular exchange of biological information, and in some clinical situations, their deleterious potential has been demonstrated or is strongly suspected. In particular these particles can influence haemostasis and vascular function in several ways. MP provide a catalytic surface for the assembly of the procoagulant enzyme complexes and can also activate platelets [10]. Thus, the procoagulant potential disseminated by these circulating particles could be responsible, at least in part, for an increased risk of associated thrombotic complications in obesity. We cannot exclude that the presence of circulating MP in these patients could reflect the existence of subclinical vascular damage related to the metabolic complications of obesity, as proposed in diabetes mellitus [6-8], even if subjects were selected on the basis of the absence of clinical vascular complication and associated cardiovascular risk factors. However, the fact that increased MP concentrations were not linked to age or insulin resistance does not favor this hypothesis. Further studies should analyze the cellular origin of these MP in obesity (platelets, mononuclear blood cells, other cell type) as different origin of metabolic data were missing in 9 patients who were excluded from the study of insulin resistance. Mean HOMA-R in obese subjects was 3.23 ± 0.35. As expected, there was an increasing trend toward the three tertiles of HOMA-R for BMI, waist circumference, energy expenditure and fat mass, but the level of MP did not differ between the three levels, indicating that elevated MP were not related to insulin resistance in the patients (table I).

Table I
Characteristics of the patients divided in tertiles of HOMA-R levels. P values correspond to ANOVA analysis between the three groups. Analysis was conducted on 49 subjects because of missing data for 9 patients.

<table>
<thead>
<tr>
<th></th>
<th>1st tertile</th>
<th>2nd tertile</th>
<th>3rd tertile</th>
<th>P</th>
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<tr>
<td>n</td>
<td>16</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.1 ± 2.2</td>
<td>35.5 ± 1.9</td>
<td>31.1 ± 2.1</td>
<td>0.333</td>
</tr>
<tr>
<td>Microparticles (nMPS/seg)</td>
<td>10.8 ± 0.9</td>
<td>10.6 ± 0.8</td>
<td>9.9 ± 1.1</td>
<td>0.786</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>40.6 ± 1.8</td>
<td>41.2 ± 1.2</td>
<td>45.4 ± 1.5</td>
<td>0.065</td>
</tr>
<tr>
<td>Waist circum (cm)</td>
<td>111.0 ± 2.7</td>
<td>114.9 ± 2.9</td>
<td>125.7 ± 4.9</td>
<td>0.020</td>
</tr>
<tr>
<td>Energy exp (kcal/d)</td>
<td>1682 ± 55</td>
<td>1630 ± 31</td>
<td>1896 ± 59</td>
<td>0.001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>49.2 ± 0.8</td>
<td>49.5 ± 0.7</td>
<td>53.1 ± 1.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>43.7 ± 4.1</td>
<td>42.3 ± 4.2</td>
<td>42.1 ± 3.1</td>
<td>0.944</td>
</tr>
<tr>
<td>Cholesterol (g/l)</td>
<td>2.01 ± 0.10</td>
<td>1.96 ± 0.10</td>
<td>2.00 ± 0.11</td>
<td>0.935</td>
</tr>
<tr>
<td>HDL chol (g/l)</td>
<td>0.59 ± 0.04</td>
<td>0.64 ± 0.06</td>
<td>0.58 ± 0.09</td>
<td>0.786</td>
</tr>
<tr>
<td>Triglycerides (g/l)</td>
<td>1.13 ± 0.10</td>
<td>0.99 ± 0.13</td>
<td>1.46 ± 0.20</td>
<td>0.089</td>
</tr>
<tr>
<td>Glucose (g/l)</td>
<td>0.98 ± 0.02</td>
<td>1.03 ± 0.04</td>
<td>1.07 ± 0.04</td>
<td>0.210</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>5.9 ± 0.3</td>
<td>9.6 ± 0.5</td>
<td>22.7 ± 2.3</td>
<td>0.000</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>1.41 ± 0.08</td>
<td>2.37 ± 0.09</td>
<td>5.96 ± 0.65</td>
<td>0.000</td>
</tr>
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</table>
venous thrombotic complications of obesity. However, elevated MP levels should be viewed as one of the necessary conditions but probably not sufficient on its own to initiate a thrombotic event. Further studies are ongoing to understand the mechanisms of this anomaly and to determine whether elevated procoagulant MP could be a reliable marker of arterial or venous thrombosis risk.

References