Arterial stiffness associates with changes in subpopulations of microparticles during postprandial conditions in males with and without the metabolic syndrome

Maarten E. Tushuizen, Rienk Nieuwland, Marcel H. Muskiet, Cees Rustemeijer, Auguste Sturk and Michaela Diamant

Submitted.
ABSTRACT

Objectives: The partly modifiable risk factors, that constitute the metabolic syndrome (MetS), do not fully account for the associated functional and structural vascular abnormalities and increased cardiovascular disease risk. Cell-derived microparticles, which reflect ongoing cellular stress but also associate with cardiovascular disease, are elevated in subjects with MetS in the fasting state. We studied the relation between arterial stiffness and circulating microparticles under postprandial conditions in males with and without MetS.

Methods: In 15 males with MetS and 15 controls, blood was collected before and following 3 consecutive meals during 24h. Arterial stiffness was measured as distensibility coefficient by carotid ultrasound. Microparticles were measured by flowcytometry.

Results: MetS versus control males (both mean age 56 years) had higher body mass index, waist, blood pressure, fasting triglycerides and lower HDL-cholesterol. Twenty-four hour area under the curve (24h-AUC) triglycerides and insulin were elevated in MetS males compared to controls (both \( P<0.01 \)), whereas 24h-AUC glucose was similar. The 24h-AUC of erythrocyte-derived, activated granulocyte and platelet-derived microparticles differed significantly (both \( P<0.05 \)), whereas 24h-AUC of total and platelet-derived microparticles did not differ. Distensibility coefficient was negatively associated with 24h-AUC of triglycerides, insulin, and erythrocyte-derived-microparticles (\( r=-0.42, P=0.02, r=-0.55, P=0.002, r=-0.48, P=0.01 \), respectively), and positively with total and platelet-derived-microparticles (\( r=0.43, P=0.02, r=0.47, P=0.02 \), respectively).

Conclusions: Postprandial changes in subpopulations of microparticles in healthy males and males with MetS were associated with arterial stiffness. Our findings suggest protective and deleterious effects of microparticles on arterial stiffness that may be associated with the cellular origin of these microparticles.
INTRODUCTION

Since their recognition in the 40’s of the previous century, the interest for cell-derived microparticles (MP) has substantially increased.\textsuperscript{1,2} MP are now known not only to contribute to a procoagulant and proinflammatory phenotype, but they may also affect endothelial functions, promote cell survival, and contribute to intercellular communication by exchanging (genetic) information in health and disease.\textsuperscript{1,2}

The prevalence of the metabolic syndrome (MetS), a concordance of insulin resistance, abdominal obesity, hypertension, hyperglycemia, and dyslipidemia, is increasing.\textsuperscript{3,4} The MetS is associated with increased type 2 diabetes and cardiovascular disease (CVD) risk.\textsuperscript{4} Elevated levels of circulating MP and various subpopulations thereof have been reported in subjects with the MetS compared to healthy subjects.\textsuperscript{5-9} In this population, compared to healthy controls, higher MP levels were found to be associated with individual fasting metabolic abnormalities,\textsuperscript{5-7} markers of oxidative stress,\textsuperscript{8} and endothelial dysfunction,\textsuperscript{9} which are all important factors in the development of CVD.

Arterial stiffness predicts the development of CVD and mortality in the general population and in vascular compromised patients.\textsuperscript{10-12} The underlying pathobiology is complex and remains to be fully elucidated. Arterial stiffness is primarily determined by the properties of the extracellular matrix (elastin, collagen) and vascular smooth muscular cell function. Arterial stiffness leads to an increased pulse pressure (increased difference between systolic and diastolic blood pressure), can be estimated locally at specific arterial sites (e.g. carotid, radial, brachial and femoral), and is most often described in terms of compliance and distensibility coefficients.\textsuperscript{10,12} Previously, increased levels of circulating CD31+/CD42-MPs have been associated with impaired systemic artery elasticity in healthy subjects.\textsuperscript{13}

Postprandial dysmetabolism, which is common in insulin resistant states, is associated with an increased risk of CVD.\textsuperscript{14} In healthy subjects, we and others found higher levels of circulating total MP and subpopulations following a high fat meal.\textsuperscript{15-17} Recently, we described elevations of endothelium-derived (CD144+) MP following meals in males with type 2 diabetes, compared to healthy controls.\textsuperscript{18} To date, postprandial studies in subjects with the MetS, focusing on meal-induced changes in MP and their association with vascular correlates are not available. In addition, most of previous studies addressing the effect of postprandial metabolism used a single meal (or rather a single fat- or glucose load), with the subject kept fasted for the subsequent 8 hours, whereas in real life, humans consume on average 3 solid, mixed meals a day. As a consequence, the metabolic responses and related effects (i.e. oxidative stress) on circulating blood cells elicited by the experimental condition may differ from those occurring in real life.
To investigate the complex interrelation of dysmetabolic changes, circulating MP and arterial stiffness in subjects with the MetS, we performed carotid vascular ultrasound and exposed these subjects to 3 consecutive high-fat mixed meals, thereby monitoring metabolic changes and MP levels longitudinally during 24 hours.

MATERIALS AND METHODS

Subjects

Thirty Caucasian males, aged 40-65 years, with the MetS (n=15), and 15 age-matched healthy males were recruited by advertisement and studied after obtaining written informed consent. Males with the MetS had to meet 3 out of 5 inclusion criteria based on NCEP/ATP III-criteria, without having hyperglycemia during a 75-g oral glucose tolerance test (OGTT). Excess alcohol intake (>20 units/wk), history of hepatitis and/or pancreatitis, abnormal liver and renal function tests (>2 times upper limits of normal), recent (<3 months) changes in weight (≥5%) and/or medication, history or current use of beta-blockers, acetylsalicylic acids, glucocorticosteroids, any blood glucose lowering therapy, were exclusion criteria. Subjects were instructed to refrain from heavy physical activities during the previous 24-h. The local ethics committee approved the study and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Study design

After an overnight fast, participants were admitted in the research unit for a 24-h period and received 3 consecutive, isocaloric (900 kcal), mixed meals (75g carbohydrates, 50g fat (60% saturated), 35g protein), at time points 9.00 AM (breakfast), 1.00 PM (lunch), and 5.00 PM (diner). Breakfast consisted of an EggMcMuffin®, croissant with butter and marmalade, 200 ml of milk, combined with 20 ml of cream, and 13 ml of syrup. The lunch consisted of a Quarterpounder®, croissant with butter, 200 ml of milk, and 16 ml of syrup. Dinner consisted of a Quarterpounder®, 90g of French fries, 175g of salad and 200 ml of water. The subjects were instructed to consume each meal within 15 minutes. Blood samples were drawn before (t=0 hours) and 2, 4, 6, 8, 12, 16, 20 and 24 hours after breakfast.

To avoid cellular (i.e. platelet, endothelium) and enzyme (i.e. lipoprotein lipase) activation artifacts by physical activity, participants remained in the supine position during the testing day.
Blood sample collection

Venous blood was collected from the left antecubital by venapuncture using a 1.0 mm Microflex blood collecting system (19 G; Vygon, France). For every following blood collection, the needle was placed at least 1 cm distal from the previous insertion and stasis was carefully avoided. To avoid artifacts of coagulation, endothelial and/or platelet activation, we used no in-dwelling canula. Plasma was prepared by centrifugation (1550 g, 20 minutes, 20°C) and plasma aliquots of 250 µL were snap frozen in liquid nitrogen within 30 minutes after withdrawal and stored at -80°C until assay. All samples from one subject were analyzed in the same series.

Biochemical measurements

Plasma glucose concentrations were measured by hexokinase-based technique (Roche diagnostics, Mannheim, Germany) and insulin concentrations by immunoradiometric assay (Centaur, Bayer Diagnostics, Mijdrecht, The Netherlands). Non-esterified fatty acids (NEFA) were assessed by ELISA (WAKO chemicals, Neuss, Germany). Plasma total cholesterol, HDL-cholesterol and triglycerides were determined by enzymatic methods (Modular, Hitachi, Japan). LDL-cholesterol was calculated by the Friedewald formula. Glycated hemoglobin (HbA1c) was measured with cation exchange chromatography (Menarini Diagnostics, Florence, Italy; reference values: 4.3-6.1%).

Reagents and assays

All chemicals were of analytical quality. Phycoerythrin (PE)-labeled anti-glycophorin A (GlycoA) (JC159, IgG1) and anti-CD61-PE (Y2/51, IgG1) were from Dako A/S (Glostrup, Denmark), and anti-CD66e-PE (CLB-gran/10, IH4Fc, IgG1) from Sanquin (Amsterdam, The Netherlands). To identify platelet-derived MP originating from activated platelets, anti-CD61-FITC plus either PE-labeled CD62p (P-selectin) or CD63 (glycoprotein 55; both antibodies from Immunotech, Fullerton, CA) was used. Anti-CD62e-PE (1.2B6, IgG1) was from Serotec Ltd (Oxford, England), allophycocyanin (APC)-labeled annexin V-APC from Pharmingen (San Jose, CA, USA) and anti-CD144-FITC (BMS 158FI) from Bender MedSystems Diagnostics GmbH (Vienna, Austria). Anti-TF (CD142)-FITC was obtained from American Diagnostica (Stamford, CT, USA) and was used for double labelling with anti-CD61-PE, CD66e-PE and GlycoA-PE. IgG1-PE (X40), IgG2b-PE (S2), and IgG1-peridinin chlorophyll-a protein (PerCP; X40) were from Becton Dickinson (San Jose, CA).
Flowcytometric analysis of MP

MP were isolated as previously described. The samples were analyzed in a FACScan flow cytometer with CellQuest software (Becton Dickinson, San Jose, CA). Fluorescence threshold were set using a calcium-containing buffer, annexin V and isotype-matched control antibody as described before. MP were identified on forward scatter (FSC) and sideward scatter (SSC) characteristics, by binding of annexin V and a monoclonal antibody (MoAb) directed against a cell type-specific antigen. MP numbers were calculated as described earlier.

Arterial stiffness measurements

Blood pressure and arterial stiffness measurements were performed in the fasting state after at least 15 min rest in the supine position. Pulse pressure (PP) was calculated as the difference between systolic and diastolic blood pressure. Pulse wave analyses were performed by radial aplanation tonometry from which the central aortic pressure and waveform are derived with the use of a Sphygmocor device (AtCor Medical, Sydney, Australia). Central augmentation index (CAIx) is an estimate of stiffness and wave reflection and therefore provides indirect information on arterial stiffness. CAIx is expressed as a percentage and a higher percentage reflects increased stiffness. In addition, arterial stiffness was estimated by measuring the relative changes in lumen area for a given change in pressure ($\Delta A/Ax\Delta P (\text{kPa}^{-1})$), expressed as distensibility coefficient (DC) at the carotid artery by arterial wall B-mode ultrasound imager with a 7.5 MHz linear-array transducer (Esaote, Maastricht, The Netherlands). The higher the change in lumen area of the carotid artery the less stiffer the arterial wall, therefore a low DC is associated with increased arterial stiffness (in contrast to CAIx). Examination took place in the supine position, with the subjects’ head turned slightly to the contralateral (left) side. When optimal longitudinal images were captured during the systole of a single heartbeat, they were stored for off-line analysis using the Wall Track System 2 software (Neurodata, Bilthoven, The Netherlands).

Measurements were performed by a single experienced investigator, reproducibility of the measurements showed a coefficient of variation <2%. Processing of arterial stiffness data and calculations were performed off-line by a different single experienced investigator, unaware of the metabolic state of the studied subject.
Statistical analysis

Results are presented as the means ± SE or medians (interquartile range). Twenty-four hour area under the curve (24h-AUC) was calculated according to the trapezoid rule. Differences between groups were calculated using independent samples t-test. Non-normally distributed data were log transformed. In order to test whether the changes of the MP levels over time are affected by group, i.e. MetS and controls, we performed analysis of variance (ANOVA) for repeated measurements with the interaction-term time x group. A P value of <0.05 was considered statistically significant. All statistical analyses were performed with SPSS version 15.0 (SPSS, Chicago, IL, USA).

RESULTS

The baseline characteristics of the participants are shown in Table 7.1. MetS and healthy males did not differ significantly with respect to age, total cholesterol, LDL-cholesterol and fasting glucose. All other measured parameters differed significantly between the two

| Table 7.1 Baseline characteristics of the study population |
|----------------|----------------|--------|
|                | MetS           | Controls | P    |
| N              | 15             | 15      | -     |
| Age (years)    | 55.7±2         | 56.1±2  | 0.91  |
| BMI (kg/m²)    | 30.5±1         | 27.2±1  | 0.003 |
| Waist circumference (cm) | 111.3±3 | 99.1±2  | 0.001 |
| SBP (mmHg)     | 139±3          | 123±2   | <0.001 |
| DBP (mmHg)     | 84±2           | 76±2    | 0.002 |
| HbA1c (%)      | 5.9±0.1        | 5.6±0.1 | 0.021 |
| Fasting glucose (mmol/l) | 5.6±0.1 | 5.5±0.1 | 0.38  |
| 2-h post load glucose (mmol/l) | 6.1±0.2 | 4.9±0.2 | <0.001 |
| Total cholesterol (mmol/l) | 5.3±0.2 | 5.1±0.2 | 0.56  |
| HDL-C (mmol/l) | 1.08±0.1       | 1.53±0.1| 0.002 |
| LDL-C (mmol/l) | 3.3±0.2        | 3.1±0.2 | 0.62  |
| Triglycerides (mmol/l) | 2.1±0.2 | 1.0±0.1 | <0.001 |

Values are means±SE or median (interquartile range), P-value is calculated by unpaired t-tests. MetS, metabolic syndrome; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; 2-h postload glucose was obtained during a 75 g oral glucose tolerance test; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.
groups. The meal-induced changes in plasma glucose, triglycerides, insulin and NEFA, including 24h-AUC, are shown in Figures 7.1A, 7.1B, 7.1C, and 7.1D, respectively. Both groups did not differ in postprandial glucose concentrations and both remained within the normoglycemic range (<7.8 mmol/L). In the MetS males, triglyceride concentrations were already elevated in the fasting state (as part of the definition), became significantly elevated following the meals and remained (prolonged) elevated in the evening hours, compared to controls (all \( P<0.05 \)). The insulin concentrations showed a significant elevation following every meal in both groups (all \( P<0.01 \) compared to baseline), although -as expected- the insulin concentrations were higher in the insulin resistant MetS males compared to controls (\( P<0.01 \)). Baseline NEFA concentrations did not differ significantly at baseline between both groups. In both groups a significant lowering following especially breakfast and dinner was observed, suggesting insulin-mediated NEFA suppression (all \( P<0.05 \), compared to baselines, Figure 7.1D). At around midnight plasma NEFA concentrations returned to baseline levels, or remained slightly higher in healthy controls (0.41±0.03 mmol/L, \( P=0.38 \) for MetS and 0.45±0.05 mmol/L, \( P<0.05 \) for controls versus \( t=0 \)).

### Figure 7.1

The 24-h courses of plasma glucose (A), triglycerides (B), insulin (C), and NEFA (D) concentrations after 3 high-fat mixed meals in MetS (asterisk) and healthy males (solid circles). Bars (grey, metabolic syndrome (MetS); black, healthy males) in the insets represent respective 24h-AUC values. Timing of meal intake is indicated by M. Night time is indicated by grey shade on timeline. The \( P \) value is given for 24h-AUC difference. Data are mean±SE.
In Figure 7.2, the concentrations of total levels of MP in plasma at baseline (t=0, before breakfast; Figure 7.2A) did not differ between subjects with MetS and healthy controls ($P=0.63$). Following the first meal, the total levels of MP increased significantly from

![Figure 7.2](image)

**Figure 7.2** The 24-h courses of total MP (A), CD61+-MP (B), GlycoA+-MP (C), CD66e+-MP (D), TF+-MP (E), and TF+/CD66e+-MP (F) concentrations after 3 high-fat mixed meals in MetS (asterisk) and healthy males (solid circles). Bars (grey, metabolic syndrome (MetS); black, healthy males) in the insets represent respective 24h-AUC values. Meal intake is indicated by M. Night time is indicated by grey shade on time line. The $P$ value is given for 24h-AUC difference. Data are mean±SE.
baseline to a similar extent in both groups (both \( P<0.05 \)), and the 24h-AUC’s were comparable (\( P=0.97 \)). The levels of CD61+-MP, i.e. MP originating from platelets or megakaryocytes and by far the largest fraction of MP in plasma (85-98%), also showed an postprandial increase following breakfast compared to baseline (\( P<0.05 \)). The 24h-AUC of these MP did not significantly differ between subjects with MetS and healthy controls (\( P=0.91 \)).

The baseline levels of erythrocyte-derived (GlycoA+-) MP were similar between subjects with MetS and controls (\( P=0.23 \)). Interestingly, plasma levels of erythrocyte-derived MP increased significantly from baseline in both groups after the meals (\( P<0.01 \) for both groups compared to baseline), reaching the highest numbers in the plasma samples of the MetS males after lunch (\( t=6h; P<0.01 \)), and returning to baseline concentrations during the night (Figure 7.2C). Twenty-four hour AUC for erythrocyte-derived MP was increased in MetS versus control subjects (\( P<0.05 \)).

Baseline plasma levels of leukocyte-derived MP (CD66e+) were non-significantly elevated in MetS versus controls (\( P=0.29 \)). Although the levels of these MP remained elevated in the MetS males during the 24h study, significant meal-induced variations could not be detected (Figure 7.2D). The level of tissue factor (TF) positive MP did not differ between the two groups, both in the fasting state as during 24-h (\( P=0.85 \); Figure 7.2E). In addition, meal-associated changes could not be detected. However, TF+-subpopulations of CD66e+-MP, i.e. TF-positive MP from granulocytes, were significantly elevated in MetS compared to controls (\( P<0.05 \); Figure 7.2D). Following breakfast and lunch, TF+/CD66e+-MP increased nearly significant in both groups (both \( P=0.07 \) on \( t=6h \) versus baseline). Plasma levels of MP from activated platelets, i.e. CD62p+/CD61+ MP as well as CD63+/CD61+ MP, were elevated in MetS compared to controls (both \( P<0.05 \); Figures 7.3A and B) and did not demonstrate meal-induced changes either. MP from activated endothelial cells, i.e. CD62e+-MP, were elevated during 24-h in MetS versus control males (\( P<0.05 \)), whereas CD144+-MP (reflecting total number of MP from endothelial cells) were similar in both groups (\( P=0.45 \)). Both CD62e+-MP and CD144+-MP did not demonstrate meal-induced changes (Figures 7.3C and D).

**Associations of MP, metabolic and arterial stiffness parameters**

Twenty-four hour AUC of total and CD61+-MP levels in plasma did not associate with metabolic changes during 24 hours. However, 24h-AUC of GlycoA+-MP were associated with 24h-AUC triglycerides (\( r=0.48, P=0.01 \)) and 24h-AUC insulin (\( r=0.41, P=0.03 \)). CD66+-MP and TF+-MP were not associated with metabolic changes. However, 24h-AUC
Figure 7.3  The 24-h courses of CD62p+/CD61+ -MP (A), CD63+/CD61+ -MP (B), CD62e+ -MP (C), and CD144+-MP (D) concentrations after 3 high-fat mixed meals in MetS (asterisk) and healthy males (solid circles). Bars (grey, metabolic syndrome (MetS); black, healthy males) in the insets represent respective 24h-AUC values. Meal intake is indicated by M. Night time is indicated by grey shade on time line. The P value is given for 24h-AUC difference. Data are mean±SE. Scatter plots representing the relationship between DC and 24h-AUCs of erythrocyte-derived MP (E) and platelet- or megakaryocyte-derived MP (F) in the whole study population (MetS (asterisk) and healthy males (solid circles)). Pearson correlation coefficients are shown.
of TF+/CD66e+-MP was associated with 24h-AUC triglycerides ($r=0.46, P=0.01$). Twenty-four hour AUC of MP from activated platelets (CD62p+ and CD63+) correlated both with 24h-AUC triglycerides ($r=0.45, P=0.02$ and $r=0.36, P=0.05$, respectively), but not with glucose and insulin AUC. In addition, 24h-AUC of endothelium-derived MP did not correlate with metabolic parameters.

Arterial stiffness, expressed as increased PP and decreased DC, differed significantly between both groups (both $P<0.01$), whereas CAIx was comparable in both groups ($P=0.52$; Table 7.2).

PP showed no association with the MP populations. DC was negatively associated with 24h-AUC triglycerides ($r=-0.38, P<0.05$), 24h-AUC insulin ($r=-0.46, P=0.01$), 24h-AUC GlycoA+-MP ($r=-0.48, P<0.01$, Figure 7.3A), 24h-AUC CD62p+/CD61+-MP ($r=-0.50, P<0.01$), and 24h-AUC CD62e+-MP ($r=-0.38, P<0.05$). Additional univariate analyses also showed a positive association of DC with 24h-AUC total MP ($r=0.42, P=0.02$) and 24h-AUC CD61+-MP ($r=0.47, P=0.02$; Figure 7.3B). CAIx was associated with 24h-AUC GlycoA+-MP ($r=0.40, P=0.04$), and negatively associated with 24h-AUC total MP ($r=-0.49, P<0.01$), 24h-AUC CD61+-MP ($r=-0.51, P<0.01$) and 24h-AUC CD63+-CD61+-MP ($r=-0.35, P<0.05$).

Multivariate analysis was performed separately in both groups and in pooled analysis to study the independent association of arterial stiffness with MP. Therefore DC and CAIx were separately entered as a dependent variable, and subsequently 24h-AUC of total MP, CD61+-MP or GlycoA+-MP, systolic blood pressure, age, BMI, and 24h-AUC triglycerides were entered as independent variables into the model (Table 7.3 for 24h-AUC GlycoA+-MP). Adjustment for these variables did not change the association of arterial stiffness parameters with 24h-AUC of these MP. Furthermore, entering these variables in a forward stepwise multivariate linear regression model showed that 24h-AUC CD61+-MP remained as a significant determinant of DC.

### Table 7.2 Parameters of arterial stiffness

<table>
<thead>
<tr>
<th></th>
<th>MetS n=15</th>
<th>Controls n=15</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP (mmHg)</td>
<td>55±3</td>
<td>47±1</td>
<td>0.01</td>
</tr>
<tr>
<td>DC (10⁻³ kPa)</td>
<td>13.1±1</td>
<td>20.0±2</td>
<td>0.002</td>
</tr>
<tr>
<td>CAIx (%)</td>
<td>136±3</td>
<td>132±4</td>
<td>0.52</td>
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</table>

Values are means±SE; $P$ value is calculated by unpaired $t$-tests. MetS, metabolic syndrome; PP, pulse pressure; CAIx, central augmentation index; DC, distensibility coefficient.
DISCUSSION

This study demonstrates that metabolic changes induced by consumption of 3 consecutive meals during 24 hours, are associated with changes in numbers of subpopulations of circulating MP and that these associate with different indicators of arterial stiffness in males with and without MetS.

We demonstrated that the number of total and CD61+-MP increase following a high-fat meal in males with and without MetS. These findings confirm our previous results in healthy young males.\(^5\) Furthermore, the numbers of CD61+-MP, i.e. MP originating from platelets or megakaryocytes, during 24-h were associated with decreased arterial stiffness. Although this finding seems to contradict with earlier reports showing that elevated numbers of MP have deleterious effects on the vasculature,\(^7\) more recently it has been proposed that circulating MP are the major factor associated with carotid artery remodeling.

### Table 7.3 Multivariate associations between DC and 24h-AUC erythrocyte-derived MP

<table>
<thead>
<tr>
<th>Model</th>
<th>B (95% CI)</th>
<th>P</th>
<th>R</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Log 24h-AUC GlycoA-MM</td>
<td>4.014 (1.822 to 6.206)</td>
<td>0.48</td>
<td>0.22</td>
<td>0.014</td>
</tr>
<tr>
<td>Model 2 (Model 1 + BP systolic)</td>
<td>Log 24h-AUC GlycoA-MM</td>
<td>4.121 (1.969 to 6.272)</td>
<td>0.52</td>
<td>0.27</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>BP systolic</td>
<td>-0.262 (-0.486 to -0.038)</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 3 (Model 2 + age)</td>
<td>Log 24h-AUC GlycoA-MM</td>
<td>3.903 (1.779 to 6.028)</td>
<td>0.58</td>
<td>0.33</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>BP systolic</td>
<td>-0.201 (-0.435 to 0.033)</td>
<td>0.090</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.003 (-0.007 to 0.001)</td>
<td>0.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 4 (Model 3 + BMI)</td>
<td>Log 24h-AUC GlycoA-MM</td>
<td>3.884 (1.744 to 6.024)</td>
<td>0.59</td>
<td>0.35</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>BP systolic</td>
<td>-0.180 (-0.421 to 0.061)</td>
<td>0.136</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.003 (-0.007 to 0.002)</td>
<td>0.202</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.007 (-0.015 to 0.002)</td>
<td>0.105</td>
<td></td>
<td></td>
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<tr>
<td>Model 5 (Model 3 + log 24h-AUC TG)</td>
<td>Log 24h-AUC GlycoA-MM</td>
<td>3.437 (1.179 to 5.695)</td>
<td>0.62</td>
<td>0.39</td>
<td>0.034</td>
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<tr>
<td></td>
<td>BP systolic</td>
<td>-0.106 (-0.377 to 0.166)</td>
<td>0.429</td>
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<tr>
<td></td>
<td>Age</td>
<td>-0.002 (-0.007 to 0.002)</td>
<td>0.244</td>
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<tr>
<td></td>
<td>BMI</td>
<td>-0.007 (-0.015 to 0.001)</td>
<td>0.085</td>
<td></td>
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<tr>
<td></td>
<td>Log 24h-AUC TG</td>
<td>-0.005 (-0.023 to 0.012)</td>
<td>0.523</td>
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DC, distensibility coefficient; MP, microparticles; BP, blood pressure; TG, triglycerides.
with high circulating levels of MP preventing compensatory remodeling in vessels with high carotid intima-media thickness values.\textsuperscript{21} Moreover, platelet-derived MP facilitate restoration of endothelial integrity after vascular injury.\textsuperscript{22} Similarly, platelet-derived MP have been shown to induce survival, proliferation and migration of endothelial cells in vitro and injection of platelet-derived MP into the myocardium may foster postischemic neovascularization after chronic ischemia in vivo.\textsuperscript{23,24} The present findings support and confirm the more recent ideas that the release of MP is not necessarily solely deleterious.

One of our interesting findings is the postprandial increase of GlycoA\textsuperscript{+}, i.e. erythrocyte-derived MP. Although we could not detect differences in baseline numbers of these MP between MetS and controls, as previously was reported,\textsuperscript{9} our results suggests that especially erythrocytes are susceptible to metabolic changes, in particular of plasma lipids and insulin. Because elevated numbers of erythrocyte-derived MP were also present in postprandial plasma samples collected from healthy males (low triglyceride and high insulin concentrations, see t=12h in Figure 7.1), it seems unlikely that the elevated levels of erythrocyte-derived MP are due to lysis of erythrocytes by increased levels of triglycerides. Because insulin induces a Ca\textsuperscript{2+}-dependent hyperpolarization of erythrocyte membranes, we hypothesize that this hyperpolarization may contribute to the release of MP which is highly calcium dependent for most cells.\textsuperscript{25-27} Furthermore, a positive association was present between the numbers of erythrocyte-derived MP and arterial stiffness. Due to their small size as compared to the erythrocyte, one may speculate that erythrocyte-derived MP localize near the endothelium and there scavenge nitric oxide (NO) as effectively as cell-free hemoglobin.\textsuperscript{28} A reduced availability of NO is known to affect vascular smooth muscle tone leading to arterial stiffening.\textsuperscript{4,10}

Previously, we reported a postprandial increase in the levels of CD144\textsuperscript{+} MP (endothelium-derived) in males with type 2 diabetes.\textsuperscript{18} This increase was absent in our males with MetS and healthy controls. Since we intended to study the impact of additional hyperglycemia versus hyperlipidemia, we selected males with MetS based on other criteria than dysglycemia. Accordingly, these individuals had normal glucose metabolism, but rather had dyslipidemia and elevated blood pressure, in addition to central obesity. As a consequence, we hypothesize that hyperglycemia, rather than other meal-related metabolic derangements play a role in the release of endothelium-derived MP. Despite the fact that we could not detect significant differences in endothelium-derived MP in our study, an association was present between activated endothelium-derived (CD62e\textsuperscript{+}) MP and arterial stiffness, which confirms earlier findings by others.\textsuperscript{13,29}

We also confirm previous findings that leukocyte-derived MP are elevated in MetS compared to healthy subjects.\textsuperscript{6,9} Although the postprandial state has been associated with
leukocyte activation, we observed no postprandial changes in the numbers of leukocyte-derived MP. However, in line with our previous findings in fasting uncomplicated type 2 diabetic patients, we did find changes in their activation status as reflected by the number of TF-positive granulocyte-derived MP, although nearly significant. A possible explanation for the lack of change in number of granulocyte-derived MP may be that we, in contrast to others, used no indwelling canula for blood collection, since this may lead to leukocyte activation. Second, we used solid, consecutive mixed meals, mimicking a real-life situation, whereas the results of previous studies were obtained following solely and rather artificial fat-enriched shakes. Of interest, after the addition of carbohydrates to the fat-enriched shakes, these authors found a decreased activation of inflammation markers. Therefore, the use of consecutive, solid mixed meals is mandatory to appreciate the real-life postprandial effects on various risk factors of CVD, including endothelial function, coagulation and inflammation.

In conclusion, we found that exposure of males with the MetS to 3 consecutive meals results in exaggerated and prolonged postprandial dyslipidemia and associated changes in (subpopulations of) MP levels during 24 hours, when compared to healthy matched controls. Our findings suggest that in addition to hypertension and postprandial dysmetabolic responses, (subpopulations of) cell-derived MP may affect arterial stiffness. Possibly, different subtypes of MP may differentially affect the endothelium and/or underlying vascular layers (i.e. media and adventitia) that contribute to arterial stiffness and development of CVD. Future studies should elucidate possible mechanisms underlying the present observation that postprandial dysmetabolism and cell-derived MP are risk factors for atherogenesis.

ACKNOWLEDGMENTS

The authors thank Mrs. J. Boerop, Mrs. A. Grootemaat and Mr. D.P. Snoeck for their technical assistance and Dr. R.K. Schindhelm for his statistical support. McDonald’s BV The Netherlands is gratefully acknowledged for providing the test-meals.

REFERENCES


4. Diamant M, Tushuizen ME. The metabolic syndrome and endothelial dysfunction: common highway to type 2 diabetes and CVD. Curr Diab Rep 2006;6:279-86


