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Postprandial changes in the phospholipid composition of circulating microparticles are not associated with coagulation activation

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ABSTRACT

Introduction: Evidence is present that the phospholipid composition of circulating cell-derived microparticles (MP) affects coagulation in vivo, and that postprandial metabolic alterations may be associated with hypercoagulable state. Our objective was to investigate whether postprandial metabolic responses affect the phospholipid composition of MP, and whether such changes are associated with coagulation activation.
Materials and Methods: Twelve healthy males were studied twice and randomly received two consecutive meals or remained fasted. Blood was collected before and at 2, 4, 6 and 8 h following breakfast. Plasma concentrations of prothrombin-F_{1+2} and thrombin-antithrombin-complexes were measured. Numbers and cellular origin of MP were determined by flowcytometry. The phospholipid composition of MP was determined by hpTLC. In vitro procoagulant activity of MP was studied by fibrin generation.
Results: During the meal visit, plasma glucose, triglyceride and insulin levels increased, compared to baseline and the fasting visit (all $P<0.05$). Postprandially, the total numbers of MP increased in time compared to the fasting visit ($P<0.05$). Erythrocyte-derived MP increased (6-fold) during the meal visit, but remained constant on the fasting day ($P<0.001$). On the meal versus fasting day circulating MP contained increased phosphatidylcholine ($P<0.05$) and decreased sphingomyelin ($P<0.05$) amounts. The amount of phosphatidylserine did not change. Concentrations of plasma F_{1+2} and thrombin-antithrombin were similar on both days, as was the ability of MP to generate fibrin in vitro.
Conclusion: Although numbers, cellular origin and phospholipid composition of MP alter during exposure to two consecutive meals in healthy subjects, this does not lead to changes in the coagulation activation in vivo.

Introduction

Clinical and epidemiological evidence indicates that postprandial elevations of plasma glucose and triglyceride-rich lipoproteins are related to the risk of atherothrombotic disease [1]. To which extent postprandial coagulation activation contributes to this risk is still under debate. Several investigators reported postprandial coagulation activation in healthy subjects and in patients suffering from cardiovascular disease, as measured by increased plasma concentrations of factor VIIa (FVIIa), prothrombin fragment F1 + 2 (F_{1+2}) or thrombin-antithrombin complexes (TAT)[2–11]. Explanations for the underlying mechanism(s) of coagulation activation in the postprandial state, however, range from activation of factor VII (FVII) and XI (FXI) by triglycerides to increased activity of plasminogen activator inhibitor (PAI). Of interest, other investigators found that postprandial FVII activation was not associated with increased concentrations of F_{1+2} and TAT [12,13]. Similarly, there is no consensus among investigators whether platelet activation occurs in the context of postprandial lipemia [14,15].

By exposing negatively charged phospholipids, mainly phosphatidylserine, but also by exposing tissue factor, cell-derived microparticles (MP) can propagate and even initiate coagulation [16–18]. Therefore, MP have been associated with coagulation activation in health and disease, and elevated numbers of circulating MP are related with an increased risk of thromboembolic events as well as with disseminated intravascular coagulation [16–18]. Recently, we demonstrated that the total number of MP in plasma from healthy males is elevated following consecutive meals, suggesting that the postprandial state induces the formation of MP [19]. At present, it is unknown to which extent quantitative and qualitative characteristics of MP are associated with coagulation in the postprandial state. Therefore, we investigated whether the postprandial state

Abbreviations: TAT, thrombin-antithrombin; F_{1+2}, prothrombin fragment F1 + 2; PAI, plasminogen activator inhibitor; MP, microparticles; BMI, body mass index; NEFA, non-esterified fatty acids; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hpTLC, high performance Thin Layer Chromatography; FGT, fibrin generation test; AUC, area under the curve.

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affects not only the numbers but also the cellular origin and phospholipid composition of circulating MP, their procoagulant phenotype and the association with coagulation activation in vivo.

Materials and Methods

Subjects

Twelve healthy males, Caucasian, aged between 20–35 years, having a body mass index (BMI) less than 27 kg/m^2 and a blood pressure <145/90 mmHg were included as previously described [19]. Current smoking, the use of drugs (except for incidental analgesic agents) and the presence of cardiovascular disease, hypertension, coagulation disorders, diabetes mellitus, or 1st degree family history of aforementioned diseases were exclusion criteria. Subjects were initially studied during a screening visit after having given written informed consent. The local ethics committee approved the study and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Study design

Each participant was studied at 2 occasions, following an overnight fast (from 8.00 PM the previous evening). Subjects had to refrain from heavy physical activities during 24 hours prior to each visit. During the meal day, the participants received 2 consecutive, isocaloric (900 kcal) test meals at time points t = 0 (breakfast) and 4 hours (lunch). Blood samples were collected before and at 2, 4, 6 and 8 hours following the first meal. During the fasting day, blood samples were collected at similar times from 8.00 AM onwards and the subjects were only allowed water at libitum. The order of the visits was randomized and the time interval between the control and meal visits was less than 4 weeks.

Test Meals

We used 2 standardized iso-caloric fat-rich mixed meals served as breakfast (8.30 AM) and lunch (12.30 PM), instead of rather artificial high-fat (ice-)cream shakes, to mimic a more real-life situation. Each meal consisted of 50 g fat, of which 60% was saturated, 55 g carbohydrates and 30 g protein. The breakfast consisted of EggMcMuffin® (McDonald's, affiliation Amsterdam-Sloten, The Netherlands), croissant with butter and marmalade, 200 mL of milk, combined with 20 ml of cream. The lunch consisted of Quarterpounder® (McDonald's, affiliation Amsterdam-Sloten, The Netherlands), croissant with butter, and 200 mL of milk. The participants were instructed to consume each meal within 15 minutes.

Blood Sample Collection

To avoid coagulation, endothelial, and platelet activation artifacts, no in-dwelling canula was used and a new blood collecting system (Microflex, size 1.0 mm - 19 G, Vygon, France) was used for each blood collection. Venous blood was collected from the left antecubital vein, the needle was placed at least 1 cm distal from the previous insertion and stasis was carefully avoided, into 0.1 volume of 105 mmol/L trisodium citrate. Plasma was recovered after centrifugation (1550 *g*, 20 minutes, 20 °C) and 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 was measured by a hexokinase-based technique (Roche diagnostics, Mannheim, Germany), HbA_{1c} by HPLC. Insulin was measured by a commercially available immunoradiometric assay (Biosource/Medgenix Diagnostics, Fleurus, Belgium). Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides were determined by enzymatic methods (Modular, Hitachi, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula. Non-esterified fatty acids (NEFA) were assessed with an enzymatic colorimetric method (WAKO chemicals, Neuss, Germany). F_{1+2} and TAT were determined by ELISA (Dade Behring, Eschborn, Germany).

Reagents and Assays

Phospholipid standards were obtained from Larodan (Malmö, Sweden): l-α-phosphatidylcholine, l-α-lyso-phosphatidylcholine, sphingomyelin, l-α-phosphatidylserine, l-α-phosphatidylinositol, l-α-phosphatidylethanolamine. The l-α-lyso- phosphatidylethanolamine standard was obtained from Sigma (St. Louis, MO). Chloroform (analytical grade) and ethylacetate, acetone, methanol, ethanol, dichloromethane, isopropanol, acetic acid (all HPLC grade) and HPTLC plates (Cat. No. 1.05641; 20 × 10 cm, Silicagel 60 Å pore size, particle size 5–17 μm, 0.2 mm layer thickness) were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical quality. Phycoerythrin (PE)-labelled anti-glycophorin A (JC159, IgG_1) and anti-CD61-PE (Y2/51, IgG_1) were from Dako A/S (Glostrup, Denmark), anti-CD4-PE (CLB-T4/2,6D10, IgG_1), anti-CD66e-PE (CLB-gran/10, IH4Fc, IgG_1) from Sanquin (Amsterdam, The Netherlands), anti-CD8-PE (SK1, IgG_1), anti-CD14-PE (MØP9, IgG_{2b}), anti-CD20-PE (L27, IgG_1), IgG_1-PE (X40), IgG2b-PE (S2) and IgG_1-peridinin chlorophyll-a protein (PerCP; X40) from Becton Dickinson (San Jose, CA), anti-CD62e-PE (1.2B6, IgG_1) from Serotec Ltd (Oxford, England), anti-CD66b-PE (80H3, IgG_1) and fluoresceinisothiocyanate (FITC)-labeled anti-CD66b (0531) from Coulter/Immunotech (Marseille, France), and allophycocyanin (APC)-labelled annexin V-APC from Pharmingen (San Jose, CA), anti-CD106-FITC (B44498) from Calbiochem-Novabiochem Corporation (San Diego, CA) and anti-CD144-FITC (BMS 158FI) from Bender MedSystems Diagnostics GmbH (Vienna, Austria).

Isolation of MP

Per time point, two 250 μl aliquots were thawed on melting ice, and centrifuged for 30 minutes at 17,590 *g* and 20 °C. The supernatant (225 μl) was removed and 225 μl phosphate-buffered saline (PBS) containing 0.32% trisodium citrate (154 mmol/L NaCl, 1.4 mmol/L phosphate and 10.9 mmol/L trisodiumcitrate, pH 7.4) was added. The MP were resuspended and again centrifuged for 30 minutes at 17,590 *g* and 20 °C. For flowcytometry, 225 μl supernatant was removed. Subsequently the MP were resuspended and diluted 4-fold with PBS/citrate buffer, of which 5 μl was used per incubation. For Bligh & Dyer extraction, the supernatant (225 μl) was removed and the pellet was washed once more. Finally, 225 μl supernatant was removed and the MP were resuspended in the remaining 25 μl.

Flowcytometric analysis of MP

The samples were analyzed in a FACScan flowcytometer with CellQuest software (Becton Dickinson, San Jose, CA). Forward scatter (FSC) and sideward scatter (SSC) were set at logarithmic gain. To distinguish MP from events due to noise, MP were identified not only on FCS and SSC characteristics, but also by binding of annexin V and a MoAb directed against a celltype-specific antigen. To identify annexin V-positive MP, a threshold was placed in a MP sample prepared in the presence of annexin V without calcium to correct for autofluorescence. To identify MP that bind cell type-specific MoAbs, the MP were incubated with identical concentrations of isotype-matched control antibodies to set the threshold. FITC-fluorescence was measured in the FL-1 channel and PE-fluorescence in the FL-2 channel.

To 35 μl of PBS/calcium buffer (154 mmol/L NaCl, 1.4 mmol/L phosphate and 2.5 mmol/L $CaCl_2$, pH 7.4), 5 μl annexin V-PE and 5 μl FITC-labeled cell-specific MoAbs or isotype-matched control antibody (IgG_1-FITC) were added. MP (5 μl) were added, and the mixtures were incubated in the dark for 15 min at room temperature. For the CD66e-PE analysis an IgG_1-PE control antibody was used. Subsequently, 900 μl PBS/calcium buffer was added to stop the labeling. All samples were analyzed for 1 minute during which the flowcytometer analyzed about 150 μl of the MP suspension. From the number of events (N_x) in the upper right (marker and annexin V-positive) quadrant of the flowcytometric analysis (FL-1 versus FL-2 corrected for isotype control and autofluorescence), the number of MP/L plasma was calculated: Number/L = $N_x[100/5]\times[950/60]\times[10^6/250]$, in which 5 (μL) is the volume of MP suspension, 100 is the total volume of washed MP suspension, 950 is the total volume in the tube before analysis, 60 is the sample volume analyzed, 10^6 is the number of μL/L, and 250 is the original volume of plasma.

1.9. Phospholipid extraction

Phospholipids were extracted according to Bligh & Dyer [20,21]. To improve the recovery for phosphatidylserine and phosphatidylinositol, the water phase was replaced by acetic acid (0.5%).

For each time point the remaining 25 μl of the two 250 μl aliquots were pooled. To these 50 μl samples, 3 ml methanol:chloroform (2:1) was added. The samples were thoroughly mixed for 30 s and 750 μl 0.5% acetic acid was added. Again the samples were mixed for 30 s. Then, chloroform (1 ml) and 0.5% acetic acid (800 μl) were added. After each addition, the samples were mixed for 30 s. Subsequently the samples were centrifuged for 10 minutes at 1560 *g* (20 °C). The chloroform fraction (at the bottom) was isolated, and the aqueous fraction was washed twice with chloroform (1 mL). The three chloroform fractions were pooled and dried under nitrogen at 40 °C. The samples were stored overnight in −20 °C, and redissolved in methanol: chloroform (2:1) to be applied to hpTLC plates.

1.10. High Performance Thin Layer Chromatography (hpTLC)

To analyze cholesterol and phospholipid contents of the samples, separate hpTLC plates were used, because the developing solvent mixtures differ as previously described [21]. All hpTLC plates were prerun (full height) with methanol:ethylacetate (6:4) in a Camag horizontal developing chamber (Merck) to remove impurities. The plates were activated for 10 minutes at 130 °C. The first plate was used for phospholipids analysis, whereas the second plate was used for cholesterol analysis.

The identification of phospholipids species was based upon a standard mixture of commercial phospholipid standards that was applied to each hpTLC plate. The density of these spots was analyzed by photodensitometric scanning by a GS-800 Calibrated Imaging Densitometer (Bio-Rad; Veenendaal, The Netherlands), quantified using Quantity One software version 4.2.2 (Bio-Rad; Veenendaal, The Netherlands) and expressed as arbitrary units.

Since plasma contains (phospho-)lipids and cholesterol, especially in the postprandial state, and the (washed) MP fraction still contains 1% plasma (final concentration), the lipid composition of MP was corrected for this confounder. Therefore, 25 μl of supernatant from the first washing step was taken and 225 μl of PBS containing 0.32% trisodiumcitrate (pH 7.4) added. These samples were also washed twice, extracted and analyzed, exactly as described for the MP preparation.

1.11. Fibrin generation test (FGT)

The ability of MP to generate fibrin was measured directly in plasma of the participants in the absence or presence of anti-human (coagulation) factor VII, anti-human factor XI or anti-human factor XII (all provided by Sanquin, Amsterdam, The Netherlands) as previously described [22]. After pre-incubation for 5 minutes at 37 °C, clotting of plasma was initiated by addition of $CaCl_2$. Fibrin formation was determined by measuring the optical density ($\lambda = 405$ nm) in duplicate on a spectrophotometer (SPECTRAmax microplate reader; Molecular Devices Corp., Sunnyvale, CA) at 37 °C.

Statistical Analysis

Based on our previous findings in detecting phospholipid changes in platelet-derived MP after stimulating with different platelet agonists, we performed a formal sample size calculation [21]. In order to detect a similar change (over 20%) in phosphatidylcholine content with a power of 0.8 and confidence level of 0.95, at least 10 subjects needed to be studied. Based on these data and taken into account this calculation is based on solely platelet-derived MPs, we expected that a number of 12 subjects in this study would yield meaningful results. Continuous variables are expressed as mean ± SE, unless otherwise stated. To estimate the overall metabolic responses and the changes in MP during both visits, area under the curve (AUC) of these parameters plotted against time were calculated. Differences between time points on both days were calculated using ANOVA and the *P*-values were corrected by the method of Bonferroni. Non-parametrical tests were performed for non-normally distributed data. Correlations were performed using the Spearman's test. A $P<0.05$ was considered statistically significant. Data were analyzed with SPSS for Windows, release 15.0.

Results

Postprandial metabolic changes

The clinical and baseline laboratory characteristics of the participants are depicted in Table 1. The baseline concentrations of all parameters did not differ between the two study days. During the meal visit, plasma glucose, triglyceride and insulin concentrations rose significantly, especially after the second meal, albeit all within the normal range, as compared to baseline and the fasting visit (both $P<0.05$, $P<0.001$, $P<0.001$, respectively; Fig. 1). During the fasting visit, plasma concentrations of NEFA rose significantly from 0.29 ± 0.1 to 0.74 ± 0.1 mmol/L ($P<0.001$ versus baseline and meals day).

Postprandial changes in circulating MP

The total numbers of MP, of which the largest fraction is platelet-derived (88-98%), increased during the meal visit compared to the

Table 1
Baseline and fasting characteristics of the study population.

	Healthy Males (n = 12)
Age, yrs	26.0 ± 0.9
BMI, kg/m^2	23.9 ± 0.4
Waist, cm	87.6 ± 1.3
Systolic blood pressure, mmHg	116 ± 2
Diastolic blood pressure, mmHg	72 ± 2
HbA1c, %	5.2 ± 0.1
Glucose, mmol/L	4.9 ± 0.1
Insulin, pmol/L	34 ± 3
Total-cholesterol, mmol/L	4.0 ± 0.2
HDL-C, mmol/L	1.36 ± 0.1
LDL-C, mmol/L	2.3 ± 0.2
Triglycerides, mmol/L	0.8 ± 0.1

Values are mean ± SE. BMI indicates body mass index; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.

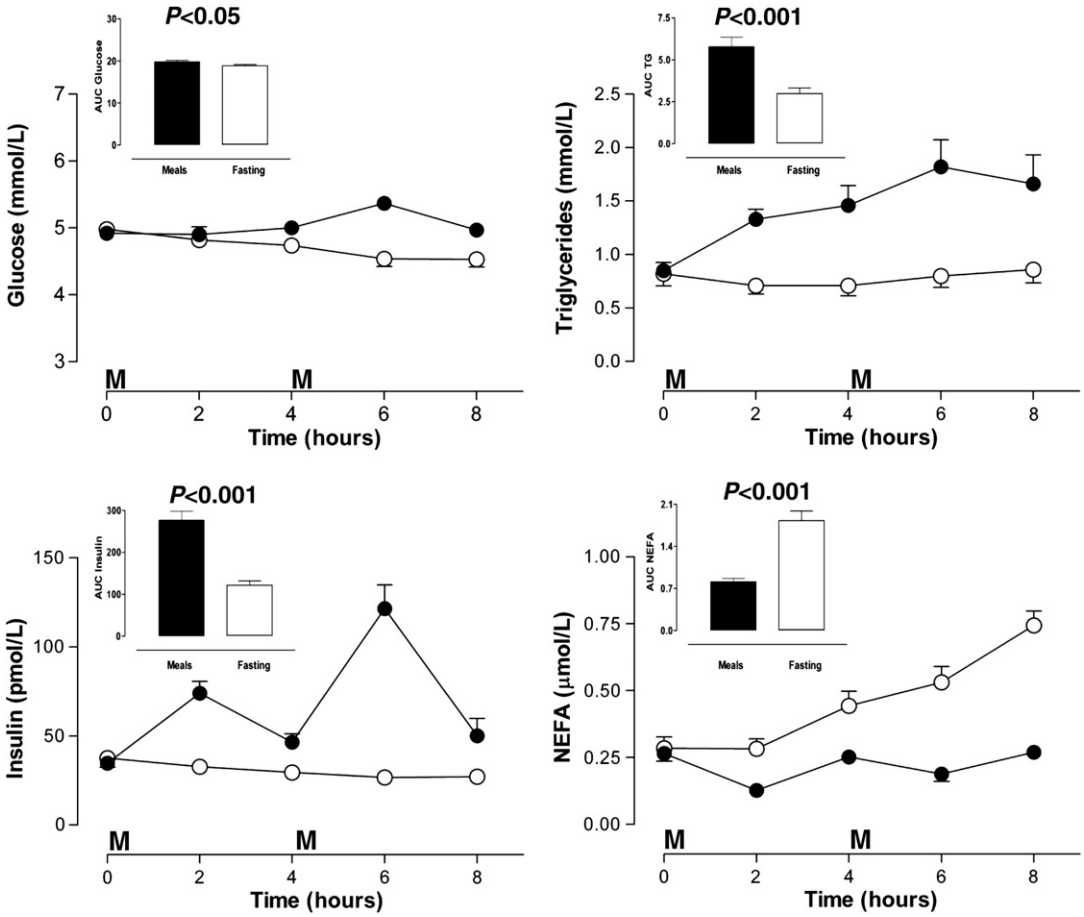

Fig. 1. Plasma concentrations of glucose, triglycerides, insulin and NEFA during the meal (solid circles) and fasting days (open circles) in healthy males. Meal intake is indicated by M. Bars (black, meals; white, fasting) in the insets represent respective AUC values. The *P* value is given for AUC difference. Data are mean ± SE.

fasting visit ($P<0.05$; Fig. 2). Mean concentrations of erythrocyte-derived MP increased to more than 6-fold after consumption of both meals ($P<0.001$, versus baseline and fasting day), and remained unaffected during the fasting day. Finally, monocyte-derived MP concentrations, constituting only 0.3% of the total numbers of circulating MP, slightly increased during the meal visit ($P=0.07$ versus fasting). MP from lymphocytes, granulocytes, and endothelial cells were not detectable. The numbers of erythrocyte-derived MP correlated significantly with the corresponding insulin concentrations measured on both days ($r=0.65$, $P<0.001$).

Postprandial changes in the phospholipid composition of circulating MP

Table 2 summarizes the overall phospholipid compositions of MP during the meal (+) and fasting (−) day, expressed as percentage of total phospholipid (mol/mol) after correction for the amount of phospholipids in MP-free plasma (see Methods). Compared to the fasting day, the relative % of phosphatidylcholine increased, whereas concurrently sphingomyelin decreased. Furthermore, the amount of phosphatidylinositol tended to increase following the first meal, although not significant. The phospholipids phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine were not detectable in plasma that had been depleted from MP.

The cholesterol : phospholipid ratio of MP during the meals day did not change significantly. During the fasting day, however, the cholesterol : phospholipid ratio increased significantly from 0.34 ± 0.1 to 0.49 ± 0.1 ($P<0.05$; Fig. 3).

Postprandial coagulation in vivo and in vitro

There were no significant differences between the meal and fasting day for both F_{1+2} and TAT concentrations (Fig. 4). Furthermore, Fig. 5 shows the results of the fibrin generation test on MP-containing plasma performed at baseline and at time point t = 6 hours (i.e. 6 hours after breakfast and 2 hours after lunch; these time intervals were chosen since the metabolic changes were maximally different from baseline). There were no significant differences between the two days in the ability of MP to trigger fibrin formation in plasma in the absence or presence of anti-human factor VII, factor XI or factor XII.

Discussion

The present study demonstrates that not only the numbers and cellular origin but also the phospholipid composition of circulating cell-derived MP alters after consumption of two consecutive high-fat mixed meals in healthy males. These alterations, however, do not lead to enhanced coagulation activation in vitro and in vivo, suggesting that these relatively minor changes in circulating MP are insufficient to cause a procoagulant state and that, under these physiological conditions, MP do not affect coagulation.

Surprisingly, in the postprandial state, the numbers of erythrocyte-derived MP markedly increased and these numbers were strongly associated with insulin concentrations. Since insulin induces a Ca^{2+}-dependent hyperpolarization of erythrocyte membranes, we hypothesize that this may be an inducer for the shedding of MP

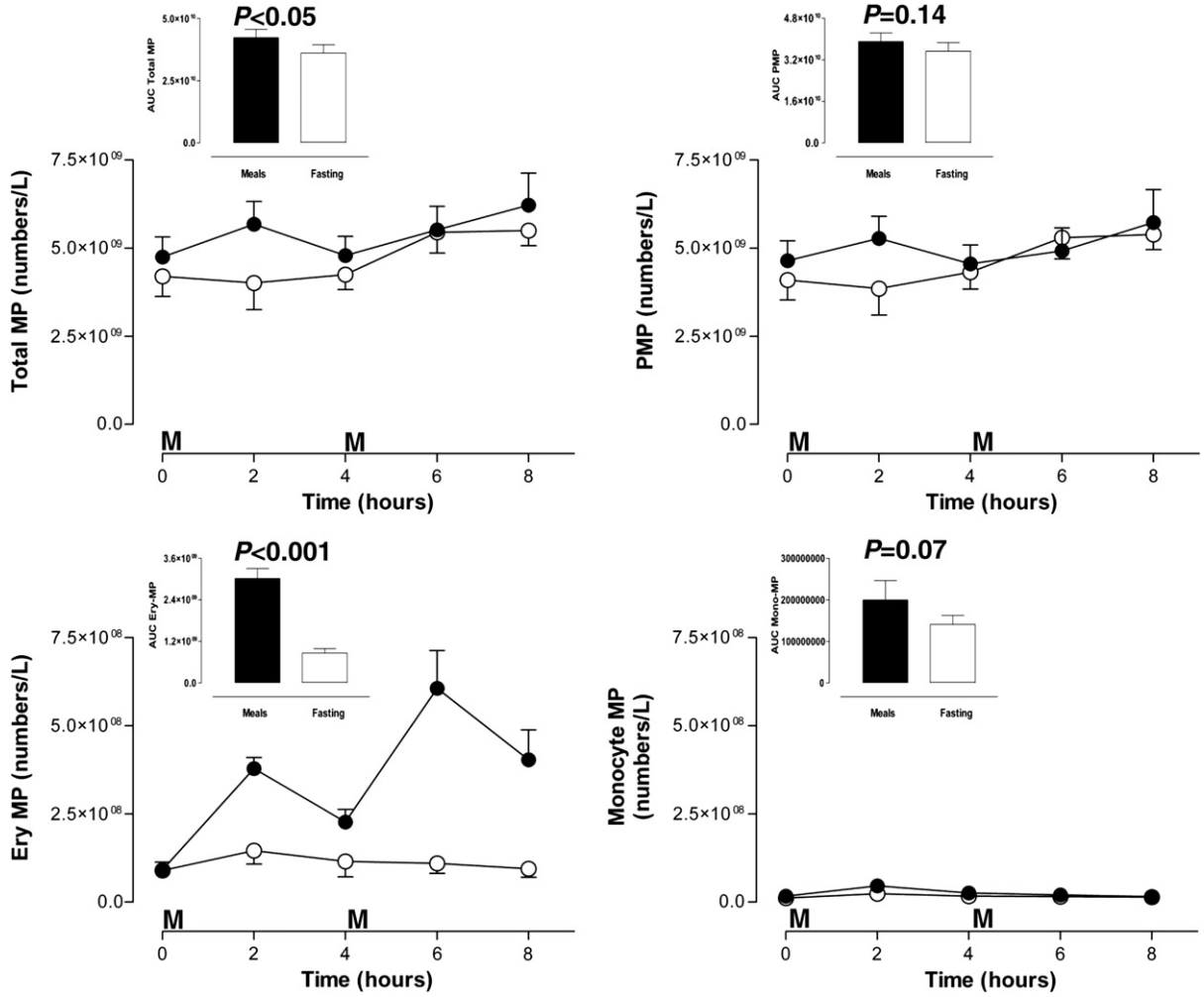

Fig. 2. The course of total MP, platelet-derived MP, erythrocyte-derived MP and monocyte-derived MP numbers/L during the meal (solid circles) and fasting (open circles) days in healthy males. Meal intake is indicated by M. Bars (black, meals; white, fasting) in the insets represent respective AUC values. The *P* value is given for AUC difference. Data are mean ± SE.

which is known to be highly calcium dependent [23–25]. Previously, we demonstrated that erythrocyte-derived MP are associated with factor XI-dependent coagulation in sickle cell disease, but we could not confirm this association in the present study. A possible explanation is that the changes in the phospholipid composition, especially the presence of oxidized and nonoxidized phospholipids, may differentially affect coagulation [26].

In healthy individuals, cell-derived MP originate predominantly from platelets and contain mostly phosphatidylcholine and sphingomyelin,

Table 2
Phospholipid composition of MP during postprandial or fasting state.

Phospholipids	Meals	Time points (hours)				
		0	2	4	6	8
L-PC	+	1.3 ± 0.8	0.9 ± 0.8	1.0 ± 0.8	1.3 ± 0.8	1.3 ± 0.8
	-	1.2 ± 1.0	1.2 ± 1.2	1.2 ± 1.2	1.1 ± 1.3	1.6 ± 0.9
SM*	+	27 ± 9.4	21 ± 3.6#	23 ± 5.5	22 ± 4.5	22 ± 4.7
	-	24 ± 5.8	23 ± 6.3	25 ± 5.0	25 ± 4.7	25 ± 5.5
PC*	+	46 ± 6.8	47 ± 7.8	46 ± 8.5	49 ± 10#	48 ± 13
	-	42 ± 12	43 ± 11	42 ± 11	43 ± 9.2	41 ± 11
PS	+	8.1 ± 6.6	12 ± 7.5	9.2 ± 7.3	8.2 ± 7.2	9.1 ± 6.7
	-	13 ± 12	13 ± 11	11 ± 7.5	11 ± 8.4	12 ± 12
PI	+	6.1 ± 3.8	6.4 ± 3.6	6.9 ± 3.6	6.2 ± 4.1	6.6 ± 4.9
	-	5.7 ± 4.0	6.9 ± 3.8	8.4 ± 7.3	6.6 ± 3.3	6.7 ± 3.1
PE	+	11 ± 5.1	13 ± 2.8	14 ± 4.5	13 ± 5.4	13 ± 5.6
	-	13 ± 6.5	12 ± 5.0	13 ± 8.3	14 ± 5.0	13 ± 3.9

Data expressed as % of total phospholipid (mol/mol) ± SE. *P<0.05 (area under the curve), #P<0.05 (individual time points). L-PC indicates lyso-phosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

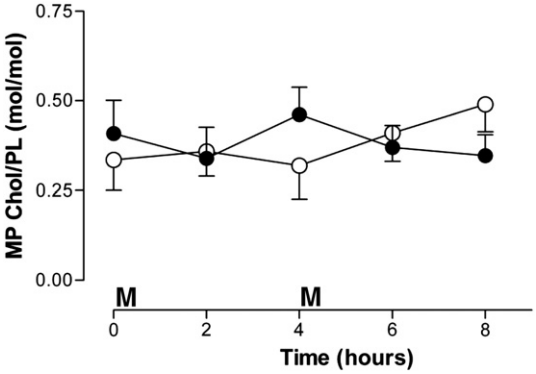

Fig. 3. The cholesterol : phospholipid (PL) ratio of MP during the meal (solid circles) and fasting (open circles) days in healthy males. Meal intake is indicated by M. Data are mean ± SE.

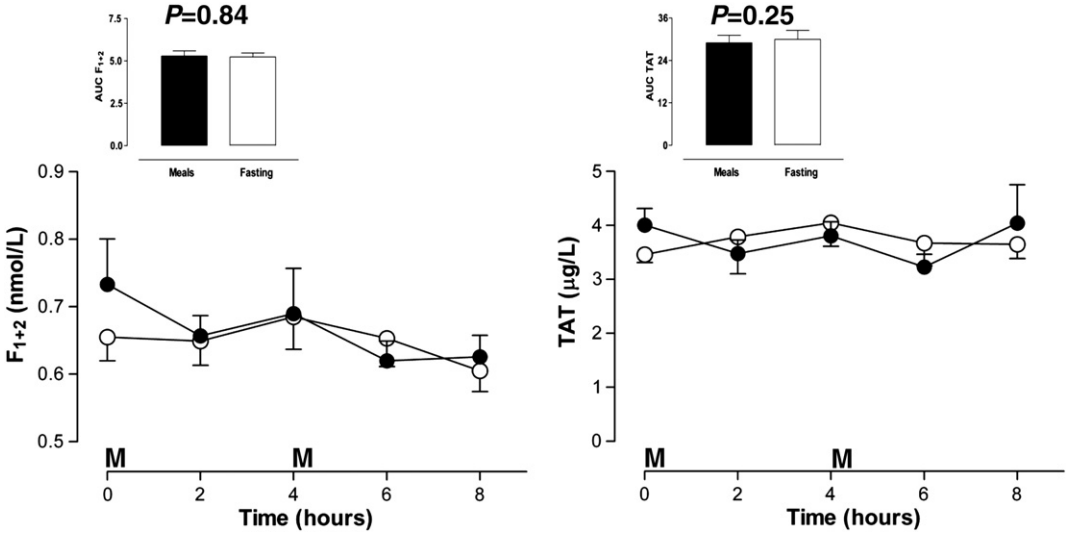

Fig. 4. Plasma concentrations of F_{1+2} and TAT during the meal (solid circles) and fasting days (open circles) in healthy males. Meal intake is indicated by M. Bars (black, meals; white, fasting) in the insets represent respective AUC values. The *P* value is given for AUC difference. Data are mean ± SE.

with smaller amounts of phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, and minute quantities of the lysophospholipids lyso-phosphatidylcholine, lyso-phosphatidylethanolamine and lyso-phosphatidylserine. We showed that following two consecutive meals the relative amount of phosphatidylcholine increases, whereas concurrently sphingomyelin decreases in MP, compared to a fasting day. These findings confirm earlier studies showing that changes in the phospholipid composition of MP vary between cell types and depend on the activation status of the parental cell [21,27,28], e.g. platelet-derived MP originating from collagen-activated platelets contained less phosphatidylcholine and more sphingomyelin, phosphatidylethanolamine and phosphatidylserine than non-stimulated platelets [27].

One of the important findings of the present study is that the total amount of phosphatidylserine present in MP fractions remained unaffected by fasting or meals in healthy subjects. Since phosphatidylserine exposed by MP or cells is believed to support the binding of (activated) coagulation factors and thus the formation of tenase and prothrombinase complexes, our present results may explain why we found neither differences in coagulation activation in vivo nor as coagulation activation potency in vitro.

In the present study, the phospholipid composition of in vivo MP was corrected for the plasma background. We found this correction to be essential, since both MP and MP-depleted plasma contained substantial quantities of phosphatidylcholine and sphingomyelin [29]. In contrast, the quantities of phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine were below the detection limit of our assay in MP-depleted plasma, whereas phosphatidylserine, phosphatidylinositol as well as phosphatidylethanolamine were clearly detectable in the corresponding fractions of MP. This finding implicates that determination of the concentrations of phosphatidylserine-exposing microparticles or their coagulant properties by capturing the MP from plasma by using ELISA plates coated with annexin V may be a suitable tool for MP detection and possibly quantification ex vivo.

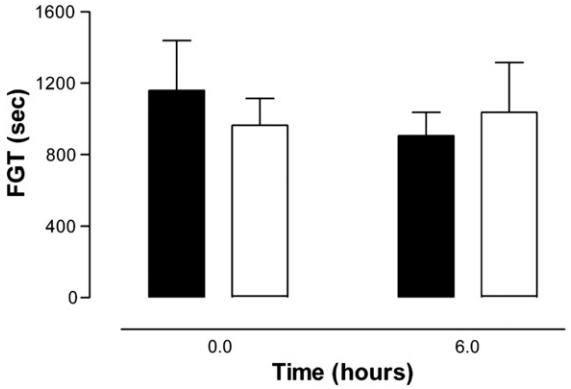

Fig. 5. Fibrin generation tests (FGT) at baseline (t = 0 hours) and at t = 6 hours during the meal (black bars) and fasting (white bars) days. Data are mean ± SE.

The observed increase in the cholesterol : phospholipid ratio of circulating MP during a prolonged episode of fasting and its association with plasma NEFA concentrations may be of interest. During the fasting day, the concentrations of NEFA in our healthy population increased almost 3-fold due to unsuppressed lipolysis, reaching levels that are found in fasting patients with type 2 diabetes [30]. Previously, it was shown that the amount of cholesterol in membranes of erythrocytes and leukocytes is associated with plasma fatty acids in patients with type 2 diabetes [31]. Furthermore, we demonstrated previously that MP released upon platelet activation have an increased cholesterol content [21]. It has been shown in several studies that growing cells in media with an altered balance of specific lipids, particularly oligo-unsaturated fatty acids and sterols such as cholesterol, affect the lipid composition of the plasma membrane [32,33]. Since cholesterol plays several structural and metabolic roles, particularly in membranes, cells may shed MP to cope with acute lipid changes in plasma to retain their membrane qualities.

In conclusion, the numbers, cellular origin and phospholipid composition of cell-derived MP is altered upon consumption of two consecutive high-fat mixed meals in healthy males. However, these alterations do not seem to affect the ability of circulating MP to activate coagulation, both in vivo and in vitro. We therefore hypothesize that the suggested differences in (phospho-)lipid composition of cell-derived MP on fasting and meal days reflect the ability to maintain membrane homeostasis, rather than to modify coagulation.

Conflict of interest statement

The authors have no financial interest with regard to the preparation of this paper.

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