Postprandial cell inflammatory response to a standardised fatty meal in subjects at different degree of cardiovascular risk

Chiara Tamburrelli; Francesco Gianfagna; Marco D’Imperio; Amalia De Curtis; Domenico Rotilio; Licia Iacoviello; Giovanni de Gaetano; Maria Benedetta Donati; Chiara Cerletti
Research Laboratories, Fondazione di Ricerca e Cura “Giovanni Paolo II”, Università Cattolica, Campobasso, Italy

Summary
A fatty meal may represent a challenge of in vivo acute inflammatory reaction. We evaluated the acute effects of a standardised fatty meal administration on leukocytes and platelets and on their interactions on 61 subjects at different degree of cardiovascular risk, without any clinical event. Before and 2 hours after a fatty meal, blood cells were counted and markers of leukocyte (intracellular myeloperoxidase [MPO] and Mac-1) and platelet (P-selectin and microparticles) activation and mixed platelet-leukocyte conjugates measured by flow-cytometry. After the fatty meal, both white blood cell and platelet count significantly increased, more markedly in subjects with lower cardiovascular risk score. Mac-1 expression too increased (from 32.2 ± 27.2% to 45.6 ± 29.0%, p=0.0016), while MPO decreased (from 83.1 ± 16.3% to 64.5 ± 23.1%, p<0.0001). A trend for increased platelet activation and interaction with leukocytes was also observed. Women were more markedly susceptible to fatty meal challenge, as compared to men, while age did not seem to affect any cell response to fatty meal. Waist-to-hip ratio and body mass index influenced polymorphonuclear cells (PMN) degranulation and platelet count increase, respectively. Cellular responses to the fatty meal, in particular PMN degranulation, were attenuated in subjects at higher degree of cardiovascular risk, who showed a basal mild inflammatory activation status. In conclusion, a fatty meal consumption may represent a model of acute inflammatory response and appears to be modulated by different demographic and cardiovascular risk degree. This model could be applied to study the effect of food-derived antioxidants or nutritional supplements, but its relevance remains to be demonstrated.

Keywords
Fatty meal, cardiovascular risk, platelet and leukocyte activation, gender, acute cell inflammatory response

Introduction
The contribution of leukocytes and other inflammatory cells to thrombosis is supported by epidemiological data and experimental studies: these cells may act either directly or through interaction with platelets. Numerous in vitro studies and experimental animal models have investigated the inflammatory mechanisms underlying the pathogenesis of thrombosis (1). Few models are available, instead, to study inflammatory responses in vivo in healthy people or in subjects at increased cardiovascular risk who have not yet developed any clinical event.

Triglycerides are able to induce leukocyte activation, both in vitro and in vivo, as observed in healthy subjects (2). The postprandial phase is characterised by a rise in plasma concentrations of triglyceride-rich lipoproteins that can induce in turn acute inflammatory reactions (3); the responsible mechanisms are not entirely clear: triglycerides would induce leukocyte activation triggering a response characterised by an increase in oxygen radical levels and the expression of adhesion molecules. The latter would then favor leukocyte interactions with platelets and endothelium, promoting cell-mediated thrombosis. The elevation in postprandial triglycerides is reportedly associated to a transient increase in blood leukocytes (4), increased expression of leukocyte activation markers, cell adhesion molecules, pro-inflammatory cytokines (2, 5) and to the up-regulation of pro-inflammatory genes in endothelial cells (6).

A fatty meal test is a model of acute triglyceride increase, which may represent a challenge of in vivo acute inflammatory reaction (7). Previous studies on the effect of acute post-prandial lipidaemia on platelet count and activation have given contradictory results (8–13).

The aim of this study was to evaluate the acute effects of the triglyceride increase induced by a standardised fatty meal on circulating leukocytes and platelets and on their mutual interactions, in volunteers at different degree of cardiovascular risk, who had not suffered, however, any cardiovascular event.

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Materials and methods

Subjects and experimental protocol

The study was performed on 61 subjects, 21 women and 40 men, selected among volunteers, coming to our clinics to participate to phase II trials, as those with a COURe risk score between 5 and 20 ([14] and http://www.cuore.iss.it/sopra/calc-rischio_en.asp) or younger subjects with hypercholesterolemia or low Mediterranean diet score. Exclusion criteria were treatment with anti-platelet and previous cardiovascular events.

Trained interviewers administered to the recruited subjects a structured questionnaire on their lifestyle and both personal and family history. Blood pressure was measured by the automatic device OMRON-HEM-705CP (15) and body weight and height on a standard beam balance scale with an attached ruler, in subjects wearing no shoes and only light indoor clothing. The ratio of circumference of the waist to that of the hip, waist-to-hip ratio (WHR), was considered high (at high risk) when ≥0.85 and ≥1 for women and men respectively, according to the WHO classification (16).

Venous blood was collected with minimal venous stasis on overnight fasting subjects; after then, all subjects ate within 15 minutes (min) a standardised test meal composed of two scrambled eggs, three slices of white bread (51 g), 30 g butter, 30 g bacon fried in butter. Water drinking was allowed during the fatty meal. The nutrient composition was 25 g protein, 26 g carbohydrate, and 52 g lipid, for a total of 675 kcal.

Two hours (h) after the fatty meal consumption (a time selected on the basis of a preliminary study as long enough to induce a significant elevation of triglyceride plasma levels, while minimizing any possible circadian effect), blood collection and measurements of blood pressure were repeated.

All subjects received explanation about the scope of the experiment and gave written informed consent. The latter as well as the study protocol and all the procedures were approved by our Institutional Ethics Committee.

Biochemical measurements, soluble markers and blood cell count

Biochemical analyses were done on fresh samples: serum lipids and blood glucose were assayed by enzymatic reaction methods using an automatic analyser (ILab 350, Instrumentation Laboratory, Milan, Italy); complete blood count, differential leukocytes and platelet values were measured in EDTA-K tubes using an automated blood counting machine (Beckman Coulter HmX Haematology Analyzer, Instrumentation Laboratory).

Cellular biomarkers

Citrated (3.8%, 1/10 v/v) whole blood samples were used to measure mixed platelet-leukocyte conjugates and markers of platelet and leukocyte activation, as described (17, 18). Briefly, 100 μl of blood was fixed by addition of Thrombofix®, as specified by the manufacturer (Instrumentation Laboratory); 1 h after fixation samples were labelled with the appropriate monoclonal antibodies to detect antigen expression for 20 min in the dark, at room temperature (RT). Then, red cells were lysed by adding the IOTest 3 lysing solution (Instrumentation Laboratory), vortexed and incubated for 10 min. The supernatant was discarded and samples re-suspended in 1 ml of phosphate-buffered saline (PBS) and acquired at flow cytometer.

Platelets and leukocytes were identified by morphological and immunological characteristics, using forward light scatter (FS) vs. side light scatter (SS) intensity (in logarithmic scale for platelets and in linear scale for leukocytes) and CD42b or CD61 (platelet population), CD14 (monocyte population) and CD45 (total leukocyte population) positivity. Specific monoclonal antibodies (MoAb) for flow cytometry (and their isotopic controls) against P-selectin (CD62P), CD42b, CD61, CD45, CD11b and Mac-1 were from Instrumentation Laboratory.

A Coulter EPICS XL flow cytometer (Beckman Coulter), daily checked by the acquisition of Flow-Check™ Fluorospheres (Instrumentation Laboratory), was used. For each measurement, 10,000 events were analysed.

To detect intracellular myeloperoxidase (MPO) in polymorphonuclear (PMN) leukocytes the FIX & PERM® kit was used to fix and permeabilise cells in whole blood and allow antibody access. Samples were processed as specified by the manufacturer (Invitrogen, Walter Occhiena, Torino, Italy).

Platelet-leukocyte mixed conjugates were quantified by double fluorescence positivity, evaluating the percentage of platelet fluorescence in the gate of the leukocyte populations, identified as above.

Platelet microparticles were identified, in logarithmic scale, by morphologic characteristics, as those events in the lowest ranges of FS and SS and of size approximately less than 1 μm, and by immuno-negativity to the platelet antigen CD42b (18). This measurement method was validated by comparing the flow cytometric gate for microparticles in whole blood with the one where two different preparations enriched in microparticles were detected: the first obtained by activation of platelet-rich plasma (PRP) by collagen-ADP or ionophore A23187 stimulation, the second by ultracentrifugation of plasma supernatant from activated PRP. Moreover, the observed expression of P-selectin on a high percentage of the identified microparticles suggests the platelet activation origin, rather than formation during thrombopoiesis from megakaryocytes (19).
**Statistical analysis**

Results are given as mean ± SD, except in figures, where mean and SEM are reported. Data were first analysed by a paired-samples t-test to determine significant differences between each time-point: before (T0) and 2 h after (T1) test meal (alpha level was 0.05).

The correlation between two variables was obtained by Pearson’s test. A general linear model (GLM) analysis was used to verify the possible interaction between the fatty meal effect and sex.

Table 1: Characteristics of study participants.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before fatty meal</th>
<th>After fatty meal</th>
<th>P paired after vs. before</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.6 ± 13.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men/women</td>
<td>40/21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26.7 ± 3.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>129.2 ± 17.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>77.0 ± 7.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>22.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>45.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycaemia (%)</td>
<td>19.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>22.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight (%)</td>
<td>65.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CUORE risk score (%)</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified-CUORE risk score</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD (n=61) or percent of subjects. *Data on 49 subjects >35 years old; range 0.2–18.2%. ** Data on 61 subjects; range 1.1–3.7. BMI, body mass index; BP, blood pressure.

Table 2: Changes of haematological parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before fatty meal</th>
<th>After fatty meal</th>
<th>P paired after vs. before</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (10^3/μl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>6.7 ± 1.7</td>
<td>7.2 ± 1.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Women*</td>
<td>6.1 ± 1.2</td>
<td>7.0 ± 1.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Men</td>
<td>7.0 ± 1.8</td>
<td>7.4 ± 2.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Granulocytes (10^3/μl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>3.9 ± 1.1</td>
<td>4.4 ± 1.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Women*</td>
<td>3.5 ± 0.9</td>
<td>4.3 ± 1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Men</td>
<td>4.0 ± 1.1</td>
<td>4.5 ± 1.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Platelets (10^3/μl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>241 ± 47</td>
<td>248 ± 49</td>
<td>0.005</td>
</tr>
<tr>
<td>Women*</td>
<td>257 ± 40</td>
<td>270 ± 40</td>
<td>0.023</td>
</tr>
<tr>
<td>Men</td>
<td>232 ± 48</td>
<td>236 ± 49</td>
<td>0.110</td>
</tr>
<tr>
<td>Mean platelet volume (fl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>8.4 ± 0.9</td>
<td>8.1 ± 1.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Women</td>
<td>8.6 ± 0.9</td>
<td>8.4 ± 1.1</td>
<td>0.173</td>
</tr>
<tr>
<td>Men</td>
<td>8.4 ± 0.9</td>
<td>8.0 ± 0.9</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD of n=61 (all subjects), n= 20 (women), n= 41 (men). *P unpaired < 0.05 for the difference (after-before) women vs. men.

Age or cardiovascular risk factors, identifying the variables to use for stratified analyses. Sex was included in the model as “fixed factor” and the age as a covariate.

Linear regression models were used to verify the associations between post-meal changes and global cardiovascular risk score, taking into account the effect of baseline values. Through a global risk assessment approach, a score evaluating the presence of more cardiovascular risk factors independent from age (“Modified-CUORE risk score”) was calculated using CUORE risk algorithm, with the exclusion of the steps related to age effect and to conversion of the score in cardiovascular event probability at 10 years. This formula allows to estimate the sub-acute impact of multiple risk factors in a specific time without considering their chronic effect, as well as to use the score also in subjects aged less than 35, in which CUORE risk score is not applicable (14). The modified Cuore risk score was normally distributed in the 61 subjects studied: Skewness value=−0.33 and Kurtosis value=−0.74; test di Kolmogorov-Smirnov= not significant.

Multiple linear regression models were built on cellular biomarkers data. The reliability of the models is given as R-squared and Durbin-Watson. The Durbin-Watson statistics is useful for evaluating the presence or absence of a serial correlation of residuals and, therefore, estimating the model’s reliability. The residual represents the difference between predicted and real values. If the residuals turn out to be independent according to the Durbin-Watson table (20), the system is extremely reliable with a good prediction capacity.

SPSS software package for Windows (version 15.0) was used to perform the analyses.

Results

The characteristics of the 61 study participants are reported in Table 1. The risk factors considered were significantly higher in men than in women (data not shown); for this reason all analyses were corrected for sex and/or the two gender subgroups were analysed separately.

As expected, after the fatty meal the triglyceride plasma levels were significantly increased (from 134.6 ± 68.8 to 181.5 ± 84.6 mg/dl, mean ± SD, p<0.0001).

Blood cell counts after fatty meal

A significant increase in white blood cell (mainly granulocytes) and platelet count was observed 2 h after the fatty meal, while the mean platelet volume was significantly decreased (Table 2).

Total white blood cells and granulocytes after the fatty meal were directly correlated with triglyceride levels after the fatty meal (Pearson coefficient 0.30 and 0.33, p=0.02 and 0.01, respectively).

In the stratified analyses performed by GLM, the basal platelet counts were similar in women and men, while leukocyte count was
lower in women; in contrast, the fatty meal-induced increases in both white cell and platelet counts were significantly greater in women than in men (0.88 ± 0.76 and 0.41 ± 0.91 white blood cell counts 10^3/μl; 13.8 ± 25.6 and 3.6 ± 14.1 platelets 10^3/μl) (▶Table 2).

Neither cell count changes after fat load differed in subjects below or over the median age (51.6 years). A higher body mass index (BMI) was associated with a smaller increase in platelet counts (1.3 ± 14.0 vs. 12.4 ± 22.3 Δ after-before fatty meal 10^3 platelets /μl in subjects with BMI above the median value of 26.2 vs. below). In contrast, the other cardiovascular risk factors individually considered (hypercholesterolaemia, hypertension, hyperglycaemia, smoking) did not appear to be related to either cell count modifications, although hypercholesterolaemic and hypertensive subjects had an age- and sex-independent greater triglyceride increase, following the fatty meal.

Considering the risk factors globally, both white blood cell and platelet counts showed a lower increase after the fatty meal in subjects with higher cardiovascular risk score (Pearson coefficients –0.25, p=0.05, and –0.26, p=0.04, respectively). However, while the results appeared to be unaffected by basal values in a linear regression model, they became non-significant introducing age as covariate.

**Leukocyte activation markers after fatty meal**

▶Figure 1 shows leukocyte function changes after the fatty meal intake: cell degranulation, measured as loss of MPO content in PMN, was significantly increased (panel A) as well as the expression of the activated form of the beta2-integrin Mac-1 both on PMN (panel B) and monocytes (panel C). ▶Table 3 reports the same parameters in women and men separately: the results are substantially similar to those obtained in the whole population, except for the loss of statistically significance for women Mac-1 expression on PMN, possibly due to the smaller number of replications in this subgroup.

MPO degranulation appeared to be directly correlated with white blood cell count increase induced by fat load (Pearson coefficient 0.31, p=0.02).

GLM analysis yielded results consistent with those for cell counts: gender subgroup analysis of the fatty meal-induced differences showed that MPO intracellular decrease was greater in women than in men (-31 ± 21 vs. -12 ± 23 % of PMN positive for MPO Δ after-before meal in women vs. men; p=0.005). Subjects with higher WHR had a significantly greater decrease in PMN intracellular MPO as compared to those with lower WHR (-27.8 ± 20.5 vs. -9.3 ± 24.3; p= 0.003). Subgroup analyses for age and other cardiovascular risk factors showed no effect on fatty meal responses.

MPO degranulation after the fatty meal appeared to be higher in subjects with lower risk score (Pearson coefficient 0.33, p=0.01), also considering basal values and age as covariates, while the increase of Mac-1 expression on PMN appeared to be unrelated to the presence of multiple cardiovascular risk factors. However, both basal values of intracellular MPO and PMN surface Mac-1 were correlated to the risk score (Pearson coefficient –0.25, p=0.05, and 0.26, p<0.05, respectively).

**Platelet activation and mixed platelet-leukocyte conjugates after fatty meal**

Data assembled in ▶Table 4 show a common trend of increase of platelet activation markers, such as P-selectin and microparticles,
and of mixed conjugates of platelets with PMN and monocytes, after the meal.

The increase in platelet P-selectin expression, as well as that in mixed conjugates were correlated with the fatty meal-induced increase in white blood cell counts (Pearson coefficient −0.28, −0.36 and −0.28, p=0.033; 0.006 and 0.031 for P-selectin, platelet-PMN and platelet-monocyte conjugates, respectively). The increase in mixed conjugates after the fatty meal was associated with increased platelet P-selectin expression (Pearson coefficient 0.36 and 0.50, p=0.007 and p>0.001 for platelet-PMN and platelet-monocyte, respectively) and with the triglyceride increase (Pearson coefficient 0.33, p=0.009, for platelet-monocyte conjugates).

Gender subgroup analysis showed that the baseline expression values of P-selectin were lower in women than in men and that the fatty meal-induced increase in P-selectin and platelet-PMN conjugates appeared to be statistically significant only in the women subgroup. On the other hand, platelet microparticle increase after the fatty meal was statistically significant only in men (Table 4).

While a direct correlation between basal platelet P-selectin expression and cardiovascular risk was observed (Pearson coefficient 0.33, p=0.01), its increase after fatty meal did not appear to vary with the different cardiovascular risk scores considered (Pearson coefficient −0.25, p=0.06).

### Multiple regression analyses

The results of multiple regression models suggested that post-meal increase in mixed (monocyte- rather than PMN-) conjugates is mainly associated with the increased expression of P-selectin on platelet microparticles and of Mac-1 on monocytes, as well as with the triglyceride increase and platelet count decrease (Table 5). These covariates explained the 85% of variance of post-meal increase in platelet-monocyte conjugates.

### Discussion

A standardised fatty meal consumption induced the expected triglyceride increase in healthy subjects, at different degree of cardiovascular risk. This acute condition was accompanied by a significant increase in white blood cell count, mainly due to granulocytes, and by leukocyte activation. Platelet count too was significantly higher after the fatty meal, while mean platelet volume was reduced; furthermore, a trend for increased platelet activation and interaction with leukocytes was observed, which became statistically significant in the women subgroup. Thus, an induced postprandial phase triggered an acute cellular inflammatory response in a group of subjects at different cardiovascular risk degree.

### Blood cell counts

The increase in white blood cell counts two hours after fatty meal confirms previous data (4, 21) and suggests a condition similar to an acute inflammatory reaction, when neutrophils are released from the marginated pool into the blood stream and accumulate at
the site of inflammation (2, 22, 23). The post-prandial increase in platelet count might result from the rapid release of senescent smaller platelets (24), stored in the reticulo-endothelial system before their elimination (25), a mechanism of platelet increase consistent with the observed mean platelet volume decrease.

The finding that white blood cell and platelet count significantly increased, more markedly in subjects with lower cardiovascular risk score can probably be explained by the higher baseline values present in patients with elevated CUORE score. These results seem to limit their applicability since the differences found as a function of the cardiovascular risk score, disappeared when age was introduced as a covariate.

### Leukocyte activation

Our finding on leukocyte MPO release triggered by fatty meal, apparently hitherto undescribed, is of interest. Indeed, MPO is a heme-containing peroxidase highly expressed by neutrophils, whose major function is the generation of hypochlorous acid as part of the neutrophil’s antimicrobial defence. This enzyme, secreted during activation of PMN, has a potent pro-atherogenic activity through low-density lipoprotein cholesterol oxidation and uptake by macrophages. In addition, it activates matrix metalloproteinases, promotes plaque destabilisation and rupture and alters the endothelial vascular properties by reducing nitric oxide bioavailability (26). A recent study has shown that in various forms of vascular damage, MPO is deposited in the vasculature after its release from neutrophils, binds to the endothelial surface and attracts other leukocytes (27).

Mac-1, the functional activated form of the leukocyte beta2-integrin, also increased upon fatty meal, plays a key role in normal and inflammatory immune responses: it is also responsible for conjugate formation with platelets and leukocyte recruitment and accumulation into the vascular wall at the site of inflammation, leading to endothelial dysfunction (28, 29).

The reported post-prandial increase of leukocyte activation confirms the acute inflammatory cell response to the fatty meal-induced metabolic changes.

### Platelet activation

In parallel with platelet count increase, a moderate, not significant, but consistent platelet activation was suggested by the increase in different markers such as P-selectin expression, circulating platelet microparticles and mixed conjugates of platelets with leukocytes. The formation of mixed conjugates is reportedly associated to platelet activation and expression of P-selectin (17), but also requires the activation of the leukocyte integrin Mac-1 (29), as observed in our sample.

### Sex, age and other determinants

Women were more susceptible to fatty meal challenge, as compared to men, in terms of cell count increase, P-selectin expression, mixed conjugate formation and leukocyte activation (MPO degranulation). This gender-related difference in the acute response to the fatty meal suggests that the lower the cardiovascular risk score, as in women, the higher the acute cell response. This observation should be further explored, as most of previous studies preferentially enrolled male subjects. Interestingly, two recent studies have reported that healthy women had significantly higher platelet counts than men (30, 31).

Age, in contrast, did not seem to affect any cellular response to fatty meal. Similarly, the individual risk factors measured did not influence any cell response to fatty meal, except for PMN degranulation and platelet count increase, which were correlated with WHR and BMI, respectively.

The correlations observed with a modified cardiovascular risk score suggest that cellular responses to the fatty meal challenge, in particular PMN degranulation, are attenuated in the presence of multiple cardiovascular risk factors. On the other hand, the basal cellular measures support a mild inflammatory activation status in subjects at higher cardiovascular risk.

The association found between post-meal increase in platelet-monocyte mixed conjugate levels and markers of platelet and leukocyte activation is plausible, since these markers increase in vivo before mixed aggregate formation. It is interesting, however, to note the association with triglyceride increase and platelet count decrease, supporting the role of platelet as an inflammatory cell

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables (equations)</th>
<th>R²</th>
<th>Durbin-Watson</th>
<th>Correlation of residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ platelet-monocyte conjugates</td>
<td>0.268 [Δ P-sel platelet-microparticles] + 0.181 [Δ monocyte Mac-1] + 0.128 [Δ platelet-PMN conjugates] + 0.024 [Δ TG] - 0.020 [platelet count T0] + 3.181</td>
<td>0.85</td>
<td>1.60</td>
<td>test is non conclusive</td>
</tr>
<tr>
<td>Δ platelet-PMN conjugates</td>
<td>1.221 [Δ platelet-monocyte conjugates] + 0.234</td>
<td>0.42</td>
<td>1.97</td>
<td>no autocorrelation</td>
</tr>
</tbody>
</table>
What is known about this topic?
- Leukocyte counts and some activation markers increase after a fatty meal in healthy subjects.
- There are contrasting results on platelet activation after a fatty meal.

What does this paper add?
- Subjects at different degree of cardiovascular risk who had not suffered any clinical event show increased platelet and leukocyte counts, leukocyte activation markers, including a marked hitherto undescribed intracellular myeloperoxidase (MPO) degranulation, and a trend for increased platelet activation (P-selectin, microparticles) and mixed platelet-leukocyte conjugates.
- Subgroup analyses show a higher susceptibility to fatty meal-induced changes in women, in respect to men and in overweight subjects.
- A fatty meal test may represent a simple, useful model of acute inflammatory response in vivo in humans and appears to be modulated by a different demographic and cardiovascular risk degree.
- This model could be applied to study the protective effect of food-derived antioxidants or nutritional supplements.

Limitations of this study
The relatively small sample size and the lack of duplicate fatty meal tests to assess its reproducibility are the main limitations of this study, which is, however, compatible with its exploratory, hypothesis-generating nature. These observations should be confirmed in larger cohorts of subjects, but in particular extended to different subgroups of subjects, women and men, bearing different single or combined cardiovascular risk factors.

Conclusions
In conclusion, the inflammatory cellular response observed as a consequence of a fatty meal may represent a simple, useful model of acute inflammatory response in vivo in humans, and appears to be modulated by different demographic and cardiovascular risk factors. This model could be applied to studies on the protective effect of food-derived antioxidants or nutritional supplements, but its relevance remains to be demonstrated (39–41).

Acknowledgements
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Conflict of Interest
None declared.

Hypertriglyceridaemia as a cardiovascular risk factor

Epidemiological data show that elevated triglyceride levels are an independent cardiovascular risk factor (33). Elevated acute postprandial levels of triglyceride-rich lipoproteins have been associated with both coronary (34) and carotid artery atherosclerosis (35). The postprandial state, a physiological phenomenon that may occur several times a day, could lead by repeated cell activation to a complex series of events that initiate and develop atherosclerosis (36). These events include the increased residence time of atherogenic lipoproteins in plasma, creating a pool of lipoproteins bound to endothelial cells, and the indirect involvement of inflammatory reactions, such as the activation of leukocytes, platelets and endothelium. The role of activation of platelets and leukocytes and their interactions in inflammation and atherothrombosis has been given more and more attention in the last years (1, 37, 38).

Previous studies observed leukocyte count increase and activation after a fatty meal in relatively small numbers of healthy male subjects only (2, 4, 5); we confirmed with other cellular markers, such as MPO release, and extended these findings to men and women at different degree of cardiovascular risk.

References


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