Circulating antioxidant defences are decreased in healthy people after a high-fat meal

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The aim of this study was to examine the responses of uric acid, antioxidant defences and pro-oxidant variables after a high-fat meal. Twenty-five healthy persons without criteria for the metabolic syndrome, underwent a high-fat meal with Supracal® (60 g fat). Measurements were made at baseline and 3 h after the meal of TAG, uric acid, HDL-cholesterol, total proteins and oxidative stress. Following the high-fat meal, we detected a significant increase in pro-oxidative variables and a decrease in antioxidative variables. The uric acid concentrations were significantly lower after the high-fat meal and the reduction correlated significantly with the oxidative stress variables. The inverse relation between reduced uric acid and increased carbonylated proteins remained in multiple regression analysis. We conclude that uric acid is a powerful antioxidant and its reduction following a high-fat meal may be related with its acute antioxidative action.

Uric acid: Oxidative stress: Postprandial hyperlipidaemia: High-fat meal

The increase in TAG after fat intake is defined as postprandial hyperlipidaemia. Clinical studies have shown that increased postprandial concentrations of TAG are associated with coronary and arterial disease(1,2) and with insulin resistance(3). Studies in healthy volunteers have shown that postprandial hyperlipidaemia causes a marked increase in oxidative stress and a worsening in endothelial function(4). Postprandial lipoproteins, especially chylomicrons and VLDL, appear to generate oxygen radicals on the endothelial surface that react with nitric oxide and reduce its availability(5).

Our group has previously detected a close association between TAG and uric acid concentrations. Hypertriacylglycerolaemia due to increased levels of VLDL is associated with hyperuricaemia with reduced renal excretion of urates(6,7) and the connection between hyperuricaemia and hypertriacylglycerolaemia has a genetic association(8,9).

Many questions remain concerning hyperuricaemia. Current debate is centred on whether high concentrations of uric acid represent a marker of cardiovascular risk or a cardiovascular risk factor itself. This debate is fuelled by the paradoxical effect of purine metabolism on oxidative stress; on one hand the action of xanthine oxidase on xanthine produces uric acid and superoxide radicals belonging to the reactive oxygen species(10); and on the other, uric acid is an abundant aqueous antioxidant that accounts for almost two-thirds of all free-radical scavenging activity in human serum(11). Allantoin, formed from the non-enzymatic oxidation of urate, is a marker of the antioxidative effect of uric acid(12).

In short, we know that a high-fat meal is accompanied by an increase in oxidative stress but we do not know the clinical importance of the concentration of uric acid or whether its antioxidative ability plays any part in the oxidative damage resulting from a high-fat meal. Accordingly, the main aim of the present study was to determine the association between uric acid and the increase in oxidative stress following a high-fat meal in healthy persons.

Material and methods

We selected twenty-five healthy persons who were accompanying patients on primary care visits, thirteen men and twelve women. These persons, who all had a normal oral glucose tolerance test, the previous 3 months, consumed a 60 g high-fat meal with a commercial liquid preparation (Supracal®; SHS International, Liverpool, UK). Only water was permitted during
the process, and no physical exercise was undertaken. The commercial preparation of 125 ml contains 60 g fat, of which 12 g are saturated, 35.35 g are monounsaturated, and 12.75 g are polyunsaturated. Each 100 ml contains < 1 g lauric acid, < 1 g myristic acid, 4.8 g palmitic acid, 1.4 g stearic acid, 27.7 g oleic acid, 9.6 g linoleic acid, 1.4 g behenic acid, and 0.5 g lignoceric acid.

Blood samples were obtained from the antecubital vein and placed in chilled vacutainer tubes (BD vacutainer™, London, UK). The serum was immediately separated by centrifugation for 30 min at 956 rpm and frozen at −80°C until analysis. The study protocol was approved by the local Bioethics Committee. All subjects gave informed consent to the study, which was approved by the Ethics Committee of Virgen de la Victoria Clinical University Hospital.

Variables evaluated

Measurements were made in all subjects of age (in years), weight, height (to calculate the BMI, calculated as the weight (kg) divided by the height squared (m²)), and waist and hip circumferences (to calculate the waist:hip ratio, calculated as the waist circumference divided by the hip circumference) (13). Measurements and corresponding calculations were made in serum samples of glucose, total protein, uric acid, cholesterol, TAG, HDL-cholesterol, apoAI, apoB, creatinine, C-reactive protein, as well as 24-h uric acid urinary excretion. Additionally, at 3 h, measurements were made of cholesterol, TAG, apoB, uric acid, and pro-oxidant and antioxidant variables. The measurements were made with a Dimension autoanalyzer from Dade Behring Inc. (Deerfield, IL, USA). Additionally, insulin and dehydroepiandrosterone sulphate levels were quantified by RIA provided by BioSource S.A. (Nivelles, Belgium) and Beckman Coulter Inc. (Fullerton, CA, USA), respectively.

Lipid peroxidation products (malondialdehyde + 4-hydroxyalkenals) and glutathione reductase (GSH-Rd) activity were estimated using reagents purchased from Oxis International (Portland, OR, USA), i.e. LPO-586 and GR-340 kits, respectively. Additionally, glutathione-S-transferase activity was evaluated using reagents purchased from BioVision Inc. (Mountain View, CA, USA), i.e. GST Colorimetric Activity Kit.

The protein carbonyl content was evaluated using the method of Levine et al. (14). Total nitrates (nitrites and nitrates; NOx) was used as a marker of nitric oxide (NO) levels and assayed following the Griess method (15) in serum. Glutathione peroxidase (GSH-Px) activity was evaluated by the Flohé and Gunzler method (16), whereas catalase and total superoxide dismutase activities were assayed following the Aebi technique (17) and Marklund method (18), respectively. The CV of the techniques were less than 10%.

Statistical analysis

Pearson correlations were made between study variables in order to evaluate their association. Comparisons between experimental conditions (baseline versus after high-fat meal) were determined using a paired-sample test (Wilcoxon). A multiple regression analysis was done to study the associations between the variables that correlated. In all cases the rejection level for a null hypothesis was a P=0.05 for two tails. Calculations were performed with SPSS software (version 11.0; Statistical Package for the SPSS Iberica, Madrid, Spain).

Results

Table 1 shows the characteristics of the study subjects; none of them fulfilled the Adult Treatment Panel III criteria for the metabolic syndrome.

Table 2 shows the associations between the oxidative stress measurements and the levels of uric acid and TAG prior to the high-fat meal. The baseline levels of plasma TAG were inversely correlated with the antioxidant variables: GSH-Rd, glutathione-S-transferase and superoxide dismutase (r = 0.57, r = 0.60, r = 0.64, respectively), and directly with the oxidant variables: NOx, lipid peroxidation and carbonylated proteins (r 0.74, r 0.49, r 0.55, respectively). Moreover, as well as a strong inverse association between NOx and GSH-Rd (r = 0.76), strong correlations were found between oxidant variables and antioxidant variables (Table 2).

Table 3 shows the comparison of the means of those variables studied both before and after the high-fat meal. There was a significant increase in the values of TAG, lipid peroxidation, carbonylated proteins, and a significant reduction in GSH-Px, GSH-Rd, NOx and plasma uric acid after the high-fat meal.

Table 4 shows the correlations of the differences in the variables before and after the high-fat meal. There was a negative association between the difference in the plasma levels of uric acid and the glutathione-S-transferase and a positive correlation with the carbonylated proteins (r = 0.59, r = 0.74, respectively).

Finally, Table 5 shows a multiple regression analysis of the difference in the plasma uric acid levels according to the other variables studied. The increase in the levels of carbonylated proteins after the high-fat meal was the only variable that predicted significantly the variance of this difference in uric acid levels.

Table 1. Baseline biological characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>13/12</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.5</td>
<td>11.25</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.678</td>
<td>0.673</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>88.95</td>
<td>10.02</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110</td>
<td>8.1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72</td>
<td>7.3</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.53</td>
<td>2.12</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>0.94</td>
<td>0.32</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.61</td>
<td>0.65</td>
</tr>
<tr>
<td>Baseline glucose (mmol/l)</td>
<td>4.96</td>
<td>0.40</td>
</tr>
<tr>
<td>ApoAI (mg/dl)</td>
<td>175.16</td>
<td>41.29</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>117.94</td>
<td>27.27</td>
</tr>
<tr>
<td>Baseline insulin (µU/ml)</td>
<td>9.67</td>
<td>4.14</td>
</tr>
<tr>
<td>Fractional excretion of uric acid (%)*</td>
<td>7.90</td>
<td>2.44</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.37</td>
<td>3.00</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.27</td>
<td>0.79</td>
</tr>
<tr>
<td>Uric acid (µmol/l)</td>
<td>299.77</td>
<td>57.10</td>
</tr>
</tbody>
</table>

* Calculated as: (urinary uric acid x serum creatinine)/(serum uric acid x urinary creatinine) x 100.
levels of TAG, even under fasting conditions, and that a high-fat meal results in an important increase in lipid peroxidation and carboxylated proteins and a decrease in both GSH-Px and GSH-Rd activity. Porter et al. (23) found that a low plasma concentration of GSH-Px constituted a risk factor for coronary disease, and low GSH-Px activity was even detected in atherosclerotic carotid plaques (24). Patients undergoing haemodialysis were found to have a reduced plasma level of GSH-Px, partially explaining the presence of atherogenesis in chronic kidney disease (25). Endogenous GSH-Px is an important antioxidant, protecting against oxidative stress in the atherogenic process; a reduction in GSH-Px levels produced by a high intake of fat could contribute to the onset of atherosclerosis associated with postprandial hypertriglyceridaemia.

The most relevant finding of the present study, though, is that a high-fat meal that increases oxidative stress produces a decrease in circulating antioxidant defences and in uric acid levels, and that this reduction has a very close association with the increase in oxidative stress.

Uric acid is the final product of purine metabolism in man. In many species, uric acid is metabolized to allantoin by urate oxidase, an enzyme activity that has been lost in man by a defect in gene transcription (26).

In the 1980s, Ames et al. proposed that uric acid may have important biological effects as antioxidants, and they showed with in vitro experiments that it is a powerful scavenger of reactive oxygen species (27). Apart from its action as a scavenger of radicals, uric acid also exerts its action chelating metals like iron or copper, converting them into poor reactive species unable to catalyse reactions (28).

Consistent with the antioxidant role of uric acid in vivo is the hypothesis that the loss of urate oxidase in man (and the accompanying rise in serum levels of uric acid) improves antioxidant defence. Watanabe et al. (29) proposed that high levels of uric acid in man provide an evolutionary advantage as the hyperuricaemia maintains blood pressure better in conditions of low dietary salt intake.

A double-blind study evaluated the effects of the systematic administration of 100 mg uric acid compared with 1000 mg vitamin C (30). The authors found a significant increase in the antioxidant capacity of the serum after administration of uric acid and vitamin C, but the effect of the uric acid was substantially greater. Another clinical study suggested an inverse relation between the concentration of uric acid and oxidative stress variables is known, as an accumulation of TAG and associated with the increase in free radicals (19). This increase in free radicals provokes a rise in oxidation and activates the antioxidant mechanisms of the organism. The increased oxidative variables after a high-fat meal and the inability of the antioxidant defences of the organism to contain this increase is known as postprandial oxidative stress (20,21). Oxidative stress has been involved in the pathogenesis of CVD and in the genesis of the metabolic syndrome (22). Moreover, oxidative stress that accompanies postprandial lipidaemia can modify the endothelial function (4).

In the present study we found an inverse association between the antioxidant defences of the organism and the plasma

Table 3. Comparisons between experimental conditions before (baseline) and after fat overload

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median Before</th>
<th>Interquartile range</th>
<th>Median After</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAG (mmol/l)</td>
<td>0.83</td>
<td>0.31</td>
<td>1.60*</td>
<td>0.90</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.18</td>
<td>1.45</td>
<td>5.42*</td>
<td>1.38</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>107</td>
<td>44.75</td>
<td>115</td>
<td>38</td>
</tr>
<tr>
<td>NOx (µmol/dl)</td>
<td>158</td>
<td>26.66</td>
<td>169.47</td>
<td>23.58</td>
</tr>
<tr>
<td>GSH-Px (µ/dl)</td>
<td>6.52</td>
<td>2.02</td>
<td>4.90*</td>
<td>3.20</td>
</tr>
<tr>
<td>CAT (µ/dl)</td>
<td>9.10</td>
<td>3.27</td>
<td>15.90</td>
<td>3.65</td>
</tr>
<tr>
<td>GSH-Rd (µ/dl)</td>
<td>25.00</td>
<td>10.88</td>
<td>7.04*</td>
<td>6.86</td>
</tr>
<tr>
<td>GST (µ/dl)</td>
<td>1.74</td>
<td>1.33</td>
<td>1.88</td>
<td>1.27</td>
</tr>
<tr>
<td>SOD (µ/dl)</td>
<td>31.79</td>
<td>11.15</td>
<td>41.41</td>
<td>17.32</td>
</tr>
<tr>
<td>Uric acid (µmol/l)</td>
<td>288.47</td>
<td>122.52</td>
<td>278.36*</td>
<td>111.22</td>
</tr>
<tr>
<td>LPO (mmol/dl)</td>
<td>298</td>
<td>95</td>
<td>407*</td>
<td>150</td>
</tr>
<tr>
<td>ProCar (mmol/dl)</td>
<td>10.07</td>
<td>1.61</td>
<td>14.59*</td>
<td>5.12</td>
</tr>
</tbody>
</table>

Median values were significantly different from those of the baseline (Wilcoxon paired-sample test): *P < 0.05.

†Antioxidant defence variables: CAT, catalase; GSH-Px, glutathione peroxidase; GSH-Rd, glutathione reductase; GST, glutathione-S-transferase; SOD, superoxide dismutase. Marker of vasodilatations: NOx, total nitrates (nitrates and nitrates). Pro-oxidant variables: LPO, lipid peroxidation; ProCar, protein carbonyl.
stress during aerobic exercise\(^{31}\). High concentrations of circulating uric acid may prevent oxidative stress in vivo during intense physical activity and protect against oxidative stress in other situations\(^{32}\). The present findings are related with this hypothesis to the extent that we show that uric acid is reduced after acute stress (after a high-fat meal) and that this reduction is supposedly because it exerts its antioxidant effect and is converted into allantoin and other products of oxidation\(^{33}\); chronic stress would probably cause an increase in uric acid in order to raise the antioxidant capacity of the plasma. Numerous studies have related uric acid with atherosclerosis\(^{34}\), but some authors suggest that the development of atherosclerosis is accompanied by a compensatory increase in antioxidant capacity, due mainly to the increased serum levels of uric acid\(^{35}\). Experimental findings in animal models found that early elevation of uric acid, during or shortly after acute ischaemic stroke, could confer significant protection against neurological deficit. This is in agreement with the protective effect of uric acid seen in other models of brain disease mediated by free radicals\(^{36}\).

The close association in the present study between the reduction in uric acid after a high-fat meal and the increase in carbonylated proteins may be explained by the role of uric acid, as it can repair tryptophan indolyl and tyrosine phenoxyl radicals\(^{37}\), and it is an oxidizable substrate for the haem protein–hydrogen peroxide system, able to protect against oxidative damage, acting as an electron donor\(^{38}\).

The present findings reinforce the direct association between uric acid and hypertriacylglycerolaemia, where oxidative stress can be the binding factor. We have previously reported the direct relation between TAG levels and uric acid figures\(^{36,7}\). The present study, and others, have shown that TAG and postprandial hyperlipidaemia are directly associated with oxidative stress. Uric acid is a powerful antioxidant, and the data from the present study suggest that the reduction seen after a high-fat meal is because it exerts its antioxidant action acutely. The hypothesis may also be completed arguing that the chronic increase in oxidative stress caused by hypertriacylglycerolaemia may produce a chronic increase in uric acid and antioxidant defences which reflects the increase in oxidative stress, thereby raising the antioxidant capacity of the plasma. If this is confirmed, it would reinforce the theory that uric acid is only a marker of risk, and that prudence would be advisable when using uric acid-lowering drugs in patients with hyperuricaemia.

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