Reduced Postprandial Serum Paraoxonase Activity After a Meal Rich in Used Cooking Fat

Wayne H.F. Sutherland, Robert J. Walker, Sylvia A. de Jong, André M. van Rij, Vicki Phillips, Heather L. Walker

Abstract—Paraoxonase is an enzyme associated with HDL in human serum that hydrolyzes oxidized phospholipids and inhibits LDL oxidation, which is an important step in atherogenesis. In animals, addition of oxidized lipids to the circulation reduces paraoxonase activity, and diets rich in oxidized fat accelerate the development of atherosclerosis. The current randomized, crossover study was designed to compare the effect of a meal rich in oxidized lipids in the form of fat that had been used for deep-frying in a fast food restaurant and a control meal rich in the corresponding unused fat on postprandial serum paraoxonase (aryl esterase) activity and peroxide content of LDL and its susceptibility to copper ion catalyzed oxidation in 12 healthy men. Four hours into the postprandial period, serum paraoxonase activity had decreased significantly after the used fat meal (−17%, \( P=0.005 \)) and had increased significantly after the meal rich in unused fat (14%, \( P=0.005 \)). These changes were significantly \( (P=0.003) \) different. A time-course study indicated that serum paraoxonase activity remained lower than baseline for up to 8 hours after the used fat meal. Serum apoA1 concentration tended to decrease after the unused fat meal and tended to increase after the used fat meal. These changes were different at a marginal level of significance \( (P=0.07) \). Also, a significantly \( (P=0.03) \) greater decrease in apoA1 content of postprandial HDL was recorded after the unused fat meal. The peroxide content of LDL tended to decrease after the used fat meal and tended to increase after the control meal. These changes were significantly \( (P=0.04) \) different. Susceptibility of isolated LDL to copper ion oxidation and plasma levels of malondialdehyde were unchanged during the study. These data suggest that in the postprandial period after a meal rich in used cooking fat, the enzymatic protection of LDL against accumulation of peroxides and atherogenic oxidative modification may be reduced, possibly due to factors associated with apoA1, without acutely affecting the intrinsic resistance of LDL to in vitro oxidation. (Arterioscler Thromb Vasc Biol. 1999;19:1340-1347.)

Key Words: paraoxonase • diet • fats, oxidized • postprandial • apoA1

Oxidative modification of LDL in the artery wall is now widely regarded as playing an important role in the development of atherosclerosis.\(^1\) Binding of LDL to proteoglycans traps LDL in the arterial intima, which provides the opportunity for oxidation of LDL lipids.\(^2\) This mildly oxidized LDL can induce endothelial cells to produce chemotactants, adhesion molecules, and colony stimulating factors for monocytes. Adhesion to the vascular endothelium followed by transmigration of circulating monocytes into the arterial intima are early steps in the formation of atheroembolic lesions. HDL inhibit LDL oxidation\(^3,4\) and the biological effect of mildly oxidized LDL on monocyte endothelial cell interaction\(^5\) in vitro. These properties of HDL may contribute to the inverse association between plasma HDL levels and risk of developing coronary artery disease in epidemiological studies.\(^6\)

There is evidence that paraoxonase activity is partly responsible for inhibition by HDL of LDL oxidation in vitro.\(^4,7\) Paraoxonase is an esterase enzyme that is synthesized by the liver and is associated with HDL in the blood.\(^8\) The enzyme hydrolyzes aromatic carboxylic acid esters,\(^8\) organophosphates,\(^8\) and oxidized phospholipids.\(^5\) Hydrolysis of oxidized phospholipids by paraoxonase destroys the biologically active lipids in mildly oxidized LDL.\(^5\) This action of paraoxonase could potentially attenuate the development of atherosclerosis. Low paraoxonase activity is present in subjects at high risk of coronary artery disease, including those with hypercholesterolemia\(^9\) and diabetes.\(^10\) Also, mice lacking serum paraoxonase activity are susceptible to atherosclerosis.\(^11\)

High levels of lipid oxidation products in the diet accelerate the development of atherosclerosis in animals\(^12,13\) and increase levels of lipid oxidation products in human chylomicrons.\(^14\) It is thought that after meals rich in oxidized fats, endogenous lipoproteins, including LDL, may also become enriched with oxidized lipid\(^14,15\) increasing the susceptibility of LDL to oxidation.\(^16\) In addition, postprandial LDL isolated after a meal rich in dairy fat has altered composition and
increased susceptibility to oxidation,17 which is believed to contribute to the increased risk of coronary artery disease that is associated with prolonged postprandial lipemia.18,19 Fats that have been heated for prolonged periods in air contain numerous compounds derived from the oxidation and breakdown of lipid.20–22 These compounds include lipid hydroperoxides and secondary oxidation products such as aldehydes, polymers, hydroxy fatty acids, hydroperoxylkenals, and hydroperoxy epoxides. Detoxification by a glutatione peroxidase cycle in the gut23 may severely limit absorption of dietary lipid hydroperoxides. However, secondary lipid oxidation products are more readily absorbed20,24 and can inhibit hepatic enzymes in animals.25 In mice that are susceptible to arterial lesion development, an atherogenic diet and injection of mildly oxidized LDL and oxidized lipids into the circulation reduces serum paraoxonase activity.26 Whether a meal rich in lipid oxidation products alters postprandial serum paraoxonase activity and susceptibility of LDL to oxidation in humans is unknown. The aim of the current study was to determine the effect of a meal rich in fat that had been used for deep-frying in a fast-food restaurant on postprandial serum paraoxonase activity, lipid oxidation products in plasma and LDL, and isolated LDL susceptibility to copper ion oxidation in healthy men.

Methods

Subjects
Twelve healthy men (aged 22 to 63 years) who were not taking antioxidant supplements or other medications were recruited from the staff of the University of Otago. Written and informed consent was obtained from each subject and the study was approved by the ethics committee of Southern Regional Health Authority.

Study Design
The study followed a crossover design. Subjects reported to the study center in the early morning after a 12 hour fast. They were randomized to receive a meal (milkshake) containing either fat that had been used for deep-frying or the corresponding unused fat. Subjects consumed the milkshake within 10 minutes. At least 1 week later, the subjects repeated this procedure with the alternate milkshake. Blood was taken at baseline and 4 hours later, which is approximately the peak of postprandial lipemia for healthy subjects. In 3 subjects, blood samples were taken at baseline, 2, 4, 6, 8, and 12 hours after the meals.

Meals
The milkshakes contained the test fat (46 g), ice-cream (100 g, 10 g fat), low-fat milk (150 mL), evaporated milk (50 mL), yogurt (10 g), egg yolks (12 g), egg whites (30 g), canned apricots without the syrup (50 g), and chocolate flavoring. The amount of test fat in the meals is approximately the quantity present in an average meal of Zealand foods.27 The test fats were obtained from a local fast-food restaurant. We obtained samples of the fat that they regularly used for deep-frying just before the weekly change of the cooking fat. A restaurant. We obtained samples of the fat that they regularly used for deep-frying in a fast-food restaurant on postprandial serum paraoxonase activity.26 Whether a meal rich in lipid oxidation products alters postprandial serum paraoxonase activity and susceptibility of LDL to oxidation in humans is unknown. The aim of the current study was to determine the effect of a meal rich in fat that had been used for deep-frying in a fast-food restaurant on postprandial serum paraoxonase activity, lipid oxidation products in plasma and LDL, and isolated LDL susceptibility to copper ion oxidation in healthy men.

Laboratory Measurements
Blood was taken into tubes containing disodium ethylenediaminetetraacetic acid (EDTA) (1.5 mg/mL), heparin (143 IU), and into plain tubes. Blood in the plain tubes was allowed to clot for 20 minutes at room temperature. The tubes containing EDTA blood were kept at 4°C during this time and then serum and plasma were separated by low-speed centrifugation at 4°C.

Serum paraoxonase (arylesterase) activity was measured using phenylacetate as the substrate.5,28 Serum diluted in 20 mmol/L Tris buffer (pH 7.4) was added to 2 mL phenylacetate (1 mmol/L in 20 mmol/L Tris buffer, pH 8), and the increase in absorbance at 270 nm was monitored at 1 minute intervals during the initial 3 minutes. Blanks to correct for spontaneous hydrolysis of phenylacetate were also included. The increase in absorbance was essentially linear during the 3 minute period, and using the molar extinction coefficient 1310 M⁻¹ cm⁻¹ the arylesterase activity was calculated. The intra-assay coefficient of variation was 5% (n=9). Inter-assay coefficient of variation for measurements on aliquots of pooled serum stored at −80°C was 12% (n=42). Plasma malondialdehyde (MDA) concentration was measured in heparin plasma stored at −80°C in the presence of butylated hydroxytoluene (0.19 μmol/mL) by a high-pressure liquid chromatography method.29 Fluorescence (excitation 350 nm, emission 460 nm) of aqueous solutions of serum proteins after delipidation with ethanol/diethyl ether (3:1, vol/vol) was measured30 as an index of circulating fluorescent Schiff’s bases (FSB). Lipid oxidation products were measured in batches of fat that had been used for deep-frying and in the corresponding unused fat using the AOCA standard iodide method (official method Cd 3a-63 for peroxide value). The carboxyl value of the fat was measured by a modification of a spectroscopic quinoidal iodine method31 with the fat and the 2,4 dinitrophenylhydrazine dissolved in isopropanol/toluene (1:1, vol/vol). A molar absorption coefficient of 18 000 M⁻¹ cm⁻¹ was used to calculate carboxyl values from absorbance at 425 nm. The acid value of these batches of fat were measured using standard methods (AOCA official method Cd 3a-63). The fatty acid composition of the cooking fats was determined by gas-liquid chromatography of fatty acid methyl esters prepared using boron trifluoride: methanol as described previously.32 The esters were chromatographed on a 3-P Sili 88 column (50 m×0.25 mm) with argon as the carrier gas and programming the column temperature from 165°C to 210°C at 3°C/min. Plasma protein-bound thiol concentration was measured spectrophotometrically33 with subtraction of absorbance due to plasma blanks.

Plasma HDL cholesterol was isolated in the supernatant after treatment of plasma with dextran sulfate/magnesium chloride34 and HDL, cholesterol was also isolated from plasma by a precipitation method.35 Plasma LDL was separated rapidly in the presence of EDTA according to the method of Gieseg and Esterbauer using a

### Table 1. Characteristics of the Used and Unused Cooking Fats

<table>
<thead>
<tr>
<th></th>
<th>Used Fat</th>
<th>Unused Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxide value, meq/kg</td>
<td>2.1 (1.4–4.0, n=7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Carboxyl value, meq/kg</td>
<td>26.3 (12.9–34.5, n=7)</td>
<td>3.5</td>
</tr>
<tr>
<td>Acid value, meq/kg</td>
<td>116 (43–193, n=7)</td>
<td>7</td>
</tr>
<tr>
<td>Fatty Acid Composition, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>2.7</td>
<td>3.2</td>
</tr>
<tr>
<td>14:0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>16:0</td>
<td>25.9</td>
<td>25.2</td>
</tr>
<tr>
<td>16:1</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>18:0</td>
<td>23.8</td>
<td>18.9</td>
</tr>
<tr>
<td>18:1</td>
<td>36.9</td>
<td>40.9</td>
</tr>
<tr>
<td>18:2</td>
<td>6.9</td>
<td>7.5</td>
</tr>
<tr>
<td>20:0</td>
<td>0.4</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Where applicable, values are mean (range).
Oxidation experiments using the method of Beuge and Aust. and 5 hours during incubation of LDL (79
measuring thiobarbituric acid reacting substances (TBARS) at 0, 2,
plasma apoA2, and apoB were measured by immunoturbidimetry. Free
commercial reagents (Boehringer Mannheim). Plasma apoA1,
transition metal ions. Cholesterol and triglycerides in plasma and
was treated with Chelex-100 resin (Biorad) to remove contaminating
previously. In addition, the oxidation of LDL was also followed by
bance at 234 nm due to conjugated diene formation as described
The oxidation was monitored by measuring the increase in absor-
mperature in an air-conditioned environment at a set temperature.

Values are given as mean±SD unless stated otherwise. The post-
prandial response (4-hour value−baseline) to the meals was com-
pared using the analysis of 2-period crossover trials described by
Hills and Armitage. Paired t tests were used to test for postprandial
changes and to compare baseline values and triglyceride area under
the curve. Repeated measures ANOVA was used to compare
the meal values of percent change paraoxonase activity and percent
change serum apoA1 during the time-course experiment and TBARS
formation during LDL oxidation. ANCOVA was used to adjust for
differences in baseline values of paraoxonase activity and LDL
peroxide content between the meals. Spearman’s rank correlation was
used to test for relationships between variables. Two-tailed tests of
significance were used and P<0.05 was considered to be statistically
significant.

Results
The mean age of the men was 40±13 years and mean body
mass index was 25.7±3.2 kg/m². Subjects consumed on
average 33.3±2.6 g fat/m² body surface area in the meals.
Serum paraoxonase activity and apoA1 concentration for
individual subjects during the study are shown in Table 2. Table 3 shows mean baseline and 4-hour postprandial
changes in serum paraoxonase activity, plasma MDA con-
centration, serum FSB, and plasma protein-bound thiol
groups during the meals. Mean paraoxonase activity
decreased significantly during the used fat meal and increased
significantly during the meal rich in the corresponding
unused fat. These changes were significantly different. There
was no significant period effect or interaction between the
meals in the postprandial response of paraoxonase activity.
Mean baseline levels of serum paraoxonase activity were
significantly (P=0.01) different between the meals. When
postprandial changes in serum paraoxonase activity were
corrected for baseline values by ANCOVA, the difference in
the changes between the meals remained significant
(P=0.04). Plasma MDA, serum FSB, and plasma protein-
bound thiol levels did not change significantly during the
meals.

Figure 1 shows the time-course in percent changes in
serum paraoxonase activity and apoA1 concentration in 3
subjects for 12 hours after the meals. Paraoxonase activity
decreased at 2 hours, remained at a lower level until 8 hours,
and returned to baseline by 12 hours after the meal rich in
used fat. An increase in paraoxonase activity was observed at
2 to 6 hours after the meal containing the unused fat and
activity returned to baseline by 8 hours and remained there for
the rest of the postprandial period. The percent change in

<p>| Table 2. Serum Paraoxonase Activity and ApoA1 Concentration in the Subjects at Baseline and 4 Hours After Meals Rich in Used Fat and Unused Fat |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Paraoxonase, μmol · ml⁻¹ · min⁻¹</th>
<th>ApoA1, g/L</th>
<th>Paraoxonase, μmol · ml⁻¹ · min⁻¹</th>
<th>ApoA1, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>195 141</td>
<td>1.32 1.38</td>
<td>110 121</td>
<td>1.45 1.37</td>
</tr>
<tr>
<td>2</td>
<td>149 141</td>
<td>1.29 1.76</td>
<td>75 100</td>
<td>1.41 1.60</td>
</tr>
<tr>
<td>3</td>
<td>187 100</td>
<td>1.39 1.63</td>
<td>208 226</td>
<td>1.55 1.42</td>
</tr>
<tr>
<td>4</td>
<td>126 125</td>
<td>0.76 0.73</td>
<td>77 96</td>
<td>0.72 0.92</td>
</tr>
<tr>
<td>5</td>
<td>138 127</td>
<td>1.04 0.96</td>
<td>112 174</td>
<td>1.26 1.21</td>
</tr>
<tr>
<td>6</td>
<td>118 89</td>
<td>1.40 1.72</td>
<td>103 111</td>
<td>1.17 1.38</td>
</tr>
<tr>
<td>7</td>
<td>227 211</td>
<td>1.52 1.47</td>
<td>218 218</td>
<td>1.53 1.44</td>
</tr>
<tr>
<td>8</td>
<td>129 126</td>
<td>1.42 1.35</td>
<td>136 140</td>
<td>1.32 1.22</td>
</tr>
<tr>
<td>9</td>
<td>155 128</td>
<td>1.26 1.34</td>
<td>124 113</td>
<td>1.60 1.35</td>
</tr>
<tr>
<td>10</td>
<td>231 174</td>
<td>1.15 1.16</td>
<td>117 157</td>
<td>1.35 1.09</td>
</tr>
<tr>
<td>11</td>
<td>162 157</td>
<td>1.77 1.73</td>
<td>133 144</td>
<td>1.54 1.47</td>
</tr>
<tr>
<td>12</td>
<td>144 124</td>
<td>1.35 1.37</td>
<td>130 153</td>
<td>1.41 1.28</td>
</tr>
</tbody>
</table>

B indicates baseline.
Baseline and 4-Hour Postprandial Change in Serum Paraoxonase Activity and Plasma Malondialdehyde, Fluorescent Schiff’s Bases, and Plasma Thiol Concentrations During the Meals

<table>
<thead>
<tr>
<th></th>
<th>Used Fat Meal</th>
<th>Unused Fat Meal</th>
<th>P&lt;sub&gt;meal&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraoxonase, μmol·mL⁻¹·min⁻¹</td>
<td>163±38 ‡</td>
<td>-27±27†</td>
<td>0.003</td>
</tr>
<tr>
<td>MDA, μmol/L</td>
<td>1.47±0.22</td>
<td>0.06±0.21</td>
<td></td>
</tr>
<tr>
<td>FSB, U/ml</td>
<td>8.5±1.7</td>
<td>0.2±0.5</td>
<td></td>
</tr>
<tr>
<td>Plasma thiols, μmol/g protein</td>
<td>5.70±0.83</td>
<td>-0.04±0.82</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD, n=12. MDA indicates malondialdehyde; FSB, fluorescent Schiff’s bases.

†Postprandial change significant at P=0.005.
‡Baseline levels significantly (P=0.01) different between the meals.

Paraoxonase activity was significantly different between the meals during the time course (ANOVA, P=0.004). Serum apoA1 levels tended to increase during the used fat meal and tended to decreased during the meal rich in unused fat. The percent change in apoA1 concentration (P=0.20) and the area under the curve of the triglyceride concentration (used fat meal: 3.81±0.65 mmol·L⁻¹·h⁻¹, unused fat meal: 4.94±1.49 mmol·L⁻¹·h⁻¹; P=0.43) were not significantly different between the meals during the time-course experiment.

Table 4 summarizes the plasma lipid, lipoprotein, and apolipoprotein levels in the subjects and changes in these variables during the meals. As expected, plasma triglycerides increased significantly during the postprandial period. Also, plasma cholesterol levels increased significantly after the unused fat meal. There was a marginally significant trend toward differential changes in serum apoA1 levels between the meals.

Baseline levels and 4-hour postprandial changes in HDL composition during the study are summarized in Table 5. The content of triglycerides increased significantly after both meals. The content of phospholipid increased and the content of apoA1 decreased significantly after the meal rich in unused fat. This decrease in apoA1 content was significantly different compared with the corresponding change after the meal rich in used fat. Baseline proportions of triglycerides were significantly higher before the used fat meal compared with the meal rich in unused fat.

Figure 2 plots the 4-hour postprandial decrease in serum paraoxonase activity against the corresponding change in serum apoA1 concentration during the used fat meal. These changes were inversely correlated at a marginal level of significance (P=0.07). When an outlier value was excluded from the analysis, a significant correlation was recorded (r=−0.764, n=11; P=0.02). These variables were not correlated significantly during the unused fat meal (r=0.210, n=12; P=0.49).

Peroxide and vitamin E content of LDL and its resistance to copper ion catalyzed oxidation are summarized in Table 6. Peroxide content of LDL tended to increase after the used fat meal and tended to decrease after the meal rich in unused fat. These changes were significantly different. This difference remained marginally significant (P=0.07) when baseline levels of LDL peroxides were taken into account by ANCOVA. The LDL vitamin E content and lag time in conjugated diene formation during oxidation with copper ions did not change significantly during the meals. Also, the time-course in TBARS formation during 5 hour incubation of LDL with copper ions was not significantly different between fasted and postprandial LDL (ANOVA, P=0.87) and between used fat and unused fat meals (ANOVA, P=0.66). The 5-hour values were 20.1±4.4 nmol/μmol LDL cholesterol and 20.2±4.6 nmol/μmol LDL cholesterol (n=12) before and after the used fat meal and 18.2±3.5 nmol/μmol LDL cholesterol and 18.5±3.6 nmol/μmol LDL cholesterol before and after the unused fat meal. Similar values were obtained at the 2-hour point in the incubation.

Discussion

The current study indicates that a meal rich in fat that has been regularly used for deep-frying in a fast-food restaurant decreases postprandial serum paraoxonase arylesterase activity, whereas a meal rich in the corresponding unheated fat has
Postprandial Paraoxonase After Oxidized Fat Meal

There was limited evidence that postprandial changes in serum paraoxonase activity did not influence the resistance of isolated LDL to copper ion catalyzed oxidation. In vivo, however, paraoxonase may be present during the oxidation of LDL, and it is possible that altered activity may modulate the oxidation rate and the level of potentially atherogenic products formed.

We measured the arylesterase activity of the paraoxonase enzyme using phenylacetate, which is a nonphysiological substrate. Phenylacetate may be more suitable than paraoxon as a substrate for paraoxonase and preferable in the context of our study. Paraoxonase catalyzed hydrolysis of phenylacetate and oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine, which may be a physiological substrate of paraoxonase, are not influenced by genetic variation at amino acid 192 in the enzyme. In contrast, paraoxon is hydrolyzed more actively by the R isozyme than the Q allele of paraoxonase. Thus, our use of a relatively nondiscriminatory substrate may reduce the effect of genetic variation on the current data.

There was limited evidence that postprandial changes in serum apoA1 and HDL composition may modulate the corresponding changes in paraoxonase activity after meals rich in used fat and unused fat. The decrease in serum paraoxonase activity was inversely associated with the trend toward an increase in serum apoA1 after the used fat meal (Figure 2). This relationship appears to be more consistent with decreased enzyme activity rather than reduced levels of enzyme during the used fat meal. However, postprandial levels of serum apoA1 may not reflect levels of the specific HDL fraction containing paraoxonase, apoA1, and clusterin. Factors other than those associated with apoA1 may also contribute to the postprandial decrease in serum paraoxonase as apoA1 levels were unchanged but paraoxonase activity was reduced at 2 hours after the used fat meal in the time-course study. After the meal rich in unused fat, HDL content of apoA1 decreased, and changes in HDL lipid fractions tended to be more prominent compared with the alternate meal. Change in HDL composition may influence the conformation of paraoxonase in the hydrophobic environment of the lipoprotein, and this in turn may alter enzyme activity. Apparently, paraoxonase is anchored in HDL lipids by its highly hydrophobic N-terminal end and is also bound to apoA1.

### TABLE 4. Baseline Concentration and 4-Hour Postprandial Change in Plasma Lipids, Lipoproteins, and Apolipoproteins During the Meals

<table>
<thead>
<tr>
<th>Component</th>
<th>Used Fat Meal</th>
<th>Unused Fat Meal</th>
<th>P&lt;sub&gt;meal&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>5.51±1.11</td>
<td>0.10±0.24</td>
<td>5.52±1.22</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.29±0.25</td>
<td>0.01±0.06</td>
<td>1.24±0.24</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;2&lt;/sub&gt;-C, mmol/L</td>
<td>0.34±0.10</td>
<td>−0.02±0.05</td>
<td>0.31±0.13</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;3&lt;/sub&gt;-C, mmol/L</td>
<td>0.95±0.20</td>
<td>0.02±0.05</td>
<td>0.93±0.16</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.43±0.56</td>
<td>0.78±0.40</td>
<td>1.62±0.86</td>
</tr>
<tr>
<td>ApoA1, g/L</td>
<td>1.31±0.25</td>
<td>0.09±0.18</td>
<td>1.36±0.24</td>
</tr>
<tr>
<td>ApoA2, g/L</td>
<td>0.50±0.12</td>
<td>0.03±0.06</td>
<td>0.51±0.09</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.83±0.31</td>
<td>0.02±0.17</td>
<td>0.86±0.27</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=12. TC indicates total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; ApoA1, apolipoprotein A1; and ApoB, apolipoprotein B.

Postprandial change significant at *P<0.05, †P<0.01. Baseline values were not significantly different between the meals.

### TABLE 5. Baseline and 4-Hour Postprandial Change in High Density Lipoprotein Composition During the Meals

<table>
<thead>
<tr>
<th>Component, wt%</th>
<th>Used Fat Meal</th>
<th>Unused Fat Meal</th>
<th>P&lt;sub&gt;meal&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>2.8±0.8</td>
<td>−0.2±0.4</td>
<td>2.7±0.8</td>
</tr>
<tr>
<td>CE</td>
<td>16.5±1.2</td>
<td>−0.8±1.19</td>
<td>15.8±1.3</td>
</tr>
<tr>
<td>TG</td>
<td>7.7±1.6§</td>
<td>0.9±0.9†</td>
<td>6.9±1.7§</td>
</tr>
<tr>
<td>PL</td>
<td>26.8±1.5</td>
<td>0.7±2.0</td>
<td>26.1±2.4</td>
</tr>
<tr>
<td>ApoA1</td>
<td>33.1±1.5</td>
<td>−0.1±2.3</td>
<td>35.5±3.7</td>
</tr>
<tr>
<td>ApoA2</td>
<td>12.8±1.8</td>
<td>−0.1±1.4</td>
<td>12.7±1.3</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=12. FC indicates free cholesterol; CE, cholesteryl esters; TG, triglycerides; and PL, phospholipids.

*Significance of the postprandial change during the used fat meal compared with the meal rich in unused fat.

Postprandial change significant at †P<0.05, ‡P<0.01. §Baseline values significantly different at P<0.05 (paired t test).
The differential changes in postprandial HDL composition and serum apoA1 levels after the meals are unexplained. Whether the used fat and unused fat have differing effects on the formation of nascent HDL in the intestine warrants consideration. Also, apoA1 catabolism may be accelerated during postprandial lipemia after the unused fat meal but not after the used fat meal. Previous studies have reported decreased, unchanged, or increased postprandial levels of apoA1 after fatty meals, which suggests that considerable variation in apoA1 catabolism in relation to synthesis may be possible during the postprandial period. The current postprandial changes in HDL lipid composition including increased triglyceride and phospholipid content and decreased cholesteryl ester content after the meals were generally in line with the corresponding postprandial changes reported previously.

Animal studies suggest that secondary lipid oxidation products, including low molecular weight carbonyl compounds, are absorbed from the gut, inhibit enzyme activities, and form conjugates with glutathione, a thiol containing compound. Derivatisation of cysteine thiol groups on paraoxonase inhibits its activity. In theory, absorbed carbonyl compounds could react with free thiol groups on cysteine residues of paraoxonase and inhibit enzyme activity. In the current study, postprandial levels of plasma protein-bound thiols were not decreased after the meal rich in used fat that contained markedly elevated levels of carbonyl compounds. This finding does not support the hypothesis that carbonyl compounds in the used cooking fat eventually react with thiol groups on plasma proteins including paraoxonase and thereby reduce the activity of the enzyme.

In mice, injection of mildly oxidized LDL or oxidized phosphatidylcholine into the blood reduces plasma paraoxonase activity at 24 hours, apparently by reducing hepatic synthesis of the enzyme. It seems doubtful that a similar mechanism was responsible for the decrease in postprandial serum paraoxonase activity after the used fat meal in the current study as serum paraoxonase activity had returned to baseline at 12 hours after the meal.

Paraoxonase hydrolyzes oxidized phospholipid fatty acids and peroxides including hydrogen peroxide. Thus, the trend toward a rise in LDL peroxides after the used fat meal and the opposite trend after the unused fat meal appear to be consistent with the concomitant changes in serum paraoxonase activity that we observed during the meals. However, these divergent trends in LDL peroxides may not be due to changes in levels of lipid hydroperoxides. Usually lipid hydroperoxide levels in native LDL are very low and may represent only a small fraction of LDL peroxides measured by the iodide method in the current study. Whether protein peroxides contribute to iodide reactive substances in LDL and are hydrolyzed by paraoxonase is unknown. Interestingly, a recent study has reported that in genetically altered mice lacking serum paraoxonase LDL lipid hydroperoxide levels are unchanged.

### TABLE 6. Baseline Levels and Postprandial Change in LDL Peroxides, Vitamin E Content, Lag Time During Copper Ion Oxidation, and Chemical Composition During the Meals

<table>
<thead>
<tr>
<th></th>
<th>Used Fat Meal</th>
<th>Unused Fat Meal</th>
<th>P&lt;sub&gt;meal&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDL peroxides, nmol/μmol C</strong></td>
<td>7.3±2.2</td>
<td>8.1±3.1</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>LDL vitamin E, nmol/μmol C</strong></td>
<td>2.56±0.46</td>
<td>2.75±0.46</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Lag-time, min</strong></td>
<td>111±27</td>
<td>114±26</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>LDL composition, wt%</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>42.3±3.3†</td>
<td>44.5±3.9†</td>
<td>0.52</td>
</tr>
<tr>
<td>FC</td>
<td>8.4±0.9</td>
<td>8.0±1.3</td>
<td>0.66</td>
</tr>
<tr>
<td>TG</td>
<td>6.7±1.4</td>
<td>6.3±1.4</td>
<td>0.77</td>
</tr>
<tr>
<td>PL</td>
<td>18.8±0.9</td>
<td>18.5±1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>PROT</td>
<td>23.8±2.0</td>
<td>23.0±2.5</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=12. LDL indicates low density lipoproteins; C, cholesterol; CE, cholesteryl esters; FC, free cholesterol; TG, triglycerides; PL, phospholipids; and PROT, protein.

*Significance of the postprandial change during the used fat meal compared with the meal rich in the corresponding unused fat.
†Baseline values significantly different at P<0.05. Postprandial change significant at †P<0.05, §P<0.01.
Paraoxonase activity is an important factor in the inhibition of LDL oxidation by HDL in vitro. The current data suggest that changes in paraoxonase activity in vivo do not appreciably alter the intrinsic resistance of isolated LDL to oxidation. Postprandial changes in serum paraoxonase activity were not accompanied by changes in susceptibility of isolated postprandial LDL to oxidation. However, this finding does not preclude the possibility that postprandial LDL after a meal rich in oxidized fat is less protected against oxidation in the artery wall in the presence of HDL that is depleted in paraoxonase activity.

Increased oxidizability of postprandial LDL isolated after a meal rich in dairy fat has been reported previously. In contrast, susceptibility of isolated LDL to oxidation by copper ions did not change noticeably after meals rich in thermally stressed cooking fat or unheated fat in the current study. Differences in the type and content of fat in the meal, the characteristics of the subjects, the techniques used to isolate LDL, and the postprandial change in LDL composition may underlie these conflicting findings. In the previous study, there were roughly equal numbers of men and women in the trial, the fat content of the meal was very high and was mainly of dairy origin, the plasma LDL fraction was isolated by zonal ultracentrifugation and required concentration, and postprandial LDL was richer in triglycerides and phospholipids and poorer in free cholesterol and cholesteryl esters. In contrast, we studied only men, the meals were markedly lower in fat that was mainly derived from lard, a single step gradient method was used for isolating LDL in a relatively concentrated form, and postprandial LDL was poorer in both triglycerides and free cholesterol and tended to be richer in cholesteryl esters.

This study has limitations. The study population was relatively small and did not include women. Thus, care should be exercised in the extrapolation of the findings to other populations. Baseline values of serum paraoxonase activity were markedly different before the 2 meals. However, the difference in postprandial response of paraoxonase activity to the meals remained when data were corrected for baseline levels. The magnitude of inter-assay variation in the paraoxonase measurements was not sufficiently large to account for the difference in paraoxonase activities between the 2 baseline points. Also, a carryover effect of the meals on baseline serum paraoxonase activities seems unlikely as there was at least a week between the test meals during which time several free-choice meals would have been consumed.

In summary, the current study indicates that a meal rich in fat that has been used for deep-frying in a fast-food restaurant reduces serum paraoxonase arylesterase activity, whereas a meal containing the corresponding unused fat has the opposite effect. This decrease in paraoxonase activity may be potentially atherogenic considering reported associations between low serum paraoxonase activity and increased risk of coronary artery disease. Furthermore, we have recently shown that a meal rich in used cooking fat markedly reduces endothelium-dependent vasodilatation, which is an early change preceeding the development of atherosclerosis. We conclude that regular consumption of fast-foods deep-fried in cooking fats rich in lard is ill advised not only in the light of the metabolic changes that we have documented here but also because diets rich in saturated fat and energy increase plasma cholesterol levels and adiposity that increase the risk of coronary artery disease.

Acknowledgments

The authors are grateful to the participants in the study and to Sheila Williams for statistical advice. The study was supported by a grant from the National Heart Foundation of New Zealand.

References


52. Williams MJ, Sutherland WHF, McCormick MP, de Jong SA, Walker RJ, Wilkins GT. Impaired endothelial function following a meal rich in used cooking fat. *J Am Coll Cardiol.* In press.
Reduced Postprandial Serum Paraoxonase Activity After a Meal Rich in Used Cooking Fat
Wayne H. F. Sutherland, Robert J. Walker, Sylvia A. de Jong, André M. van Rij, Vicki Phillips and Heather L. Walker

_Arterioscler Thromb Vasc Biol_. 1999;19:1340-1347
doi: 10.1161/01.ATV.19.5.1340

_Arteriosclerosis, Thrombosis, and Vascular Biology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/5/1340

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Arteriosclerosis, Thrombosis, and Vascular Biology_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Arteriosclerosis, Thrombosis, and Vascular Biology_ is online at:
http://atvb.ahajournals.org//subscriptions/