Effects of two lipid-lowering, carotenoid-controlled diets on the oxidative modification of low-density lipoproteins in free-living humans

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ABSTRACT

This study compares the effects of two lipid-lowering diets [a diet enriched in MUFAs (monounsaturated fatty acids) and a HCLF (high-carbohydrate/low-fat) diet] with a controlled carotenoid content on risk factors for coronary heart disease, including in vitro copper-induced LDL (low-density lipoprotein) oxidation and serum lipid levels. A randomized crossover dietary intervention study, with two diets each consumed for 14–16 days, was conducted in 18 women and 13 men aged 20–70 years, recruited via personal contacts and advertisements in newspapers. Both diets (MUFA-enriched diet and HCLF diet) contained the same basic foods and had a controlled carotenoid content, high in lycopene. The in vitro copper-induced oxidation of isolated LDL showed a longer lag phase (mean difference 7.4 min in women and 7.34 min in men) after the MUFA-enriched diet compared with the HCLF diet. Serum total cholesterol, LDL cholesterol and carotenoid levels were similar after the two diets. Serum triacylglycerol levels were significantly lower and those of HDL (high-density lipoprotein) cholesterol were significantly higher at the end of the MUFA-enriched diet compared with the HCLF diet. It is concluded that the significantly longer lag phase for oxidation of LDL, the higher HDL cholesterol level and the lower triacylglycerol level in subjects following a carotenoid-controlled, MUFA-enriched diet may decrease the risk of coronary heart disease.

INTRODUCTION

Replacement of saturated fat in the diet with other sources of energy is required in order to reduce the risk of CHD (coronary heart disease). However, debate continues as to which source of energy, i.e. monounsaturated fat, polyunsaturated fat or carbohydrate, should replace this saturated fat. All three substitutes seem to lower serum levels of total cholesterol and LDL (low-density lipoprotein) cholesterol. Meta-analyses suggest a neutral effect of replacement of saturated fat with unsaturated fat on levels of HDL (high-density lipoprotein) cholesterol and triacylglycerols (triglycerides) [1–3]. However, when carbohydrates replace the saturated fat, HDL is decreased and triacylglycerols are increased. Still, there is no epidemiological evidence for a relationship between an increased risk of CHD and the decreased levels of serum HDL or high levels of serum triacylglycerol achieved by HCLF (high-carbohydrate/low-fat) diets [3,4].

In addition to serum lipid and lipoprotein levels, increased lipid oxidative stress has been suggested as a cause of an increased risk of cardiovascular disease [5,6]. Increased oxidation of LDL can lead to the formation of foam cells and subsequent arteriosclerosis [7]. As well as being a major determinant of serum LDL concentration, dietary fat intake can also alter the susceptibility of LDL oxidation.

Key words: carotenoid, dietary intervention, low-density lipoprotein oxidation, monounsaturated, saturated fat.

Abbreviations: CHD, coronary heart disease; HCLF, high-carbohydrate/low-fat; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acid.

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to oxidative modification. Various measures of oxidative susceptibility have been used and all have limitations [8], but a number of studies have used an in vitro stimulation test. Polyunsaturated fat, especially n-6, in the LDL particles increases the susceptibility of LDL to oxidation, as displayed in vitro by the shorter lag phase before the start of LDL oxidation [9,10]. On the other hand, LDL particles enriched with MUFAs (monounsaturated fatty acids) appear to be resistant to oxidation for a longer time, with lower production of conjugated dienes and a longer lag phase [11–14].

Studies that have compared the effects of MUFAs and carbohydrates on the oxidation of LDL in vitro [15–17] have shown a beneficial effect of MUFA-enriched diets in increasing the lag phase, indicating increased resistance of LDL to oxidation. However, none of these studies have clearly indicated whether the amount and type of carotenoid intake was controlled or not. Differences in carotenoid intake may well influence the results. Studies on the effects of sequential changes in the levels of antioxidants on the copper-induced oxidative modification of LDL have shown that the disappearance of carotenoids is preceded by the disappearance of vitamin E and ubiquinol-10 [6,18]. Following the disappearance of carotenoids, the rate of lipid peroxidation increases rapidly. However, experimental studies aimed at establishing the relationship between supplemental β-carotene and the oxidative modification of LDL in vitro failed to show any significant effect [19–22]. In contrast, the enrichment of LDL with β-carotene in vitro has been shown to reduce the susceptibility of LDL to oxidative modification [23,24]. A possible link between lycopene and myocardial infarction has also been reported [25], but intervention studies of the effects of lycopene supplementation on the oxidative modification of LDL have shown equivocal results [26–29].

The aim of the present dietary intervention study was to compare the effects of two diets, i.e. a MUFA-enriched diet and an HCLF diet, with the amount of carotenoids in the two diets carefully controlled, on CHD risk factors, such as susceptibility of LDL to oxidation and serum lipid and lipoprotein levels, in healthy free-living women and men.

METHODOLOGY

Subjects
A total of 20 women and 13 men were recruited by personal contacts and newspaper advertisements. All subjects were healthy, were non-smokers and were not taking any antibiotics or mineral and/or vitamin supplements. Pregnant, lactating and perimenopausal women and women on hormone replacement therapy were excluded from the study. Deakin University Ethics Committee, Melbourne, Australia, approved the study protocol, and each subject gave written, informed consent.

Study design
A randomized crossover design was used to compare the effects of the two diets, i.e. MUFA-enriched diet and HCLF diet. Both diets contained the same basic foods and a controlled total carotenoid content, high in lycopene. A computer-generated randomization sheet was used; ten female and seven male subjects started on the MUFA-enriched diet first, and ten female and six male subjects started on the HCLF diet. The study protocol was slightly different in the two groups, but the study aims were the same. The duration of the dietary periods was 14 days in men and 16 days in women. A ‘washout’ period of 6 weeks was allowed between the two diets, during which subjects returned to their habitual diets. This washout period was to allow serum lycopene levels to return to baseline. It was also to synchronize pre-menopausal women in the same phase of the menstrual cycle at the start of both dietary periods in order to avoid any effects on serum lipids and/or serum carotenoid levels [30].

Dietary design
A set menu plan was designed for each person for each of the two dietary periods (MUFA-enriched and HCLF diets), providing meals that were isocaloric to his or her habitual diets (analysed from 4-day weighed food records collected before the start of the study and analysed using NUTTAB 95). All subjects were asked to consume a low-carotenoid diet for 2–3 days prior to starting the two dietary periods, to allow a blood measurement unaffected by the acute peaks of serum carotenoid concentration that may occur 10–12 h after the intake of carotenoid-rich food [31].

The diets were designed to provide approx. 36 % of energy from fat in the MUFA-enriched diet (mainly from MUFAs) and approx. 16 % of energy from fat in the HCLF diet. An oleate-enriched variant of sunflower oil (Sunola™ oil), provided by Meadowlea Foods Ltd (Mascot, Australia), was used for the MUFA-enriched diet. Sunola™ oil contains more oleic acid and less saturated fatty acids than olive oil [32]. The diets were designed to have a similar content of carotenoid, protein, cholesterol, fibre and vitamin C.

Energy balance was maintained for the two diets using non-carotenoid-containing foods, such as white bread, carbonated beverages and Polyjoule (Nutricia), a glucose polymer. The two diets differed in their content of vitamin E, mainly due to the different amounts and types of oil used in the diets.

To control carotenoid intake, subjects were allowed a limited amount and variety of fruits and vegetables. Each subject consumed 100 g of frozen peas and corn mix in every evening meal. Fruit intake was restricted to
one piece per day, either an apple or a banana. Intake of alcoholic drinks was limited to one drink a day, and subjects were asked to avoid the consumption of red wine. The diets were high in lycopene (> 90% of total carotenoid intake per day), which was achieved by the consumption of tomato soup and tomato paste or canned tomatoes. Table 1 lists the lycopene-containing foods for both the men’s and women’s diets. Women were given a lower amount because of their lower energy intake, and to allow assessment of the effects of the pattern (three meals for women and two larger intakes for men) of different intakes. The difference in the lycopene protocol for the two groups (women and men) was that the women consumed tomato soup every other day, whereas men ate tomato soup every day. In addition, canned tomatoes were consumed by women, whereas men consumed tomato paste. Guava nectar was given only to women. The same batch and brand of tomato products (donated by H. J. Heinz, Melbourne, Australia) were used, to avoid seasonal and processing differences in the carotenoid content of the foods [33,34]. Tomato products were cooked, according to food and hygiene regulations, in the university food laboratory, divided into portions, frozen and given to the subjects for the dietary periods. Written as well as verbal instructions were given for heating the tomato soup, and thawing and reheating of the tomato dishes, prior to consumption.

Each subject’s height was measured at the start of the first diet. Body weight was measured at the start and monitored throughout the two dietary periods, using electronic scales. To attain good compliance, subject selection was performed judiciously, and highly motivated people were selected. Subjects were given full information on the restriction of diets and the need for compliance. Subjects were telephoned at least twice a week, for encouragement and to check if they were experiencing any problems with the diet. Subjects were also asked to complete 4-day dietary records at the end of each dietary period, which were used for analysing dietary intake as well as for assessing compliance.

### Blood collection and analysis
Fasting venous blood samples were taken at the start (day 1) and after the last day (day 17 for women and day 15 for men) of the two dietary periods. All subjects were asked to fast for 10–12 h, during which time only drinking of water was allowed. Venous blood samples were collected between 07.30 and 09.30 hours. To separate the plasma, blood collected in EDTA tubes was centrifuged at 1200 g at 4 °C for 20 min immediately after collection. For serum separation, the blood was allowed to coagulate for 1 h (protected from natural light) and then centrifuged at 1200 g at 4 °C for 20 min. Plasma and serum were aliquotted and stored at −70 °C for later analysis. All biochemical analyses were subsequently carried out in the same run to reduce inter-assay variability.

### Biochemical analysis
Fasting serum levels of total cholesterol, triacylglycerols and HDL cholesterol were measured using an enzymic colorimetric test on a centrifugal autoanalyser (Hitachi Boehringer Mannheim Automatic Analyser 704), using commercially available enzymic colorimetric kits (Boehringer Mannheim, Melbourne, Australia). Levels of LDL cholesterol were calculated using the Friedewald equation [35]. LDL was isolated and copper-induced oxidative modification was measured spectroscopically using the method described by Ashton and Ball [36]. Briefly, LDL was isolated by ultracentrifugation at 15 °C at 416 640 g for 2 h in a Beckman Optima TLX-100 centrifuge. The LDL band was removed through the side of the tube by needle and then dialysed in deoxygenated PBS (pH 7.4) overnight. CuCl (1 mM) was added to the isolated LDL to induce the oxidation reaction, which was monitored at 234 nm at 37 °C on a UV-1601 spectrophotometer (Shimadzu, Tokyo, Japan). Maximum diene formation was calculated from the molar absorption coefficient for conjugated dienes (295 000 l·mol⁻¹·cm⁻¹), as described by Abbey et al. [12]. The lag phase (min) was determined as the intercept between linear portion of the lag and propagation phase.
Serum carotenoids were analysed using the HPLC method described by Su et al. [37]. Briefly, an internal standard of \( \alpha \)-tocopherol acetate in 95 % (v/v) ethanol solution was added to serum samples to denature the proteins. Hexane was added to separate the phase containing carotenoids, which was then dried under nitrogen. The sample was reconstituted with 40 \( \mu \)l of chloroform and then with 80 \( \mu \)l of acetonitrile/methanol (1:1, v/v), and transferred to amber-coloured vials for analysis by HPLC. The HPLC system included Waters Alliance 2690 separations, a Waters 996 Photodiode Array Detector and Millennium chromatography manager PDA software (Waters, Melbourne, Australia). The HPLC mobile phase was acetonitrile/methanol/chloroform (45:45:10, by vol.), containing 0.5 % ammonium acetate in methanol and 0.1 % triethylamine in acetonitrile. The flow rate was 1 ml/min, and the run time was 20 min. Individual carotenoids (lutein + zeaxanthin, \( \beta \)-carotene, \( \alpha \)-carotene, trans-lycopene, cis-lycopene and \( \beta \)-cryptoxanthin) were monitored at 450 nm. The results generated were compared with a standard curve to calculate the concentration of serum carotenoids. Isolated LDL cholesterol was also analysed for carotenoid content, in samples from men after the two dietary periods, using the same method as for serum carotenoid levels.

**Statistical analysis**

SPSS software (version 9, 1999; SPSS, Chicago, IL, U.S.A.) was used for the analysis of all data obtained. The data were first assessed for normality of distribution, and logarithmic transformation was performed on non-normally distributed data (lipids, carotenoids and LDL oxidation). Non-parametric tests were performed if data were not normally distributed even after logarithmic transformation. Spearman’s correlation coefficient was used to identify any relationship between the lag phase of LDL oxidation and serum lycopene levels. All data are presented as mean ± S.D. A \( P \) value of \(< 0.05\) was taken as statistically significant.

**RESULTS**

Of the 20 women, 18 completed both dietary periods; two female subjects withdrew from the study on the fourth day of the first diet for personal reasons. All 13 men completed the two dietary periods. The basic characteristics of the study groups are presented in Table 2.

The dietary intake of macronutrients on the two diets for both women and men is presented in Table 3. Although the diets were designed to be isocaloric, the energy intake of a few subjects was higher on the MUFA-enriched diet compared with the HCLF diet; hence there was a significant difference in mean energy intake between the two diets. In accordance with the study design, carbohydrate and total fat intake were significantly different between the MUFA and HCLF diets; vitamin C, alcohol and cholesterol intake were similar on the two diets. Although the diets were designed to have a similar fibre content, the 4-day dietary analysis in women showed a slightly higher intake of dietary fibre on the HCLF diet (\(< 10 \%\)) compared with the MUFA-enriched diet (\( P = 0.01\)). This was due to a higher intake of white bread, which was used to make up the energy intake on the HCLF diet, and it is unlikely that this would have had any effect on the parameters measured. In men, no significant difference in fibre intake was noted. Total intake of sugar (\( P < 0.01\)) and starch (\( P < 0.01\)) were also higher on the HCLF diet. The vitamin E content of the MUFA-enriched diet was higher than that of the HCLF diet in both men and women.

Serum levels of total cholesterol and LDL cholesterol were similar after the two diets (Table 4). Levels of HDL cholesterol were higher, and the LDL/HDL ratio and triacylglycerols were lower, after the MUFA-enriched diet compared with the HCLF diet. Serum levels of trans-lycopene, cis-lycopene and total lycopene were not significantly different at the end of the two dietary periods. The lycopene content of isolated LDL cholesterol was also similar at the end of the two diets (measured in samples from men). The lag phase for the oxidation of LDL was longer by 7.4 min in women and 7.34 min in men following the MUFA-enriched diet compared with the HCLF diet. There were no significant differences in the rates of oxidation or conjugated diene formation at the end of MUFA and HCLF diets in men or women. No correlation was detected between the lag phase of LDL oxidation and serum lycopene levels.

**DISCUSSION**

Previous studies [15–17] comparing the effects of monounsaturated fat and carbohydrate on the oxidation of isolated LDL cholesterol do not clearly indicate if the intake of carotenoids (i.e. the antioxidants from fruits and vegetables) was controlled or not, yet this may affect the results [23,24,38]. In fact, the study by O’Byrne et al. [17]

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Table 2  Baseline characteristics of the study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 18)</th>
<th>Men (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40 ± 16</td>
<td>39 ± 11</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23 ± 4</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.05 ± 1.26</td>
<td>5.54 ± 0.85</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.52 ± 0.67</td>
<td>1.07 ± 0.22</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.10 ± 1.02</td>
<td>3.84 ± 0.88</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>0.96 ± 0.32</td>
<td>1.65 ± 1.06</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>2.28 ± 0.96</td>
<td>3.65 ± 0.87</td>
</tr>
</tbody>
</table>
Table 3  Nutrient intake on the HCLF and MUFA diets
Values are means ± S.D., and were calculated from 4-day weighed food intake records, obtained during the final 4 days on each diet. *P < 0.05 compared with HCLF diet (Wilcoxon signed ranks test).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Women (n = 18)</th>
<th>MUFA-enriched diet</th>
<th>Men (n = 13)</th>
<th>MUFA-enriched diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCLF diet</td>
<td></td>
<td>HCLF diet</td>
<td></td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>7.6 ± 1.4</td>
<td>8.4 ± 1.3*</td>
<td>10.2 ± 1.2</td>
<td>11.4 ± 1.9*</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>89.1 ± 17.5</td>
<td>85.5 ± 17.2</td>
<td>109.6 ± 19.7</td>
<td>106.3 ± 20.8</td>
</tr>
<tr>
<td>% energy from protein</td>
<td>19.8 ± 1.5</td>
<td>17.4 ± 1.8</td>
<td>18.1 ± 1.8</td>
<td>15.9 ± 1.2</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>33.6 ± 4.7</td>
<td>86.0 ± 11.8*</td>
<td>39.2 ± 8.0</td>
<td>116.4 ± 18.1*</td>
</tr>
<tr>
<td>% energy from fat</td>
<td>16.6 ± 1.9</td>
<td>38.2 ± 2.1*</td>
<td>14.2 ± 2.8</td>
<td>37.9 ± 2.2*</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>286.6 ± 56.8</td>
<td>219.7 ± 37.5</td>
<td>412.9 ± 50.9</td>
<td>303.8 ± 57.4*</td>
</tr>
<tr>
<td>% energy from carbohydrate</td>
<td>60.0 ± 2.9</td>
<td>42.0 ± 2.2*</td>
<td>64.6 ± 3.3</td>
<td>42.6 ± 2.7*</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>9.0 ± 2.0</td>
<td>14.3 ± 2.4*</td>
<td>12.1 ± 3.4</td>
<td>19.4 ± 2.9</td>
</tr>
<tr>
<td>% of total fat</td>
<td>30.4 ± 3.2</td>
<td>17.1 ± 1.3*</td>
<td>34.3 ± 4.6</td>
<td>17.4 ± 1.7</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>10.3 ± 1.8</td>
<td>59.3 ± 8.2*</td>
<td>11.6 ± 2.6</td>
<td>74.9 ± 12.8*</td>
</tr>
<tr>
<td>% of total fat</td>
<td>35.2 ± 2.3</td>
<td>71.9 ± 1.4*</td>
<td>33.4 ± 1.8</td>
<td>66.6 ± 2.0*</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>10.1 ± 0.9</td>
<td>9.1 ± 1.2</td>
<td>11.1 ± 2.4</td>
<td>18.0 ± 2.5*</td>
</tr>
<tr>
<td>% of total fat</td>
<td>34.4 ± 4.3</td>
<td>11.0 ± 0.6*</td>
<td>32.3 ± 4.7</td>
<td>16.1 ± 2.2*</td>
</tr>
</tbody>
</table>

Table 4  Serum lipids, lipoproteins, lycopene concentrations and LDL oxidation for women and men at the end of two diets
N/A, not available. Values are means ± S.D. *P < 0.05 compared with HCLF diet (Wilcoxon signed ranks test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Women (n = 18)</th>
<th>MUFA-enriched diet</th>
<th>Men (n = 13)</th>
<th>MUFA-enriched diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.34 ± 1.12</td>
<td>4.27 ± 1.04</td>
<td>4.86 ± 0.63</td>
<td>4.89 ± 0.77</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.22 ± 0.38</td>
<td>1.36 ± 0.44*</td>
<td>0.96 ± 0.20</td>
<td>1.13 ± 0.23*</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.68 ± 1.08</td>
<td>2.55 ± 0.83</td>
<td>3.13 ± 0.63</td>
<td>3.16 ± 0.68</td>
</tr>
<tr>
<td>LDL/HDL cholesterol</td>
<td>2.36 ± 1.15</td>
<td>1.99 ± 0.68*</td>
<td>3.39 ± 0.85</td>
<td>2.96 ± 0.94*</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.01 ± 0.40</td>
<td>0.84 ± 0.23*</td>
<td>1.78 ± 0.77</td>
<td>1.37 ± 0.50*</td>
</tr>
<tr>
<td>trans-Lycopene (µmol/l)</td>
<td>0.21 ± 0.11</td>
<td>0.26 ± 0.12</td>
<td>0.60 ± 0.13</td>
<td>0.59 ± 0.25</td>
</tr>
<tr>
<td>cis-Lycopene (µmol/l)</td>
<td>0.12 ± 0.07</td>
<td>0.13 ± 0.05</td>
<td>0.35 ± 0.14</td>
<td>0.41 ± 0.24</td>
</tr>
<tr>
<td>Total lycopene (µmol/l)</td>
<td>0.33 ± 0.17</td>
<td>0.38 ± 0.18</td>
<td>0.95 ± 0.26</td>
<td>0.99 ± 0.40</td>
</tr>
<tr>
<td>LDL lycopene (µmol/L of LDL)</td>
<td>N/A</td>
<td>N/A</td>
<td>0.04 ± 0.04†</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>Lag phase of LDL oxidation (min)</td>
<td>33.72 ± 6.00</td>
<td>41.13 ± 6.09*</td>
<td>37.16 ± 4.62</td>
<td>44.50 ± 6.05*</td>
</tr>
<tr>
<td>Maximum diene formation (nmol of diene/mg of LDL)</td>
<td>248.30 ± 55.57</td>
<td>229.24 ± 41.73</td>
<td>218.70 ± 40.11</td>
<td>202.33 ± 71.68</td>
</tr>
<tr>
<td>Oxidation rate (nmol of diene · min⁻¹ · mg⁻¹ of LDL)</td>
<td>10.90 ± 2.93</td>
<td>10.00 ± 2.35</td>
<td>6.00 ± 1.21</td>
<td>5.60 ± 2.13</td>
</tr>
</tbody>
</table>

†n = 12 men.

clearly indicated a different intake of β-carotene (mean 9668 µg on an HCLF diet and 4741 µg on a MUFA-enriched diet). In addition, in that study, LDL oxidation was compared between baseline and the end of each diet, and not at the end of the two diets [17]. These diets were not well controlled, as the group on the low-fat diet consumed less polyunsaturated fat than at baseline, and the group on the MUFA-enriched diet consumed less saturated fat and polyunsaturated fat compared with baseline. As content of polyunsaturated fat in the diet may affect the oxidation results [9,10,39], it cannot be ascertained whether the decreased susceptibility of LDL to oxidation seen was due to a reduced intake of polyunsaturated fat or to the higher intake of MUFAs in the diet.

In the study by Ashton et al. [16], the energy content of the HCLF diet was maintained using lemonade, yoghurt and fruit. Fruit and vegetables are the major sources of carotenoids, and hence there is a possibility that the carotenoid content of the two diets was different. Berry et al. [15] apparently controlled for the intake of fruits and vegetables, but carotenoid levels in blood were not
measured to confirm that they were the same. The present investigation is unique because not only the intake of carotenoids, but also factors that may have an effect on their bioavailability, such as type of fruit and vegetables, heating temperature and date of canning of tomato products, as well as the cooking time of food [33,34], were controlled, as discussed in the Methods section. In addition, serum levels of lycopene and other carotenoids were analysed and found to be similar at the end of the two dietary periods, confirming that the dietary intake of these carotenoids was similar on the two diets in both men and women. Although the lycopene levels in the women were lower on both the MUFA and HCLF diets, the differential effect of the diets on in vitro oxidation was still seen. Only the intake of vitamin E was not controlled, because the foods that contain large amounts of vitamin E are also rich in fat. Controlling vitamin E would have required supplements of vitamin E in the HCLF diet, which would have changed the emphasis of the study, as the aim was to compare diets containing the same basic foods and different amounts of oils, and not food with supplements. Moreover, epidemiological evidence suggests that supplements of vitamin E do not exert similar effects in reducing CHD risk as foods rich in vitamin E [40,41].

The fact that isolated LDL obtained from subjects after the MUFA-enriched diet resisted in vitro oxidation for a longer time than that obtained after the HCLF diet would have an important physiological consequence of decreasing the risk of CHD if it reflects the situation in vivo. A lower maximum level of conjugated diene formation after a MUFA-enriched diet, possibly because of a lower content of polyunsaturated fat in the LDL particles, was observed by Ashton et al. [16]. In the present study, there was no statistically significant difference in the maximum level of conjugated dienes between the two diets, but the mean level on the MUFA-enriched diet was 7–8 % lower than that on the HCLF diet for both study groups (men and women). The reason for the non-significant difference could be that the polyunsaturated fat content of the LDL was insufficiently replaced by MUFA [11,21,42], probably because the dietary periods in the present study were shorter than in the study by Ashton et al. [16]. Analysis of the fatty acid content of the LDL may have been useful in assessing this, but was not undertaken.

The endogenous antioxidant content of the LDL particle can influence the oxidation of LDL [43]. The carotenoid content of LDL isolated after the two diets (MUFA and HCLF) was also analysed in men and found to be similar. This suggests that the increase in the lag phase before LDL oxidation after the MUFA-enriched diet compared with the HCLF diet was due to one or a combination of the following factors: high content of MUFAs, vitamin E content or unidentified components of the Sunola™ oil.

The effects of the two diets on serum total cholesterol and LDL cholesterol were in accordance with the findings of the meta-analysis by Clarke [1], although the dietary periods were relatively short. The HCLF diet resulted in higher triacylglycerol and lower HDL levels, probably because of the enhanced endogenous synthesis of palmitic acid, which Knopp et al. [44] suggests “may negate the benefits of saturated fat restriction, if the total dietary fat is reduced to less than 25 % of energy”.

In conclusion, in this randomized crossover study, in which carotenoid intake was controlled, the lower susceptibility of LDL to oxidation, higher HDL cholesterol levels and lower triacylglycerol levels in subjects following the MUFA-enriched diet indicates that a diet enriched in Sunola™ oil may specifically decrease the risk of CHD compared with a HCLF diet.

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