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The effect of quality and amount of dietary fat on the susceptibility of low density lipoprotein to oxidation in subjects with impaired glucose tolerance

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Objectives: We examined the effects of a high fat diet rich in monounsaturated fat (MUFA-diet) and a moderate fat diet rich in polyunsaturated fat (PUFA-diet) on the susceptibility of LDL to oxidation.
Subjects: 29 subjects with impaired glucose tolerance.
Methods: After consuming a run-in diet [37% of energy (E%) fat, 18 E% saturated fat] for three weeks, subjects were randomly assigned either to a MUFA-diet (40 E% fat, 19 E% monounsaturated fatty acids) or a PUFA-diet (34 E% fat, 10 E% polyunsaturated fat) for eight weeks. The susceptibility of LDL to oxidation was measured by challenging LDL with hemin and H_2O_2 and measuring the time for the reaction to reach maximum velocity. Results are expressed as lag time to oxidation in minutes.
Results: In the PUFA-diet group ($n = 15$) lag time tended to decrease during the experimental diet (97 ± 28 *vs* 90 ± 25 min, mean ± s.d., $P = 0.073$), whereas in the MUFA-diet group ($n = 14$) there was no significant change (lag time 96 ± 24 *vs* 100 ± 16 min, $P = 0.408$). The mean change in lag time was -7 ± 14 min (-7.2%) in the PUFA-diet group and $+4 \pm 16$ min ($+4.0\%$) in the MUFA-diet group ($P = 0.029$, PUFA-diet group *vs* MUFA-diet group). The α-tocopherol concentration in LDL increased significantly ($P < 0.01$) in both diet groups relative to the run-in diet period, but LDL particle score did not change in either of the diet groups during the dietary intervention.
Conclusions: In subjects with impaired glucose tolerance a PUFA-rich diet with a moderate amount of fat tended to increase the susceptibility of LDL to oxidation as compared to a higher fat diet rich in MUFA. Furthermore, the negative mean change in lag time to oxidation found in the PUFA-diet group differed significantly from the slightly positive mean change found in the MUFA-diet group.
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Descriptors: diet; impaired glucose tolerance; LDL; oxidation; saturated fat; monounsaturated fat; polyunsaturated fat; cholesterol

Introduction

Low density lipoprotein (LDL) is considered to be an atherogenic particle and oxidative modification seems to further increase the atherogenicity of this particle (Esterbauer *et al*, 1990; Steinberg *et al*, 1989). There is strong evidence that oxidative modification of LDL occurs *in vivo* (Palinski *et al*, 1989; Ylä-Herttuala *et al*, 1989). Oxidized LDL is not recognized by LDL receptors, but it is taken up by scavenger receptors on macrophages (Parthasarathy, 1987; Rohrer *et al*, 1990; Sparrow *et al*, 1989). This leads to foam cell formation due to the inability of intracellular cholesterol to downregulate the activity of the scavenger receptors (Brown & Goldstein, 1983; Steinberg *et al*, 1989). In addition, oxidized LDL has been shown to be cytotoxic, chemotactic for monocytes, and alters vascular tone and gene expression in arterial wall cells (Cathcart *et al*, 1989; Cushing *et al*, 1990; Liao *et al*, 1991; Quinn *et al*, 1987).

Factors that have been demonstrated to affect the susceptibility of LDL to oxidation include the fatty acid composition and antioxidant content of the particle, particle size and the degree of glycosylation of the particle. LDL particles seem to be more susceptible to oxidation after consumption of a polyunsaturated fat enriched diet than after consumption of a monounsaturated fat enriched diet both in normocholesterolemic and hypercholesterolemic subjects (Abbey *et al*, 1993; Berry *et al*, 1991; Bonanome *et al*, 1992; Reaven *et al*, 1991, 1993, 1994). Furthermore, small dense particles have been found to be more susceptible to oxidation than large buoyant particles (Reaven *et al*, 1994; DeGraaf *et al*, 1991; Tribble *et al*, 1992; Chait *et al*, 1993; Dejager *et al*, 1993). Increasing the α-tocopherol content of LDL via supplementation has been reported to decrease the susceptibility of LDL to oxidation (Esterbauer *et al*, 1991; Reaven & Witztum, 1993; Jialal & Grundy, 1992; Jialal *et al*, 1995; Princen *et al*, 1995). However, variations due to naturally occurring α-tocopherol in the diet has not (Babiy *et al*, 1990; Esterbauer *et al*, 1991; Esterbauer *et al*, 1992; Jessup *et al*, 1990). Glycosylation of LDL has been shown to increase the susceptibility of this particle to oxidation. This has been considered as one possible mechanism for the increased incidence of atherosclerosis in diabetic patients (Bowie *et al*, 1993; Campbell *et al*, 1994; Kawamura *et al*, 1994; Kimura *et al*, 1995; Sobenin *et al*, 1993), especially those patients that are not

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under optimal glycemic control.

The optimal diet with respect to the total fat and fatty acid composition of the diet for NIDDM subjects remains controversial. Diets relatively low in fat [< 30% of energy (E%)] and high in carbohydrates (> 55 E%) may increase concentrations of serum triglycerides and VLDL cholesterol, and decrease HDL cholesterol as compared to a lower carbohydrate diet (40–45 E%) (Coulston *et al*, 1989; Garg *et al*, 1992; Garg *et al*, 1994). A diet rich in monounsaturated fatty acids seems to decrease the concentration of serum triglycerides and increase the concentration of HDL cholesterol, and may also have beneficial effects on glucose metabolism compared to a diet high in carbohydrates (Garg *et al*, 1988; Parillo *et al*, 1992). A diet with > 30 E% fat with the amount of monounsaturated fatty acids up to 20 E% has recently been recommended for NIDDM patients who have elevated concentrations of serum triglycerides and LDL cholesterol (Position Statement, 1994).

The aim of the present study was to examine the effects of the recently recommended type of diet high in fat (40 E%) and rich in monounsaturated fatty acids (MUFA-diet) and a moderate fat (34 E%) diet rich in polyunsaturated fat (PUFA-diet) on the susceptibility of LDL to oxidation in subjects with impaired glucose tolerance (IGT). These subjects, as those with NIDDM, are at increased risk for atherosclerotic vascular diseases.

Methods

Subjects

Twenty-nine subjects (17 males, 12 females) were recruited for this study from a Finnish FinMonica Survey (Salomaa *et al*, 1989). The primary inclusion criterion for the study was impaired glucose tolerance according to the World Health Organisation (WHO) criteria. An oral glucose tolerance test (OGTT) with a 75 g glucose load was performed twice before the beginning of the study: the first OGTT about one year and the second OGTT four weeks before the beginning of the study. Subjects whose results indicated IGT in both measurements were included in the present study. None of the subjects had a history of thyroid, kidney or liver disease, previously diagnosed diabetes or present use of lipid lowering medication. Six of the subjects used β-blockers and three used diuretics for hypertension. Their medication was kept unchanged during the study period. There were no differences in the use of medication between the two diet groups. The whole study group consisted of 31 subjects, however, samples for measurement of the susceptibility of LDL to oxidation were not available on two subjects and for this reason results of 29 subjects are presented in this paper. Results of the whole study group regarding serum lipids, fatty acids, plasma CETP activity, glucose tolerance and clotting factors have been previously reported (Sarkkinen *et al*, 1996; Niskanen *et al*, 1997).

Study design

Prior to random assignation to one of the experimental diet periods all subjects participated in a three-week run-in period during which they consumed a baseline diet (Sarkkinen *et al*, 1996; Niskanen *et al*, 1997). After this period the subjects consumed the experimental diet for 8 weeks; 15 subjects (8 males, 7 females) were on the PUFA-diet and 14 (9 males, 5 females) were on the MUFA-diet. Samples for assessment of the susceptibility of LDL to oxidation were drawn at the end of the run-in period and at the end of the experimental diet period. Each subject gave their informed consent for the study and the study plan was approved by the Ethics Committee of the University of Kuopio.

Diets

All diets in this study were isocaloric. The goals for the fatty acid composition of the baseline diet consumed during the run-in period were 18 E% saturated (S), 12 E% monounsaturated (MU), and 6 E% polyunsaturated (PU) fatty acids (FA). For the MUFA-diet the goals were 10 E% SFA, 18 E% MUFA, and 8 E% PUFA and for the PUFA-diet 10 E% SFA, 10 E% MUFA, 10 E% PUFA (Sarkkinen *et al*, 1996). A nutritionist gave detailed written and oral instructions to the subjects on the dietary regimen, specifying the amount and quality of all items by food groups: fats, milk and milk products, meat and fish, bread and cereals, potato, rice or pasta, vegetables, fruit and berries. The subjects were instructed to minimize the amount of invisible fat and use as visible fat the spreads and oils provided to them by the study. During the baseline diet subjects were provided with butter and a small amount of low erucic acid rapeseed (LEAR) oil. The PUFA-diet group consumed sunflower oil and margarine based on sunflower oil. During the experimental diet period the MUFA-diet group consumed LEAR oil, margarine based on LEAR oil and salad dressing made of Trisun®-oil (high-oleic acid sunflower oil). Dietary fats and salad dressing were provided free of charge, on a single blind basis for the subjects. Diets were isocaloric. The individual energy intake levels were estimated by using a four day food record kept by the subjects before the study.

Dietary compliance was monitored by repeated four day food records kept once during the run-in period and three times during the experimental diet period. During the experimental diet period the subjects were given immediate feedback by a nutritionist based on the food records. Nutrient intake was calculated using the Micro-Nutrica® software package for dietary analysis (Rastas *et al*, 1993). Fatty acid composition of serum cholesteryl esters was used as an objective marker of compliance to the diets.

Laboratory measurements

Blood was collected in tubes containing EDTA after a 12 h fast and plasma was separated by centrifugation at 3000 rpm at 4°C. Serum lipid and lipoprotein concentrations were analysed immediately after centrifugation. For other measurements the samples were immediately frozen and stored at −80°C until analysed.

Isolation of LDL. LDL was isolated by single discontinuous density gradient ultracentrifugation in a near-vertical rotor (NVT90, Beckman Instruments, Palo Alto, CA) (Chung *et al*, 1980) as previously described (Cuchel *et al*, 1996). Briefly, plasma density was adjusted to 1.35 g/mL with solid KBr and the sample was layered under 0.9% NaCl with 0.5 M phenylmethylsulphonyl fluoride in Beckman Optiseal polyallomer tubes. Samples were centrifuged in a Beckman L8-80 ultracentrifuge at 339 000 × g (70 000 rpm) at 4°C for 90 min with a sample pool of plasma used as a control. Immediately after isolating the LDL fraction by a gradient fractionator (Hoefer Scientific Instruments, San Francisco, CA) cholesterol concentration in samples was analysed by an enzymatic method previously described (Shireman & Durieux, 1993).

Susceptibility of LDL to oxidation. Immediately after analysis of cholesterol concentration, susceptibility of LDL to oxidation was assessed as previously described (Cuchel *et al*, 1996). Briefly, LDL was challenged with hemin and H_2O_2 (Balla *et al*, 1991). Each sample was analysed in quadruplicate. Oxidation of LDL was monitored by measuring the decreasing absorbance of hemin at 405 nm using an MR60 Microplate reader (Dynatech Laboratories, Chantilly, VA). Susceptibility of LDL to oxidation is expressed as lag time to oxidation, which was assessed by calculating the time required for the reaction to reach maximum velocity (Belcher *et al*, 1993).

α-Tocopherol concentration. For the measurement of α-tocopherol concentration in LDL, isolated LDL was cryopreserved with sucrose as previously described (Rumsey *et al*, 1992). Sucrose as 50% (w/v) solution was added to the LDL sample so that the concentration of sucrose was 10% (w/v) in the sample. Samples were stored at −70°C until analysis of α-tocopherol concentration by high-performance liquid chromatography (HPLC) (Meydani *et al*, 1989). Tocol (Hoffman-LaRoche) was used as an internal standard. Eluted peaks were detected with a 650-15 Perkin-Elmer Fluorescence Spectrophotometer (Perkin-Elmer, Norwalk, CT) set at 292 nm excitation and 330 nm emission wavelengths. Results are expressed as μg per mg of LDL cholesterol.

LDL particle size. LDL particle size was analysed by separating LDL subclasses using 2–16% polyacrylamide agarose gel electrophoresis (Pharmacia, Piscataway, NJ) as previously described (Campos *et al*, 1992). An LKB Ultrascan XL laser densitometer (LKB Instruments, Paramus, NJ) was used to scan the gels, and LKB GSXL software for peak integration. The LDL score represents the relative area under each LDL peak. A smaller particle score corresponds to larger LDL particle size.

Serum lipids and lipoproteins. The VLDL fraction was isolated from plasma by ultracentrifugation for 18 h at density of 1.006 kg/L. HDL in the infranatant was separated from LDL by precipitation of LDL with dextran sulfate and magnesium chloride (Penttilä *et al*, 1981). LDL cholesterol was calculated as the difference between the cholesterol concentration in the infranatant and HDL. Enzymatic colorimetric methods with commercial kits (Monotest® Cholesterol and Triglyceride GPO-PAP, Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) were used for the determination of cholesterol and triglycerides from the whole serum and separated lipoprotein fractions with Kone Specific Clinical Analyzer (Kone Ltd, Espoo, Finland).

Fatty acid composition of serum cholesteryl esters. In the analysis of the fatty acid composition of serum cholesteryl esters, serum samples were extracted with chloroform-methanol (2:1) and the lipid classes were separated by solid phase extraction with an aminopropyl column (Ågren *et al*, 1992). Fatty acids were analysed with Carlo Erba Vega 6130 gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with NB-351 silica capillary column (HNU-Nordion Ltd, Helsinki, Finland). The standards used to determine recovery and to identify individual fatty acids were purchased from Sigma Chemical (St. Louis, MO) and used as previously described in detail (Ågren *et al*, 1992).

Statistical analysis

Statistical analyses were performed with SPSS 4.0 statistics program (Norusis, 1990). Paired *t*-test was used for two-tailed comparisons within a diet group and the differences between the two diet groups were analysed with Student *t*-test. Pearson's correlation coefficient was used to explore the relationship between variables. The results are given as mean ± s.d. except in figures in which the results are given as mean ± s.e.m.

Results

The baseline characteristics of the subjects are presented in Table 1. The age range of the subjects was 48–64 y (median 56 y) in the PUFA-diet group and 47–62 y (median 58 y) in the MUFA-diet group. The subjects had elevated serum total and LDL cholesterol concentrations according to the NCEP criteria (1994). All of the subjects were overweight: BMI 27.8–35.5 kg/m^2 (median 30.1 kg/m^2) in the PUFA-diet group and 25.5–32.5 kg/m^2 (median 29.4 kg/m^2) in the MUFA-diet group.

According to the results of repeated food records the dietary fat modification was successful (Table 2). The total fat content of the MUFA-diet was 6 E% higher than the PUFA-diet. This difference was primarily contributed by a higher level of MUFA in this diet (19 E% *vs* 10 E%). Body weight of the subjects decreased by 1.0% (85.5 ± 7.2 *vs* 84.5 ± 7.1, $P = 0.013$) in the PUFA-diet group and by 0.9% (78.2 ± 11.4 *vs* 77.5 ± 11.3, $P = 0.030$) in the MUFA-diet group. However, the mean change in body weight did not differ between the groups ($P = 0.691$).

The results of the fatty acid composition of serum cholesteryl esters confirmed the results of the repeated food records regarding the fat modification: in the PUFA-diet group the proportion of oleic (C18:1, n-9) and α-linolenic (C18:3, n-3) acids was significantly lower ($P < 0.01$) and the proportion of linoleic acid was significantly higher ($P < 0.01$) at the end of the experimental diet period than at the end of the baseline diet (Table 3). In the MUFA-diet group the proportion of oleic and α-linolenic acids was significantly higher ($P \leq 0.05$) at the end of the experimental diet than at the end of the baseline diet. The linoleic to oleic acid ratio increased in the PUFA-diet group ($P = 0.0001$) and decreased in the MUFA-diet group ($P = 0.025$). These data provide indirect evidence that there was good compliance of the subjects with the dietary modification.

Table 1 Baseline characteristics of the subjects

	PUFA (n = 15)	*MUFA (n = 14)*
Age (y)	56.3 ± 5.2	56.1 ± 5.6
BMI (kg/m^2)[a]	31.0 ± 2.9	29.1 ± 2.3
Plasma glucose (mmol/L)[b]		
0 h	6.47 ± 0.85	6.49 ± 1.27
2 h	9.79 ± 2.62	8.06 ± 1.75
Serum total and lipoprotein lipids (mmol/L)		
Total C[c]	6.38 ± 1.00	6.27 ± 1.25
HDL-C	1.32 ± 0.25	1.16 ± 0.20
LDL-C	4.34 ± 0.95	4.07 ± 1.13
VLDL-C	0.71 ± 0.41	1.04 ± 0.92
Triglycerides	1.81 ± 0.92	2.61 ± 2.72

[a] BMI indicates body mass index.
[b] Based on the oral glucose tolerance test.
[c] C indicates cholesterol.

Table 2 Proportion of energy nutrients and fatty acids, and intake of cholesterol and fiber during the baseline diet and the study diets according to repeated food records

	PUFA-group (n = 15)		*MUFA (n = 14)*	
	Baseline diet [a]	*PUFA-diet* [b]	*Baseline diet* [a]	*MUFA-diet* [b]
Fat (E%)[c]	37 ± 6	34 ± 5	37 ± 5	40 ± 4
Fatty acids (E%)				
Saturated	18 ± 4	11 ± 2	17 ± 2	11 ± 1
Monounsaturated	11 ± 2	10 ± 2	11 ± 2	19 ± 2
Polyunsaturated	5 ± 1	10 ± 2	6 ± 2	8 ± 1
Cholesterol (mg/MJ)	42 ± 10	30 ± 7	43 ± 9	30 ± 6
Carbohydrates	44 ± 5	46 ± 5	45 ± 7	42 ± 5
Fiber (g/MJ)	3.1 ± 0.6	3.2 ± 0.5	2.8 ± 0.7	2.7 ± 0.6
Protein (E%)	18 ± 4	18 ± 3	16 ± 2	16 ± 3

[a] Mean of 4 days.
[b] Mean of 12 days.
[c] E% indicates percent of energy.

Table 3 Fatty acid composition of serum cholesteryl esters in the diet groups

	PUFA (n = 15)		*MUFA (n = 14)*		
	0 wk	*8 wk*	*0 wk*	*8 wk*	*P*[a]
			mol% of total		
16:0	11.46 ± 1.38	11.24 ± 0.85	12.18 ± 0.85	12.50 ± 3.13	0.093
18:0	1.09 ± 0.17	1.01 ± 0.21	1.08 ± 0.25	1.11 ± 0.61	0.049
18:1	19.70 ± 2.05	16.45 ± 2.23[b]	18.52 ± 1.63	22.10 ± 5.54[c]	0.208
18:2, n-6	51.62 ± 4.00	56.71 ± 5.85[b]	52.36 ± 3.65	48.29 ± 9.33	0.623
18:3, n-3	0.68 ± 0.23	0.48 ± 0.15[d]	0.73 ± 0.23	1.20 ± 0.61[c]	0.094
20:4, n-6	5.70 ± 1.39	5.33 ± 1.24	5.73 ± 1.42	5.48 ± 2.08	0.146
			ratio		
18:2/18:1	2.66 ± 0.46	3.54 ± 0.78[e]	2.86 ± 0.40	2.33 ± 0.65[c]	0.280

Values are means ± s.d.
[a] *P*-value for the difference between the diet groups at week 8.
[b,c,d] Difference within a diet group.
[b] $P < 0.001$; [c] $P \leq 0.05$; [d] $P < 0.01$; [e] $P < 0.0001$.

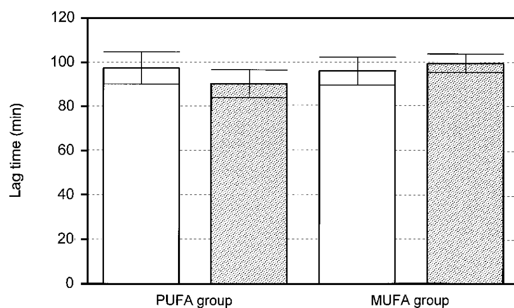

Figure 1 Lag time to oxidation at the end of the baseline diet and at the end of the experimental diet in the diet groups; white bars = baseline diet, dotted bars = experimental diet. Values are mean ± s.d., $n = 15$ in the PUFA-diet group and $n = 14$ in the MUFA-diet group.

Lag time to oxidation tended to decrease in the PUFA-diet group, although the difference did not reach statistical significance (97 ± 28 *vs* 90 ± 25 min, $P = 0.073$) (Figure 1). In the MUFA-diet group there was no significant change in the lag time to oxidation (96 ± 24 *vs* 100 ± 16 min, $P = 0.408$). The difference in lag time between the diet groups at the end of the experimental periods did not reach statistical significance ($P = 0.114$). However, the mean change in lag time during the experimental periods differed significantly between the diet groups: −7 ± 14 min (−7.2%) in the PUFA-diet group *vs* 4 ± 16 min (4.0%) in the MUFA-diet group ($P = 0.029$).

Table 4 α-Tocopherol concentration in LDL (μg/mg LDL-C) and LDL particle size expressed as particle score in the diet groups

	PUFA (n = 15)		*MUFA (n = 14)*	
	0 wk	*8 wk*	*0 wk*	*8 wk*
α-Tocopherol (μg/mg LDL-C)	3.94 ± 0.48	4.49 ± 0.66[a]	4.09 ± 0.74	4.74 ± 1.19[a]
LDL particle score	2.77 ± 0.89	2.81 ± 0.79	3.43 ± 0.73	3.43 ± 0.67

[a] Difference within a diet group ($P \leq 0.01$).

In the PUFA-diet group the proportion of oleic acid in serum cholesteryl esters correlated positively ($r = 0.62$, $P = 0.014$) and the proportion of linoleic acid approached significant negative correlation ($r = -0.49$, $P = 0.066$) with lag time to oxidation at the end of the experimental period. The linoleic to oleic acid ratio correlated significantly with lag time to oxidation ($r = -0.61$, $P = 0.015$). In the MUFA-diet group only the proportion of α-linolenic acid in serum cholesteryl esters correlated significantly with lag time to oxidation ($r = -0.57$, $P = 0.034$).

During the experimental diet period the α-tocopherol concentration in LDL increased significantly in both diet groups. Between the groups there was no difference in α-tocopherol concentration (Table 4). LDL particle size did not change during the experimental periods and no difference between the diet groups were found (Table 4). No significant correlations were found between the

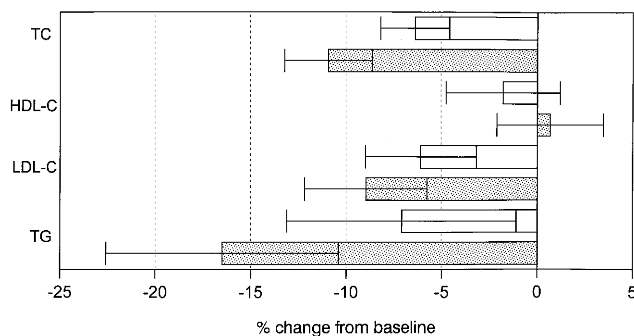

Figure 2 Mean changes as percents in concentrations of serum total, HDL and LDL cholesterol and total triglycerides; white bars = PUFA-diet group ($n = 15$), dotted bars = MUFA-diet group ($n = 14$). No significant differences between the diet groups.

α-tocopherol concentration of LDL or LDL particle size and lag time.

There were no significant differences in any of the serum lipid variables between the diet groups after the experimental periods (Figure 2) (Sarkkinen *et al*, 1996). The mean serum total and LDL cholesterol concentrations were significantly lower after consumption of the experimental diets as compared to the baseline diet in both the PUFA- and the MUFA-diet groups. There was no significant change in serum HDL cholesterol concentrations in either of the diet groups. The concentration of serum total triglycerides decreased by 13% in the PUFA-diet group and by 22% in the MUFA-diet group. However, neither of these differences reached statistical significance.

Discussion

There is evidence that in diabetic patients the susceptibility of LDL to oxidation is increased due to glycosylation of the particles (Bowie *et al*, 1993; Campbell *et al*, 1994; Kawamura *et al*, 1994; Kimura *et al*, 1995; Tsai *et al*, 1994) and the same may be true in subjects with IGT. Studies in subjects with IGT are scarce and there are no studies examining the effect of the fatty acid composition of a diet on the susceptibility of LDL to oxidation in these subjects. For this reason we examined the effect of a high fat diet rich in monounsaturated fat (MUFA-diet) and a moderate fat diet rich in polyunsaturated fat (PUFA-diet) on the susceptibility of LDL to oxidation in middle aged subjects with well characterized impaired glucose tolerance.

The subjects consumed one of the experimental diets after a three week run-in period during which a baseline diet was consumed (Sarkkinen *et al*, 1996; Niskanen *et al*, 1997). The baseline diet was relatively high in fat (37 E%) derived mainly (49%) from saturated fatty acids. Although exceeding the recommendation for total fat the fatty acid and cholesterol content of the PUFA-diet resembled that recommended for the National Cholesterol Education Program Step 1 diet (NCEP 1994). Approximately one third (29%) of the fat in the PUFA-diet was contributed by polyunsaturated fatty acids. The MUFA-diet contained somewhat more fat than the PUFA-diet (40 E% *vs* 34 E%, respectively). Approximately half of the fat (48%) in this diet was monounsaturated fat. Compared to the PUFA-diet, the MUFA-diet contained about twice as much as MUFA than the PUFA-diet (19 E% *vs* 10 E%). The intake of saturated fat, cholesterol and dietary fiber was similar in the two diet groups. Therefore, the only differences between the experimental diets were in the amount of fat (6 E%) and in the amount of monounsaturated fatty acids (9 E%).

The results of the fatty acid composition of serum cholesteryl esters indicate that compliance of the subjects to the dietary fat modification in this study was very good. The levels of fatty acids in serum cholesteryl esters at the beginning of the study resembled the ones we have found in our previous studies using the same method (Sarkkinen *et al*, 1994; Schwab *et al*, 1996). During the experimental diet the proportion of linoleic acid increased and the proportions of oleic and α-linolenic acids decreased in the PUFA-diet group reflecting increased intake of sunflower oil. On the contrary, in the MUFA-diet group the proportions of oleic and α-linolenic acids increased indicating increased intake of rapeseed oil.

The susceptibility of LDL to oxidation was assessed in the present study by challenging LDL with hemin and H_2O_2. This assay assesses the effect of the total LDL particle composition on the susceptibility of LDL to oxidation (Tribble *et al*, 1995) since hemin has been demonstrated to readily intercalate into LDL particles and cause rapid oxidation (Balla *et al*, 1991). The results of this assay have been demonstrated to correlate well with the more widely used $CuSO_4$ mediated oxidation method (Belcher *et al*, 1993).

Lag time to oxidation decreased in the PUFA-diet group after consumption of the experimental diet for eight weeks whereas there was no significant change in lag time in the MUFA-diet group. The mean change in lag time between the diet groups differed significantly: in the PUFA-diet group lag time decreased by 7 ± 14 min whereas in the MUFA-diet group it increased by 4 ± 16 min. In the PUFA-diet group lag time to oxidation correlated positively with the proportion of the monounsaturated oleic acid and tended to correlate negatively with the proportion of the polyunsaturated linoleic acid in serum cholesteryl esters. Lag time to oxidation correlated negatively with the linoleic to oleic acid ratio. In the MUFA-diet group the proportion of the polyunsaturated α-linolenic acid correlated negatively with lag time. These results are in accordance with previously published data in normocholesterolemic and hypercholesterolemic subjects (Abbey *et al*, 1993; Berry *et al*, 1991; Bonanome *et al*, 1992; Reaven *et al*, 1991, 1993, 1994).

The α-tocopherol concentration in the LDL particle increased in both diet groups. The probable reason for this is the increased intake of this vitamin during the experimental diets due to the larger amount of vegetable oils in these diets compared to the subjects' habitual diet. There was no significant difference between the diet groups after consumption of the experimental diet. The significance of α-tocopherol to the susceptibility of LDL to oxidation is controversial: α-tocopherol has been reported to be the most powerful antioxidant in the LDL particle (Esterbauer *et al*, 1991) and α-tocopherol supplementation has been reported to decrease the susceptibility of LDL to oxidation (Reaven & Witztum, 1993; Jialal & Grundy, 1992; Jialal *et al*, 1995; Princen *et al*, 1995). However, the α-tocopherol concentration in LDL particles has not been found to correlate with the susceptibility of LDL to oxidation in subjects who have not been taking α-tocopherol supplements (Babiy *et al*, 1990; Esterbauer *et al*, 1991, 1992; Jessup *et al*, 1990).

LDL particle size has been reported to affect the susceptibility of LDL to oxidation so that the small, dense particles are more susceptible to oxidation than the

large buoyant ones (Chait *et al*, 1993; DeGraaf *et al*, 1991; Dejager *et al*, 1993; Reaven *et al*, 1994; Tribble *et al*, 1992). In the present study LDL particle size did not change in either of the diet groups and there was no significant difference in this variable between the groups.

Conclusions

In subjects with impaired glucose tolerance a moderate fat diet rich in PUFA tended to increase the susceptibility of LDL to oxidation as compared to a high fat diet rich in MUFA. Furthermore, the negative mean change in lag time to oxidation found in the PUFA-diet group differed significantly from the slightly positive mean change in lag time found in the MUFA-died group. Hence, these data would suggest that, regarding the susceptibility of LDL to oxidation, the high fat diet rich in MUFA would be more beneficial to patients with IGT than a moderate fat diet rich in PUFA.

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