# Dietary Unsaturated Fatty Acids in Type 2 Diabetes

## Higher levels of postprandial lipoprotein on a linoleic acid–rich sunflower oil diet compared with an oleic acid–rich olive oil diet

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OBJECTIVE — The present study was undertaken to examine the effect of a polyunsaturated fat diet compared with an isocaloric Mediterranean-style monounsaturated fat diet.

RESEARCH DESIGN AND METHODS — This was a randomized 2-week crossover study on either a high-polyunsaturated or a high–monounsaturated fat diet in 11 well-controlled diabetic men. Blood was taken fasting and for up to 8 h after a high fat meal. Lipoproteins were isolated by sequential ultracentrifugation. Apolipoprotein (apo) B48 and apo B100 were separated by PAGE. Fatty acids were analyzed by gas-liquid chromatography.

RESULTS — Fasting blood glucose and insulin levels were significantly higher on the linoleic acid diet compared with the oleic acid diet ($P < 0.01$ and $P < 0.002$, respectively). Plasma cholesterol and LDL cholesterol levels were also significantly higher on the linoleic acid diet ($P < 0.001$). Likewise, fasting chylomicron apo B48 and apo B100 ($P < 0.05$) and postprandial chylomicron and VLDL apo B48 and B100 ($P < 0.05$) were also higher on the linoleic acid diet.

CONCLUSIONS — This study suggests that, in type 2 diabetes, an oleic acid–rich Mediterranean-type diet versus a linoleic acid–enriched diet may reduce the risk of atherosclerosis by decreasing the number of chylomicron remnant particles.

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Dyslipidemia is considered a major cause for the increased atheroma found in diabetes, especially because strong evidence linking hyperglycemia and atheroma remains elusive (1–4). Postprandial lipoproteins are thought to be particularly atherogenic (5–8), and disturbance of postprandial lipoproteins is a common finding in diabetes (9–11). The major postprandial lipoprotein is the chylomicron, which has apolipoprotein (apo) B48 as its structural protein. Recently apo B–specific binding sites, which bind to apo B48, have been discovered on the human monocyte/macrophage, which suggests a mechanism that might explain the atherogenicity of these particles (12). The other postprandial particle is the apo B100–containing VLDL, which is produced by the liver. We and others (11,13–15) have shown very considerable disturbance in both the amount and the size of these particles in diabetes. We have demonstrated in the experimental animal that clearance and synthesis of these particles account for the abnormalities found in diabetes (16,17). The understanding of the regulation of hepatically derived VLDL has advanced with the discovery of the peroxisome proliferator–activated receptors (PPARs) and their effect on gene regulation (18). This has provided an explanation of how the fibrates reduce VLDL and lower serum triglyceride levels (19). It has become clear that fatty acids also act as regulators of gene expression (19). Many studies have examined the effect of change from a carbohydrate-rich to a fat-rich diet in diabetes (20,21) or a saturated to an unsaturated fat diet in both diabetic and nondiabetic subjects (22). Comparison of a polyunsaturated to a monounsaturated fat diet is of considerable interest in view of the interest in the benefits of a Mediterranean-type diet (23). van Greevenbroek et al. (24) have shown different effects of polyunsaturated compared with monounsaturated fat in the ability to stimulate the secretion of apo B48 in Caco-2 cells. However, we have been unable to find any information on the effect of a polyunsaturated diet compared with a monounsaturated fat diet on postprandial lipoproteins in diabetes and, in particular, on the intestinally derived apo B48–containing chylomicron. The present study was undertaken to examine the effect of a polyunsaturated fat diet compared with an isocaloric Mediterranean-style monounsaturated fat diet on postprandial lipoproteins.

## RESEARCH DESIGN AND METHODS

### Subjects

A cohort of 11 well-controlled (mean $HbA_{1c}$ 5.7 ± 0.8%) type 2 diabetic men (mean age 56.0 ± 2.5 years) was randomly recruited for the study from those patients who regularly attended the diabetes clinic. Patients with renal or hepatic dysfunction, patients with triglyceride levels >4 mmol/l, and patients on lipid-lowering agents or other drugs that would interfere with lipid metabolism were excluded. Patients were euthyroidal and

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Abbreviations: apo, apolipoprotein; AUC, area under the curve; MTP, microsomal triglyceride transfer protein; PPAR, peroxisome proliferator–activated receptor; sf, Suedberg Units.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Subject characteristics*

| | Linoleic acid diet | Oleic acid diet |
|---|---|---|
| Age (years) | 56.0 ± 2.5 | — |
| BMI (kg/m$^2$) | 27.7 ± 2.6 | 27.8 ± 2.2 |
| Fasting glucose (mmol/l) | 8.5 ± 0.8* | 7.6 ± 0.7 |
| Fasting insulin (mmol/l) | 13.4 ± 2.9† | 11.8 ± 2.3 |
| Plasma cholesterol (mmol/l) | 5.3 ± 0.6‡ | 4.9 ± 0.5 |
| Plasma triglyceride (mmol/l) | 1.9 ± 0.9 | 1.7 ± 0.6 |
| Median | 1.4 | 1.3 |
| Range | 1.1–3.6 | 1.0–2.5 |
| LDL cholesterol (mmol/l) | 3.2 ± 0.5‡ | 3.0 ± 0.5 |
| HDL cholesterol (mmol/l) | 1.1 ± 0.3 | 1.1 ± 0.3 |

Data are means ± SD unless otherwise indicated. *$P < 0.01$ vs. oleic acid diet; †$P < 0.002$ vs. oleic acid diet; ‡$P < 0.001$ vs. oleic acid diet.

their diabetes and weight had been stable for at least 3 months. One patient was treated with diet alone, and 10 were treated with diet and sulfonylurea. Approval from the hospital ethics committee was obtained, and all subjects gave informed consent.

### Study design

This was a randomized 2-week crossover study with subjects on either a high–polyunsaturated or a high–monounsaturated fat diet. We used a 2-week period because the chylomicron and VLDL particles mostly reflect the fatty acids of the previous meal. On the other hand, if the fatty acids affect the regulation of particle synthesis or turnover, this may be through the regulation of gene expression via the PPAR system (19), perhaps necessitating a few days on each diet. Patients were interviewed by a qualified dietitian and were randomly instructed on an isocaloric diet either high in monounsaturated fat, including 30 ml olive oil daily, or high in polyunsaturated fat, including 30 ml sunflower oil daily. After 2 weeks, patients were again seen by the dietitian and switched to the other diet.

At the end of each dietary period, fasting blood was taken, and patients were given a high-fat breakfast containing a 50-g glucose load, which they consumed over 30 min. The meal consisted of a standard serving of orange juice containing 50 g glucose, 30 g cereal with 0.15 g milk, two slices of white bread fried in a commercial sunflower or olive oil (30 ml Sunflower [Flora] or olive oil [Don Giovanni], depending on diet), two fried eggs, two fried tomatoes, and a cup of tea or coffee. The meal contained 1,100 kcal, of which 55% of the calories were fat. Patients on oral hypoglycemic agents took their medication as usual, and blood was sampled every 2 h over the next 8 h.

### Methods

**Lipoprotein isolation.** Blood was centrifuged to separate plasma and cells, and the following preservatives were added to prevent degradation of apo B: D-Phe-Pro-Arg-chloromethyl ketone (PPACK) (1 mmol/l), phenylmethyl-sulfonyl fluoride (PMSF) (0.1 mmol/l), aprotinin (0.005%), sodium azide (0.02%), and EDTA (0.1 g/l). A chylomicron-rich sample was isolated as previously described (13) by layering the plasma under an equal volume of saline (density 1.006 kg/l) containing the above preservatives and then centrifuging at 20,000 rpm for 30 min at 4°C. Chylomicrons were washed and concentrated by relayering under the same solution, and they were reisolated by centrifugation for 30 min at 20,000 rpm at 4°C. VLDL (density <1.006 kg/l) was isolated from the infranate by ultracentrifugation at 40,000 rpm for 24 h at 4°C. The density of the fasting sample infranate was raised to 1.025 kg/l and centrifuged for 24 h to remove intermediate-density lipoprotein, and fasting LDL (density 1.025–1.063 kg/l) was isolated from the infranate by sequential ultracentrifugation (25). Chylomicron and VLDL apo B48 and apo B100 were analyzed within 2 days, and chylomicron, VLDL, and LDL samples were stored at 4°C. Lipoprotein composition was measured within 1 week. LDL was stored at −20°C for fatty acid determination.

Plasma and lipoprotein cholesterol levels were measured by enzymatic colorimetric methods using commercially available kits (Boehringer Mannheim, Mannheim, Germany). Plasma and lipoprotein triglycerides and phospholipids were measured with kits from BioMerieux (Charbonnieres les Bains, France). Chylomicron, VLDL, LDL, and HDL protein was estimated by a modification of the Lowry method (26). Blood glucose was measured by an enzymatic method (Boehringer Mannheim), and $HbA_{1c}$ was determined using an enzyme immunoassay method (Novo Nordisk, Cambridge, England) (normal value <4.9%). Serum total insulin was measured using a microparticle enzyme immunoassay, which does not cross-react with proinsulin (Abbott, North Chicago, IL).

**Chylomicron and VLDL apo B48 and B100 determination.** Chylomicron and VLDL apo B48 and B100 were separated by SDS-PAGE using 4–15% gradient gels (Bio-Rad, Hercules, CA) as previously described (13,14). Nondelipidated lipoprotein samples (~0.4 g protein/l) were reduced for 4 min at 96°C in Tris-HCL (0.1 mmol/l, pH 6.8, final concentration), mercaptoethanol (1%), SDS (1%), bromophenol blue (0.01%), and glycerol (10%). Aliquots of 35 µl were applied to the gel and run at a 60-mA constant current in 0.019 mol/l Tris plus 0.192 mol/l glycine. Gels were stained for 1 h with Coomassie Brilliant Blue (0.1% in methanol:acetic acid:water 4:1:5) and were destained with several changes of the same solvent. Because the chromogenicity of apo B48 has been shown to be similar to that of apo B100 (27), a protein standard was prepared from LDL (density 1.025–1.063 kg/l) of a single individual and was stored at −20°C and used throughout the study for quantification of apo B48 and apo B100. Apo B48 and apo B100 staining was linear within the range of 0.1–20 µg protein. Four concentrations of LDL apo B100 within this range, depending on the expected apoprotein concentration, were applied to all gels. The bands were quantified by densitometry using Vilber Lourmat equipment (Marne La Valee, France). Video images of the gels were generated and imported into Bio1D version 6.32 software (Viber) for analysis. Density values were assigned to the apo B100 bands of the human LDL, and a standard curve was constructed. The values were recalculated by linear regression, and curves with a correlation coefficient of >0.95 were accepted. The concentration of apo B48 and apo B100 were determined from this standard. The inter- and intra-assay variations ($n = 6$) for apo B48 were 6.0 and 3.9%, and they were 4.5 and 3.1% respectively, for apo B100.

Table 2—*Fasting chylomicron and VLDL in diabetic men*

| | Linoleic acid diet | Oleic acid diet |
|---|---|---|
| Chylomicron | | |
| Apo B48 | 3.1 ± 1.5* | 1.6 ± 1.7 |
| Apo B100 | 6.3 ± 4.1† | 2.6 ± 2.2 |
| Cholesterol | 14.0 ± 13.2 | 9.6 ± 8.1 |
| Triglyceride | 65.7 ± 58.0 | 35.6 ± 26.6 |
| Phospholipid | 15.5 ± 13.6 | 9.5 ± 2.5 |
| VLDL | | |
| Apo B48 | 1.7 ± 1.6 | 1.3 ± 0.8 |
| Apo B100 | 21.0 ± 13.5 | 15.3 ± 9.1 |
| Cholesterol | 66.0 ± 37.2 | 78.1 ± 88.4 |
| Triglyceride | 388.0 ± 250.0 | 278.9 ± 160.9 |
| Phospholipid | 104.7 ± 55.7† | 64.2 ± 30.7 |

Data are means ± SD and are expressed as micrograms per milliliter of plasma. *$P < 0.05$ vs. oleic acid diet; †$P < 0.02$ vs. oleic acid diet.

LDL fatty acid composition. Heptadecanoic acid (100 µg) was added as the internal standard to LDL (1 g/l LDL protein), and lipids were extracted by a modification of the method of Folch et al. (28). The organic phase was dried under nitrogen and transmethylated as previously described (29). Fatty acid methyl esters were extracted into hexane, dried under nitrogen, and reconstituted in iso-octane immediately before chromatography. The fatty acids were analyzed using a Shimadzu GC-14A gas chromatograph (Kyoto, Japan) and expressed as the percent of major fatty acids or as micrograms per milligram of LDL protein. Intra- and interassay variations of the method were 1.8 and 2.6%, respectively.

### Statistical analysis

Statistical analysis was performed using the paired Student's *t* test for comparison of fasting levels before and after treatment. Area under the curve (AUC) measurements were obtained using Graphpad Prism 2 for Macintosh (Graphpad Software, San Diego, CA). AUC analyses were made incrementally from fasting because fasting values are sometimes different in diabetic patients (15). Correlation coefficients were calculated for regression analysis. Results are expressed as mean ± SD. Inter- and intra-assay variation is expressed as the SD/mean × 100. $P < 0.05$ was considered statistically significant.

RESULTS — The characteristics of the subjects on the 2 diets are given in Table 1. Diabetic patients were well controlled with a mean $HbA_{1c}$ of 5.7% (normal value <4.9%). When comparing linoleic and oleic acid diets, we found no difference in BMI between diets, but fasting glucose was significantly higher on the linoleic acid diet than on the oleic acid diet ($P < 0.01$). Fasting insulin was also significantly higher on the linoleic acid diet ($P < 0.002$). Plasma cholesterol and LDL cholesterol levels were significantly higher on the linoleic acid diet ($P < 0.001$), whereas plasma triglyceride and HDL cholesterol levels were similar on the two diets.

### Fasting chylomicron and VLDL

Fasting chylomicron and VLDL apolipoproteins and lipids are shown in Table 2. All fasting chylomicron components were higher on the linoleic acid diet, although only the increase in apo B48 ($P < 0.05$) and apo B100 ($P < 0.02$) were statistically significant. VLDL apo B48 and apo B100 were higher on the linoleic acid diet, but the increase was not significant. VLDL phospholipid levels were significantly higher on the linoleic acid diet ($P < 0.02$), whereas the other VLDL components were similar.

### Postprandial chylomicron and VLDL (AUC)

Chylomicron apo B48 and apo B100 AUCs (Table 3 and Fig. 1) were nearly twice as high on the linoleic acid diet compared with the oleic acid diet ($P < 0.05$). These AUCs were determined incrementally from fasting, and when the fasting differences were taken into consideration, the differences in postprandial AUCs were considerably magnified ($P < 0.001$). There was no significant difference in postprandial chylomicron triglyceride, cholesterol, or phospholipid AUCs from fasting or baseline between the diets.

Examination of VLDL apo B48 and B100 (Table 3 and Fig. 2) also showed an approximate twofold increase in both VLDL apo B48 and apo B100 ($P < 0.05$) on the linoleic acid diet compared with the oleic acid diet. VLDL phospholipid levels were significantly higher on the linoleic acid diet ($P < 0.01$) with no change in AUC for triglyceride or cholesterol.

The only significant difference in fasting LDL composition between the diets was an increase in phospholipids on the linoleic acid diet ($P < 0.01$) and an increase in total fatty acids (1,294 ± 223 vs. 1,117 ± 309 µg/mg LDL protein, $P < 0.05$). This reflected a significant increase in linoleic acid (512 ± 73 vs. 383 ± 124 µg/mg LDL protein, $P < 0.005$) on the linoleic acid diet. There was significantly more oleic acid in LDL on the oleic acid diet, and there was an increase in the ratio of linoleic acid to oleic acid on the linoleic acid diet (2.0 ± 0.7 vs. 1.6 ± 0.04, $P < 0.02$), confirming adher-

Table 3—*Postprandial chylomicron and VLDL AUCs*

| | Linoleic acid diet | Oleic acid diet |
|---|---|---|
| Chylomicron | | |
| Apo B48 | 38 ± 21* | 22 ± 10 |
| Apo B100 | 69 ± 42* | 34 ± 19 |
| Cholesterol | 180 ± 100 | 140 ± 136 |
| Triglyceride | 853 ± 926 | 1,371 ± 1,781 |
| Phospholipid | 223 ± 171 | 275 ± 280 |
| VLDL | | |
| Apo B48 | 11 ± 9* | 4 ± 2 |
| Apo B100 | 74 ± 58† | 42 ± 38 |
| Cholesterol | 355 ± 301 | 465 ± 668 |
| Triglyceride | 2,028 ± 1,260 | 1,545 ± 1,545 |
| Phospholipid | 380 ± 251† | 209 ± 166 |

Data are means ± SD. *$P < 0.05$ vs. oleic acid diet (paired *t* test); †$P < 0.01$ vs. oleic acid diet (paired *t* test).

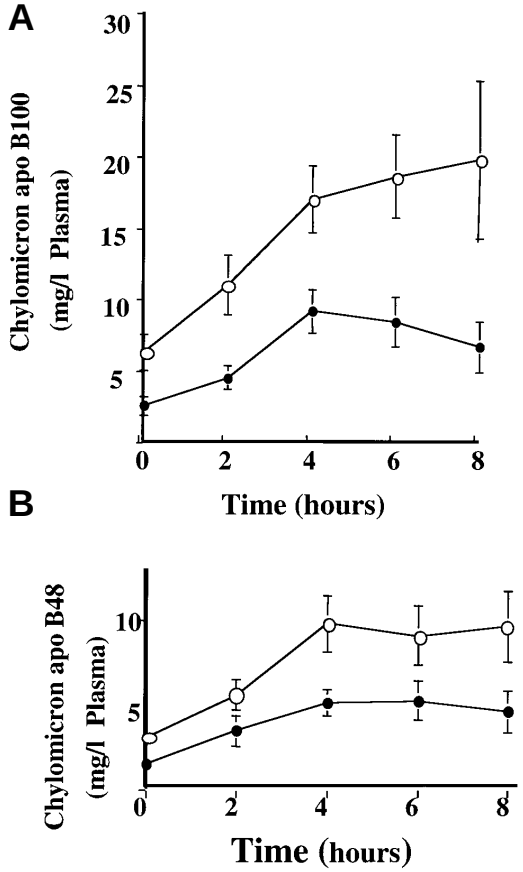


**Figure 1—***Postprandial response to a high-fat meal of chylomicron apo B100 (*A*) and apo B48 (*B*) in type 2 diabetic subjects after 2 weeks on a linoleic acid–rich (○) or an oleic acid–rich diet (●). Apo B48 and apo B100 AUCs were significantly different between the linoleic and oleic acid diets (*$P < 0.05$ *by Student's paired* t *test).*

ence to the dietary program. There was no significant change in the other fatty acids between diets.

CONCLUSIONS — Chylomicron remnant particles, especially the small particles, are thought to be particularly atherogenic, and the recent finding of an apo B48 receptor in macrophages (12) adds considerable weight to the aforementioned theory. It was originally thought that the polyunsaturated fat diet was more effective in lowering cholesterol than a monounsaturated fat diet (30,31), but more recent studies have suggested that there is probably no difference between the diets in their effect on LDL levels (32). With regard to the postprandial effect, there is little information on changing between linoleic and oleic acids in nondiabetic subjects, yet it has been shown experimentally that the rate of lipolysis of chylomicrons may depend on the type of fatty acid (33). We and others have shown that not only fasting but also postprandial chylomicrons are elevated in diabetes, and we have been particularly struck by our studies, which show considerable amounts of apo B48 floating in the VLDL density range (13–15). We have calculated AUC from fasting levels (34), because it has been argued that this is a more valid measurement because fasting triglycerides are so frequently raised in diabetes. Recalculating the data to include the raised fasting level further exaggerates the differences. The meal we used reflected the intervention fatty acids used during the previous 2-week period, thus altering the fatty acid composition of the chylomicrons and VLDL, which are known to reflect the previous meal (35). We used the 2-week intervention period because we felt this was a reasonable period of time to elapse if part of the investigative parameters were altered because of the effect of fatty acids on gene regulation through the PPAR system (19).

Examination of LDL composition showed that diet had little impact on the major lipid components, although phospholipid was a little higher ($P < 0.01$) on the linoleic acid diet.

The major finding in this study is that a linoleic acid diet in diabetic patients increases the number of chylomicrons and VLDL, apo B48, and apo B100 particles, with each particle containing less lipid. Simons et al. (36) have shown that the relative enrichment of apo B48 compared with apo B100 in the Suedberg Units (sf) 60 fraction 4 h after fat intake was significantly greater in patients with coronary artery disease than in control subjects. It has also been shown that postprandial concentrations of small chylomicron remnants (apo B48 in the sf 20–60 lipoprotein fraction) correlates with the progression of coronary atherosclerosis (5). Tkac et al. (6) examined whether there was a relationship between the severity of coronary heart disease and the concentration of the major classes of lipoproteins. They found that the number of particles, as reflected by the apo B levels of the triglyceride-rich lipoproteins, were greater in individuals with moderate and severe disease than in individuals with mild disease. Analysis of triglyceride levels did not show a significant correlation with coronary artery disease. That study may be important in highlighting the role of apo B in the development of atherosclerosis. Our study has demonstrated that diet altered both apo B48 and apo B100 in the chylomicrons and VLDL in the diabetic patients we studied. The linoleic acid diet appears to have the particular ability to increase these levels in diabetes. Examination of the lipid/protein content of the postprandial apo B–containing particles demonstrated that the linoleic acid diet was particularly associated with a lipid-depleted chylomicron particle. These changes were also apparent in the VLDL fraction. This suggests that postprandial lipoprotein particles were smaller because each particle has only one molecule of apo B. There is good evidence that the smaller remnant particles are particularly atherogenic at least in nondiabetic subjects (8). The triglyceride-depleted particle that we found in our study (AUC triglyceride/apo B48 22.4 on linoleic acid vs. 62.3 on oleic acid) is in keeping with the findings in studies using Caco-2 cells (24).

We have recently examined the role of apolipoproteins in postprandial lipoprotein clearance in diabetes and have demonstrated a decrease in the amount of apo E per particle postprandially in VLDL with no

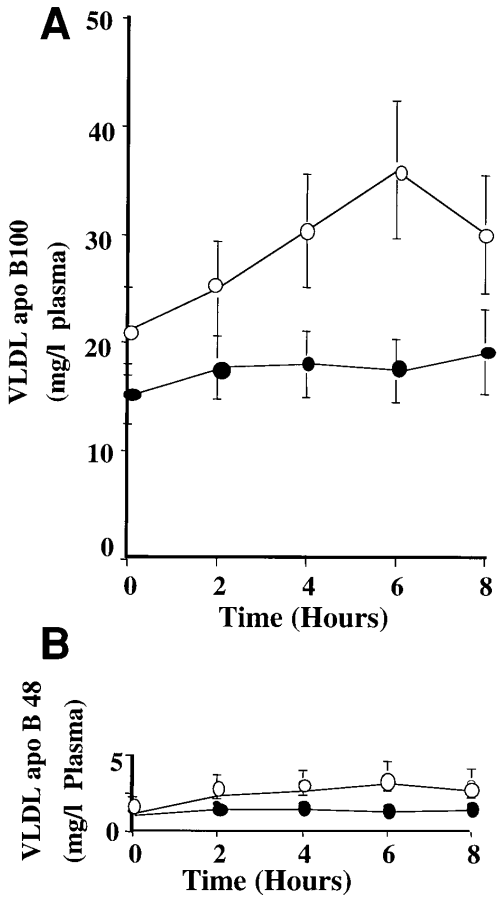


**Figure 2—***Postprandial response to a high-fat meal of VLDL apo B100 (*A*) and apo B48 (*B*) in type 2 diabetic subjects after 2 weeks on a linoleic acid–rich (○) or an oleic acid–rich diet (●). VLDL apo B48 and apo B100 AUCs were significantly different between diets (*$P < 0.01$ *and* $P < 0.05$*, respectively, by Student's paired* t *test).*

change in apo C111 on a linoleic acid diet compared with an oleic acid diet (37). Because triglyceride-rich lipoprotein clearance is mediated through apo E and inhibited by apo C111, a mechanism for the delay in clearance of postprandial lipoprotein particles on the linoleic acid diet is suggested. Apo C11, which is a cofactor for lipoprotein lipase, an insulin-sensitive enzyme, was not different between diets in our previous study, but the reduction in insulin sensitivity in the present study may help to explain why a linoleic acid–rich diet would be associated with increased postprandial apo B48 and B100.

Microsomal triglyceride transfer protein (MTP) regulates lipoprotein particle formation and is increased in the liver in experimental diabetes (38). We have shown, in diabetic animal experiments, a large increase in MTP mRNA expression in the intestine (39). It has also been shown in animal studies that the nature of dietary fat may be important in the regulation of MTP expression (40). It is possible that another underlying mechanism in the alteration of postprandial lipoproteins may be related to the different effects of oleic and linoleic acid on the intestinal formation of chylomicron particles. Fatty acids are known to regulate PPARs, which regulate insulin action through gene expression. This may be the link between dietary fatty acids and the regulation of postprandial lipoproteins.

The results of our study suggest that a linoleic acid diet may not be the best option for people with type 2 diabetes. In this study, a linoleic acid–rich diet was associated with increased fasting insulin and glucose levels, increased postprandial lipoproteins, and significantly higher plasma and LDL cholesterol levels, all of which are associated with atherosclerosis risk. In conclusion, an oleic acid–rich diet appears to be a more suitable option for type 2 diabetic patients.

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