Effects of medium-chain fatty acids and oleic acid on blood lipids, lipoproteins, glucose, insulin, and lipid transfer protein activities1–3

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ABSTRACT
Background: Dietary medium-chain fatty acids (MCFAs) are of nutritional interest because they are more easily absorbed from dietary medium-chain triacylglycerols (MCTs) than are long-chain fatty acids from, for example, vegetable oils. It has generally been claimed that MCFAs do not increase plasma cholesterol, although this claim is poorly documented.

Objective: We compared the effects of a diet rich in either MCFAs or oleic acid on fasting blood lipids, lipoproteins, glucose, insulin, and lipid transfer protein activities in healthy men.

Design: In a study with a double-blind, randomized, crossover design, 17 healthy young men replaced part of their habitual dietary fat intake with 70 g MCTs (66% 8:0 and 34% 10:0) or high-oleic sunflower oil (89.4% 18:1). Each intervention period lasted 21 d, and the 2 periods were separated by a washout period of 2 wk. Blood samples were taken before and after the intervention periods.

Results: Compared with the intake of high-oleic sunflower oil, MCT intake resulted in 11% higher plasma total cholesterol (P = 0.0005), 12% higher LDL cholesterol (P = 0.0001), 32% higher VLDL cholesterol (P = 0.080), a 12% higher ratio of LDL to HDL cholesterol (P = 0.002), 22% higher plasma total triacylglycerol (P = 0.0361), and higher plasma glucose (P = 0.033). Plasma HDL-cholesterol and insulin concentrations and activities of cholesteryl ester transfer protein and phospholipid transfer protein did not differ significantly between the diets.

Conclusions: Compared with fat high in oleic acid, MCT fat unfavorably affected lipid profiles in healthy young men by increasing plasma LDL cholesterol and triacylglycerol. No changes in the activities of phospholipid transfer protein and cholesteryl ester transfer protein were evident.

KEY WORDS Medium-chain triacylglycerols, medium-chain fatty acids, oleic acid, LDL cholesterol, lipoproteins, glucose, insulin, cholesteryl ester transfer protein, phospholipid transfer protein

INTRODUCTION
Medium-chain triacylglycerols (MCTs) are of nutritional interest because they are more readily degraded in the intestine than are triacylglycerols containing long-chain fatty acids. The released medium-chain fatty acids (MCFAs) are absorbed and preferentially β-oxidized (1, 2). It has been persistently claimed that MCTs do not increase cholesterol, although this claim is poorly documented. However, a study by Cater et al (3) showed that, in comparison with oleic acid, MCTs have a cholesterol-increasing potential one-half of that of palmitic acid in mildly hypercholesterolemic, middle-aged men, which corroborates results reported in the 1960s (4, 5). In contrast with their effect on plasma cholesterol, MCTs increase plasma triacylglycerol in comparison with the effect of long-chain triacylglycerols (LCTs) (6, 7). Evaluation of the effects of MCTs on glucose metabolism has been limited. One study found no difference between the effects of MCFAs and oleic acid on fasting glucose and insulin concentrations (7), whereas another study found that glucose metabolism was increased by MCTs (8).

Cholesterol ester transfer protein (CETP) participates in the transfer of cholesterol ester (CE) and triacylglycerol between lipoproteins. Although CETP plays a role in reverse cholesterol transport, which has an apparent antiatherogenic effect, the evidence suggests that lipoprotein changes resulting from CETP activity are typically proatherogenic (9). Fasting CETP activity is affected by dietary fatty acid composition: lower CETP activity occurs after the intake of cis monounsaturated fatty acids than after the intake of polyunsaturated fatty acids (10), trans fatty acids (elaidic acid) (11–14), or palmitic acid (15).

Phospholipid transfer protein (PLTP) is a key modulator of HDL size and composition (16). The main functions of PLTP are the transfer of phospholipids between lipoprotein particles (17–19) and the conversion of HDL to larger and smaller particles, including pre-β-HDL (20). Besides being a prerequisite for HDL modeling (21), PLTP was recently shown to be involved in VLDL synthesis (22). Some results indicate an antiatherogenic potential of PLTP (18, 23), possibly by enhancing reverse cholesterol transport (18) and transfer of antioxidants to endothelial cells (24), whereas other results have shown that PLTP can be proatherogenic in transgenic mice (22). PLTP activity is elevated in insulin-resistant subjects with a high plasma triacylglycerol.

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concentration, patients with type 2 diabetes mellitus, and subjects with an elevated body mass index (25–28).

Although the study by Cater et al (3) found that MCTs significantly increased plasma cholesterol concentrations, another study by Temme et al (29) found that the cholesterol-increasing effect was not significantly higher than that of oleic acid. Because it may still be assumed that dietary MCTs are neutral with regard to increasing plasma cholesterol, we found it relevant to compare the effect of MCFAs with that of oleic acid, a fatty acid not regarded to affect plasma cholesterol concentrations. Plasma lipoprotein concentrations and CETP and PLTP activities were measured in healthy young men to examine the effect of MCTs on lipid metabolism. Fasting plasma glucose and insulin concentrations were analyzed to provide information on potential glycemic effects of MCTs.

SUBJECTS AND METHODS

We performed a double-blind, randomized, dietary intervention study with a crossover design. The study included 2 intervention periods of 3 wk each separated by a 2-wk washout period. During the intervention periods, sandwich spreads and foods providing 70 g of their daily fat intake were provided by our department during the 3-wk intervention periods. Before the study, the subjects with 70 g of the specific test fat, which was intended to replace 70 g of their habitual fat intake. Before the study, the subjects’ habitual diets were assessed from 4-d weighed-food records, and on the basis of these records, the subjects were instructed in how to change their diet to consume the foods provided by our department during the 3-wk intervention periods without increasing the total fat content of their diet.

We tested adherence to the dietary advice by assessing each participant’s diet from a 4-d weighed-food record after the first week during both dietary periods. All dietary calculations were performed by using a national database (Dankost; National Food Agency, Søborg, Denmark).

Blood sampling

After the subjects fasted overnight for 12 h, venous blood was drawn on the morning before the intervention period (day 1) and at the end of the study on day 21. Blood for lipoprotein and fatty acid analyses was collected in tubes containing EDTA and was centrifuged at 3000 × g for 15 min at 20 °C. EDTA plasma (3 mL) was stored at 5 °C, and ultracentrifugation was started within a maximum of 72 h. VLDL [density (d) < 1.006 kg/L], intermediate-density lipoprotein (d = 1.006–1.019 kg/L), LDL (d = 1.019–1.063 kg/L), and HDL (d = 1.063–1.210 kg/L) were separated by ultracentrifugation as described previously (30).

Lipid and apolipoprotein analysis

Cholesterol and triacylglycerol concentrations were measured in plasma lipoprotein fractions by using enzymatic kits (MPR and GPO-PAP, respectively; Boehringer Mannheim GmbH, Mannheim, Germany) on a Cobas Mira analyzer (Roche AG, Basel, Switzerland). Fatty acid profiles of plasma triacylglycerol and CE were determined by using a method described previously (31).

Lipid transfer protein analyses

Plasma CETP activity was measured after removal of endogenous VLDL and LDL by phosphotungstate–magnesium chloride precipitation as described previously (32). PLTP activity was quantitated as the transfer of radioactively labeled phosphatidylcholine from phosphatidylcholine-liposomes to HDL₃.
according to the procedure of Damen et al (33), with minor modifications (19, 34).

**Statistical analysis**

For comparison of the 2 diets, a two-factor analysis of covariance with a value for each diet and for each subject was performed. The respective baseline values were used as covariates, and the analyses were thereby adjusted for the baseline value of each variable. The baseline values were pooled and renamed “habitual” for the purpose of presentation in the tables and figures. Because of variance heterogeneity, the values for all lipids, lipid transfer proteins, and lipoproteins were logarithmically transformed. We did not add the result of a power analysis based on CETP activity values because the actual sample size calculation was based on a 10% change in LDL cholesterol, as found in the study by Cater et al (3). For assessment of associations, Pearson’s correlation coefficients \( r \) were calculated by using log-transformed variables. Statistical significance was set at \( P < 0.05 \), and \( P \) values are reported as two-sided hypotheses. The SAS statistical package (version 8.0; SAS Institute Inc, Cary, NC) was used for all statistical analyses.

**RESULTS**

Compared with the intake of HOSO, the intake of MCTs resulted in 12% (0.30 mmol/L) higher LDL-cholesterol concentrations (\( P < 0.0001 \)), 32% higher (0.10 mmol/L) VLDL-cholesterol concentrations (\( P = 0.080 \)), a 12% higher ratio of LDL to HDL cholesterol (\( P = 0.002 \)), and 22% (0.23 mmol/L) higher plasma total triacylglycerol concentrations (\( P = 0.0361 \)).

However, no significant effect was observed for plasma HDL cholesterol (Figure 1).

Compared with the intake of HOSO, the intake of MCTs resulted in significantly higher plasma glucose concentrations (\( P = 0.033 \)) (Table 3). Insulin concentrations and CETP and PLTP activities did not differ significantly between the 2 diets (Table 3).

**Incorporation of fatty acids into plasma lipids**

After the intake of MCTs, there was no incorporation of 8:0 into plasma triacylglycerol, CEs, or phospholipids. In addition, only traces of 10:0 could be found in triacylglycerol, and no incorporation of 10:0 was seen in CEs (Table 4). Concentrations of 16:0, 16:1n-7, and 18:0 in plasma triacylglycerol were sig-

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**FIGURE 1.** Mean (± SE) plasma lipid and lipoprotein concentrations in 17 healthy young men after 3 wk of dietary intervention with high-oleic sunflower oil (HOSO) or medium-chain triacylglycerols (MCTs) or after consumption of the habitual diet. Each habitual value is the mean of the baseline values from the 2 dietary intervention periods. The fatty acid composition of the test fats is presented in Table 2. * **Significantly different from HOSO (two-factor analysis of covariance with a value for each diet and for each subject and with the baseline value used as a covariate): * \( P < 0.0001 \), ** \( P = 0.036 \). There was a tendency for higher VLDL cholesterol (32%; \( P = 0.080 \)) after MCTs than after HOSO.
significantly higher after MCTs than after HOSO \((P < 0.001\) for 16:0 and 16:1n-7, and \(P < 0.05\) for 18:0), which indicates that de novo synthesis of long-chain fatty acids may have taken place. Incorporation of 18:1n-9 into plasma triacylglycerol was significantly greater after HOSO than after MCTs \((P < 0.001)\), whereas concentrations of 18:1n-7, 18:2n-6, and 18:3n-3 in plasma triacylglycerol were significantly higher after MCTs than after HOSO (Table 4). In CEs the incorporation pattern was similar except for 18:0, for which concentrations were not significantly higher after MCTs than after HOSO. In addition, concentrations of 18:2n-6, 18:3n-3, 20:3n-6, and 20:4n-6 in CEs were significantly higher after MCTs than after HOSO (Table 4).

**Correlations**

After the HOSO intervention, CETP activity was correlated with HDL triacylglycerol \((r = 0.57, P = 0.017)\) and with VLDL cholesterol \((r = 0.51, P = 0.038)\), but these associations were not significant after the MCT intervention (HDL triacylglycerol: \(r = 0.288, P = 0.263;\) VLDL cholesterol: \(r = -0.014, P = 0.957)\). CETP activity was not correlated with total triacylglycerol or PLTP activity after either dietary intervention. After the MCT intervention, no significant associations with CETP were observed, but a significant inverse association was observed between PLTP activity and insulin \((r = -0.51, P = 0.046)\). However, this association was not significant after the HOSO intervention \((r = -0.47, P = 0.069)\). There were no significant correlations when the baseline values were analyzed \((n = 17)\).

**Energy intake**

Total fat intake was significantly higher during the MCT and HOSO intervention periods than during the habitual diet \((P < 0.0001; 118.1 \pm 5.7, 127.2 \pm 6.6, \text{and } 79.6 \pm 6.7 \: \text{g}, \text{respectively})\). The intakes of MCTs and HOSO did not differ significantly as assessed by the weighed food records.

**DISCUSSION**

We found that in comparison with HOSO, MCTs had a cholesterol-increasing effect in young normolipidemic men. Our results support the findings of Cater et al (3), who reported an MCT-facilitated cholesterol-increasing effect of about one-half that of palmitic acid in middle-aged, mildly hypercholesterolemic men, and are in line with the findings of Temme et al (29), who reported that in comparison with oleic acid, MCFAs test fat (60% MCFAs compared with 99% MCFAs in the present study) had a slight, but not significant, LDL-increasing effect. In early studies, the effect of MCTs was compared with that of fats having

### Table 3

Activities of cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) and glucose and insulin concentrations after 3 wk of dietary intervention

<table>
<thead>
<tr>
<th></th>
<th>Habitual</th>
<th>HOSO</th>
<th>MCTs</th>
<th>(P^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CETP activity (nmol \cdot mL^{-1} \cdot h^{-1})</td>
<td>21.91 ± 2.50</td>
<td>24.03 ± 2.66</td>
<td>25.28 ± 2.26</td>
<td>0.58</td>
</tr>
<tr>
<td>PLTP activity (µmol \cdot mL^{-1} \cdot h^{-1})</td>
<td>5.90 ± 0.58</td>
<td>6.11 ± 0.52</td>
<td>6.23 ± 0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.04 ± 0.06</td>
<td>4.99 ± 0.07</td>
<td>5.19 ± 0.07</td>
<td>0.033</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>32.2 ± 3.5</td>
<td>31.6 ± 3.6</td>
<td>34.8 ± 3.7</td>
<td>0.14</td>
</tr>
</tbody>
</table>

1. All values are \(\bar{x} \pm SE; n = 17\). HOSO, high-oleic sunflower oil; MCTs, medium-chain triacylglycerols. Because the baseline values of the variables did not differ significantly between the 2 diet periods, the 2 baseline values for each variable were pooled, renamed the “habitual” value, and used as a covariate in the analysis.

2. Two-factor analysis of covariance with a factor for subject and a factor for diet.

### Table 4

Fatty acid composition of plasma triacylglycerol and cholesterol esters after 3 wk of dietary intervention

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Plasma triacylglycerol</th>
<th>Cholesterol ester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOSO</td>
<td>MCTs</td>
</tr>
<tr>
<td>10:0</td>
<td>0.00 ± 0.00</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>12:0</td>
<td>0.32 ± 0.14</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>14:0</td>
<td>2.02 ± 0.30</td>
<td>2.16 ± 0.19</td>
</tr>
<tr>
<td>16:0</td>
<td>22.20 ± 0.74</td>
<td>25.61 ± 0.61²</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>3.10 ± 0.20</td>
<td>4.41 ± 0.28²</td>
</tr>
<tr>
<td>18:0</td>
<td>2.81 ± 0.24</td>
<td>3.27 ± 0.17³</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>47.75 ± 1.23</td>
<td>37.88 ± 0.82²</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>2.15 ± 0.16</td>
<td>2.74 ± 0.20⁴</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>11.18 ± 0.47</td>
<td>13.75 ± 0.73⁴</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.89 ± 0.09</td>
<td>1.47 ± 0.20²</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.26 ± 0.05</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.99 ± 0.07</td>
<td>1.07 ± 0.09</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.24 ± 0.05</td>
<td>0.24 ± 0.05</td>
</tr>
</tbody>
</table>

1. All values are \(\bar{x} \pm SE; n = 17\). HOSO, high-oleic sunflower oil; MCTs, medium-chain triacylglycerols.

2. Significantly different from HOSO (paired t test): \(^2P < 0.001, ^3P < 0.05, ^4P < 0.01\).
either a cholesterol-increasing effect [e.g., butter or coconut oil (5, 35)] or a cholesterol-decreasing effect [corn oil (5)], which led to the interpretation that MCTs were neutral in regard to cholesterol. On the basis of these early findings, the idea that MCTs were cholesterol neutral was generally accepted until a cholesterol-increasing effect of MCTs was shown in 1997 (3). In comparison with HOSO, MCTs had an LDL cholesterol–increasing effect of 12% in the present study and 13% in the study by Cater et al (3), which indicates that young normolipidemic men and mildly hypercholesterolemic men respond to dietary MCTs similarly. This is in agreement with previous findings showing a similarity in response to dietary fatty acid composition in young normolipidemic men (36) and hypercholesterolemic patients (37).

Cater et al (3) suggested that, because plasma triacylglycerol does not contain MCFAs, the mechanism for the cholesterol-increasing effect of MCTs is the utilization of acetyl-CoA MCFA oxidation for synthesis of long-chain fatty acids. These then enter the hepatic long-chain fatty acid pool and thus behave like dietary long-chain fatty acids. A suggested hepatic de novo synthesis of long-chain triacylglycerols after the intake of MCTs is in agreement with the results of the present study, in which there was no incorporation of 8:0 into plasma triacylglycerol, only traces of 10:0 were incorporated into plasma triacylglycerol, and significantly higher concentrations of the 2 long-chain fatty acids palmitic acid and stearic acid and of 16:1n-7 were observed after MCT intake than after HOSO intake (Table 4). Our findings agree with those of others (7, 38, 39), which indicate that excessive MCT feeding stimulates both de novo fatty acid synthesis of long-chain fatty acids and, to a minor degree, desaturation. The significantly higher content of oleic acid in plasma triacylglycerol and CE after HOSO than after MCTs confirmed the good compliance of the subjects.

The triacylglycerol-increasing effect of MCTs that was observed by others (6, 7) was supported by the results of the present study but was not, however, confirmed in a study in which lower doses of MCFAs were tested (29). A suggested mechanism for a triacylglycerol-increasing potential of high MCT intake is stimulation of insulin secretion and of anabolic-related processes (7). Thus, increased de novo fatty acid synthesis would lead to an increase in hepatic triacylglycerol production and thereby to VLDL secretion as shown in animals (40) and humans (7, 41).

Our finding that, in comparison with long-chain fatty acids given as oleic acid, MCFAs increased fasting plasma glucose is in accordance with the observed similarity between MCT and carbohydrate metabolism (42), with enhanced glycogen storage and de novo fatty acid synthesis from acetate supplied by enhanced glycolytic flux (7). However, in the very few studies on MCTs and fasting plasma glucose (most studies examined plasma glucose postprandially), no effect of MCTs on fasting blood glucose concentrations was observed (7). Only a single study in patients with type 2 diabetes showed improved insulin-mediated glucose metabolism after MCTs (8).

We observed no significant difference in CETP activity after the intake of the 2 test diets, which is in agreement with the results of another study showing no effect of oleic acid on CETP activity (15). Increased plasma triacylglycerol concentrations enhance CETP activity (15, 43), as seen in dyslipidemic patients (44). However, the 22% higher plasma triacylglycerol concentrations observed after MCTs than after HOSO were not followed by an increase in CETP activity. We measured CETP activity by using an assay that is not influenced by endogenous triacylglycerol-rich lipoproteins, and therefore the results of the assay probably reflected true increases in CETP activity. A comparison between the CETP activities measured after the 2 diets in the present study and those reported by Lagrost et al (15) after dietary intervention is difficult to perform because unlike us, Lagrost et al (15) used a method in which CETP activity was measured in the presence of triacylglycerol-rich lipoproteins. In healthy subjects, VLDL concentrations determine the rate of net CE transfer (45). Because we did not determine VLDL triacylglycerol concentrations in the subjects in the present study, whether a correlation between VLDL triacylglycerol and CETP activity exists is difficult to assess. However, the observed correlation between VLDL cholesterol and CETP activity suggests that a correlation between VLDL triacylglycerol and CETP activity does exist.

In contrast with observations made by others (15), we did not observe any relation between PLTP and CETP activities after the HOSO diet. Although dietary change may affect CETP and PLTP activities, extreme differences in dietary compositions seem to be required to change the relation between lipoproteins and lipid transfer protein activities.

A strength of the present study is that we compared MCFAs fat with a test fat high in oleic acid, which is considered cholesterol neutral. Thus, in contrast with earlier studies in which either cholesterol-increasing or cholesterol-decreasing dietary fats were used for comparison, we could determine to a higher degree the effect of MCTs per se.

In conclusion, in comparison with the cholesterol-neutral oleic acid given in the form of HOSO, MCFAs in the form of MCTs significantly increased plasma triacylglycerol and LDL-cholesterol concentrations and the ratio of LDL to HDL cholesterol and thereby resulted in a less beneficial lipid profile overall. No changes in PLTP and CETP activities were evident.

REFERENCES


