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# Hepatic fatty acid metabolism in rats fed diets with different contents of $C_{18:0}$, $C_{18:1}$ *cis* and $C_{18:1}$ *trans* isomers

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In the present study the effects of some $C_{18}$ fatty acids on hepatic fatty acid metabolism have been compared. Male rats were fed cholesterol-free diets containing either $C_{18:0}$, $C_{18:1}$ *cis* or $C_{18:1}$ *trans* isomers as the variables. In accordance with previous work, oleic acid in the diet caused an increase in cholesterol concentration in the liver and in the lipoprotein fraction of density (*d*; kg/l) $< 1{\cdot}006$. Oleic acid also reduced the triacylglycerol:cholesterol value in this fraction. Surprisingly, the $C_{18:1}$ *trans* isomers diet induced a decrease in the amount of cholesterol in total plasma as well as in the $1{\cdot}019 < d < 1{\cdot}063$ lipoprotein fraction. Both oleic acid and $C_{18:1}$ *trans* isomers increased the concentration of triacylglycerols in the liver. The two $C_{18:1}$ fatty acids differently influenced the hepatic activities of carnitine palmitoyltransferase-I and 3-hydroxy-acyl-CoA dehydrogenase; both enzymes were inhibited by $C_{18:1}$ *trans* isomers, while no change was induced by oleic acid. The activity of the citrate carrier was lower in the oleic acid- and $C_{18:1}$ *trans* isomers-fed rats, when compared with the rats fed stearic acid. No diet effects were seen for the activities of acetyl-CoA carboxylase, fatty acid synthase, diacylglycerol acyltransferase, citrate synthase and phosphofructokinase. The results are interpreted in that oleic acid raised liver triacylglycerol by reducing the secretion of it with the $d < 1{\cdot}006$ lipoprotein fraction whereas the $C_{18:1}$ *trans* isomers enhanced liver triacylglycerol by lowering the hepatic oxidation of fatty acids.

**Dietary fatty acids: Hepatic enzymes: Liver: Plasma lipoproteins**

A number of clinical studies have shown that dietary *trans* fatty acids have an adverse effect on the plasma lipid profile. In human subjects, *trans*-monounsaturated fatty acids, when compared with *cis*-monounsaturated fatty acids (for example, oleic acid), increase among other things plasma levels of triacylglycerols (TAG) (Khosla & Hayes, 1996; Katan, 1998; Nelson, 1998). The mechanism responsible for the effect of *trans* fatty acids on lipid metabolism is as yet unknown.

Studies with isolated rat-liver mitochondria (Lawson & Holman, 1981) and hepatocytes (Guzmán *et al.* 1999) have shown that the two geometrical isomers, oleic and elaidic acid, are metabolized differently. Surprisingly, elaidic acid is preferentially oxidized whereas oleic acid is preferentially esterified (Guzmán *et al.*, 1999). The addition of elaidic acid to the incubation medium of isolated hepatocytes increased total acid-soluble products as well as the mass of ketone bodies when compared with the addition of oleic acid (Guzmán *et al.* 1999). There seems to be a discrepancy between short-term *in vitro* and long-term *in vivo* effects. It would be anticipated that the consumption of elaidic acid *v*. oleic acid affects the expression of enzyme activities in the pathways of fatty acid metabolism differently from the effects on the activities observed *in vitro*. The authors are not aware of feeding trials in which elaidic acid and oleic acid were the only variables and in which the activities of key hepatic enzymes of fatty acid oxidation, esterification and *de novo* synthesis have been measured.

The present study with rats was undertaken to investigate the effects of dietary elaidic acid *v*. oleic acid on the hepatic fate of fatty acids. To avoid interference of *de novo* synthesized fatty acids, high concentrations of dietary fatty acids were employed. For assessing the specificity of the effects of oleic and elaidic acid, control rats were used that were fed a diet containing stearic acid. The diets were

**Abbreviations:** ACC, acetyl-CoA carboxylase; CPT, carnitine palmitoyltransferase; *d*, density; DGAT, diacylglycerol acyltransferase; 3-HAD, 3-hydroxy-acyl-CoA dehydrogenase; TAG, triacylglycerol.

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formulated so that the contents of stearic, oleic and elaidic acid were the variables. To determine the effect of the dietary fatty acids on lipogenesis, the study measured the activities of acetyl-CoA carboxylase (ACC) and fatty acid synthase, key enzymes in hepatic fatty acid synthesis, and diacylglycerol acyltransferase (DGAT), the only enzyme exclusively involved in the formation of TAG. The tricarboxylate carrier activity in mitochondria was also determined as this carrier protein plays an important role in fatty acid biosynthesis. It is responsible for the transfer of acetyl-CoA, condensed with oxaloacetate in the form of citrate, from the mitochondria to the cytosol where lipogenesis occurs (Fritz *et al.* 1973; Schiller *et al.* 1974). To study fatty acid oxidation, the activities of carnitine palmitoyltransferase (CPT)-I, 3-hydroxy-acyl-CoA dehydrogenase (3-HAD) and citrate synthase were measured. The process of hepatic fatty acid oxidation is controlled by the specific activity and/or the sensitivity to malonyl-CoA of CPT-I. Thus, hepatic CPT-I sensitivity to inhibition by malonyl-CoA was determined.

## Materials and methods

### *Chemicals*

[1,5-$^{14}$C]citrate, L-[*methyl*-$^{3}$H]carnitine, [1-$^{14}$C]palmitoyl-CoA and [1-$^{14}$C]acetyl-CoA were purchased from New England Nuclear (Dreieichenhain, Germany). Other chemicals were obtained from Roche (Mannheim, Germany) or Baker (Deventer, The Netherlands).

### *Animals and diets*

The experimental design was approved by the animal experiments committee of the Utrecht Faculty of Veterinary Medicine. Male outbred Wistar rats (HsdCpb:Wu; Harlan-CPB, Zeist, The Netherlands), aged 6 weeks, were used. They were housed two per cage in a room with a 12 h light–dark cycle (lights on 07·00–19·00 h). During a 1-week pre-experimental period, all animals were fed a pre-experimental diet. The pre-experimental diet contained 180 g animal fat/kg instead of the experimental fats, but was otherwise identical to the experimental diets. At the end of the pre-experimental period, the rats were divided into three groups of twelve rats each, the groups being stratified for body weight, plasma TAG and cholesterol concentrations. The groups either received a diet with stearic acid, oleic acid or elaidic acid. The experimental period lasted 14 d. The composition of the experimental diets is presented in Table 1. The experimental diets were formulated using hydrogenated soyabean-oil preparations and olive oil (Table 2) so that in the diets the contents of stearic, oleic and $C_{18:1}$ *trans* isomers, of which elaidic acid is a major component, were the important variables (Table 2). The animals had free access to feed and tap water.

### *Collection and preparation of samples*

Blood and liver samples were taken between 10·00 and 12.00 hours as described previously (Geelen *et al.* 1995*b*).

**Table 1.** Composition of the diets (g/kg)

| | Stearic-acid diet | Oleic-acid diet | $C_{18:1}$-*trans* isomer diet |
|---|---|---|---|
| Components | | | |
| Casein | 200 | 200 | 200 |
| Maize oil | 20 | 20 | 20 |
| Hydrogenated soyabean oil | 120 | – | – |
| Olive oil | 60 | 180 | – |
| Hydrogenated, fractionated soyabean oil | – | – | 180 |
| Maize starch | 441 | 441 | 441 |
| Molasses | 50 | 50 | 50 |
| Cellulose | 50 | 50 | 50 |
| $CaCO_3$ | 12 | 12 | 12 |
| $MgCO_3$ | 2 | 2 | 2 |
| $NaH_2PO_4.2H_2O$ | 15 | 15 | 15 |
| KCl | 8 | 8 | 8 |
| Vitamin mix* | 12 | 12 | 12 |
| Mineral and trace element mix* | 10 | 10 | 10 |
| Calculated dietary content | | | |
| Stearic acid | 102 | 6 | 8 |
| Oleic acid | 50 | 141 | 48 |
| $C_{18:1}$ *trans* isomers | 0 | 0 | 95 |

* The composition of these two premixes has been described by Verbeek *et al.* (1993).

Before sampling, the rats had free access to feed and water. Lipoproteins were isolated from fresh plasma by density gradient centrifugation (Terpstra *et al.* 1981) and the following fractions were collected on the basis of their densities ($d$; kg/l) as previously (Beynen *et al.*, 1984): $d < 1{\cdot}006$; $1{\cdot}006 < d < 1{\cdot}019$; $1{\cdot}019 < d < 1{\cdot}063$; $d > 1{\cdot}063$. Based on the feeding schedule relative to the time of killing of the animals, it was estimated that TAG in the $d < 100{\cdot}6$ fraction represents about 70 % VLDL and 30 % chylomicrons TAG (ten Hoor *et al.* 1980; Groot *et al.* 1988). Isolated lipoprotein fractions were frozen and stored at −20°C until analyses. Subcellular liver fractions

**Table 2.** Fatty acid composition of the dietary fats (g methyl ester/100 g methyl esters)

| Fatty acid | Maize oil* | Hydrogenated soyabean oil† | Olive oil* | Hydrogenated, fractionated soyabean oil*‡ |
|---|---|---|---|---|
| $C_{16:0}$ | 10·4 | 10·4 | 9·3 | 6·3 |
| $C_{18:0}$ | 1·8 | 87·4 | 3·3 | 4·4 |
| $C_{18:1}$ | 28·2 | 0·1 | 79·3 | 83·7§ |
| $C_{18:2}$ | 57·2 | 0·1 | 4·1 | 0·7‖ |
| $C_{18:3}$ | 0·9 | 0·0 | 0·5 | 0·0 |

* Analysed composition.
† Product (B065) and composition obtained from Loders Croklaan B.V., Wormerveer, The Netherlands (melting point 65°C).
‡ Product (fraction isolated from B039) (melting point 20°C).
§ The $C_{18:1}$ fraction consisted of (g/100 g): $C_{18:1}$ 7 + 8*trans*, 12·1; $C_{18:1}$ 9*trans*, 9·3; $C_{18:1}$ 10*trans*, 8·9; $C_{18:1}$ 11*trans*, 8·6; $C_{18:1}$ 12*trans*(+5-*cis* + 7*cis*), 13·4; $C_{18:1}$ 9 *cis*(+13*trans*), 10·9; $C_{18:1}$ 10 *cis*, 6·7; $C_{18:1}$ 11*cis*(+5*trans*), 5·8; $C_{18:1}$ 12*cis*, 3·1; $C_{18:1}$ 13*cis*, 1·7; $C_{18:1}$ 14*cis*, 1·2; $C_{18:1}$ 16*trans*, 0·6; $C_{18:1}$ 15*cis*, 1·5 (total $C_{18:1}$*trans*: 52·9 %).
‖ The $C_{18:2}$ fraction consisted of (g/100 g): $C_{18:2}$ 12*trans trans*, 0·4; $C_{18:2}$ 12*cis trans*, 0·3; $C_{18:2}$ 12*trans cis*, 0·0; $C_{18:2}$ 12*cis cis*, 0·2 (total $C_{18:2}$*trans*: 0·7 %).

were prepared by homogenization and differential centrifugation exactly as reported previously (Geelen *et al.* 1995*a*). Isolated mitochondria were used immediately for the analyses of citrate carrier and CPT-I activity. Isolated microsomes were stored at −80°C until analysis a few weeks later. Samples of the hepatic homogenate were also used to measure the levels of TAG (Sundler *et al.* 1974) and glycogen (Hassid & Abraham, 1957). One part of the liver sample was homogenized immediately with a loose-fitting Dounce homogenizer (five strokes) in a medium containing (mmol/l): N′-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid (pH 7·5), 50; mannitol, 0·25; citrate, 4; EDTA, 6·16; β-mercaptoethanol, 5. The crude homogenate was centrifuged at 12 000 ***g*** for 5 min and the supernatant fraction was frozen quickly in liquid $N_2$ and stored at −80°C until analysed for the activities of fatty acid synthase and ACC.

*Enzyme assays*

Citrate carrier activity in freshly isolated mitochondria was assayed essentially as reported by Zara & Gnoni (1995). Briefly, freshly isolated rat-liver mitochondria were re-suspended in 100 mmol KCl/l, 20 mmol N′-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid/l, 1 mmol ethylene-glycol-bis(a-aminoethyl)-N,N,N′,N′-tetra-acetic acid/l, 2 μg rotenone/ml (pH 7·0), and loaded with L-malate as described previously (Palmieri *et al.* 1972). The rate of exchange between [$^{14}$C]citrate and malate catalysed by the carrier was measured at 9°C. The transport was started by the addition to the mitochondrial suspension of 0·5 mmol [$^{14}$C]citrate/l and stopped by the addition of 12·5 mmol 1,2,3-benzene tricarboxylic acid/l. The mitochondria were then re-isolated by centrifugation. The radioactivity, extracted from mitochondria after their osmotic disruption, was counted.

CPT-I activity was assayed in freshly isolated mitochondria as the incorporation of radiolabelled carnitine into acylcarnitine as reported by Guzmán *et al.* (1994). CPT activity that was insensitive to 100 μmol malonyl-CoA/l was always subtracted from the CPT activity experimentally determined.

Measurement of ACC, fatty acid synthase and DGAT was performed as described previously (Tijburg *et al.* 1988). The activities of citrate synthase, 3-HAD and PFK were determined spectrophotometrically as described by Geelen *et al.* (2001).

*Chemical analyses*

TAG (triacylglycerols–GB), total cholesterol (CHOD–PAP method) and phospholipids (enzymic colorimetric method) in plasma, lipoprotein fractions and liver tissue were determined with test kit combinations from Roche as specified. Hepatic lipids were extracted with chloroform–methanol (1:2, v/v) (Bligh & Dyer, 1959). Total lipids of the hydrogenated, fractionated soyabean oil were saponified and methylated according to Metcalfe *et al.* (1966) and the fatty acid composition was determined by GLC. Protein was determined by using the Lowry *et al.* (1951) method with bovine serum albumin as the standard.

*Statistical analysis*

The results were computed with Excel (Microsoft 7). Comparison was made using one-way ANOVA (Williams, 1993; Bailey, 1995). When a statistical effect was uncovered on the basis of the ANOVA analysis, the data were also subjected to the Student's *t* test. All statistical analyses were performed using an SPSS/PC computer program (SPSS, Chicago, IL, USA). Differences were considered statistically significant at $P<0·05$.

## Results

*Feed intake, body weight, liver weight, growth rates and feed efficiency*

Feed intake in the $C_{18:1}$ *trans* isomers-fed group differed significantly from that in the other two groups. Body weights, relative liver weights and growth rates did not differ significantly among the three dietary groups (Table 3). Feed efficiency was lowest on the $C_{18:1}$ *trans* isomers diet. Stearic acid is poorly digested (Smits *et al.* 2000) which explains why feed intake was higher and body weight and growth rate were somewhat lower when compared with the groups fed monounsaturated fatty acids.

*Liver lipids and glycogen concentrations*

Group mean liver glycogen content was not influenced by the type of dietary fatty acid (Table 4). The oleic and $C_{18:1}$ *trans* isomers diets caused a significant increase in hepatic TAG content. The hepatic cholesterol content was significantly increased by oleic acid administration when compared with stearic acid feeding. Liver phospholipid concentrations were not modified by diet (Table 4).

**Table 3.** Body weights, relative liver weights, feed intake, growth rates and feed efficiency of rats fed a diet containing either stearic acid, oleic acid or $C_{18:1}$ *trans* isomers for 14 d‡

(Mean values and standard deviations for twelve rats per group)

| | Stearic acid diet | | Oleic acid diet | | $C_{18:1}$ *trans* isomer diet | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Body weight (g) | | | | | | |
| Day 0 | 256 | 28 | 268 | 28 | 262 | 32 |
| Day 7 | 287 | 24 | 308 | 27 | 300 | 29 |
| Day 14 | 322 | 26 | 341 | 28 | 334 | 30 |
| Relative liver weight (g/100 g body weight) | | | | | | |
| Day 14 | 4·0 | 0·3 | 4·0 | 0·3 | 4·2 | 0·3 |
| Feed intake (g/d) | | | | | | |
| Day 0–14 | 28·0 | 4·3 | 26·4 | 3·6 | 22·0*† | 1·6 |
| Growth (g/d) | | | | | | |
| Day 0–14 | 4·7 | 1·2 | 5·6 | 1·3 | 5·2 | 0·9 |
| Feed efficiency (g feed/g growth) | | | | | | |
| Day 0–14 | 6.4 | 2.4 | 5.0 | 1.2 | 4.3* | 0.7 |

* Mean value was significantly different from that for the stearic acid diet ($P<0·01$).

† Mean value was significantly different from that for the oleic acid diet ($P<0·05$).

‡ For details of diets and procedures, see Tables 1 and 2 and p. 888.

**Table 4.** Hepatic contents (nmol/mg protein†) of glycogen, triacylglycerols, cholesterol and phospholipids of rats fed a diet containing either stearic acid, oleic acid or $C_{18:1}$ *trans* isomers for 14 d‡
(Mean values and standard deviations for twelve rats per group)

| | Stearic acid diet | | Oleic acid diet | | $C_{18:1}$ *trans* isomer diet | |
|---|---|---|---|---|---|---|
| Parameter | Mean | SD | Mean | SD | Mean | SD |
| Glycogen | 990 | 327 | 912 | 347 | 1080 | 125 |
| Triacylglycerols | 68 | 16 | 136** | 40 | 85* | 20 |
| Cholesterol | 29·6 | 3·0 | 53·5** | 14·7 | 29·4 | 2·4 |
| Phospholipids | 106 | 7 | 117 | 8 | 107 | 6 |

Mean value was significantly different from that for the stearic acid diet: *$P < 0{\cdot}05$, **$P < 0{\cdot}001$.
† 216 mg protein/g wet weight of liver.
‡ For details of diets and procedures, see Tables 1 and 2 and p. 889.

*Plasma lipids and lipoproteins*

As shown in Table 5, the serum total cholesterol and TAG content at the beginning of the experimental period (day 0) did not differ between the groups. At the end of the experimental period (day 14), serum cholesterol and TAG were not significantly affected by the oleic acid diet compared with the stearic acid diet. Feeding $C_{18:1}$ *trans* isomers decreased the plasma level of cholesterol when compared with either the stearic acid- or oleic acid-fed groups (Table 5). No diet effects were observed for the plasma content of phospholipids.

As shown in Table 6, rats fed oleic acid had significantly more cholesterol and phospholipids in the $d < 1{\cdot}006$ lipoprotein fraction when compared with the group fed stearic acid. In addition, the TAG:cholesterol value in the $d < 1{\cdot}006$ lipoprotein fraction was significantly decreased by the oleic acid diet. Feeding $C_{18:1}$ *trans* isomers caused a significant decrease in cholesterol in the $1{\cdot}019 < d < 1{\cdot}063$ lipoprotein fraction.

*Key enzymes of hepatic metabolism*

As shown in Table 7, the transport activity of the citrate carrier protein was significantly reduced in the oleic acid-fed and the $C_{18:1}$ *trans* isomers-fed rats when compared with their counterparts fed stearic acid. Consumption of the oleic acid or the $C_{18:1}$ *trans* isomers diets *v.* the stearic acid diet did not affect the specific activities of ACC, fatty acid synthase, DGAT or PFK (Table 7).

The activity of CPT-I was significantly reduced by $C_{18:1}$ *trans* isomers (Table 8). The sensitivity of CPT-I to inhibition by malonyl-CoA was not changed by dietary treatment (Table 8). No difference was observed in the citrate synthase activity between the three diets (Table 8). The activity of 3-HAD was decreased in the $C_{18:1}$ *trans* isomers-fed group when compared with either the stearic acid- or the oleic acid-fed groups (Table 8).

**Table 5.** Cholesterol, triacylglycerol and phospholipid concentrations (mmol/l) in plasma of rats fed a diet containing either stearic acid, oleic acid or $C_{18:1}$ *trans* isomers for 14 d*
(Mean values and standard deviations for twelve rats per group)

| | Stearic acid diet | | Oleic acid diet | | $C_{18:1}$ *trans* isomer diet | |
|---|---|---|---|---|---|---|
| Parameter | Mean | SD | Mean | SD | Mean | SD |
| Cholesterol | | | | | | |
| Day 0 | 2·55 | 0·32 | 2·53 | 0·17 | 2·55 | 0·26 |
| Day 14 | 2·24[a] | 0·17 | 2·35[a] | 0·28 | 1·92[b] | 0·22 |
| Triacylglycerols | | | | | | |
| Day 0 | 1·58 | 0·53 | 1·70 | 0·61 | 1·65 | 0·51 |
| Day 14 | 1·82 | 0·68 | 2·36 | 1·11 | 2·18 | 1·16 |
| Phospholipids | | | | | | |
| Day 14 | 2·22 | 0·21 | 2·46 | 0·36 | 2·20 | 0·32 |

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
* For details of diets and procedures, see Tables 1 and 2 and p. 889.

## Discussion

Compared with the stearic acid diet, the feeding of diets containing either oleic acid or $C_{18:1}$ *trans* isomers resulted in a significantly higher liver TAG concentration. The increase was most pronounced in the group fed oleic acid in which the TAG level was almost double that of the value seen in the animals fed stearic acid. In accordance with previous observations (Geelen & Beynen, 2000), the animals fed the oleic acid-containing diet had accumulated cholesterol in their livers, probably reflecting the preferential esterification of cholesterol with oleic acid (Beynen, 1988). The increase in liver cholesterol in the rats fed oleic acid was not associated with an increase in plasma total cholesterol, but there was a significant increase of cholesterol in the $d < 1{\cdot}006$ lipoprotein fraction. The concomitant decrease of cholesterol in the $1{\cdot}019 < d < 1{\cdot}063$ lipoprotein fraction, when compared with the rats fed stearic acid, explains why oleic acid failed to change the total plasma cholesterol concentration. In the light of studies with human subjects (Khosla & Hayes, 1996; Katan, 1998; Nelson, 1998) it was a surprising outcome of the present study with rats that feeding $C_{18:1}$ *trans* isomers reduced the amount of total cholesterol in plasma. The lowering of plasma cholesterol was associated with a reduction in cholesterol in the $1{\cdot}019 < d < 1{\cdot}063$ lipoprotein fraction. The present study focused on hepatic fatty acid metabolism and therefore the enzyme measurements do not shed light on the mechanisms underlying the differential effects of oleic acid and $C_{18:1}$ *trans* isomers on hepatic and plasma cholesterol metabolism.

In the present fatty acid-consumption trial with rats, fat-rich diets were used. High amounts of dietary fat will

**Table 6.** Cholesterol, triacylglycerol and phospholipid levels (μmol/l plasma) in lipoproteins of rats fed a diet containing either stearic acid, oleic acid or $C_{18:1}$ *trans* isomers for 14 d*

(Mean values and standard deviations for twelve rats per group)

| | Stearic acid diet | | Oleic acid diet | | $C_{18:1}$ *trans* isomer diet | |
|---|---|---|---|---|---|---|
| Density (kg/l) | Mean | SD | Mean | SD | Mean | SD |
| Cholesterol | | | | | | |
| $d<1{\cdot}.006$ | 199[a] | 95 | 475[b] | 205 | 204[a] | 97 |
| $1{\cdot}006<d<1{\cdot}019$ | 77 | 53 | 114 | 47 | 45 | 24 |
| $1{\cdot}019<d<1{\cdot}063$ | 559[a] | 139 | 480[a] | 129 | 417[b] | 129 |
| $d>1{\cdot}063$ | 1171 | 182 | 1051 | 163 | 1021 | 119 |
| Triacylglycerols | | | | | | |
| $d<1{\cdot}006$ | 1380 | 627 | 2079 | 1127 | 1838 | 1033 |
| $1{\cdot}006<d<1{\cdot}019$ | 351 | 395 | 396 | 298 | 329 | 315 |
| $1{\cdot}019<d<1{\cdot}063$ | 145 | 60 | 133 | 125 | 150 | 60 |
| $d>1{\cdot}063$ | 68 | 21 | 89 | 44 | 65 | 18 |
| Phospholipids | | | | | | |
| $d<1{\cdot}006$ | 362[a] | 195 | 817[b] | 440 | 475[a] | 344 |
| $1{\cdot}006<d<1{\cdot}019$ | 89 | 83 | 122 | 89 | 80 | 89 |
| $1{\cdot}019<d<1{\cdot}063$ | 317 | 82 | 268 | 66 | 217 | 85 |
| $d>1{\cdot}063$ | 910 | 151 | 905 | 113 | 712 | 128 |
| Triacylglycerols:cholesterol | | | | | | |
| In $d<1{\cdot}006$ | 7·43[A] | 2·10 | 4·36[B] | 1·65 | 8·70[A] | 1·68 |

*d*, Density.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
[A,B]Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}01$).
* For details of diets and procedures, see Tables 1 and 2 and p. 888.

depress lipogenesis. Despite this, TAG synthesis was still regulated, as hepatic TAG levels were different in the three dietary groups. This suggests that other than lipogenic enzymes, such as for instance citrate carrier, CPT-I and 3-HAD, were responsible for these differences.

Short-term studies with isolated hepatocytes have shown that stearic acid, oleic acid and $C_{18:1}$ *trans* isomers exhibit a fatty acid-specific pattern of oxidation and esterification (Woldseth *et al.* 1998; Guzmán *et al.*, 1999). In line with these observations, elaidic acid was shown to be a poorer substrate than oleic acid for the *in vivo* synthesis of hepatic TAG (Guzmán *et al.* 1999). In comparison with stearic acid, $C_{18:1}$ *trans* isomers may be a better substrate for hepatic TAG synthesis (see Table 4). Contrary to the *in vitro* outcome, the activity of CPT-I was inhibited in $C_{18:1}$ *trans* isomers-fed rats relative to stearic acid- and oleic acid-fed animals. The reason for which CPT-I is activated by $C_{18:1}$ *trans* isomers in the short term (Guzmán *et al.* 1999) and inhibited in the long term (Table 8) is not obvious. It could represent an adaptation to diminished expression of enzyme activities in the pathway of fatty acid oxidation in the face of enhanced fatty acid esterification. Inhibition of 3-HAD activity by $C_{18:1}$ *trans* isomers *v.* oleic and stearic acid is consistent with this explanation.

Dietary $C_{18:1}$ *trans* isomers may reduce the conversion of fatty acids into acetyl-CoA. This notion is based on the observed decrease in the activities of CPT-I and 3-HAD. Such a decrease will lead to the accumulation of fatty acids that enter the pathway of esterification, and as a result the amount of hepatic TAG will increase as was indeed found (Table 4). $C_{18:1}$ *trans* isomers feeding did not affect the activity of DGAT, but the increase in the amount of substrate, i.e. fatty acids, may by itself raise the rate of esterification. However, such a substrate-driven metabolic flow is uncommon as DGAT is known to be activated by fatty acid availability (Haagsman *et al.* 1982). *De novo* synthesized fatty acids may also be utilized for TAG synthesis, but this is not very probable as the activity of the rate-controlling enzyme of *de novo* fatty acid synthesis, ACC (Geelen *et al.* 1979), was unaffected by $C_{18:1}$ *trans* isomers consumption.

When compared with $C_{18:1}$ *trans* isomers, the consumption of oleic acid caused even more hepatic accumulation of TAG (Table 4). However, in the case of oleic acid feeding, fatty acid oxidation was unaffected and *de novo* fatty acid synthesis probably was decreased as indicated by the reduction in citrate carrier activity. It is unlikely that the increase in hepatic TAG was secondary to an increase in fatty acid mobilization from adipose tissue. In that case it

**Table 7.** Hepatic activities of the citrate carrier, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), diacylglycerol acyltransferase (DGAT) and phosphofructokinase (PFK) of rats fed a diet containing either stearic acid, oleic acid or $C_{18:1}$ *trans* isomers for 14 d†

(Mean values and standard deviations for twelve rats per group)

| Enzyme activity (nmol/min per mg protein) | Stearic acid diet | | Oleic acid diet | | $C_{18:1}$ *trans* isomer diet | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Citrate carrier | 10·7 | 2·1 | 8·4** | 1·6 | 6·8*** | 1·2 |
| ACC | 0·27 | 0·14 | 0·24 | 0·09 | 0·29 | 0·11 |
| FAS | 4·6 | 1·4 | 3·6 | 1·2 | 3·8 | 1·0 |
| DGAT | 0·57 | 0·10 | 0·49 | 0·08 | 0·48 | 0·09 |
| PFK | 14·3 | 2·5 | 14·6 | 2·2 | 13·5 | 2·3 |

Mean value was significantly different from that for the stearic acid diet: **$P<0{\cdot}01$, ***$P<0{\cdot}001$.
† For details of diets and procedures, see Tables 1 and 2 and p. 889.

**Table 8.** Hepatic activities of carnitine palmitoyltransferase (CPT)-I, citrate synthase and 3-hydroxy-acyl-CoA dehydrogenase (3-HAD) of rats fed a diet containing either stearic acid, oleic acid or $C_{18:1}$ *trans* isomers for 14 d§
(Mean values and standard deviations for twelve rats per group)

| Enzyme activity (nmol/min per mg protein) | Stearic acid diet | | Oleic acid diet | | $C_{18:1}$ *trans* isomer diet | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| CPT-1 | 3·04 | 0·40 | 2·93 | 0·41 | 2·59*† | 0·30 |
| CPT-1 + 10 μM-malonyl-CoA | 2·06 | 0·41 | 2·07 | 0·39 | 1·82 | 0·30 |
| Inhibition by malonyl-CoA (%) | 32·5 | 7·2 | 29·5 | 7·3 | 30·3 | 8·9 |
| Citrate synthase | 210 | 30 | 199 | 18 | 204 | 20 |
| 3-HAD | 1487 | 219 | 1597 | 211 | 690‡ | 246 |

* Mean value was significantly different from that for the stearic acid diet ($P < 0·01$).
† Mean value was significantly different from that for the oleic acid diet ($P < 0·05$).
‡ Mean value was significantly different from those for the stearic acid and oleic acid diets ($P < 0·001$).
§ For details of diet and procedures, see Tables 1 and 2 and p. 889.

would be expected that oleic acid feeding had mediated an increase in DGAT activity (Haagsman *et al.*, 1982), which was not observed. However, the increase in hepatic TAG may be explained by secretion of less TAG in the $d < 1·006$ lipoprotein fraction. Indeed, the TAG:cholesterol value in this fraction was significantly decreased after oleic acid feeding (Table 6), pointing to diminished hepatic secretion of TAG.

In conclusion, both oleic and $C_{18:1}$ *trans* isomers *v.* stearic acid increased the hepatic concentration of TAG. The underlying mechanisms, however, are probably quite different. Oleic acid may have induced a decrease in the secretion of TAG in the $d < 1·006$ lipoprotein fraction whereas $C_{18:1}$ *trans* isomers may have inhibited the hepatic oxidation of fatty acids.

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