Nutrient Metabolism

Stereospecific Incorporation of Palmitoyl, Oleoyl and Linoleoyl Moieties into Adipose Tissue Triacylglycerols of Rats Results in Constant sn-1:sn-2:sn-3 in Rats Fed Rapeseed, Olive, Conventional or High Oleic Sunflower Oils, but Not in Those Fed Coriander Oil

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ABSTRACT We report the stereospecific (sn-1, sn-2, sn-3) distribution of fatty acids in subcutaneous adipose tissue triacylglycerols of male weaned Wistar rats fed either a standard diet or diets containing, in addition to 20 g corn oil/kg feed, 120 g/kg feed, each, of canola-type rapeseed oil, olive oil, conventional or high oleic sunflower oil or high petroselinic coriander oil for 10 wk. The regiospecific distribution of the major acyl moieties in the sn-1 (3) vs. sn-2 positions of the adipose tissue triacylglycerols broadly reflected that of the dietary oils. The saturated palmitoyl and stearoyl moieties were more abundant in the sn-1 and sn-3 positions compared with the sn-2 position of the adipose tissue triacylglycerols, and both occurred at a higher proportion in the sn-1 than in the sn-3 position. Oleoyl moieties were abundant in all the three positions of the adipose tissue triacylglycerols, whereas petroselinoyl moieties were more abundant in the sn-1 and sn-3 positions compared with the sn-2 position. Linoleoyl moieties occurred predominantly in the sn-2 position compared with the sn-1 and sn-3 positions of the adipose tissue triacylglycerols; however, they were more abundant in the sn-3 than in the sn-1 position. Despite widely varying proportions of the palmitoyl, oleoyl and linoleoyl moieties at the three positions of the dietary triacylglycerols, the ratios of each of these acyl moieties at the sn-1, sn-2, and sn-3 positions in adipose tissue triacylglycerols were essentially constant for all groups, with the exception of the group fed coriander oil, indicating a rigid stereospecific incorporation. J. Nutr. 133: 435–441, 2003.

KEY WORDS: • rapeseed oil • olive oil • sunflower oil • stereospecific acyl pattern of triacylglycerols • rat subcutaneous adipose tissue

The effect of the fatty acid composition of dietary fat on body fat accumulation in adipose tissues and its mobilization is of great interest. The deposition and mobilization of triacylglycerols in adipose tissues depend on the chain length, degree of unsaturation and positional isomerism of their constituent fatty acids. Monounsaturated and (n-6) polyunsaturated fatty acids (PUFA)2 are preferentially incorporated into the triacylglycerols of adipose tissues compared with saturated and (n-3) PUFA (1,2). Moreover, lipolysis of adipose tissue triacylglycerols and mobilization of fatty acids seem to depend on the polarity of the molecular triacylglycerol species, i.e., the number of double bonds of the acyl moieties present in an individual triacylglycerol molecule (3–5).

Dietary fats affect the fatty acid composition of adipose tissue triacylglycerols of humans (2,6,7), rats (8,9) and rabbits (10); however, little is known concerning the effects of dietary fats on the molecular species of adipose tissue triacylglycerols (1,11–14). A few studies have described the regiospecific distribution in the (sn-1 + sn-3) positions and sn-2 position of adipose tissue triacylglycerols after feeding different oils by using regiospecific lipase for the determination of regiospecific distribution of acyl moieties (12,13). According to our knowledge, no studies using Grignard degradation and chiral phase liquid chromatography have been published to date on the complete stereospecific distribution of acyl moieties in the sn-1, sn-2, and sn-3 positions of adipose tissue triacylglycerols after feeding triacylglycerol mixtures with well-defined stereospecific distribution.

In a continuation of our earlier studies on the effects of dietary oils on the tissue lipid composition of rats (13–17), we report here the effects of dietary canola-type rapeseed oil (RAP), olive oil (OLI), conventional sunflower oil (SF), high-oleic sunflower oil (HOS) and high-petroselinic coriander oil (COR) on the stereospecific distribution of acyl moieties in triacylglycerols of subcutaneous adipose tissues of rats compared with those of the dietary triacylglycerols. A standard diet (ALT) was fed for comparison.

The aim of our study was to determine the stereospecific distribution of acyl moieties in the sn-1, sn-2- and sn-3 positions of adipose tissue triacylglycerols after feeding various

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2 Abbreviations used: ALT, rat standard laboratory diet; COR, coriander oil diet; FAME, fatty acid methyl esters; GC, gas chromatography; HOS, high oleic sunflower oil diet; OLI, olive oil diet; PUFA, polyunsaturated fatty acids; RAP, rapeseed oil diet; SF, sunflower oil diet.
MATERIALS AND METHODS

Materials. Triacylglycerols and fatty acid methyl ester (FAME) standards, ethyl magnesium bromide (1 mol/L solution in methanol tert-butyl ether) as well as 3,5-dinitrophenyl isocyanate were obtained from Sigma-Aldrich (Deisenhofen, Germany). Trimethyl sulfoxonium hydroxide reagent was purchased from Macherey-Nagel (Düren, Germany). Analytical grade and HPLC-grade solvents were products of Merck (Darmstadt, Germany).

Diets. Refined, bleached and deodorized rapeseed oil and high oleic sunflower oil were kindly provided by W. Holtmeyer, Noble & Thorl, Hamburg, Germany. Native olive oil and refined sunflower oil as well as corn oil were purchased from a local supermarket. Refined, bleached and deodorized coriander oil was a generous gift of A. Westfichtel, Henkel Cie & KG, Düsseldorf, Germany.

Semi-purified isocaloric pelleted feeds3 (metabolizable energy 13.1 kJ/g) containing recommended levels of protein, carbohydrates, vitamins and mineral nutrients (18) and semi-purified Altromin standard diet (Kontroldiät C 1000) for rats and one of the above experimental oils (120 g/kg diet) plus corn oil (20 g/kg diet) were prepared by Altromin International, Lage, Germany.

Animal experiments. Weaned male Wistar rats (Lippische Versuchstierzucht, Extertal, Germany) weighing 85–90 g were caged individually and divided into groups of 10 rats each. The rats were kept in rooms with adequate ventilation at a temperature of 22°C and a relative humidity of 60% with a 12-h light:dark cycle. The rats had free access to feed and water at all times until 12 h before they were killed.

Rats were fed for 10 wk. At the end of the feeding, the rats were killed by subjecting them to ether narcosis followed by section of the aorta. All procedures for the rat experiments were approved by the official commission for animal experimentation [Der Regierungspräsident Münster, permission no. 26.0834 (48/90) of November 29, 1990]. Subcutaneous adipose tissue was rapidly removed and kept frozen at −60°C until the lipids were extracted.

Lipid analysis. Lipids were extracted from subcutaneous adipose tissue according to Bligh and Dyer (19) and stored in dichloromethane/methanol (9:1, v/v) under nitrogen at −20°C. Lipids were also extracted from the various feed fats. Total lipids were fractionated by TLC on Silica Gel H (Merck) using i-hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as the solvent system. The fractions of triacylglycerols that had been identified by chomatography with a standard were scraped out, extracted from silica gel with water-saturated diethyl ether, blown with nitrogen until dry and stored in i-hexane under nitrogen at −20°C. Aliquots of the triacylglycerols extracts (−0.5 mg) dissolved in 40 μL methyl i-butyl ether were treated with 20 μL trimethyl sulfoxonium hydroxide reagent to prepare methyl esters of their constituent fatty acids. The mixture was shaken vigorously for 1 min and kept at room temperature for 15 min before direct injection onto the gas chromatograph (20).

Methyl esters were analysed by gas chromatography (GC) in a Hewlett-Packard 5890 instrument (Böblingen, Germany) on a 60-m DB-23 (0.25 μm/50% crosslinked methyl silicone, J & W, Ass-Chrom, Bad Homburg, Germany) fused silica capillary column (0.25 mm i.d., 0.25-μm film thickness). Hydrogen was used as the carrier gas at a column pressure of 100 kPa (20°C) and the split ratio was 1:10. The temperature was programmed from 150 to 180°C at 6°C/min (18 min isothermal) and from 180 to 250°C at 20°C/min (2 min isothermal). Injector and detector temperatures were set at 280°C. Peaks were integrated using a Hewlett-Packard GC ChemStation software. Aliquots of triacylglycerols from subcutaneous adipose tissues and feeds were analyzed for their stereospecific composition of acyl moieties by chiral HPLC as follows. Aliquots (~30 mg) from adipose tissues or feeds were subjected to Grignard degradation and the enantiomeric (sn-1 + sn-3)-monoyacylglycerols and sn-2-monoyacylglycerols were separated from the other degradation products by chromatography on layers of Silica Gel 60 G impregnated with boric acid (5 g boric acid/100 g silica gel) using first i-hexane/diethyl ether (7:3, v/v) and then dichloromethane/acetone/methanol (75:25:0.5, v/v/v) as the solvent system (21). The fractions of (sn-1 + sn-3)-monoyacylglycerols, −5 to 10 mg each, were extracted from silica gel and converted to their 3,5-dinitrophenylurethane derivatives by treatment with 10 mg of 3,5-dinitrophenyl isocyanate in a mixture of 0.5 mL dry toluene and 0.04 mL dry pyridine (22). The reaction products were purified by preparative TLC on Silica Gel H using dichloromethane/methanol (99:1, v/v). The margins of the chromatograms were stained with iodine vapor and the fraction corresponding to the 3,5-dinitrophenyl ether derivatives (R0.39) was scraped off and eluted with diethyl ether. HPLC separations of the 3,5-dinitrophenyl ether derivatives of the enantiomeric (sn-1 + sn-3)-monoyacylglycerols were conducted in a 5-μm Suplechiral OA-4100 column, 25 cm × 4 mm i.d. (Chrom Tech, Hägersten, Sweden) using n-hexane/dichloromethane/ethanol (40:12:1, v/v/v) 1 mL/min at 20°C for elution (22). The HPLC system consisted of a Merck-Hitachi pump L-6200 equipped with a column oven and a Kontron (Kontron Instruments, Milan, Italy) UV/Vis HPLC 332 detector set to a wavelength of 254 nm. The UV trace was monitored and evaluated in a Kromosystem 2000 data acquisition unit (Kontron Instruments). The column oven temperature was set at 20°C; the flow rate was 1 mL/min. Injections [−3 to 4 mg 3,5-dinitrophenylether derivatives of (sn-1 + sn-3)-monoyacylglycerols] were carried out with a Rheodyne 7161 sample injector (Cotati, CA) equipped with a 20-μL sample loop. Synthetic standards of 3,5-dinitrophenylether derivatives of (sn-1 + sn-3)-monoyacylglycerols were prepared from synthetic triacylglycerols by Grignard degradation and derivatization of the enantiomeric (sn-1 + sn-3)-monoyacylglycerols as described above, were used for comparison. Figure 1 shows a typical chiral HPLC separation of a mixture of enantiomeric sn-1- and sn-3-monoyacylglycerols.

After chiral HPLC separation of the 3,5-dinitrophenylether derivatives of sn-1- and sn-3-monoyacylglycerols, FAME were prepared by acid-catalyzed transesterification (23) and purified by preparative TLC on Silica Gel H using i-hexane/diethyl ether (80:20, v/v). The fraction of FAME was scraped off and eluted with water-saturated diethyl ether, dried and analyzed by GC as described above. Methyl esters derived from sn-2-monoyacylglycerols were prepared by the reaction with trimethylsulfoxonium hydroxide reagent and analyzed by GC as described above.

Statistical analysis. Triacylglycerols of subcutaneous adipose tissues from four randomly selected rats of each feeding group were used. Statistical evaluation of the data were conducted using a Statsoft one-way ANOVA computer program (STSC, Rockville, MD); a probability value (P) of <0.01, unless stated otherwise, was considered to be significant in the multiple range analysis. Cochran’s C test and Bartlett’s test were used as statistical tests for homogeneity of variance (24). If ANOVA was significant, Tukey’s honest significant differences test (24) was used for pairwise comparisons between groups using the above computer program.

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3 The composition of the semi-purified experimental diets was as follows (per kg diet): crude protein, 170.8 g; disaccharides, 111.4 g; poly saccharides, 151.3 g; crude fiber, 29.9 g; crude ash, 75.8 g; moisture, 53.8 g; p-aminobenzoic acid, 100.0 g; biotin, 0.20 mg; choline chloride, 1.0 mg; folic acid, 10.0 mg; niacin, 111.0 g; nicotinic acid, 50.2 mg; pantothenic acid, 50.1 mg; α-tocopherol, 4.5 mg; thiamine, 20.0 mg; riboflavin, 20.3 mg; pyridoxine hydrochloride, 15.0 mg; cyanocobalamin, 0.04 mg; ascorbic acid, 20.0 mg; cholecalciferol, 0.0125 mg; α-tocopherol, 193.6 mg; mendainone, 10.0 mg; available phosphorus, 7.16 g; calcium, 9.26 g; phytin, 3.6 g; copper, 0.13 mg; magnesium, 5.5 mg; fluoride, 4.17 mg; iodine, 0.45 mg; iron, 0.18 mg; manganese, 0.10 mg; molybdenum, 0.20 mg; potassium, 7.01 mg; selenium, 0.32 mg; sodium 2.44 mg; sulfur, 2.0 g; zinc, 28.8 mg. In addition, the RAP diet contained 120 g rapeseed oil, the OLI diet 120 g olive oil, the SF diet 120 g sunflower oil, the COR diet 120 g coriander oil, and the HOS diet 120 g high oleic sunflower oil. Moreover, all experimental diets contained 20 g corn oil/kg diet. The fatty acid composition of the triacylglycerols of the various diets is given in Table 1. The semi-purified rat standard diet (ALT) contained 40 g fat/kg diet with the fatty acid composition given in Table 1.
RESULTS

The fatty acid composition of the triacylglycerols of the standard laboratory diet (ALT) and diets containing rapeseed oil (RAP), olive oil (OLI), conventional sunflower oil (SF), high-oleic sunflower oil (HOS), and high-petroselinic coriander oil (COR), given in Table 1, shows that the major differences between the dietary triacylglycerols were in the level as well as stereospecific distribution of oleic acid [18:1(n-9)],4 petroselinic acid [18:1(n-12)], linoleic acid [18:2(n-6)] and α-linolenic acid [18:3(n-3)]. Thus, the dietary triacylglycerols of the RAP, OLI and HOS diets was distinctly abundant in the sn-1 and sn-3 positions compared with the sn-2 position of all the diets. The α-linolenoyl moieties, major constituents of the dietary triacylglycerols of the COR diet only, were distinctly abundant at the sn-1 and sn-3 positions compared with the sn-2 position. The linoleoyl moieties were present in substantially higher proportions at the sn-2 position compared with sn-1 and sn-3 positions of the dietary triacylglycerols of all diets, whereas the α-linolenoyl moieties were rather evenly distributed in the OLI and ALT diets; in the RAP diet, they were more abundant in the sn-2 position than in the sn-1 and sn-3 positions.

In earlier studies in which we fed RAP, OLI, SF, COR and HOS diets to rats (15), body weights of the rats fed the different diets did not differ significantly. Food consumption over the 10-wk period for the groups fed the RAP, OLI, SF, COR and HOS diets were 1867, 1900, 2000, 1908 and 1933 g/rat, respectively. The corresponding weight gains were 36.7, 32.1 35.6, 32.2 and 33.2 g/wk, respectively. No significant differences were observed in heart weight for the various experimental groups or in heart lipid content. Similarly, no significant differences were observed among the three groups of rats in plasma total cholesterol or triacylglycerols.

Our present data show that the regiospecific distributions of the major acyl moieties in the triacylglycerols of subcutaneous adipose tissues of the experimental rats broadly reflect those of the triacylglycerols of the corresponding dietary oils (Table 1). Thus, the saturated palmitoyl and stearoyl moieties, which were more abundant in the sn-1 and sn-3 positions of the dietary triacylglycerols compared with the sn-2 position, were also significantly enriched in the corresponding primary positions of the adipose tissue triacylglycerols of rats fed all diets except COR in which the proportion of palmitoyl moieties at the sn-2 position did not differ significantly from that at the sn-3 position. In particular, the palmitoyl moieties, and to some extent the stearoyl moieties, were significantly more abundant in the sn-1 position than in the sn-3 position of adipose tissue triacylglycerols of all groups of rats.

The oleoyl moieties, which were more abundant at the sn-1 and sn-3 positions compared with the sn-2 position of the dietary triacylglycerols of the RAP and SF diets, were found to be rather evenly distributed in the sn-2 and sn-3 positions of adipose tissue triacylglycerols of the rats fed the RAP diet and in sn-1 and sn-2 positions of those fed the SF diet (Table 1). In rats fed the OLI, COR, and HOS diets, the oleoyl moieties were more abundant at the sn-2 position compared with the sn-1 and sn-3 positions, of both dietary triacylglycerols and adipose tissue triacylglycerols. In those fed the ALT diet, the oleoyl moieties were evenly distributed in both dietary triacylglycerols and adipose tissue triacylglycerols. It is noteworthy that the proportion of oleoyl moieties at the sn-3 position of adipose tissue triacylglycerols was significantly higher than at the sn-1 position of all diet groups.

The petroselinoyl moieties were somewhat more abundant at the sn-1 and sn-3 positions of dietary triacylglycerols of rats fed the COR diet compared with the sn-2 position, and they were distinctly enriched in the sn-1 and sn-3 positions of the corresponding adipose tissue triacylglycerols with no significant preference for either of the two primary positions (Table 1). Similarly, cis-vaccenoyl moieties were distinctly more

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4 Fatty acids are designated by number of carbon atoms:number of double bonds as well as (n-x) nomenclature giving the position of double bonds, e.g., 18:1(n-9), oleic acid; 18:1(n-12), petroselinic acid; sn-1, sn-2 and sn-3 describe the stereospecific numbering (sn) of the glycerol backbone.

COMPOSITION OF ADIPOSE TISSUE TRIACYLGLYCEROLS

FIGURE 1 Separation by preparative chiral HPLC of enantiomeric sn-1 and sn-3 monoacylglycerols as their bis-(3,5-dinitrophenylurethane) derivatives using a Sumichiral OA-4100 column (25 cm × 4 mm i.d.) as described in Materials and Methods. The monoacylglycerols were derived from triacylglycerols of subcutaneous adipose tissue of rats fed a conventional sunflower oil diet (SF) for 10 wk.
### TABLE 1

**Stereospecific distribution of acyl moieties of triacylglycerols in subcutaneous adipose tissues of rats fed diets containing various plant oils for 10 wk**

<table>
<thead>
<tr>
<th>Acyl moiety</th>
<th>Stereospecific distribution</th>
<th>Pooled SEM (feeding groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acyl composition of dietary triacylglycerols</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>sn-1</td>
</tr>
<tr>
<td>16:0</td>
<td>6.5</td>
<td>8.7</td>
</tr>
<tr>
<td>18:0</td>
<td>1.6</td>
<td>3.1</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>51.0</td>
<td>53.6</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>3.4</td>
<td>5.8</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>29.0</td>
<td>21.8</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>8.6</td>
<td>6.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acyl moiety</th>
<th>Stereospecific distribution</th>
<th>Pooled SEM (feeding groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acyl composition of subcutaneous adipose tissue triacylglycerols</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sn-1</td>
<td>sn-2</td>
</tr>
</tbody>
</table>

1. Values given are means ± pooled SEM (n = 4).
2. Values in each row within a particular feeding group with different superscripts (a–c) are significantly different (P < 0.01), values with different superscripts (x–z) are significantly different (P < 0.05).
3. Values in each column within the various feeding groups with different superscripts (a–z) are signifi cantly different (P < 0.01).
4. Pooled SEM of values of the various positions (sn-1, sn-2 or sn-3) at the glycerol backbone within the same feeding group (comparison of data in rows).
5. Pooled SEM of values of the various feeding groups within the same stereospecific position (sn-1, sn-2 or sn-3) at the glycerol backbone (comparison of data in columns).

abundant at the sn-1 and sn-3 positions compared with the sn-2 position, of both dietary and adipose tissue triacylglycerols of all groups. There was no significant preference in the incorporation of cis-vaccenoyl moieties into either sn-1 or sn-3 positions in rats fed the OLI and COR diets or preference for their incorporation into the sn-1 position over the sn-3 posi-
tion for those fed the RAP, SF, HOS and ALT diets (Table 1). Very small proportions of cis-4-hexadecenoyl [16:1(n-12)] moieties, which were formed in rats from petroselinic acid by catabolic processes (13), were detected only in dietary triacylglycerols of those fed the COR diet, and the corresponding adipose tissue triacylglycerols contained distinctly enriched proportions of cis-4-hexadecenoyl moieties at the sn-1 and sn-3 positions (Table 1).

Linoleoyl moieties were distinctly more abundant at the sn-2 position compared with the sn-1 and sn-3 positions, of the dietary triacylglycerols of all diet groups and the corresponding adipose tissue triacylglycerols (Table 1). It is noteworthy that the proportion of linoleoyl moieties at the sn-3 position of adipose tissue triacylglycerols was significantly higher than at the sn-1 position of rats fed the RAP, OLI, SF, HOS and ALT diets, whereas in those fed the COR diet, there was no significant difference in the proportion of linoleoyl moieties at the sn-1 and sn-3 positions.

Linolenoyl moieties were distinctly more abundant at the sn-2 position compared with the sn-1 and sn-3 positions, of the dietary triacylglycerols of the RAP diet, but in the corresponding adipose tissue triacylglycerols, the proportion of ω-linolenoyl moieties was not significantly higher at the sn-2 position compared with the sn-1 and sn-3 positions (Table 1). For rats fed the OLI and ALT diets, the ω-linolenoyl moieties were evenly distributed at the sn-1, sn-2 and sn-3 positions of both dietary triacylglycerols and adipose tissue triacylglycerols. The remaining diet groups contained only traces of ω-linolenoyl moieties in both dietary triacylglycerols and adipose tissue triacylglycerols.

Table 2 shows the ratios of palmitoyl, oleoyl, linoleoyl and petroselinoyl moieties at the sn-1, sn-2, and sn-3 positions of dietary triacylglycerols and subcutaneous adipose tissue triacylglycerols of rats fed different plant oils. For the subcutaneous adipose tissue triacylglycerols of all diet groups, with the exception of the COR diet, the mean ratio of palmitoyl moieties at the sn-1, sn-2, and sn-3 positions was close to 4:1:2, and the corresponding mean ratios of oleoyl and linoleoyl moieties were ~1:1.2:1.2 and 1:2.5:1.5, respectively. In contrast, the ratios of palmitoyl, oleoyl, and linoleoyl moieties at the sn-1, sn-2, and sn-3 positions of the subcutaneous adipose tissue triacylglycerols was close to 2:1:1 and the corresponding ratios of oleoyl and linoleoyl moieties were ~1:1.3:1.2 and 1:4.6:1.5, respectively; the ratio of petroselinoyl moieties at the sn-1, sn-2, and sn-3 positions was close to 3:1:3.

**DISCUSSION**

The fatty acid composition of the dietary fat affects the composition of the acyl moieties of adipose tissue triacylglycerols in many animal species; however, the asymmetrical regiospecific distribution of saturated and unsaturated acyl moieties at the sn-1,3- and the sn-2-positions, respectively, of adipose tissue triacylglycerols is generally retained irrespective of the composition and positional distribution of acyl moieties in the dietary triacylglycerols (25).

Under normal (nonfasting) dietary conditions, a substantial part of adipose tissue triacylglycerols is derived from triacylglycerols of the intestinal mucosa, which in turn results from dietary triacylglycerols via lipolysis at the sn-1,3-positions in the intestinal lumen and reacylation of the resulting

**TABLE 2**

<table>
<thead>
<tr>
<th>Acyl moieties</th>
<th>Feeding group</th>
<th>Dietary triacylglycerols</th>
<th>Subcutaneous adipose tissue triacylglycerols</th>
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<tr>
<td></td>
<td></td>
<td>Ratio of acyl moieties in positions</td>
<td>Mean ratios&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sn-1</td>
<td>sn-2</td>
</tr>
<tr>
<td>16:0</td>
<td>RAP</td>
<td>4.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OLI</td>
<td>7.7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SF</td>
<td>5.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>HOS</td>
<td>3.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>5.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>COR</td>
<td>3.1</td>
<td>1</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>RAP</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>OLI</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>SF</td>
<td>2.4</td>
<td>1</td>
</tr>
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<td>HOS</td>
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<tr>
<td></td>
<td>ALT</td>
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<td>COR</td>
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<td>1.8</td>
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<td>18:2(n-6)</td>
<td>RAP</td>
<td>1.5</td>
<td>2.9</td>
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<td>OLI</td>
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<td>3.2</td>
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<tr>
<td>18:1(n-12)</td>
<td>COR</td>
<td>1.5</td>
<td>1</td>
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<sup>1</sup> Diet abbreviations: RAP, rapeseed oil; OLI, olive oil; SF, sunflower oil; HOS, high oleic sunflower oil; ALT, standard laboratory diet; COR, coriander oil.

<sup>2</sup> Excluding COR group; values rounded.
sn-2-monoacylglycerols in the intestinal mucosa (26). The sn-2-monoacylglycerols in intestinal cells are converted to triacylglycerols by monoacylglycerol acyltransferase and diacylglycerol acyltransferase (27). The triacylglycerols of intestinal mucosa are transported to various tissues, including adipose tissue, via chylomicrons of blood (26). Circulating triacylglycerols are hydrolyzed by tissue-specific lipoprotein lipase (28); the fatty acids released enter the adipocytes (29) and are then converted to triacylglycerols of the adipose tissues, possibly via the glycerol-3-phosphate pathway (30–32). Together with the transfer of endogenous fatty acids, this may result in adipose tissue triacylglycerols with a stereospecific distribution of acyl moieties different from that of the dietary triacylglycerols. Therefore, the composition and stereospecific distribution of acyl moieties of mucosal triacylglycerols, and consequently, of adipose tissue triacylglycerols may be determined by the composition and stereospecific distribution of acyl moieties in dietary triacylglycerols, the substrate specificity, endogenous activities of the acyltransferases in intestine and adipose tissues, as well as substrate specificity of lipoprotein lipase (26,27).

We showed recently in feeding studies with rats that most of the major acyl moieties and molecular species of dietary triacylglycerols from rapeseed oil, olive oil, as well as conventional sunflower oil (14) and high oleic sunflower and to some extent coriander oil (13) are also found as predominant acyl constituents and molecular species of the corresponding adipose tissue triacylglycerols. In the present study, we showed that when rats were fed these oils, which have widely varying fatty acid and triacylglycerol composition, the regiospecific distribution of the major acyl moieties of subcutaneous adipose tissues broadly reflected that of the dietary triacylglycerols (Table 1).

Distinct enrichment of palmitoyl and stearoyl moieties at the sn-1 position of adipose tissue triacylglycerols of all diet groups compared with the sn-2 and sn-3 positions, can be attributed in part to preferential incorporation of both endogenous and dietary palmitic and stearic acids into adipose tissue triacylglycerols during the first step of the glycerol-3-phosphate pathway (30–32), i.e., the formation of sn-1-acylphosphatidic acid.

The oleoyl moieties, which were abundant in each of the sn-1, sn-2 and sn-3 positions of most of the dietary triacylglycerols, except those of the COR diet, were also found in large proportions in all three positions of the corresponding adipose tissue triacylglycerols (Table 1). It seems that both dietary and endogenously synthesized oleic acid is readily accepted as a substrate by all of the enzymes involved in the biosynthesis of adipose tissue triacylglycerols. Significantly higher proportions of oleoyl moieties at the sn-3 position of adipose tissue triacylglycerols of rats fed the RAP, OLI, SF and HOS diets, compared with the sn-1 position, can be attributed in part to preferential incorporation of oleic acid into the sn-3 position of the glycerol backbone, mediated by diacylglycerol acyltransferase, in the last step in the biosynthesis of triacylglycerols via the glycerol-3-phosphate pathway (26,27).

Petroselinoyl moieties, which were more abundant in the sn-1 and sn-3 positions of dietary coriander oil triacylglycerols compared with the sn-2 position were distinctly enriched in the sn-1 and sn-3 positions of the corresponding adipose tissue triacylglycerols (Table 1), thus behaving like saturated acyl moieties due a higher melting point of petroselinic acid compared with oleic acid. Preferential incorporation of saturated acyl moieties into the sn-1 and sn-3 positions of adipose tissue triacylglycerols is well documented (27).

Linoleoyl moieties, which were more abundant in the sn-2 position than in the sn-1 and sn-3 positions of all the dietary triacylglycerols, were also found to be distinctly enriched in the sn-2 position of the corresponding adipose tissue triacylglycerols (Table 1), which may be attributed to preferential incorporation of linoleic acid into the sn-2 position of the glycerol backbone during conversion of sn-1-acylphosphatidic acid to phosphatidic acid, the second step during the biosynthesis of triacylglycerols via the glycerol-3-phosphate pathway. The abundance of linoleoyl moieties at the sn-2 position might also result from regioselective hydrolysis of the sn-1,3 acyl groups of dietary triacylglycerols and reacylation of the resulting sn-2-monoacylglycerols in the intestine (26,27).

In all of the adipose tissue triacylglycerols, the linoleoyl moieties were more abundant in the sn-3 position than the sn-1 position (Table 1). It is conceivable that diacylglycerol acyltransferase, which is involved mainly in the incorporation of acyl moieties at the sn-3 position of triacylglycerols, readily accepts linoleoyl-CoA as a substrate (26,27).

The distinctly different pattern of stereospecific distribution of the palmitoyl moieties in the adipose tissue triacylglycerols of rats fed the COR diet (Table 2) is likely due to the presence of large proportions of the unusual petroselinic acid in the dietary coriander oil, which seems to modulate the stereospecificity of the acyl transferases toward oleic, linoleic and α-linolenic acids.

It appears from our data that despite alterations by the endogenous fatty acid pool and numerous other physiologic processes occurring during the transport of ingested long-chain triacylglycerols and their derivatives via intestinal mucosa, lymph is and blood to subcutaneous adipose tissue, the overall regiospecific distribution of the individual acyl moieties of the dietary triacylglycerols was broadly reflected by the regiospecific distribution of the adipose tissue triacylglycerols (Table 1). Despite the above resemblance of regiospecific distribution of the major acyl moieties of the dietary triacylglycerols to those of the adipose triacylglycerols (Table 1), the rigid stereospecific distribution of palmitoyl, oleoyl, and linoleoyl moieties in the sn-1, sn-2 and sn-3 positions of adipose tissue triacylglycerols, irrespective of the dietary oils. It seems from these data that the biosynthesis of adipose tissue triacylglycerols might involve a strict stereospecific incorporation of individual acyl moieties to ensure the structure and function of triacylglycerols in animal fat storage tissues.
as rapeseed oil, olive oil and sunflower oil (14). Although only the subcutaneous adipose tissues were examined in the present investigation, it is likely that the triacylglycerols of the epididymal and perirenal adipose tissues within the same feeding group also had a similar stereospecific distribution of acyl moieties as those of the subcutaneous adipose tissues. This is evident from the stereospecific analysis of selected individual samples of triacylglycerols of other adipose tissues (epididymal and perirenal) showing similar results (data not shown). These results suggest that common physiologic and biochemical pathways determine the overall stereospecific distribution of acyl moieties in triacylglycerols found in the different adipose tissues irrespective of the stereospecific distribution of fatty acids of the dietary triacylglycerols.

Our studies on stereospecific distribution of fatty acids in adipose tissue triacylglycerols should provide evidence concerning the substrate specificity and stereospecificity of acyltransferases in vivo, especially of monoacylglycerol acyltransferases and diacylglycerol acyltransferases, which ultimately determine the positioning of individual fatty acids in triacylglycerols. Fats containing higher proportions of saturated fatty acids were not studied here, and they should be included in future work to determine their stereospecific pattern of incorporation under more competitive conditions, i.e., against unsaturated fatty acids. Moreover, the work conducted here using weaning rats should be extended to adult animals.

LITERATURE CITED