The effect of triacylglycerol-fatty acid positional distribution on postprandial metabolism in subcutaneous adipose tissue

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We hypothesized that fatty acids at the sn-2 position of chylomicron triacylglycerol are preferentially released into the venous plasma (rather than being taken up and stored in the adipocytes) after hydrolysis by lipoprotein lipase (EC 3.1.1.34) in adipose tissue. Arterio-venous differences across adipose tissue were studied in eight healthy subjects on two occasions for 6 h after ingestion of different structured triacylglycerols rich in palmitic acid either at the sn-2 or the sn-1,3 positions. In particular the specific fatty acids making up lipoprotein fractions and plasma non-esterified fatty acids were analysed. After the different meals there were no differences between either postprandial arterialized or venous plasma metabolite concentrations. Chylomicron triacylglycerol extraction in adipose tissue was the same following the two types of fat. There was no difference between the specific fatty acid composition of the postprandial non-esterified fatty acid release from adipose tissue after ingestion of the two triacylglycerols, indicating that there was no preferential release of a saturated fatty acid at the sn-2 position.

Triacylglycerols: Adipose tissue: Lipoprotein lipase: Postprandial metabolism

Evidence is growing that impaired postprandial triacylglycerol (TAG) metabolism may play an important role in the development of atherosclerosis (Patsch, 1994). In particular, the degree and duration of postprandial triacylglycerolaemia may be directly related to the evolution of atherogenic particles, such as small, dense LDL and loss of cholesterol from the HDL pool (Ebenbichler et al. 1995). It is of interest, therefore, to understand nutritional influences on the postprandial lipaemic response.

One such influence may be the molecular structure of dietary TAG. It has been suggested that TAG enriched with saturated fatty acids at the sn-2 position may be absorbed more rapidly in the gut in human infants (Filer et al. 1969; Carnielli et al. 1995) and cleared from the circulation more slowly in rats (Redgrave et al. 1988) than TAG containing saturated fatty acids at the sn-1 and -3 positions. TAG with a saturated fatty acid in the sn-2 position are not well hydrolysed by lipoprotein lipase (EC 3.1.1.34; LPL) in vitro (Tuten et al. 1993) or in vivo in rats (Mortimer et al. 1988). However, it has been shown previously that the positional distribution of fatty acids in dietary TAG has no effect on postprandial systemic plasma metabolite and hormone responses in normal adult men (Zampelas et al. 1994), suggesting that such effects are not after all important in human subjects.

Differences in the postprandial handling of dietary TAG may be more readily observed at the level of tissue metabolism. Adipose tissue is an important site for the initial hydrolysis of chylomicron TAG by LPL in the postprandial period. For some years it has been known from animal (Bergman et al. 1971; Scow, 1977) and human (Heimberg et al. 1974) studies that not all fatty acids released by the action of LPL are taken up for esterification and storage. Recent work with human subjects has shown that up to 70% of the fatty acids released by LPL action in adipose tissue were released into the venous plasma 4-5 h after a typical mixed meal when LPL action is maximal (Frayn et al. 1994). In the postprandial period LPL hydrolyses chylomicron TAG to release fatty acids. The fatty acids at the sn-1 and -3 positions are the first to be

Abbreviations: ATBF, adipose-tissue blood flow; LPL, lipoprotein lipase (EC 3.1.1.34); MAG, monoacylglycerol; NEFA, non-esterified fatty acid; SUU, structured TAG with saturated fatty acid at sn-1,3 position; TAG, triacylglycerol; USU, structured TAG with saturated fatty acid at sn-2 position; V-A, venous-arterial.

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released. The fatty acid at the sn-2 position has to isomerize to the sn-1 or -3 position in order to be hydrolysed, or there may be uptake of the resulting 2-monoacylglycerol (MAG; Braun & Severson, 1992). Whichever occurs, there appears to be no net production of plasma MAG during rapid LPL action in human adipose tissue in vivo (Fielding et al. 1995).

We hypothesized that, as the fatty acid at the sn-2 position has to isomerize to the sn-1 or -3 position before complete hydrolysis can occur, it will be the last chylomicron TAG-fatty acid to be hydrolysed and is likely to be released at the venous end of the adipose-tissue capillary; a fatty acid at the sn-2 position, therefore, may be particularly likely to enter the plasma non-esterified fatty acid (NEFA) pool. Furthermore, as saturated fatty acids at the sn-2 position are not well hydrolysed (Mortimer et al. 1988; Tuten et al. 1993), we hypothesized that a saturated fatty acid at the sn-2 position would be preferentially released into the adipose-tissue venous plasma. In order to test this hypothesis we have looked at the release of specific fatty acids into the adipose-tissue venous NEFA pool following meals containing structured TAG with a saturated fatty acid at either the sn-1 or -2 position. However, our experiments would equally test the opposite hypothesis of tissue uptake of the 2-MAG resulting from incomplete LPL action with preferential release of the sn-1 fatty acids into the plasma. The experiments also allowed us to examine the effects of TAG structure on chylomicron-TAG clearance in adipose tissue. This is the first study of the action of LPL on structured TAG in vivo in human subjects.

Some of the results have previously been published in abstract form (Summers et al. 1997) and some of the measurements of adipose-tissue blood flow (ATBF) have been presented as part of a more detailed study of blood flow regulation (Summers et al. 1996).

**Methods**

**Subjects**

Studies were conducted on eight healthy subjects (two male) aged 18–55 years (median 30.5 years), BMI 19–30 kg/m² (median 24 kg/m²) on two occasions. Their median fasting total cholesterol concentration was 4.3 (3.3–8.2) mmol/l with a median HDL-cholesterol concentration of 1.1 (0.72–1.4) mmol/l and TAG of 1.1 (0.56–3.7) mmol/l. Subjects were asked to refrain from smoking, alcohol and ‘unaccustomed exercise’ for 24 h before the study. They were asked to eat a fat-free meal on the evening before the study and then to fast from 20.00 hours, also avoiding caffeinated drinks. Premenopausal female subjects were studied at the same phase of their menstrual cycle on each visit. The studies were approved by the Central Oxford Research Ethics Committee and all subjects gave informed consent.

**Experimental methods**

A 100 mm, 22-gauge Secalon Hydrocath catheter (Ohmeda, Swindon, UK) was introduced over a guide wire into a superficial vein on the anterior abdominal wall and threaded towards the groin so that its tip lay just superficial to the inguinal ligament. As described previously (Frayn et al. 1989), this provided access to the venous drainage from the subcutaneous abdominal adipose tissue, uncontaminated by muscle drainage and with a relatively minor contribution from skin. This adipose tissue depot has been shown to be representative of whole-body adipose tissue (Frayn et al. 1993). In one subject it proved impossible to cannulate an abdominal vein on the second attendance, so the study was continued with only arterial samples being taken and, therefore, only arterial concentrations for this subject were used in analysis of the results. Also, with two further subjects it proved impossible to obtain sufficient adipose venous samples to be able to perform analysis of the specific fatty acids in the plasma NEFA and lipoprotein fractions.

A cannula was inserted in a retrograde fashion into a vein draining a hand heated in a box at 60°, to provide arterialized samples. Both the arterial and the venous cannulas were kept patent by continuous infusion of isotonic saline (9 g NaCl/l). Simultaneous blood samples were taken from the arterialized vein (for simplicity, referred to as arterial) and the abdominal vein at –20, 0, 30, 60, 90, 120, 180, 240, 300 and 360 min. A meal consisting of a milk shake with bread, containing 85 g carbohydrate, 60 g fat and 13 g protein was given at 0 min. The fat consisted of a structured TAG, either predominantly with a saturated fatty acid at the sn-1,3 position (SUU; Unilever Research, Sharnbrook, Beds., UK) or predominantly with a saturated fatty acid at the sn-2 position (USU; Betapol; Unilever Research), given in random order (Table 1). ATBF was measured immediately after each blood sample using the ¹³¹¹Xe washout method (Larsen et al. 1966) as described in Summers et al. (1996).

**Analyses**

A portion of each blood sample was rapidly deproteinized with perchloric acid (70 g/l). The remainder was heparinized and was used for blood gas analysis, packed cell volume estimation (arterial samples only) and to prepare plasma. Plasma NEFA, glucose and whole-blood lactate,

<table>
<thead>
<tr>
<th>Table 1. Specific fatty acid composition (mol/100 mol) of the total triglyceride (TAG) content and of the fatty acid at the sn-2 position of structured TAG with saturated fat at sn-2 position (USU) or sn-1,3 position (SUU)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific fatty acids</td>
</tr>
<tr>
<td>Fatty acid composition of total TAG</td>
</tr>
<tr>
<td>14:0</td>
</tr>
<tr>
<td>16:0</td>
</tr>
<tr>
<td>16:1</td>
</tr>
<tr>
<td>18:0</td>
</tr>
<tr>
<td>18:1</td>
</tr>
<tr>
<td>18:2</td>
</tr>
<tr>
<td>18:3</td>
</tr>
</tbody>
</table>

* Unilever Research, Sharnbrook, Beds., UK.
glycerol and 3-hydroxybutyrate concentrations were measured using enzymic methods on an IL Monarch centrifugal analyser (Instrumentation Laboratory (UK) Ltd, Warrington, Cheshire, UK). Plasma TAG concentrations were also measured enzymically (with correction for free glycerol; Humphreys et al. 1990). Plasma insulin was measured in the arterial samples using a double-antibody radioimmunoassay method (Kabi Pharmacia Ltd, Milton Keynes, Bucks, UK).

Analysis of chylomicron TAG was performed at seven time points: at 0 min and hourly after this. VLDL-TAG analysis was performed at 0, 180 and 360 min. TAG-rich lipoprotein fractions were prepared from plasma as described previously. All equipment was supplied by Beckman Instruments (UK) Ltd, High Wycombe, Bucks, UK. Chylomicron-rich fractions were prepared by layering 0.75 ml plasma prepared as described previously, which was transferred into 13 x 51 mm bell-topped centrifuge tubes and centrifuged at 4°C in a rotor type TLA 100-4 at 100 000 rev/min for 2 h 30 min. The VLDL-rich fraction was separated by slicing using a ‘CentriTube’ tube slicer. The VLDL-rich fraction was prepared using the infranate from 1.5 ml plasma prepared as described previously, assuming equal esterification of hormone-sensitive lipase (EC 3.1.1.3) and LPL-derived fatty acids, has been described previously (Frayn et al. 1994).

Repeated-measures ANOVA was used to compare metabolite concentrations in each subject, analysing the effects of both the two different meals and also time. Where appropriate a paired t test was used to compare maximum or minimum values with the basal value (taken as the value at 0 min). Basal and postprandial areas under curves were calculated for arterial metabolite concentrations. Total area under curve was calculated for total TAG (from 0 min), chylomicron TAG and VLDL-TAG extraction.

Results

Insulin concentrations

Arterial insulin concentrations rose significantly after both meals (P < 0.005), reaching a peak at 30–60 min. There was no difference in insulin concentrations between meals.

Adipose tissue blood flow measurements

As previously reported (Samra et al. 1995; Summers et al. 1996), there was a significant rise in ATBF postprandially (P < 0.005), with a peak at 30–60 min. There were no significant differences in the ATBF between the two studies.

Metabolite concentrations in the two sampling sites

There were no significant differences between the two sites for either arterial or venous plasma glucose, glycerol, lactate and 3-hydroxybutyrate concentrations. Arterial concentrations were shown in Table 2. As expected, arterial glucose concentrations were slightly higher than venous concentrations throughout both experiments, but these differences were not significant (data not shown). However, the increase in both arterial and venous glucose concentrations with time after both meals was significant (P < 0.005), the peak occurring at 60–90 min. Glycerol concentrations were consistently higher in the adipose-tissue venous effluent than in arterial blood. Arterial and venous glycerol concentrations decreased significantly postprandially (P < 0.005) and were lowest at about 60–90 min following both meals. Arterial and venous lactate and 3-hydroxybutyrate concentrations also changed significantly with time (P < 0.005), lactate concentrations reaching a peak at 60–90 min and 3-hydroxybutyrate reaching a trough at 90–120 min.

Triacylglycerol

Arterial and venous total TAG concentrations changed significantly with time (P < 0.005), reaching a peak at 180–240 min. The arterial TAG concentrations were consistently
Table 2. Arterial metabolite concentrations for healthy subjects after meals containing structured triacylglycerol with saturated fatty acid at sn-2 position (USU) or sn-1,3 position (SUU)*
(Time-averaged values, based on mean basal and postprandial areas under curve, with their standard errors for eight subjects)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Meal containing</th>
<th>Basal Mean</th>
<th>SE</th>
<th>Postprandial Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SUU</td>
<td></td>
<td></td>
<td>USU</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.0</td>
<td>0.1</td>
<td>5.0</td>
<td>0.10</td>
<td>5.3</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>6.3</td>
<td>3.8</td>
<td>4.41</td>
<td>0.67</td>
<td>13.6</td>
</tr>
<tr>
<td>Lactate (pmol/l)</td>
<td>650</td>
<td>140</td>
<td>640</td>
<td>61</td>
<td>790</td>
</tr>
<tr>
<td>3-Hydroxybutyrate (µmol/l)</td>
<td>120</td>
<td>35</td>
<td>110</td>
<td>30</td>
<td>190</td>
</tr>
<tr>
<td>Glycerol (µmol/l)</td>
<td>74.1</td>
<td>7.1</td>
<td>78.4</td>
<td>8.1</td>
<td>59.4</td>
</tr>
</tbody>
</table>

* For details of subjects and procedures, see pp. 142–143 and Table 1.

Table 3. Fractional extraction of total triacylglycerol (TAG) and chylomicron TAG across adipose tissue for healthy subjects after meals containing structured TAG with saturated fatty acid at sn-2 position (USU) or sn-1,3 position (SUU)*
(Time-averaged values, based on mean total areas under curves from 0 min, with their standard errors for seven subjects for total TAG and for five subjects for chylomicron TAG)

<table>
<thead>
<tr>
<th>Meal containing</th>
<th>USU Mean</th>
<th>SE</th>
<th>SUU Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total TAG</td>
<td>6.7</td>
<td>1.0</td>
<td>5.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Chylomicron TAG</td>
<td>21.6</td>
<td>3.1</td>
<td>18.8</td>
<td>8.9</td>
</tr>
</tbody>
</table>

* For details of subjects and procedures, see pp. 142–143 and Table 1.

higher than the venous concentrations. Adipose-tissue TAG clearance increased postprandially, being maximal at 240 min following the meal containing USU and at 180 and 360 min following the meal containing SUU. These increases in TAG clearance were significant (P < 0.05).

There were no differences between the two meals for arterial or venous total TAG (arterial values shown in Fig. 1), chylomicron TAG (Fig. 2) or VLDL-TAG concentrations (not shown). The fractional extraction of total TAG and chylomicron TAG did not differ between meals (Table 3). There were also no differences in total TAG or chylomicron TAG clearance between the two meals.

Chylomicron TAG (Fig. 2) and VLDL-TAG concentrations changed significantly with time at both sites (P < 0.005), reaching a maximum at 180 min after both meals. Arterial chylomicron TAG was consistently higher than adipose venous chylomicron TAG after both meals (Fig. 2). Arterial VLDL-TAG concentrations were also higher than venous concentrations for both meals. There
Triacylglycerol structure and metabolism

60 120 180 240 300 360
Period after meal (min)

Fig. 3. Specific fatty acid composition (mol/100 mol) of arterial chylomicron triacylglycerol in eight healthy subjects compared with composition of structured triacylglycerol with saturated fatty acid at sn-1,3 position (SUU), or at sn-2 position (USU). (■), C16:0; ( ), C18:1; ( ), other fatty acids. For details of subjects and procedures, see pp. 142-143 and Table 1.

was no difference between the arterial and venous concentrations of palmitic and oleic acid relative to total chylomicron TAG-fatty acid content over time with either meal (Fig. 3).

Non-esterified fatty acids

NEFA concentrations at the arterial site and in the adipose venous effluent changed significantly with time ($P < 0.005$) with maximal NEFA suppression occurring at 60-90 min. As in previous mixed-meal studies (Coppack et al. 1990) there was a large venous-arterial (V–A) difference for NEFA both before and after the meal, indicating net release of fatty acid from adipose tissue. The V–A difference changed significantly with time ($P < 0.005$) and was lowest at the times of maximal NEFA suppression (Fig. 4). There was no difference between the two meals in the V–A differences. When comparing the two meals there were no differences between arterial or venous plasma NEFA concentrations. The proportions of LPL-derived fatty acids released into the venous plasma (rather than being re-esterified and stored in adipose tissue) were similar after both meals and increased significantly ($P < 0.005$) in the postprandial period, reaching a maximum of at least 70 % after 4–6 h.

There were no differences between the meals in palmitic or oleic acid concentrations in the arterial and venous plasma NEFA fractions at any time point, nor any difference in their concentrations when expressed relative to total NEFA released from adipose tissue (Table 4). There was also no difference in oleic acid : palmitic acid ratio in the V–A difference of the plasma NEFA fraction with time following either meal and no differences between the two meals.

Table 4. Composition of total adipose-tissue non-esterified fatty acid release (mol/100 mol) for healthy subjects from 4–6 h after meals containing structured triacylglycerols with saturated fatty acid at sn-2 position (USU) or sn-1,3 position (SUU)*

<table>
<thead>
<tr>
<th>Meal containing ...</th>
<th>USU</th>
<th>SUU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific fatty acids</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>14:0</td>
<td>1.93</td>
<td>0.45</td>
</tr>
<tr>
<td>16:0</td>
<td>28.2</td>
<td>6.07</td>
</tr>
<tr>
<td>16:1</td>
<td>3.42</td>
<td>1.86</td>
</tr>
<tr>
<td>18:0</td>
<td>3.66</td>
<td>1.90</td>
</tr>
<tr>
<td>18:1</td>
<td>47.1</td>
<td>7.09</td>
</tr>
<tr>
<td>18:2</td>
<td>15.1</td>
<td>0.76</td>
</tr>
<tr>
<td>18:3</td>
<td>0.98</td>
<td>1.01</td>
</tr>
<tr>
<td>18:1/16:0</td>
<td>1.96</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* For details of subjects and procedures, see pp. 142–143 and Table 1.

Discussion

We have assumed that the fatty acid at the sn-2 position of dietary fat is mainly conserved at the same position in the chylomicron TAG. There is good evidence that this does occur *in vivo*. When dietary TAG is ingested it is acted on by pancreatic lipase in the small intestine to form a 2-MAG and two fatty acids. These are then taken up into the intestinal cells where chylomicron TAG is manufactured.
There are two pathways for the production of chylomicron TAG; the phosphatidic acid pathway and the 2-MAG pathway. Following fat ingestion, most chylomicron TAG synthesis occurs via the 2-MAG pathway. In any case TAG arising by the MAG or the phosphatidic acid pathway are closely similar to each other and to the TAG ingested (Myher et al. 1987). It has been shown that at least 85% of the fatty acid at the sn-2 position of dietary TAG is conserved at the sn-2 position of chylomicron TAG following ingestion and absorption in the intestine (Åkesson et al. 1978; Yang & Kuksis, 1991; Pufal et al. 1995).

As expected from the study by Zampelas et al. (1994) there were no differences between the arterial metabolite or insulin concentrations after the two meals. We found no difference in palmitic acid release across adipose tissue after the two meals. A power calculation shows that there is a 90% chance in this study of being able to detect a true difference of 15% between the adipose tissue V-A difference of the NEFA released from adipose tissue variably similar after the two meals. These results suggest that our original hypothesis was incorrect; there is no evidence against the alternative hypothesis of tissue uptake or use of dietary TAG containing fatty acids that are less commonly found in the plasma NEFA fraction or to use $^{13}$C-labelled dietary fats.

Acknowledgements

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uptake by human adipose tissue in vivo. Biochemical Society Transactions 23, 487S.


