The effect of triacylglycerol fatty acid positional distribution on postprandial plasma metabolite and hormone responses in normal adult men

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(Received 14 September 1992 – Revised 4 February 1993 – Accepted 27 April 1993)

The present study has examined the possibility that the positional distribution of fatty acids on dietary triacylglycerol (TAG) influences the postprandial response to a liquid meal in adult subjects. Postprandial TAG, non-esterified fatty acids (NEFA), ketones, glucose, insulin and gastric inhibitory polypeptide (GIP) responses were monitored in sixteen normal adult male subjects over 6 h following consumption of test meals containing dietary TAG in which palmitic acid was predominantly on the sn-1 (Control) or sn-2 positions (Betapol). Plasma total TAG, chylomicron-rich TAG and chylomicron-poor TAG concentrations were identical in response to the two test meals. The peak increase (mean (SD)) in chylomicron TAG was 0.85 (0.46) mmol/l after the Control meal and 0.85 (0.42) mmol/l after the Betapol meal. Plasma glucose, insulin, GIP, NEFA and ketone concentrations were also very similar following the two meals. It is concluded that dietary TAG containing saturated fatty acids on the sn-2 position appear in plasma at a similar level and over a similar timescale to TAG in which saturated fatty acids are predominantly located on sn-1 or sn-3 positions. The results reported in the present study demonstrate that the positional distribution of fatty acids on dietary TAG is not an important determinant of postprandial lipaemia in adult male subjects, but do not exclude the possibility that different responses may occur when these dietary TAG are given long term.

Triacylglycerols: Postprandial lipaemia: Insulin: GIP

Recognition of the importance of postprandial lipoproteins in the aetiology of atherosclerosis (Zilversmit, 1979; Mahley, 1982) has led to interest in the characteristics of dietary triacylglycerols (TAG) which may influence the extent and duration of postprandial lipaemia. That the positional distribution of fatty acids on dietary TAG may be a determinant of postprandial lipaemia is suggested by evidence that fatty acid positional distribution can affect both rates of absorption and clearance of dietary TAG. During digestion and absorption the acyl chain on the sn-2 position of dietary TAG is relatively resistant to the lipolytic actions of pancreatic lipase (EC 3.1.1.3) so that approximately 75% of fatty acids at sn-2 remain intact as monoacylglycerols (Mattson & Volpenheim, 1964). This positional specificity of pancreatic lipase may result in advantage for the absorption of saturated fatty acids located at sn-2 since there is evidence from studies in rats (Tomarelli et al. 1968; Mattson et al. 1979) and human infants (Filer et al. 1969) to suggest

* For reprints.
that saturated fatty acids located at sn-2 are more readily absorbed in their monoacylglycerol form than are non-esterified saturated fatty acids released from sn-1 and sn-3 positions.

Because monoacylglycerols provide the basic structure for the resynthesis of chylomicron TAG in the enterocyte, sn-2-located saturated acyl chains present in ingested dietary TAG will be conserved and incorporated into circulating chylomicron TAG. This may be relevant to the clearance of such TAG since kinetic studies in rats have shown that when dietary TAG containing saturated fatty acids at the sn-2 position are administered orally or intravenously, rates of TAG hydrolysis and removal, and rates of chylomicron remnant clearance, are slower than when a saturated fatty acid is located at sn-1 or sn-3 (Mortimer et al. 1988; Redgrave et al. 1988). Thus TAG enriched with saturated fatty acids at sn-2 may be absorbed more rapidly and cleared from the circulation more slowly than TAG containing saturated fatty acids at sn-1 and sn-3, so that feeding these dietary TAG may result in a more pronounced postprandial lipaemia.

The picture is further complicated by potential effects of TAG structure on the secretion of the hormones insulin and the insulinotropic hormone gastric inhibitory polypeptide (GIP). The secretion of GIP is brought about by the absorption of dietary TAG into the enterocyte (Ebert & Creutzfeldt, 1985), so that factors which increase the rate of TAG absorption may also enhance the secretion of GIP, and thereby insulin. Because both insulin and GIP are hormone stimulators of lipoprotein lipase, (LPL; EC 3.1.1.34; the enzyme which regulates TAG hydrolysis in circulation), effects of fatty acid positional distribution on rates of absorption may result in secondary effects on rates of clearance due to modulation of LPL by hormones of the enteroinsular axis.

The availability of synthetic TAG of known fatty acid composition and positional distribution means that it is now possible to study effects of TAG structure on postprandial lipoprotein metabolism in adult human subjects. Such studies are required because present knowledge is based on extrapolation from studies in animals (Mortimer et al. 1988; Redgrave et al. 1988) and metabolic studies in newborn infants (Filer et al. 1969). In the present study two fat blends of identical fatty acid composition, but differing in the positional distribution of their major fatty acids (oleic and palmitic acids), were incorporated into a liquid meal in order to study effects of TAG structure on postprandial lipaemia. Measurements of postprandial NEFA, ketones, insulin, GIP and glucose, as well as TAG concentrations, have been made in order to obtain as complete a picture as possible of effects of TAG structure on postprandial metabolism in man.

SUBJECTS AND METHODS

Subjects

Sixteen male volunteer subjects were recruited from the University of Surrey student and staff population. Blood low-density-lipoprotein (LDL)- and high-density-lipoprotein (HDL)-cholesterol, TAG and glucose levels were screened before entry into the study to exclude the possibility of undiagnosed hyperlipidaemia or diabetes in any of the volunteers. Other exclusion criteria included: presence of endocrine, liver or alcoholic disease; body mass index (BMI) < 20 or > 27; current adherence to an exclusion diet; habitual undertaking of > 8 h vigorous exercise/week; habitual consumption of > 30 units alcohol/week.

The mean (sd) age and BMI of subjects recruited into the study were 24.8 (2.6) years and 22.7 (2.4) kg/m² respectively.

All subjects were asked to provide written consent to participate in the study which was approved by the Ethics Committee of the University of Surrey.
Table 1. Fatty acid composition and sn-2 positional distribution of Control and Betapol fat blends

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Composition (g/100 g total fatty acid)</th>
<th>sn-2 Position</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Betapol</td>
</tr>
<tr>
<td>14:0</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>16:0</td>
<td>29.9</td>
<td>30.4</td>
</tr>
<tr>
<td>16:1</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>18:0</td>
<td>3.4</td>
<td>3.2</td>
</tr>
<tr>
<td>18:1</td>
<td>50.0</td>
<td>51.5</td>
</tr>
<tr>
<td>18:2</td>
<td>13.4</td>
<td>13.3</td>
</tr>
<tr>
<td>18:3</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Fat blends

Two fat blends were prepared (Unilever Research) with identical fatty acid profiles but differing in the positional distribution of the major fatty acids (Table 1). The Control blend was a blend of natural vegetable oils (mainly palm oleine) in which only 6% of the palmitic acid present was esterified to the sn-2 position of the TAG. The Betapol (tradename) blend was derived by enzymic interesterification of palm stearine with sunflower, and high oleic acid sunflower acids. Of the palmitic acid in this blend, 73% was esterified at the sn-2 position.

Meal preparation and composition

The liquid meal consumed by subjects in the present study was prepared by mixing 84 g powdered meal replacement diet (Carnation Slender) with 200 ml water and 40 g of the fat blend. The mixture was prepared freshly on the day of use and was homogenized immediately before consumption. The calculated nutrient composition of the meal is shown in Table 2.

Procedure

Postprandial responses to the two meals containing either the Control or the Betapol fat blends were measured over 6 h on two occasions separated by 2 weeks. Subjects were randomized to receive either the Control or the Betapol oil on the first visit and received the other oil on the second visit. Subjects fasted overnight and refrained from alcohol consumption on the day before the study. On each morning of the study the subjects reported to the study centre where they were seated quietly before the insertion of an indwelling cannula into the antecubital vein for the purpose of blood collection. Room temperature was maintained at 22°C.

Following the collection of two fasting blood samples the subjects were asked to take the liquid meal, which all consumed within 5 min.

Blood samples were taken at -10 and 0 min before the meal, at 15 min intervals for the first hour, 30 min intervals for the second hour and at hourly intervals thereafter until 6 h. Each blood sample (20 ml) was divided into two equal portions for the preparation of 5 ml volumes of plasma. One portion was used to prepare chylomicron-rich (supernatant) and chylomicron-poor (infranatant) fractions using the ultracentrifugation method of Grundy & Mok (1976). TAG analysis was carried out on each of these fractions as well as...
Table 2. *Nutrient composition* of the liquid meal (meal replacement plus Betapol or Control fat blend)

<table>
<thead>
<tr>
<th></th>
<th>g</th>
<th>kJ (kcal)</th>
<th>% Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>40.0</td>
<td>1506 (360)</td>
<td>54</td>
</tr>
<tr>
<td>Protein</td>
<td>15.1</td>
<td>251 (60)</td>
<td>9</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>60.5</td>
<td>1012 (242)</td>
<td>37</td>
</tr>
</tbody>
</table>

*Figures calculated from manufacturer's ingredient compositions.*

on the intact plasma (see below). The other sample was subdivided for the measurement of plasma glucose, TAG, NEFA and ketones (the latter on eight subjects only). In the case of the −10 and 0 min samples (fasting), measurements of LDL- and HDL-cholesterol and apoproteins B and A1 were also carried out. These measurements were made to ensure that differences in lipoprotein metabolism, reflected by changes in lipoprotein and apoprotein measurements, did not occur between the two study days.

**Analyses**

**Metabolites.** Glucose, TAG, cholesterol, NEFA and apoprotein analyses were carried out on a Boehringer Hitachi 704 EC analyser at 37°C. Glucose was analysed by the glucose oxidase (EC 1.1.3.4)–peroxidase (EC 1.11.1.7) coupled enzymic procedure, TAG content was measured by a standard coupled enzymic method using (EC 3.1.1.3)–lipase–glycerol kinase (EC 2.7.1.30), total cholesterol was measured using a standard coupled enzymic procedure using cholesterol esterase (EC 3.1.1.13)–cholesterol oxidase (EC 1.1.3.17), all supplied as test kits (Boehringer Mannheim GmbH, Mannheim, Germany). NEFA content was also measured using a Boehringer Mannheim test kit. HDL-cholesterol was determined using phosphotungstic acid–MgCl₂ precipitation followed by analysis of cholesterol as above. LDL-cholesterol was calculated from the measurement of total cholesterol, HDL-cholesterol and TAG, as described by Freidwald et al. (1972). Apoprotein B (apo B-100 and apo B-48) and apoprotein A1 were measured by immunoturbidimetric methods using test kits (Boehringer Mannheim). Total ketone (acetoacetate and β-hydroxybutyrate) concentrations were measured on fresh plasma by an enzymic method using an Encore centrifugal analyser (Pearse et al. 1987).

**Hormones.** Measurements of plasma immunoreactive insulin and GIP concentrations were performed on venous blood by double-antibody techniques as described previously (Morgan et al. 1978). Antisera were provided by Guildhay Antisera Ltd (Guildford). The sensitivity of the insulin assay was 2.5 mU/l and the GIP assay 110 ng/l.

**Statistical analyses**

The mean and standard deviation for each variable for each time point were calculated and plotted as postprandial response curves. Differences in postprandial responses between Control and Betapol fat blends were tested using a repeated measures design analysis of variance and by calculation of total and incremental areas under the postprandial response curves taking the −10 min sample as the baseline. Differences between individual time points were tested where differences in mean values suggested that effects may be evident, using the unpaired Student’s t test. All procedures were carried out using the SPSS-PC statistical package. The level of statistical significance for each test was taken as P < 0.05.
Table 3. Fasting concentrations of metabolites and apoproteins in subjects before consumption of Control and Betapol fat blends*

(Mean values and standard deviations for sixteen subjects)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Betapol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.00 ± 0.40</td>
<td>5.01 ± 0.36</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>0.76 ± 0.19</td>
<td>0.83 ± 0.27</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.75 ± 0.47</td>
<td>4.63 ± 0.49</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.00 ± 0.49</td>
<td>2.80 ± 0.43</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.30 ± 0.21</td>
<td>1.27 ± 0.23</td>
</tr>
<tr>
<td>Apo B (mg/100 ml)</td>
<td>61.8 ± 15.9</td>
<td>56.0 ± 22.2</td>
</tr>
<tr>
<td>Apo A1 (mg/100 ml)</td>
<td>134.9 ± 34.3</td>
<td>125.6 ± 20.2</td>
</tr>
</tbody>
</table>

TAG, triacylglycerol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo, apoprotein.
* For details of test meals and procedures, see Tables 1 and 2, and pp. 402-404.

RESULTS AND DISCUSSION

Table 3 shows that fasting concentrations of glucose, TAG, cholesterol and apoproteins were identical on each of the study days.

Following administration of the Control and Betapol meals the total plasma TAG concentration (Fig. 1a) rose from fasting levels of about 0.8 mmol/l to postprandial levels of about 1.3 mmol/l at 180 min after the meals. Chylomicron-rich TAG concentration (Fig. 1b) rose from a fasting level of about 0.5 mmol/l to a postprandial level of approximately 0.9 mmol/l. There was no significant rise in the chylomicron-poor TAG concentration (Fig. 1c). The method of Grundy & Mok (1976) was used to separate chylomicron-rich lipoproteins (supernatant) from chylomicron-poor lipoproteins (infra-natant). The former include lipoproteins originating largely from the intestine during absorption, the latter consist largely of TAG secreted from the liver (mainly in very-low-density lipoproteins (VLDL)). The results indicate that during the first 6 h after a meal the postprandial rise in TAG is largely due to an increase in TAG in the chylomicron fraction with no change in the TAG concentration arising from secretion of VLDL. If part of the plasma TAG increase had been due to increased secretion of VLDL, for example due to NEFA recycling in the second part of the postprandial phase, then a rise in TAG concentration in the chylomicron-poor fraction might have been expected.

Similar postprandial TAG responses to the Betapol and Control meals were seen in each of the plasma fractions (total plasma, chylomicron-rich, chylomicron-poor). There were no significant differences in the areas under the postprandial TAG curves for total (Fig. 1a), chylomicron-rich (Fig. 1b) or chylomicron-poor (Fig. 1c) TAG concentrations. The peak chylomicron-rich TAG concentration (mean (sd)) was 0.85 (0.46) mmol/l after the Control blend and 0.85 (0.42) mmol/l after the Betapol blend, with the times to peak concentration (mean (sd)) being 208 (111) and 260 (104) min respectively. Because the extent of postprandial lipaemia is determined by both rates of absorption and clearance of dietary TAG, the fact that we found no difference in the peak chylomicron TAG concentration or the time to maximal TAG concentration following consumption of the two fat blends suggests either that rates of both uptake and clearance of dietary TAG were similar for both oils, or that differences in rates of absorption were compensated for by opposite but equivalent effects on rates of clearance. However, the fact that the TAG response curves were virtually superimposable, together with the similar GIP responses (which suggest
Fig. 1. Postprandial concentrations of triacylglycerol (TAG) in (a) plasma, (b) a chylomicron-rich plasma fraction and (c) a chylomicron-poor plasma fraction of male subjects, following consumption of Control (○) and Betapol (●) fat blends. Values are means with their standard errors for sixteen subjects. For details of meals and procedures, see Tables 1 and 2, and pp. 402–404.

similar rates of absorption as discussed below), makes the latter possibility extremely unlikely.

Previous studies in rats (Tomarelli et al. 1968; Mattson et al. 1979) and in human newborn infants (Filer et al. 1969) have shown that saturated fatty acids present on the sn-2 position of dietary TAG are more readily absorbed than saturated fatty acids on the sn-1 or -3 positions. Differences in absorptive efficiency between adults, children and newborn infants for certain dietary fats (Deuel, 1955; Southgate et al. 1969) suggest that the newborn infant may have a limited capacity to absorb saturated fatty acids located at sn-1 or -3 positions because these are hydrolysed during digestion and are less well absorbed in their non-esterified form. Adults on the other hand appear to absorb most dietary fats very efficiently whether in non-esterified or monoacyl form (Deuel, 1955) and this may explain why there were no apparent differences in the uptake of the two fat blends used in the present study of adult subjects. The fact that the newborn infant has a different capacity from the adult to absorb dietary fat and other differences in the gastrointestinal tract of the adult and the newborn mean that the findings reported here cannot be assumed to apply to the newborn. The poor absorption of non-esterified saturated fatty acids by the neonate, and the fact that in human breast-milk palmitic acid is esterified largely at the sn-2 position
offering an advantage for absorption of this fatty acid, suggest that the availability and use in infant milk formulas of structured TAG enriched with saturated fatty acids at sn-2 may be of nutritional benefit.

Because the chylomicron remnant particle has been strongly implicated in atherogenesis, and because animal studies have suggested impaired clearance of remnant particles following ingestion of TAG enriched with saturated fatty acids at sn-2 (Redgrave et al. 1988), measurement of remnant as well as TAG clearance in the present study may have provided useful additional information. However, considerable concern regarding the validity of the retinyl palmitate labelling technique, the only method currently available for monitoring remnant clearance in humans (Kransinski et al. 1990), and the technical difficulty of the procedure precluded the application of this method in this preliminary assessment of effects of TAG structure on postprandial metabolism in man.

The postprandial NEFA responses (Fig. 2a) are reciprocally related to those of the TAG responses described above so that levels of NEFA fell during the first 2 h after the meal, returning to baseline values at approximately 5 h after the meals. This fall in NEFA is a characteristic postprandial response and probably reflects decreased lipolysis as a consequence of increased substrate availability and plasma insulin levels after the meal (van Amelsvoort et al. 1989). The fall in NEFA concentration tended to be less marked in response to the Betapol compared with the Control fat blend, although differences in NEFA response to the two fat blends were not statistically significant. The total ketone concentration (Fig. 2b) showed a similar pattern to the NEFA concentration over the time course of the experiment, with an initial decrease in concentration in the first 3 h after the meal and thereafter an increase up to 6 h. There was a notably sharp rise in ketone concentration between 5 and 6 h following consumption of the Control fat blend which was due to moderate rises in five subjects and marked rises in three others, but differences in ketone levels within the Control group between 3 and 6 h after the meal did not reach a level

Fig. 2. Postprandial concentrations of (a) non-esterified fatty acids (NEFA) and (b) total ketones in subjects, following consumption of Control (○) and Betapol (●) fat blends. Values are means with their standard errors for sixteen subjects (NEFA) and eight subjects (ketones). For details of meals and procedures, see Tables 1 and 2, and pp. 402-404.
Fig. 3. Postprandial concentrations of (a) glucose, (b) insulin and (c) gastric inhibitory polypeptide (GIP) in subjects, following consumption of Control (○) and Betapol (●) fat blends. Values are means with their standard errors for sixteen subjects. For details of meals and procedures, see Tables 1 and 2, and pp. 402-404.

of statistical significance. Although there were differences in the mean values for Betapol and Control ketone levels at 6 h, these differences were not found to be statistically significant.

**Glucose, insulin and GIP responses**

The postprandial plasma glucose, insulin and GIP levels are shown in Fig. 3. Following ingestion of the meal, plasma glucose concentration increased sharply reaching a peak at 40 min after the meal and returning to baseline values within 140 min (Fig. 3a). Similar responses were seen in subjects given the Control and Betapol blends. Peak areas were not significantly different after the two meals with virtually identical responses for most time points. The only exception to this was the 90 min time point where a second small rise in glucose concentration was seen following the Control meal but not the Betapol meal. It is interesting to note that a second peak was also observed in the insulin response at the 90 min time point following the Control but not the Betapol fat blend. Insulin responses (Fig. 3b) were generally very similar for the two fat blends and the peak areas did not
differ between the two meals. The reason for the second small peak in glucose and insulin concentrations at 90 min following the Control but not the Betapol meal is not known, since the carbohydrate administered was identical for both meals. The only possibility that can be considered is that stomach emptying may have differed slightly in response to the two fat blends. However, in the context of the whole time course the effects observed are extremely small and are unlikely to be of physiological significance.

Plasma GIP levels (Fig. 3c) rose sharply after the meal, peaking at about 45 min then declining steadily up to 120 min and then more slowly for the remaining 4 h. GIP levels were still above fasting concentrations 6 h after the meal. Since GIP secretion is stimulated by both fat and carbohydrate ingestion, this postprandial profile is probably a reflection of early stimulation by carbohydrate (0–60 min) with later stimulation by the more slowly digested fat component of the meal (40–360 min). However, there was no effect of TAG structure on the plasma GIP response. Fat-induced GIP secretion is stimulated by both saturated and unsaturated fatty acids via a process believed to require chylomicron formation (Ebert & Creutzfeldt, 1980). The identical GIP responses to the two fat blends in the present study therefore support the tentative conclusion that there was a similar rate of uptake of the two oils from the intestine (Tomarelli et al. 1968).

CONCLUSION
The present study has examined the possibility that the positional distribution of fatty acids in dietary TAG modifies the postprandial response to a liquid meal in adult male subjects, and is the first study to be able to investigate specifically the effects of TAG structure in human subjects. The results demonstrate that enrichment of the sn-2 position of dietary TAG with a saturated fatty acid had no effect on postprandial lipaemia when dietary TAG is fed in amounts normally consumed as part of a standard meal and suggest that, in adults, TAG structure is not an important determinant of postprandial lipaemia.

Differences in TAG structures did not influence the secretion of hormones of the entero-insular axis and blood glucose responses to the two meals were essentially very similar. Because this was an acute study only, the possibility that TAG structure may influence lipoprotein metabolism and hormone secretion when consumed as a significant component of total dietary TAG on a long-term basis remains to be investigated.

We are grateful to Dr R. J. Howland for his advice on the statistical analysis of the data and to the volunteers who willingly gave up their time to participate in the study.

REFERENCES


