ABSTRACT Studies have shown several beneficial effects of dietary diacylglycerol oil (DAG oil), but the mechanism behind these effects is still not clear. One hypothesis is that an increase in portal vein transport of nonesterified fatty acids (NEFA) with subsequent oxidation in the liver might be responsible for the positive effects. We examined the portal vein transport of NEFA and other lipid related variables, in response to DAG and triacylglycerol (TAG) bolus feeding and a bolus of standard pig feed in 4 portal vein and mesenteric artery catheterized pigs. Also, the effect of the boluses on postprandial lipid variables was examined. Portal vein transport of NEFA did not differ when pigs were administered the 2 oil bolus diets, consistent with the similar portal plasma concentrations of oleic and linolenic acids during h 1 after feeding. Glycerol, on the contrary, was transported by the portal vein to a much higher degree after intake of DAG oil ($P < 0.001; 20, 40, and 60$ min). The postprandial arterial TAG response at 5 and 6 h postprandially was significantly lower after the DAG bolus intake. Analysis of AUC for lipid absorption showed a lower concentration of vaccenic acid ($P = 0.002$) after the DAG bolus diet. In conclusion, DAG bolus feeding did not increase the portal transport of NEFA, but it did increase the portal transport of glycerol and lower the postprandial lipid concentration in arterial plasma. J. Nutr. 136: 1800–1805, 2006.

KEY WORDS: • diacylglycerols • portal vein transport • nonesterified fatty acids • lipid absorption • postprandial lipid response

Obesity has become a major problem worldwide with more than 1 billion overweight adults of whom at least 300 million can be classified as clinically obese (1). Obesity is a risk factor for lifestyle-related diseases such as type II diabetes, hypertension, stroke, and cardiovascular diseases. Recent studies in both animals and humans (healthy and overweight) have shown that intake of dietary oil with a high content of diacylglycerol (DAG) results in a reduction of bodyweight gain and fat deposition in adipose tissue, which are very beneficial effects for the prevention and management of obesity (2–5). The positive effects of DAG oil on the prevention of atherosclerosis and coronary artery disease are suggested to be the result of lower postprandial levels of serum triacylglycerols (TAG) and of TAG in remnant lipoproteins (6,7). Also, postprandial hyperlipidemia in diabetic subjects was shown to be reduced after DAG intake (8).

DAG is a minor natural component of various edible oils (9). Today a dietary DAG oil is sold in Japan (since 1999) (10) and has been sold nationwide in the US since 2005. It consists of ~80 wt % DAG, and the remaining is ordinary TAG oil. DAG exists in 2 isomeric forms: sn1,3-DAG and sn1(3),2-DAG, which are naturally found in a ratio of ~7:3 (11).

Although it is generally agreed that the 1,3-DAG isomer is responsible for the observed beneficial effects (2,12), the mechanism behind these effects is still speculative. It has been shown that the absorption coefficient of DAG oil and TAG oil is similar (13). Hence, one likely overall explanation could be attributed to a difference in the utilization of sn1,3-DAG, which causes a divergence in energy metabolism (14)
that the lymphatic transport after DAG oil infusion might be slower, and that β-oxidation may increase after the intake of DAG oil both in the intestinal mucosa and in hepatocytes.

One hypothesis for the mechanism of DAG action is that more nonesterified fatty acids (NEFA) are transported by the portal vein to the liver where they undergo β-oxidation (5,14). Another is that DAG oil delays the lymphatic transport of TAG and therefore delays the entry of TAG-rich chylomicrons into blood circulation. In the late postprandial phase, insulin levels are decreased, which causes a decrease in the activity of lipoprotein lipases (18). It has been hypothesized that, as a result of the late TAG entry to the systemic circulation, lipid deposition in adipose tissue is decreased, thereby increasing the utilization of lipids for energy (16).

This study examined the transport pathway after DAG oil vs. TAG oil bolus feeding and the effect on postprandial lipid variables in catheterized pigs.

MATERIALS AND METHODS

**Bolus diets.** The 2 test oils were rapeseed oil (TAG oil, from Aarhus United) and DAG oil produced from glycerolysis of rapeseed oil (19) of same origin as the TAG oil. The DAG oil contained 93% DAG with an isomeric ratio of 1,3-DAG:1,2-DAG of (63:37) and the remaining was monoglycerol [MAG, mainly 1-(3)-MAG]. Standard feed was 40% rye, 33% oatmeal, 20% animal fat (2%), molasses (1%), necessary amino acids (0.6%), and minerals and vitamins (2.3%).

**Housing and surgery of pigs.** Four female crossbred [LY(D)] growing pigs (initial weight 35.2 ± 2.3 kg) were obtained from the swineherd of the Danish Institute of Agricultural Sciences in Foulum, Denmark. The pigs were surgically fitted with 2 catheters, one placed in the portal vein (1.25 mm internal diameter; Tygon, polyvinyl chloride, from Cole Parmer) and the second in the mesenteric artery (1.00 mm internal diameter; Tygon, polyvinyl chloride, from Cole Parmer), and with an ultrasonic blood flow probe (14 mm or 16 mm; from Transonic Systems) around the portal vein. A flowmeter (Transonic T201D flowmeter with P-option; from Transonic Systems) was used to monitor the blood flow.

The pigs were housed in individual pens covered with raised plastic gratings, and the temperature in the room was 16–18°C. Standard feed was offered at 0800 and 1430 on the days without bolus feeding. On the days of bolus feeding, the pigs were fed at 0830 and again when sampling was completed at ~1500. Equal amounts of feed were given morning and afternoon. Standard feed portions were 650 g during the first wk, 750 g during wk 2, and 850 g during the final week.

The catheters were checked daily throughout the experiment and rinsed approximately 2 times/wk with a 100 IU heparinized saline solution. All aspects of the protocol were approved by the Danish Animal Inspectorate, Ministry of Justice, Copenhagen, Denmark.

At the termination of the experiment the pigs were killed by an overdose of pentobarbital in the portal vein and a post mortem autopsy was performed.

**Analytical methods.** The concentrations of TAG, total cholesterol, nonesterified fatty acids (NEFA), and glycerol were analyzed in all plasma samples on an automatic analyzer (Cobas Mira Plus System from Roche Diagnostic) using enzymatic kits (TAG and total cholesterol from ABX Diagnostics, NEFA from Wako Chemicals GmbH, and glycerol from R-Biopharm AG).

TABLE 1

<table>
<thead>
<tr>
<th>Acylglycerol and fatty acid composition of bolus diets</th>
<th>STD bolus</th>
<th>TAG bolus</th>
<th>DAG bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat, g/kg diet</td>
<td>39</td>
<td>135(^1)</td>
<td>135(^1)</td>
</tr>
<tr>
<td>Acylglycerol composition, g/100 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>100</td>
<td>100</td>
<td>26</td>
</tr>
<tr>
<td>DAG</td>
<td>n.d.(^3)</td>
<td>n.d.</td>
<td>69</td>
</tr>
<tr>
<td>MAG</td>
<td>n.d.</td>
<td>n.d.</td>
<td>5</td>
</tr>
<tr>
<td>Fatty acids, g/100 g FA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>0.6</td>
<td>0.2(^3)</td>
<td>0.2(^3)</td>
</tr>
<tr>
<td>16:0</td>
<td>30.4</td>
<td>11.4</td>
<td>11.6</td>
</tr>
<tr>
<td>18:0</td>
<td>3.4</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>25.6</td>
<td>48.9</td>
<td>47.1</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>2.7</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>32.8</td>
<td>24.1</td>
<td>26.4</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>3.4</td>
<td>7.6</td>
<td>7.1</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>0.4</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Other</td>
<td>0.7</td>
<td>1.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\(^1\) Calculated value based on amount of fat in STD bolus and amount of oil added.

\(^2\) n.d., not detected.

\(^3\) FA profile of the oil bolus diets are calculated based on the amount of fat and the FA profile of STD pig feed and of the respective oil.

**Bolus feeding and collection of blood samples.** The pigs were allowed 12 d of recovery before the bolus feeding experiment was initiated. The bolus feeding experiment was conducted as a randomized design (3 diets and 4 pigs). Bolus feeding with TAG and DAG oil, respectively, was performed a total of 8 times and the STD bolus feed was performed a total of 6 times. The bolus feedings were conducted twice a wk (Tuesdays and Fridays) for 3 wk. When the oils were tested, 10% of the weight of the standard feed portion was replaced by the test oil. The test oils were essentially added on top of half of the portion of standard pig feed and, after the pigs had finished the standard feed-oil mixture (~10 min), the second half of the standard feed portion was offered. On the remaining days, pigs consumed only standard feed.

During bolus feeding, blood samples were withdrawn simultaneously from the portal vein and mesenteric artery and blood flow was measured for 1 min before sampling. Samples were collected at 30 min before bolus feeding, then at 0 min and every 20 min until 120 min after bolus feeding, and then again every 60 min until 360 min after bolus feeding. Approximately 9 mL blood was withdrawn, and, subsequently, a similar volume of physiologic saline solution was injected through the catheters to avoid dehydration. The blood samples were collected in sodium heparin–coated plastic tubes and glucose concentration was measured immediately (Accu-check Compact, Roche), then the tubes were centrifuged (1300 × g, 10 min, 4°C) and plasma was separated and frozen (~80°C) until analyzed.

**Nutritional value.** The nutritional value of the test oils was determined in a multipurpose animal feeding study at the Department of Animal Nutrition, University of Copenhagen, Denmark. The pigs were given the test oils in a modified commercial pig feed calculated for 10% of the weight of the standard feed portion. The test oils were essentially added on top of half of the portion of standard pig feed and, after the pigs had finished the standard feed-oil mixture (~10 min), the second half of the standard feed portion was offered. On the remaining days, pigs consumed only standard feed.

Beside the test oils, the pigs received drinking water ad libitum.

**Analytical methods.** The concentrations of TAG, total cholesterol, nonesterified fatty acids (NEFA), and glycerol were analyzed in all plasma samples on an automatic analyzer (Cobas Mira Plus System from Roche Diagnostic) using enzymatic kits (TAG and total cholesterol from ABX Diagnostics, NEFA from Wako Chemicals GmbH, and glycerol from R-Biopharm AG).

Total lipids were extracted from plasma samples after the addition of an internal standard (TG 15:0) according to the modified method by Folch et al. (20). This method was modified by exchanging the NaCl solution with 1 mol/L HCl, which enabled the extraction of albumin-bound NEFA as well. The concentration of selected and total fatty acids (FA) in total lipid extract was determined after methylation with BF3. The BF3 method was a modification (21) of the procedure originally established by Hamilton et al. (22). FAME were analyzed by GLC, as described previously (19). The fatty acids were identified by comparing the retention time with standards of known fatty acid composition (Nu-Check-Prep). Peak areas were calculated on an automatic analyzer (Cobas Mira Plus System).

**Summary.** This study examined the transport pathway after DAG oil vs. TAG oil bolus feeding and the effect on postprandial lipid variables in catheterized pigs.
The concentration of total fatty acids in the TAG fraction of the total lipid extract was determined for the plasma samples taken from −30 to 80 min. Total lipids were separated on TLC plates and was followed by the extraction of TAG fractions and methylation with BF₃ (22) and GLC, as described above.

Calculations and statistical analysis. The time courses of plasma variables are based on the incremental change (Δ) relative to the background level (mean of −30 and 0 min). An estimate for the quantitative absorption (net influx) of glycerol to the portal vein after DAG bolus was calculated from the portal-arterial differences and the portal flow measurement, as described by Rerat et al. (23); although the procedure was modified by using an approximate mean portal blood flow rate. The incremental areas under the curves (ΔAUC) were calculated according to the trapezoidal rule for the 6-h time course.

All data were analyzed by using the MIXED procedure of SAS version 8 (SAS Inst.). Effects of bolus diets on Δ-values of NEFA, glycerol, TAG, glucose, cholesterol, and selected and total FAs were analyzed using the following normal mixed model:

\[ Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \nu_l + \delta_{ijkl} + \epsilon_{ijkl}. \]

\( Y_{ijkl} \) is the observed independent variable, \( \mu \) is the overall mean of the observations, \( \alpha_i \) is the main effect of bolus diet (i = TAG, DAG, STD), \( \beta_j \) is the repeated effect of time (j = 0, 20, ..., 360), \( \gamma_k \) is the effect of repeating a bolus diet in the same pig (k = bolus repeat: 1, 2, 3), \( \nu_l \) is interaction of bolus diet and time, \( \delta_{ijkl} \) is the random effect of pig (l = pig: 1, 2, 3, 4), and \( \epsilon_{ijkl} \) is the residual error component. The random pig effect was imposed to account for the repeated feeding of the 4 pigs (bi, 1, 2, 3, 4), and \( \epsilon_{ijkl} \) is the residual error component.

To analyze the statistical significance of the ΔAUC data following model was used:

\[ Y_{ijkl} = \mu + \alpha_i + \delta_{ijkl} + \epsilon_{ijkl}. \]

\( Y_{ijkl} \) is the observed independent variable, \( \mu \) is the overall mean of the observations, \( \alpha_i \) is the main effect of bolus diet (i = TAG, DAG, STD), \( \delta_{ijkl} \) is the random effect of pig (l = pig: 1, 2, 3, 4), and \( \epsilon_{ijkl} \) is the residual error component.

Reported values are means ± SEM and differences were considered significant at \( P < 0.05 \).

RESULTS

Animal experiments. The 4 pigs were healthy throughout the experiment and the catheters worked without problems. The flow measurements, on the contrary, showed a decrease in blood flow during the study period (from bolus feedings 1 to 6) for 3 of the pigs. We do not think this reflects an actual blood flow decrease in the pigs, but was due, rather, to the formation of scar tissue, which disturbed the measurements. Therefore, we decided not to use any of the flow measurements for calculations of portal transport, because they would only add additional noise to the analytical results.

The postmortem autopsy revealed that one of the pigs had the portal vein catheter bent into a small branch of the portal vein. The bolus feeding data from this pig were therefore discarded. One pig had trouble eating the whole bolus portion twice and these samples were also discarded. To summarize, samples from 6 bolus feedings with TAG and DAG oil, respectively, and from 3 bolus feedings with STD feed were usable for analyses.

Background blood variables and portal vein flow rate. The background levels of cholesterol, NEFA, glycerol and glucose, determined as the mean of values from the −30 and 0 min samples, did not differ significantly before the 3 different bolus feedings (data not shown). The background level of TAG in the mesenteric artery was higher before STD bolus feeding (\( P = 0.039 \)). We attributed this to the biological variation between both sampling days and pigs, because bolus diets had no influence on the background blood variables and STD bolus feeding was performed once in each pig.

Although all blood flow measurements were discarded for use in calculations, we were able to evaluate the curve shape for the individual bolus diets from the first 2 measurements. The blood flow rate in the portal vein was not influenced by the bolus diets but varied with time after feeding. The blood flow was ~1.2 L/min before feeding and increased until 60–100 min to ~1.8 L/min, after which time it decreased again (data not shown).

Portal vein absorption. The incremental time courses for NEFA concentrations in the portal vein did not differ after feeding the 3 bolus diets. (Fig. 1A). The NEFA time courses were almost identical for the 2 oil bolus diets, and for the STD bolus diet the NEFA level was more or less stable throughout the sampling period. Glycerol levels in the portal vein after bolus feedings were, on the contrary, significantly different after the bolus diets (Fig. 1B). After DAG bolus feeding, the glycerol level increased dramatically between 0 and 20 min, whereafter

![FIGURE 1](https://academic.oup.com/jn/article-abstract/136/7/1800/4664634)
it slowly decreased again and reached a similar level at 180 min as after the TAG bolus treatment. The glycerol level at 20, 40, and 60 min after DAG bolus feeding was significantly higher than after feeding TAG and STD boluses.

The incremental time courses of TAG levels in the portal vein differed significantly between bolus diets with the STD bolus time course being significantly different from the time courses for the TAG and DAG treatments (Fig. 1C). The TAG levels increased more rapidly after DAG bolus feeding, and at 60 min the TAG level for the DAG bolus treatment was significantly different from that in the TAG and STD bolus treatments.

With respect to glycerol levels in the portal vein the different bolus diets did not result in any significant differences between the incremental time courses. The 2 oil bolus treatments resulted in a peak glucose concentration in the portal vein at 20 min, and again at 100 min, reaching an approximate maximum level of 2.5 mmol/L above the background level (data not shown). Also, the levels of total cholesterol in the portal vein did not differ significantly after bolus treatments and the incremental time courses from background level were negative for all bolus diets (data not shown).

The portal transport was calculated approximately by the Δ AUC for the portal-arterial differences of NEFA, glycerol, TAG and glucose levels after 1 h and after the total sampling period of 6 h. Only the portal transport of glycerol was significantly different after DAG bolus feeding compared with both TAG and STD bolus feedings. At 1 h, 4.64 ± 1.30 mmol·min⁻¹·L⁻¹ glycerol was transported by the portal vein after DAG bolus feeding, whereas only 0.92 mmol·min⁻¹·L⁻¹ and 0.72 mmol·min⁻¹·L⁻¹ glycerol was transported after feeding TAG and STD bolus diets, respectively (P = 0.017). During the 6-h sampling period, 15.83 ± 2.82 mmol·min⁻¹·L⁻¹ glycerol was transported after feeding the DAG bolus diet and 5.54 mmol·min⁻¹·L⁻¹ and 4.46 mmol·min⁻¹·L⁻¹ glycerol after the TAG and STD bolus treatments, respectively (P = 0.003).

Another way of examining the portal transport of lipids is to examine the total level of specific FAs (from total lipid extract). The selected FAs were oleic acid as the major FA in the bolus oils (≈48 wt %) and linolenic acid, which is a more or less purely exogenous FA (24) and accounts for ≈7.4 wt % in the bolus oils. The background concentrations of oleic- and linolenic acids were 0.70 mmol/L and 0.03 mmol/L, respectively (data not shown). The incremental changes from background concentrations for oleic- and linolenic acids in the portal vein did not differ after TAG and DAG bolus feeding (P > 0.5) and until 120 min, the time courses for the DAG and TAG bolus treatments were almost identical (data not shown).

Postprandial effects. The postprandial time courses of NEFA, glycerol, TAG, glucose, and cholesterol levels in the mesenteric artery in response to the different bolus diets were established. The NEFA, glycerol, glucose, and cholesterol time courses in the arterial plasma did not differ significantly between bolus diets (data not shown). The time courses for TAG arterial levels (Fig. 2) were more or less comparable to the portal vein curves (Fig. 1C) for all bolus diets and they were significantly different with the STD bolus diet being different from the 2 oil bolus diets. There was a significantly more rapid increase in TAG level (at 60 and 80 min) after DAG bolus feeding as compared with TAG and STD bolus diets. On the contrary, at 300 and 360 min, the TAG levels were significantly higher after TAG bolus feeding than after DAG and STD bolus diets (Fig. 2).

The Δ AUC values, based on the incremental time courses from background level of the major FA of the TAG and DAG bolus oils (palmitic, oleic, vaccenic, linoleic, and linolenic), were calculated (Table 2). The Δ AUC for vaccenic acid was significantly lower after DAG feeding than after the TAG bolus diet.

**Fig. 2** Incremental (Δ) change from background level of plasma TAG postprandially in the mesenteric artery after bolus feeding with TAG, DAG, and STD feed in catheterized pigs. Values are means ± SEM, n = 6 for DAG and TAG boluses and n = 3 for STD bolus. P-values for treatment effects are shown on the figures. Asterisks indicate different from TAG and STD bolus diets at that time: *P < 0.05. Hatch symbols indicate different from DAG and STD bolus diets at that time: †P < 0.05.

**DISCUSSION**

**Portal vein absorption.** Portal transport is commonly associated with transport of short chain FA and medium chain FA, however long chain FA are also capable of being transported by the portal vein bound to albumin, although at a lower degree (25,26). Of long chain FA, the polyunsaturated long chain FA were shown to be more disposed toward portal transport, which is explained by a higher affinity of fatty acid binding protein toward these FA (27). The mechanism responsible for the beneficial effects, observed after DAG oil intake, was suggested to be the result of a higher transport of NEFA by the portal vein to the liver for subsequent β-oxidation, because less 2-MAG is available for resynthesis of TAG in the enterocytes (14); hence, more NEFA is accumulated in the enterocytes after 1,3-DAG intake (12).

The findings in this study, however, did not show a higher level of NEFA in the portal vein after DAG bolus feeding compared with TAG bolus feeding (Fig. 1A). Also, when examining total concentration in the portal vein of the major FA in the 2 bolus oils, oleic acid, and the almost purely exogenous polyunsaturated FA, linolenic acid, no difference indicating portal transport was observed. However, measurements

**TABLE 2**

 Δ AUC for total and selected FA in the total lipid extract from arterial plasma from TAG or DAG bolus-fed catheterized pigs

<table>
<thead>
<tr>
<th></th>
<th>TAG bolus</th>
<th>DAG bolus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol·min⁻¹·L⁻¹</td>
<td>mmol·min⁻¹·L⁻¹</td>
</tr>
<tr>
<td>16:0</td>
<td>29.9 ± 9.0</td>
<td>15.7 ± 8.0</td>
</tr>
<tr>
<td>18:1 (n-9)</td>
<td>153.7 ± 27.3</td>
<td>105.1 ± 23.2</td>
</tr>
<tr>
<td>18:1 (n-7)</td>
<td>24.6 ± 2.5</td>
<td>13.8 ± 1.6</td>
</tr>
<tr>
<td>18:2 (n-6)</td>
<td>118.0 ± 15.4</td>
<td>84.4 ± 22.0</td>
</tr>
<tr>
<td>18:3 (n-3)</td>
<td>46.8 ± 4.7</td>
<td>36.9 ± 3.8</td>
</tr>
<tr>
<td>Total FA</td>
<td>416.0 ± 54.4</td>
<td>298.8 ± 41.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 6. *Different from TAG, P = 0.002.
2 All samples are from the mesenteric artery after bolus feeding with TAG and DAG. Δ AUC values are calculated from the 6-h time course.
of lipids in portal plasma also reflect, in part, NEFA and TAG of endogenous sources (28), which may minimize the potential difference in transport of exogenous lipids.

The amount of NEFA available for portal transport should also be considered. Full hydrolysis, of FA from 1,3-DAG and the MAG fraction of the DAG bolus diet, would yield ~0.152 mol (FA in total lipid 0.323 mol), but some of these FA will be used for TAG resynthesis from 1,2-DAG digestion products, because 1 FA/mol of 1,2-DAG will be lacking. Hence, 34% of the total FA in the DAG bolus diet will be in excess because of FA inability to be used for TAG resynthesis through the normal MAG pathway. This may not be an amount large enough to cause a measurable difference in NEFA transport after the DAG bolus diet if only a minor fraction of these NEFAs are exposed for portal transport. Further studies using DAG oil with labeled FA will therefore be needed to reveal whether a smaller part of the FA from 1,3-DAG hydrolysis are preferentially transported by the portal vein.

On the other hand, portal transport of glycerol was clearly seen in response to DAG bolus feeding (Fig. 1B). This finding supports the conclusion that some 1,3-DAG are fully hydrolyzed by pancreatic lipase to free glycerol and 2 FA (12) and that digestion products of DAG oil may be metabolized through a different pathway in the enterocytes compared with TAG oil. Free glycerol has been reported to be seldom utilized in the synthesis of acylglycerols and to be readily transported by the portal vein to the liver (29,30). If a full hydrolysis of 1,3-DAG and MAG in the DAG bolus diet occurs, 56 mol % of total glycerol (present in total acylglycerols) will be found as free glycerol after DAG bolus feeding. A total of 15.83 mmol · min⁻¹ · L⁻¹ of glycerol equals the Δ AUC for the porta-arterial difference in glycerol level. To obtain an approximate accumulated transport of glycerol, the blood flow rate needs to be multiplied. Thus, if the average flow rate is ~1.5 L/min, the accumulated portal transport of glycerol for 6 h is 24 mmol, which equals 30 mol % of the potential amount of free glycerol. The remaining 70 mol % of glycerol from 1,3-DAG and MAG may have remained inside the enterocytes or have been utilized by different pathways. Free glycerol can be phosphorylated by the action of glycerol kinase and thus be utilized in the glycerol-3-phosphate pathway (31), although the level of glycerol kinase activity has been reported to be low in the intestinal mucosa (32). Another possibility is utilization of sn1-MAG as an intermediate for the glycerol-3-phosphate pathway. Phosphorylation of sn1-MAG has been suggested to yield lysophosphatidic acid, which can be further metabolized through the glycerol-3-phosphate pathway (33). However, a more likely explanation is that acyl migration of either sn1-MAG to sn2-MAG, or sn1,3-DAG to sn1,2-DAG, has taken place to some degree, resulting in the metabolism of this fraction through the MAG pathway.

The TAG response to the 3 diets was significantly different, with the STD bolus diet different from the 2 oil boluses. This was an expected result of the low lipid content in the STD bolus diet. The STD feed only contained 29% of the fat load in the oil diets, which the TAG time course reflects with only a minor increase from background level. As expected, no significant portal transport of TAG was observed for any of the bolus diets (Fig. 1C), although a significantly higher TAG level at 60 min was observed after DAG bolus feeding. This significantly faster increase in TAG level after the DAG bolus is ascribed to a possible faster digestion and absorption of 1,2-DAG, which accounts for 23 g/100 g of the fat content in the DAG bolus diet. The 1,2-DAG is a more polar molecule than TAG and has amphiphilic properties because of its free alcohol group. This property may contribute to a more rapid hydrolysis in the stomach and small intestine (15). As a consequence, plasma TAG levels may rise more quickly in response to 1,2-DAG intake.

Postprandial effects. Some of the postprandial effects have already been discussed based on the portal vein time courses, because the arterial and portal curves are relatively similar if no marked portal transport of the nutrient occurs.

The postprandial NEFA curves after oil bolus feedings corresponded with the normal postprandial plasma NEFA pattern in humans (34), and the intake of DAG oil, therefore, had no acute effect on the plasma NEFA status. Results from a human study, where lipid emulsions of either DAG or TAG oil were ingested by normolipemic men (7), are comparable to the present study with regard to postprandial NEFA and glucose concentrations.

The postprandial arterial TAG response showed a faster appearance of TAG at 60 and 80 min after DAG bolus, as also seen from the portal vein TAG curve (Fig. 1C and Fig. 2). This was ascribed to faster digestion of the 1,2-DAG fraction of the DAG bolus diet. On the contrary, a significantly lower TAG level after DAG bolus feeding was observed at 5 and 6 h (Fig. 2). The lower postprandial lipid content after DAG feeding was further supported in this study by the Δ AUC analysis of total and individual FA (Table 2). Similar results were found in 2 human studies where lipid emulsions of either DAG or TAG oil were ingested by normolipemic men (7) or creamed test meals, prepared with either DAG or TAG oil, were taken orally by 6 men after a minimum 12-h fast (6). The findings indicated that serum TAG Δ AUC (8 h) was significantly lower after intake of a DAG emulsion compared with the TAG emulsion, and that serum TAG concentrations were significantly lower at 2, 3, and 8 h after loading of DAG as compared with TAG, respectively.

An increased portal transport of NEFA can, based on our results, be excluded as the explanation for a lower postprandial plasma lipid concentration in response to a DAG diet. However, another explanation for the observed lower TAG concentration may be that TAG are released to the lymphatic system more slowly than, therefore, more fat is remaining inside the enterocytes at 6 h postprandial to DAG oil intake. A study examining lymphatic recoveries of radioactive activity after administration of lipid emulsions containing 1,3-di[14C]oleoylglycerol or tril[14C]oleoylglycerol in rats found only an approximate difference in 24-h lymphatic recovery of 5% (16). However, the DAG group showed a significant reduction in lymphatic recovery in the first fraction, 0–1 h, but a higher recovery in the last fraction, 8–24 h, compared with the TAG group. The reduced TAG transport during the first hour is opposite to this study where a high TAG transport was seen, but it confirms that the observed early TAG increase most likely is a result of the 1,2-DAG fraction, insofar as the lymphatic study used pure 1,3-DAG. The results from the lymphatic study suggest that the fat does not remain inside, or is subjected to oxidation, in the enterocytes after DAG intake in a 24-h time span, but that it is released more slowly to the lymphatic system, hence confirming the possibility of more lipids remaining inside the enterocytes at 6 h postprandial to DAG bolus feeding in this study.

The explanation for a possible slower release of TAG to the lymphatic system after DAG bolus feeding, which results in a lower lipid content at 6 h postprandially, may be that of differing metabolic processes in enterocytes of the 1,3-DAG fraction of the DAG bolus diet compared with 1,2-DAG and TAG (12,15). TAG resynthesis in the enterocytes has been shown to primarily take place through the glycerol-3-phosphate pathway when 1,3-DAG is ingested, because of the lack of 2-MAG (12,35). The glycerol-3-phosphate pathway produces both TAG and phospholipids (36), and it has been shown that TAG resynthesis occurs at a slower rate than through the MAG
pathway (37). The shift from the MAG pathway to the glycerol-3-phosphate pathway requires an upregulation of enzymes involved in the pathway, which may also cause a delay in the TAG resynthesis.

In conclusion, this study demonstrates that FA released from lipolysis of 1,3-DAG are not transported by the portal vein as previously hypothesized. In contrast, portal transport of free glycerol was shown to be significantly increased after DAG bolus intake compared with intake of TAG bolus, which, we believe, is a novel finding in the research related to mechanisms of DAG oil. Furthermore, this work has shown that the post-prandial TAG level, as well as the level vaccenic acid in the arterial plasma for 6 h after DAG oil intake, is reduced when compared with the intake of TAG oil.

ACKNOWLEDGMENTS

We thank Benny Thomesen and Sun Tielu for assistance with the pig experiment, and Nina Kjeldsen and Jannie Agersten for technical assistance. We thank Sarah Linderoth-Lang for setting up the glycerol method and subsequently performing the glycerol measurements. Furthermore, the authors thank Xuebing Xu for skillful help with the planning of the DAG oil production.

LITERATURE CITED