Enteral administration of soyabean lecithin enhanced lymphatic absorption of triacylglycerol in rats

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As the physiological roles of dietary lecithin have not yet been clearly defined, we examined the effects of lecithin on lipid absorption in male Wistar rats with a mesenteric lymph cannula. Lymphatic absorption was observed after the infusion of 1 ml emulsion containing 100 mg test oil emulsified with sodium taurocholate (10 g/l) in three separate experiments. Test oils (100 mg) were: soyabean oil (triacylglycerol (TG) source, SO) and soyabean oil + lecithin (75 mg soyabean oil + 25 mg lecithin, LE) in Expt 1; SO, LE or soyabean oil + lysolecithin (75 mg soyabean oil plus 25 mg lysolecithin, LY) in Expt 2; hydrolysed soyabean oil (HSO) or HSO + lysolecithin (75 mg HSO + 25 mg lysolecithin, HLY) in Expt 3. After LE and LY infusions, lymph flow and the lymphatic output of TG was higher than after SO infusion at 0–30 min and 0–90 min respectively (Expts 1 and 2). Lecithin-induced increases in lymph TG output remained constant when HSO was infused (Expt 3). There were no differences in the TG:phospholipid ratio in the lymph after infusion among the groups; nevertheless, the lymphatic output of TG was much higher after infusion with LE than with SO. Fatty acid was released more efficiently from SO than from LE and LY by in vitro digestion with rat bile–pancreatic juice. These present results demonstrate that a TG emulsion containing soyabean lecithin or its hydrolysates promote lymphatic TG output and suggest that the increases in TG absorption do not depend on TG digestion.

Soyabean lecithin: Lipid absorption: Rats

Phosphatidylcholine (PC) from biliary sources is known to play an important role in intestinal lipid absorption. O’Doherty et al. (1973) first demonstrated that the removal of biliary PC results in decreases in the uptake of fatty acids (lipids) and the repletion of PC restores the absorption in bile-diverted rats. Another study showed that dietary PC did not increase triacylglycerol (TG) absorption (Jiang et al. 2001). In addition, PC inhibits the intestinal uptake of cholesterol; this was observed using intestinal segments (Hollander & Morgan, 1980; Reynier et al. 1985) and Caco-2 cells (Homan & Hamelehle, 1998). However, the inhibitory mechanism is not yet sufficiently known. These previous studies show that the role of dietary PC on TG absorption and other lipophilic compounds is not yet sufficiently clarified.

Efficient lipid absorption is useful for enteral nutrition. Sufficient supply of energy, essential fatty acids and fat-soluble vitamins are needed for patients with impaired digestive function, for example, before and after surgical operation, in inflammatory bowel disease etc. (Bamba et al. 2003). In addition, it is beneficial for patients to have a supply of n-3 fatty acids: in clinical studies these have been shown to improve immunity (Alexander, 1998).

Our preliminary study showed that the oral or intestinal administration of TG with PC from soyabean increased plasma TG concentrations more rapidly than that without PC (M Nishimukai, H Hara and Y Aoyama, unpublished results). This suggests that dietary PC enhanced TG digestion and/or absorption. This effect of PC may be beneficial to enable patients with impaired digestive function to metabolize energy and lipids efficiently. The aim of the present study was to determine whether PC enhances lymphatic absorption of TG and whether lipid digestion is involved in the PC-induced increase in absorption, using conscious rats with a lymphatic cannula.

Materials and methods

**Emulsified test solution used in the in vivo experiments**

The test solution consisted of 100 g test lipid/l; the test lipid was emulsified with sodium taurocholate (10 g/l) using a homogenizer (dial 6 for 90s, Polytron® PT10/35; Kinematica, Lucerne, Switzerland) (Expt 1) or a sonicator (150 W for 1·5 min, Sonicator® 5202; Ohtake Seisakusyo, Tokyo, Japan) (Expts 2, 3 and 4). The lipids

**Abbreviations:** HLY, hydrolysed soyabean oil + lysolecithin; HSO, hydrolysed soyabean oil; LE, soyabean oil + lecithin; LY, soyabean oil + lysolecithin; PC, phosphatidylcholine; PL, phospholipid; SO, soyabean oil; TG, triacylglycerol.

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in the 1 ml test solution for the three separate experiments were as follows: 100 mg soyabean oil (SO; Wako Pure Chemical, Osaka, Japan) or 75 mg SO + 25 mg lecithin (LE) in Expt 1; SO, LE or 75 mg SO + 25 mg lysolecithin (LY) in Expt 2; 100 mg hydrolysed SO (HSO) or 75 mg HSO + 25 mg lysolecithin (HLY in Expt 3). Lecithin used in the present study contained: 950 g soyabean PC; 20 g other phospholipids (PL); 10 g moisture; 10 g oil/kg (Epikuron 200; Lucas Meyer, Hamburg, Germany). Lysolecithin used in the present study contained 870 g hydrolysed soyabean lecithin/kg, in which the fatty acids had been completely removed (Taiyo Kagaku, Yokkaichi, Japan).

The predigested SO (HSO) used in Expt 3 was prepared as follows: a lipid emulsion containing 50 mg SO and 5 mg taurocholate in 2.5 ml 50 mM-Tris buffer (pH 8.2, containing 20 mM-CaCl$_2$) was hydrolysed by porcine pancreatic lipase (pancreatin activity for TG hydrolysis, 750–1400 units/g; Wako Pure Chemical) at 37°C for 2.5 h. The hydrolysis products were extracted with 5 vol. chloroform–methanol (2:1, v/v) according to the method of Folch et al. (1957). The complete hydrolysis of the TG in the procedure was confirmed by TLC.

**Measurement of total surface area of lipid emulsions (size of lipid droplets)**

We measured the total surface area of the emulsion particles in order to examine the effects of lipid emulsion size on lipid absorption. The 1 ml test emulsion contained 10 mg sodium taurocholate and 100 mg test lipids. The SO (Wako Pure Chemical):lecithin (soyabean PC; Epikuron 200) ratios in the test lipids were 10:0, 9:1, 7:1, 5:1, 3:1, 1:1 or 0:10. The emulsions were prepared using a sonicator (Ohtake Seisakusyo; as for Expts 2, 3 and 4). The total surface area of lipid emulsions was measured with a laser diffracton particle size distribution analysys (Coulter LS130; Beckman Coulter, Fullerton, CA, USA). We also measured the size of the emulsion particles prepared by a Polytron homogenizer (Kinematica; see p. 565).

**Animals**

Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), aged 9 weeks, were fed a semipurified casein–sucrose–based diet (AIN 76 formula) for 5 d. After a 24 h fast, a vinyl catheter (0.5 mm internal diameter, 0.8 mm outer diameter; Silascon SH no. 00, Kaneka Medix Co., Osaka, Japan) were implanted into the main mesenteric lymph duct (Bollman et al. 1948) and the duodenum respectively under anaesthesia (sodium pentobarbital, 40 mg/kg body weight).

After the operation, the rats were placed in individual restraining cages. During the 1 d recovery period, an iso-osmotic solution containing 139 mM-glucose and 85 mM-NaCl was infused continuously at a rate of 3 ml/h until the end of the lymph collection through the duodenal catheter. After the collection of lymph for 30 min (initial lymph) on the day following the operation, rats were administered 1 ml emulsified test solution through the duodenal tube. The lymph was collected in a tube at 0.5 h intervals during the first 2 h and at 1 h intervals during the next 2 h following the administration of the test solution. The collected lymph was frozen immediately and kept at −40°C until subsequent analyses (TG and PL concentrations in Expts 1, 2 and 3, fatty acid composition in Expt 2). In the present in vivo study, all rats before infusion of the emulsion were continuously infused with an iso-osmotic solution overnight via the duodenal catheter; this led to a similarly non-stimulated condition for digestive enzymes among all the groups of rats.

The present study was approved by the Hokkaido University Animal Committee and animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

**In vitro digestion of emulsified test solutions by rat bile–pancreatic juice (Expt 4)**

The emulsified test solutions (SO, LE and LY) were hydrolysed with bile–pancreatic juice (lipase resources) using the method described by Luddy et al. (1964). Briefly, 0.5 ml lipid emulsion, consisting of 5 mg sodium taurocholate and 50 mg of each lipid in saline (9 g NaCl/l), was mixed with 3 ml 50 mM-Tris buffer (pH 8.2, containing 20 mM-CaCl$_2$ and 50 µl bile–pancreatic juice that was collected from a rat fed a semipurified basal diet) and incubated at 37°C for 0–3 h. The reaction was terminated by boiling and the non-esterified fatty acids released during the reaction were measured.

**Analyses**

TG and PL concentrations in the lymph were measured by enzymatic procedures (TG-EN and PL-EN; Kainos Laboratories, Tokyo, Japan). The test lipids and the lymph collected from rats at 60–90 min after administration of test lipids were hydrolysed by KOH in ethanol and fatty acid composition in these solutions were measured by GLC (Shimadzu GC-14A; Shimadzu, Tokyo, Japan) with a CBP20-M25-025 capillary column (25 m, inner diameter 0.25 mm; Shimadzu), flame-ionization detection and He as a carrier gas after methylization detection and He as a carrier gas after methylation with methanol containing HCl (50 mg HCl/kg methanol). The initial temperature was 170°C and this was increased by 2°C per min to 250°C. With regard to the test diet, the oleic acid/other fatty acids ratios were higher in SO than in lecithin and lysolecithin. Non-esterified fatty acids produced during the in vitro digestion test were also measured by an enzymatic procedure (SCFA-test; Wako Pure Chemical).

**Statistical analysis**

Data were analysed by one-way or two-way ANOVA. Duncan’s multiple range test was used to determine whether mean values were significantly different ($P<0.05$). Values are shown as means with their standard errors.
Results

Total surface area of lipid emulsions

Table 1 shows the total surface area (m²/ml) of the lipid emulsions (100 g test lipid/l). As the amount of lecithin in the solution was increased, the total surface area decreased. When the TG:PL ratio was 3:1, as used in the present study, the total surface area was 3·39 m²/ml and smaller than that of SO (3·99 m²/ml).

Expt 1 (effects of lecithin)

The lymphatic output of TG in rats given LE was higher during the first 2 h after the injection than in those given SO (Fig. 1(A)). Total outputs of TG over the 4 h were estimated at 39·1 (SEM 4·5) and 70·9 (SEM 9·8) % (P<0·01, n 6 and 7 respectively) of the total amount of administered TG in rats given SO and LE respectively. Changes in lymphatic PL output were much smaller than those of lymphatic TG (Fig. 1(B)). However, the output of PL in the LE group was higher during the first 2 h after the injection than in the SO group. Lymph flow rate was much higher in rats given LE than in those given SO at 0–30 min after the injection (Fig. 1(C)). The flow rates decreased rapidly after 30 min in both groups, and there was no difference between groups at 30–240 min after the administration. There were no differences in the TG:PL ratio in the lymphatic output among the groups and the values remained constant (Fig. 1(D)).

Table 1. Total surface area of lipid emulsions*

<table>
<thead>
<tr>
<th>Soybean Oil/Lecithin</th>
<th>Soybean Oil 9:1</th>
<th>Soybean Oil 7:1</th>
<th>Soybean Oil 5:1</th>
<th>Soybean Oil 3:1</th>
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<td>3·38</td>
<td>3·39</td>
<td>2·59</td>
<td>2·63</td>
</tr>
</tbody>
</table>

*The emulsions were varied according to the ratio soybean oil:lecithin and prepared using a sonicator (Sonicator® 5202; Ohtake Seisakusyo, Tokyo, Japan). Total surface area of the lipid emulsions was measured with a laser diffraction particle size distribution analyzer (Coulter LS130; Beckman Coulter, Fullerton, CA, USA). Values are the total surface area (m²/ml) of the lipid emulsions (100 g test lipid/l).

Expt 2 (comparison between lecithin and lysolecithin)

The lymphatic output of TG in rats given LE and LY was higher 0–30 min after the infusion than in those given SO (Fig. 2(A)). The total lymphatic outputs of TG over the 4 h were estimated at 39·1 (SEM 4·5) and 70·9 (SEM 9·8) % (P<0·01, n 6 and 7 respectively) of the total amount of administered TG in rats given SO and LE respectively. Changes in lymphatic PL output were much smaller than those of lymphatic TG (Fig. 1(B)). However, the output of PL in the LE group was higher during the first 2 h after the injection than in the SO group. Lymph flow rate was much higher in rats given LE than in those given SO at 0–30 min after the injection (Fig. 1(C)). The flow rates decreased rapidly after 30 min in both groups, and there was no difference between groups at 30–240 min after the administration. There were no differences in the TG:PL ratio in the lymphatic output among the groups and the values remained constant (Fig. 1(D)).

Expt 3 (absorption of predigested triacylglycerol)

The lymphatic output of TG in rats given HLY was higher during the first 1·5 h after infusion than in those given HSO (Fig. 3). The total output over the 4 h in rats given HLY was also higher than in rats given HSO (P<0·01). Changes and differences in lymph flow, lymphatic output of PL and TG:PL ratio in lymphatic output after the infusion of emulsions containing predigested TG (HSL) were very similar to those after the infusion of emulsified solutions containing intact TG in Expts 1 and 2 (results not shown) and pre-digested TG with lysolecithin (HLY).

Expt 4 (in vitro digestibility of lipid emulsions)

Fatty acids released from SO by hydrolysis with rat bile–pancreatic juice were two times greater than those from LE and LY at 30–240 min after the start of the reaction. The rate of release of fatty acids from LY was very similar to that from LE (Fig. 4).

Discussion

Several studies (O’Doherty et al. 1973; Tso et al. 1981; Ahn & Koo, 1995; Koo & Noh, 2001) have suggested that luminal dietary and biliary PC enhance lymphatic transport of TG in the bile-diverted rats. However, other studies have suggested that luminal PC does not enhance the lymphatic transport of TG (Noh & Koo, 2001; Wang et al. 2001) or only slightly increases it (Ahn & Koo, 1995). In the latter case, the TG:PL ratio was between 16:1 and 7:1. In the present study, we showed that a higher PC intake (the TG:PL ratio was 3:1) clearly enhanced the lymphatic output of TG. Moreover, we showed that lysophosphatidylcholine has a similar but somewhat lower effect to that of PC (Fig. 2). These results revealed that dietary PC enhanced lymphatic TG absorption, and that lysophosphatidylcholine, a product of the digestion of lecithin by pancreatic enzyme, is also an enhancer for the lymphatic output of TG. PC may affect the promotion of TG output after hydrolysis to lysophosphatidylcholine; however, this step is not a critical point for the promotive effect of PC, because the effect of lysophosphatidylcholine was not greater than that of PC.
There are several rate-limiting steps in TG absorption and possible mechanisms underlying the promotive effect of PC on TG absorption. First, we examined emulsion particle size by the measurement of the total surface area of the TG emulsion with or without PC, which may greatly affect TG absorption through changes in the TG hydrolysis rate. As the PC:TG ratio in the test emulsion was increased, the surface area of the emulsion decreased (Table 1). This result suggests that emulsion size is not involved in the enhancement of TG absorption by PC. Actually, we clearly showed that the hydrolytic rate of the emulsions containing PC (LE) or lysophosphatidylcholine (LY) was lower than that of the emulsions without these PL in vitro digestion with rat bile–pancreatic juice (Fig. 4). Borgström (1980) also showed that the digestibility of core TG in a lipid emulsion was inhibited by egg PC in vitro digestion.

Several fatty acid moieties in the emulsion without PC (SO) were present in greater concentrations than in the emulsion containing PC (LE) or lysophosphatidylcholine (LY) was lower than that of the emulsions without these PL in in vitro digestion with rat bile–pancreatic juice (Fig. 4). Borgström (1980) also showed that the digestibility of core TG in a lipid emulsion was inhibited by egg PC in in vitro digestion.

Several fatty acid moieties in the emulsion without PC (SO) were present in greater concentrations than in the emulsion containing PC (SO 339 μmol fatty acid, LE 318 μmol fatty acid). The much higher lymphatic output of TG by infusion of PC emulsion despite lower TG digestion and lower fatty acid content suggests that PC dramatically enhanced TG absorption via steps other than TG digestion. We also examined the effects of PC on predigested TG absorption and showed that PC also enhanced predigested TG absorption. This result shows that PC increases lymphatic TG output at stages after TG digestion.

In Expt 3, we expected a much higher level of lyssolecithin-promoted absorption of predigested TG; however, the enhancement of predigested TG was of a similar degree to that of intact TG (Fig. 3(A) vs. Fig. 2(A)). Some factors, such as products of TG hydrolysis, in the predigested TG may slow lymphatic TG output. Murota et al. (2001), for example, showed that monoacylglycerol inhibited fatty acid uptake into rat intestinal epithelial cells. Thus, large amounts of monoacylglycerol in the HSO may delay the lymphatic output of TG.

Another possible mechanism for PC-enhanced TG absorption is the packaging and secretion of chylomicrons. In the present study, we showed that there were no intergroup differences in the TG:PL ratio in the lymph (Figs 1(D) and 2(D)). Nevertheless, the lymph output of TG was much higher in rats given LE than in those given SO. This finding indicates that the TG:PL ratio in newly formed chylomicron remained at a constant value.
Fig. 2. The outputs of triacylglycerol ((A), TG) and phospholipid ((B), PL), lymph flow (C) and TG:PL ratio (D) in rats given soyabean oil (SO; O), SO + lecithin (LE; ■) and SO + lysolecithin (LY; ●). Values are means with their standard errors shown by vertical bars for eight, six and seven rats respectively. For details of procedures, see p. 566. (A), TG output for 4 h as % lipid administered was 43·9 (SEM 7·7), 72·3 (SEM 3·1) and 64·5 (SEM 3·0) when SO, LE and HY were given respectively. \( P \) values for TG (A) estimated by two-way ANOVA were 0·0001 for lipid, 0·0001 for time and 0·0282 for lipid \( \times \) time. \( P \) values for phospholipid (B) estimated by two-way ANOVA were 0·0001 for lipid, 0·0001 for time and 0·0956 for lipid \( \times \) time. \( P \) values for lymph flow (C) estimated by two-way ANOVA were 0·1406 for lipid, 0·0001 for time and 0·0057 for lipid \( \times \) time. \( P \) values for TG:PL ratio (D) estimated by two-way ANOVA were 0·8103 for lipid, 0·2654 for time and 0·9991 for lipid \( \times \) time.

There were significant differences in the lymphatic output of phospholipid (B) between the groups (results not shown). \( a \),\( b \) Mean values with unlike superscript letters within the same time period were significantly different (\( P \) \( < \) 0·05).

Fig. 3. The output of triacylglycerol (TG) in rats given hydrolysed soyabean oil (HSO; O) and HSO + hydrolysed lecithin (HLY; ●). Values are means with their standard errors shown by vertical bars for ten and eight rats respectively. For details of procedures, see p. 566. TG output for 4 h as % lipid administered was 38·5 (SEM 5·5) and 59·2 (SEM 4·5) when HSO and and HLY were given respectively. \( P \) values for TG estimated by two-way ANOVA were 0·0001 for lipid, 0·0001 for time and 0·0143 for lipid \( \times \) time. \( a \),\( b \) Mean values with unlike superscript letters within the same time period were significantly different (\( P \) \( < \) 0·05).

Fig. 4. *In vitro* digestibility of lipid emulsion: fatty acid released from lipids by hydrolysis with rat bile—pancreatic juice. O, soyabean oil; ■, soyabean oil + lecithin; ●, soyabean oil + lysolecithin. Values are means with their standard errors shown by vertical bars for five samples. For details of procedures, see p. 566. \( P \) values for non-esterified fatty acids estimated by two-way ANOVA were 0·0001 for lipid, time, and lipid \( \times \) time. \( a \),\( b \) Mean values with unlike superscript letters within the same time period were significantly different (\( P \) \( < \) 0·05).
and possibly shows that the supply of PC is the rate-limiting step in the formation of chylomicron and the absorption of luminal TG. Furthermore, Davidson et al. (1986) suggested that biliary PC plays a role in the intestinal synthesis of apolipoprotein B-48, and Wang et al. (2001) suggested that dietary PC regulated intestinal synthesis of apolipoprotein A-I. These results suggest that luminal lecithin has an important role in chylomicron formation in the intestine. Recently, the role of microsomal TG transfer protein is receiving attention with regard to the association of apolipoprotein B with lipid during intestinal lipoprotein formation (Hamilton et al. 1998; van Greevenbroek et al. 1998). The effect of PC on this stage should be clarified in the future.

The changes in lymphatic PL output were much smaller than those of TG in all of the present experiments. This finding is consistent with those of previous studies that showed that exogenous PL does not greatly influence the lymphatic output of PL (Noh & Koo, 1997; Wang et al. 2001). It has also been reported (Le Kim & Betzing, 1976) that lysophosphatidylcholine is further hydrolysed to fatty acids and glycerol-3-phosphocholine in the enterocytes. These results suggest that a large part of the fatty acids derived from PC were not reconstructed as PC and possibly shows that the supply of PC is the rate-limiting step in the formation of chylomicron and the absorption of luminal TG. Furthermore, Davidson et al. (1986) suggested that biliary PC plays a role in the intestinal synthesis of apolipoprotein B-48, and Wang et al. (2001) suggested that dietary PC regulated intestinal synthesis of apolipoprotein A-I. These results suggest that luminal lecithin has an important role in chylomicron formation in the intestine. Recently, the role of microsomal TG transfer protein is receiving attention with regard to the association of apolipoprotein B with lipid during intestinal lipoprotein formation (Hamilton et al. 1998; van Greevenbroek et al. 1998). The effect of PC on this stage should be clarified in the future.

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