Comparison of the effects of medium and long chain triglyceride containing liquid meals on gall bladder and small intestinal function in normal man

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SUMMARY We investigated the effects of medium (MCT) and long (LCT) chain triglyceride test meals on gall bladder contraction (using ultrasonography) and small intestinal bile acid concentrations and ileal flow rates (using intubation techniques) in normal individuals. Comparing the effects of ingesting medium chain triglyceride and long chain triglyceride meals, the gall bladder volume was reduced by $23.4 \pm 33.6\%$ and $59.0 \pm 17.1\%$ respectively (mean $\pm$ SD, $p < 0.01$, $n = 13$), the jejunal bile acid concentrations increased significantly only after the ingestion of long chain triglyceride ($p < 0.05$, $n = 5$), while the volume of the postprandial jejunal contents was not significantly different (540 ± 150 , 522 ± 169 ml, medium chain triglyceride vs long chain triglyceride, $p = 0.2$, $n = 5$). The postprandial colonic inflow was 48.5 ± 12.5 and 123.4 ± 35.2 ml/h (medium chain triglyceride vs long chain triglyceride, $p < 0.01$, $n = 5$). Thus medium chain triglyceride, compared with long chain triglyceride, produces a smaller input of bile acids into the small intestine and a smaller volume of fluid delivered to the colon. These observations may be relevant to the beneficial effects of medium chain triglyceride substitution for long chain triglyceride in the treatment of diarrhoea in patients with small intestinal disease.

In many patients with a reduced small intestinal mucosal area due to resection or disease, the replacement of dietary long chain triglyceride by medium chain triglyceride in the diet has been shown to reduce both steatorrhoea and diarrhoea,¹⁻⁵ as well as faecal electrolyte excretion.¹⁻⁴ These effects are believed to be because of the rapid absorption of medium chain triglyceride, which occurs independently of the micelle formation, intraluminal hydrolysis and mucosal re-esterification,⁶⁻⁸ and to the absence of the secretory stimulus of unabsorbed long chain fatty acids in the intestinal lumen.⁴ Medium chain triglyceride also differs from long chain triglyceride in its effect on bile salt metabolism. This may be of considerable physiological importance because both bile acids and long chain fatty acids interfere with water and electrolyte absorption from the small intestinal lumen.^{9,10}

In the rhesus monkey, medium chain triglyceride feeding decreased bile salt synthesis, pool size and

biliary secretion.¹¹ There have been no similar long term comparisons made in man, but we have found lower jejunal bile salt concentrations after a medium chain triglyceride meal compared with a long chain triglyceride meal.¹² This led us to test the hypothesis that the ingestion of medium chain triglyceride rich as compared with long chain triglyceride rich meals might lead to a weaker gall bladder contraction,¹³ resulting in lower luminal bile acid concentration and a lower postprandial ileal flow.

We therefore measured gall bladder contraction using ultrasound and ileal flow and using intestinal perfusion after ingestion of long chain triglyceride rich and medium chain triglyceride rich test meals.

Methods

SUBJECTS

Eighteen healthy volunteers aged 22-65 years old (12 men and six women) ingested the medium chain triglyceride and long chain triglyceride test meals on two separate occasions. In 13 of these subjects, the changes of gall bladder volume were assessed by ultrasound. In the other five subjects, changes in

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luminal bile acid concentrations and jejunal and ileal aqueous volumes were assessed by jejuno-ileal intubations. Written informed consent was obtained from all subjects. The protocol was approved by the Guy's Hospital Committee on Ethical Practice (January 1982). Women of child bearing age were not studied by intubation.

TEST MEALS

Each meal diluted in tap water had a final volume of 300 ml and provided 1.7 MJ (400 Kcal). Thirty six per cent of the calories were derived from fat, 48% from carbohydrate and 16% from protein. In both meals the carbohydrate was a mixture of mono-, oligo- and polysaccharides, and the protein was derived from whey, casein and soy hydrolysates. Eighty per cent (w/w) of the fat in the medium chain triglyceride meal was medium chain triglycerides (C_6 - C_{12}) compared with only 31% (w/w) in the long chain triglyceride meal. The medium chain triglyceride meal had an osmolality of 460 mOsm/kg and sodium concentration of 60 mmol/l, while those of the long chain triglyceride were 350 mOsm/kg and 24 mmol/l respectively. The meals were bland tasting and did not produce symptoms.

GALL BLADDER ULTRASOUND STUDY

After an overnight fast (14 hours) the volunteers drank the contents of a numbered test meal containing either the medium chain triglyceride or the long chain triglyceride test meal. The test was repeated on a second day using the other meal at random order. The gall bladder volume was assessed blindly by one of us (PETI) at time zero, 30, and 60 minutes using a real time ultrasound system (system 285, Diagnostic Sonar Ltd, Livingston, Scotland) equipped with a 128 mm 3.0 MHz transducer positioned to obtain a scan showing an axial section of the gall bladder at maximum length (L) at each time interval and a single scan across the axis of the gall bladder to obtain the transverse diameter (w) and antero-posterior diameter (D). Assuming that the gall bladder is cylindrical, the volume was calculated as $V = \pi L [(w/2) \times (D/2)]$.¹⁴⁻¹⁶ This method tends to reproducibly overestimate the absolute gall bladder volume,¹⁵ but should show changes in response to different meals. (Fig. 1).

JEJUNO-ILEAL INTUBATION STUDIES

After an overnight fast, the volunteers swallowed a weighted polyvinyl radio-opaque three lumen tube which was positioned so that its aspiration ports were located 10 to 30 cm beyond the ligament of Treitz and at the terminal ileum (mercury bag in the caecum). Twenty four hours were necessary for the tube to reach the desired position which was

Fig. 1 Fasting (F*) and smallest postprandial (P**) gall bladder volumes estimated by ultrasound in 13 normal subjects after ingestion of long chain triglyceride and medium chain triglyceride test meals. Lines indicate individual subjects. Vertical lines are mean $\pm$ 1SD.

confirmed fluoroscopically at the beginning and the end of each study. Twenty five centimetres proximal to the ileal aspiration point a plasma like solution (sodium 135 mmol/l, potassium 5 mmol/l, chloride 105 mmol/l, bicarbonate 35 mmol/l, osmolality 280 mOsm/kg) containing 10 μ Ci/l of 14 C-PEG as non-absorbable marker and 2 g/l of PEG 4000 as carrier was infused at a rate of 0.3 ml/min using a Harvard peristaltic pump model 1203A (Southnatick, Mass). After a one hour equilibration period, fasting samples of jejunal and ileal contents were collected for two half hour periods using a syringe pump (perfusor MK IV, Braun AG Melsungen) set at an aspiration rate of 0.2 ml/min. The volumes obtained from the jejunum and ileum in 30 minutes were between one and five millilitres

(median: 3 ml). The volunteers then drank either the long chain triglyceride or the medium chain triglyceride test meal, which contained 150 mg of phenol red (PR) as a non-absorbable marker. Half hour samples were continuously aspirated with the syringe pump from the jejunum and terminal ileum for eight periods. The test was repeated on the next day with the other meal in random order. Estimated gonadal irradiation at fluoroscopy was 50–70 mrad and colonic radiation by infused ^{14}C was 70 mrad per study.

Samples obtained were stored at -20°C and estimations were made of phenol red by absorption at 560 nm in the sample adjusted to pH 9.2,¹⁷ of sodium by flame photometry and of total bile acid concentrations by enzymatic fluorimetric method¹⁸ using uncentrifuged specimens. Duplicate 100 μl aliquots of each sample were added to 10 ml of NE 260 scintillation cocktail (Nuclear Enterprises, Sighthill, Edinburgh) and counted on a liquid scintillation counter (Rack-Beta 1215, LKB-Wallac, Finland). A colour quench correction curve was made using a series of ileal aspirates to which were added a fixed amount of ^{14}C -PEG and increasing amounts of phenol red within the range observed in the samples.

The volume of the meal as it passed the upper jejunum (V_j) was estimated as:

$$V_j = V_m \times [\text{PR}_m] \times [\text{PR}_j]^{-1}$$

where V_m is the original volume of the meal (300 ml), $[\text{PR}_m]$ the phenol red concentration in the meal (0.5 mg/ml) and $[\text{PR}_j]$ the maximum phenol red concentration observed in the jejunum postprandially. The volume of the postprandial jejunal contents calculated in this way is only an estimate of the minimal volume of fluid passing the jejunal aspiration port of the tube, but should reflect the relative dilution and transit of the two meals in the upper intestine.¹⁹

The ileal flowrate (F_i) was calculated from the change in concentration of the infused marker (^{14}C -PEG) according to the formula:

$$F_i = F_{\text{in}} \times [\text{PEG}_{\text{in}}] \times [\text{PEG}_{\text{out}}]^{-1}$$

where F_{in} is the infusion rate (0.3 ml/min), $[\text{PEG}_{\text{in}}]$ the disintegrations per minute in the infusate and $[\text{PEG}_{\text{out}}]$ the disintegrations per minute in the ileal sample. The above formula assumes that there was complete mixing of the infusate with the ileal contents and that the small volume (18 ml/h) of the infusate was completely absorbed from the terminal 25 cm of ileum. These assumptions are not entirely justified but, as discussed by Levitt and Bond²⁰ the choice of a slow rate of marker infusion, though giving rise to possible inaccuracies during changing states, is most appropriate to the ileum where the

volume of fluid is usually very small. Small bowel transit time was taken as the time interval between the first visible appearance of phenol red in the jejunal and ileal aspirates.

STATISTICAL ANALYSES

To compare the different local responses to the arrival of the two meals, variability of transit has been eliminated and the results were grouped to give parallel time concentration curves of phenol red. Subsequently, using probability graph paper, it was determined whether the frequency distribution of the results could be closely approximated by a normal distribution.²¹ Results with a normal distribution were tested for significance by analysis of variance appropriate to an experiment with repeated measures of a single factor,² and results were expressed as mean $\pm$ 1 SD. Non-normally distributed results were expressed as median and range and differences examined by Wilcoxon's signed rank test.

Results

GALL BLADDER

The fasting gall bladder volumes before medium chain triglyceride (21.2 ± 12.5 ml) and long chain triglyceride meals (26.6 ± 18.0 ml) were not significantly different ($F_{1,12}=4.13$, $p>0.07$). The minimum postprandial volume was significantly less than the fasting volume for both medium chain triglyceride meals (14.3 ± 6.0 ml, $F_{1,12}=6.16$, $p<0.005$) and long chain triglyceride meals (10.3 ± 7.7 ml, $F_{1,12}=21.35$, $p<0.001$). The minimum gall bladder volume observed after the long chain triglyceride meal, however, was significantly greater than that after the long chain triglyceride meal ($F_{1,12}=9.81$, $p<0.01$). The mean reduction of gall bladder volume [(fasting-minimum postprandial volume/fasting volume) $\times$ 100] was greater after the long chain triglyceride meal ($59.0 \pm 17.1\%$) than after the medium chain triglyceride meal ($23.4 \pm 33.6\%$, $F_{1,12}=12.33$, $p<0.005$).

UPPER JEJUNUM

After the ingestion of the long chain triglyceride meal the jejunal bile acid concentration rose significantly ($p<0.001$, $F_{9,36}=4.64$) (Fig. 2a), but was unchanged after the ingestion of the medium chain triglyceride test meal ($p=0.2$, $F_{9,36}=1.54$). The differences between the jejunal bile acid concentration after the two meals were significant at one hour ($p<0.05$, $F_{1,4}=9.24$), $1\frac{1}{2}$ ($p<0.01$, $F_{1,4}=29.80$) and two hours ($p<0.05$, $F_{1,4}=12.39$) postprandially, although the estimated volumes of

Fig. 2 (a) Jejunal bile acid concentrations in each half hour samples after medium chain triglyceride (●) and long chain triglyceride (○) meals. (b) Phenol Red concentrations (mean $\pm$ 1SD) in jejunal samples after medium chain triglyceride (●—●) and long chain triglyceride (○---○) meals containing 0.5 mg/ml of phenol red. NB Variation of mouth to jejunum transit time between individuals and meals has been eliminated (see statistical analysis).

the postprandial jejunal contents were not significantly different (540 ± 150 and 522 ± 169 ml, medium chain triglyceride vs long chain triglyceride, $p=0.80$, $F_{1,4}=0.09$). Furthermore, the meal marker (phenol red) concentration curves were almost identical (Fig. 2b), strongly suggesting that the two meals were being diluted in the upper jejunum to the same extent. Finally, there was no significant difference between the postprandial sodium concentrations after the long chain triglyceride meal (106 ± 15 mmol/l) and those after the medium chain triglyceride test meal (101 ± 17 mmol/l $F_{1,78}=1.70$, $p=0.8$).

TERMINAL ILEUM

The small bowel transit time of phenol red was 66 ± 12 and 54 ± 12 minutes for the medium chain

triglyceride and long chain triglyceride test meals respectively ($F_{1,4}=1$, $p=0.4$). As shown in Fig 3a, the ileal flow rate did not increase above the fasting value when the medium chain triglyceride meal marker (phenol red) reached the terminal ileum (Fig. 3b). The arrival of the long chain triglyceride meal marker (phenol red) in the terminal ileum, however, coincided with a consistent increase in the ileal flow rate which lasted for one hour (Fig. 3a). The postprandial ileal flow was 48.5 ± 12.6 and 123.4 ± 35.2 ml/h for the medium chain triglyceride and long chain triglyceride test meals respectively ($F_{1,4}=28.95$, $p<0.01$). The bile acid concentrations were higher after the long chain triglyceride meal (1.0, 0.5–30.0 mmol/l median and range) than after the medium chain triglyceride meal (0.5 0.2–1.3

Fig. 3 (a) Ileal flow during each half hour period after medium chain triglyceride (●) and long chain triglyceride (○) meals. (b) Phenol Red concentrations (mean $\pm$ 1SD) in ileal samples after medium chain triglyceride (●—●) and long chain triglyceride (○---○) meals containing 0.5 mg/ml of phenol red. NB Variation of mouth to ileum transit time between individuals and meals has been eliminated (see statistical analysis).

mmol/l), but this difference was not statistically significant. The postprandial bile acid colonic input, however, was significantly higher after the long chain triglyceride meal (0.10, 0.03–2.79 mmol/h) than after the medium chain triglyceride meal (0.02, 0.01–0.04 mmol/h) ($p < 0.05$, Wilcoxon's signed rank test). Though the postprandial sodium concentrations were not significantly different after the two meals (139 ± 11 and 133 ± 8 mmol/l long chain triglyceride vs medium chain triglyceride, $p = 0.07$), the total amount of sodium passing down the ileum postprandially was significantly higher after the long chain triglyceride meal (17.20 ± 5.30 mmol/h) than after the medium chain triglyceride meal (6.40 ± 1.60 mmol/h, $F_{1,4} = 26.57$, $p < 0.01$).

Discussion

This study has shown several important differences in the gut and gall bladder responses to medium chain triglyceride rich compared with long chain triglyceride rich liquid meals in normal man. We would attribute these differences to the nature of the fat ingested, although the meals differed in relation to ionic composition and osmolality, which might affect gastric emptying and intestinal transit. The dilution of the meal marker in the jejunum, the jejunal sodium concentrations and small bowel transit time, however, were not significantly different for the two meals and therefore differences in gastric emptying or transit cannot explain our results. It seems likely that the different effect of the two meals on the gall bladder is the key to the other phenomena.

Because the phenol red concentration curves in the jejunum indicate that the transit and dilution of the meals was similar, the greater concentration of bile acids in the duodenum after the long chain triglyceride is probably caused by a great input of bile acid into the duodenum. A more vigorous gall bladder emptying in response to long chain triglyceride can be inferred from the studies of Malagelada *et al*¹³ who perfused fatty acids through the human duodenum and noted that the bilirubin input increased with increasing chain length of the fatty acid infused. In the present study, medium chain triglyceride and long chain triglyceride rich meals clearly produced markedly different gall bladder responses. If gall bladder contraction is mainly influenced by cholecystokinin it seems likely that only a small release of cholecystokinin was triggered by the presence of medium chain triglyceride in the duodenum and further studies examining cholecystokinin response are now being undertaken.

From the estimated final volume of either meal in

the jejunum of 540 ml and the observed bile acid concentrations of 15.2 and 5.0 mmol/l (long chain triglyceride and medium chain triglyceride respectively), it may be estimated that an average of about 7.6 and 2.5 mmol of bile acid were delivered into the duodenum after the long chain triglyceride and medium chain triglyceride meals respectively. If gall bladder bile after an overnight fast contains approximately 0.2 mmol/ml of bile acid,²³ the observed gall bladder ejection of 16 ml after one long chain triglyceride meal would contain 3.2 mmol of bile acid and account for less than half of the bile acid delivered into the jejunum after the long chain triglyceride meal. The remainder is presumably derived from a stimulated hepatic cholerisis. The amount of bile acid delivered into the upper jejunum after the medium chain triglyceride meal (2.5 mmol), however, was close to that similarly estimated from the gall bladder ejection volume ($7 \text{ ml} \times 0.2 \text{ mmol/ml} = 1.4 \text{ mmol}$), suggesting that the medium chain triglyceride meal had a lesser effect on hepatic bile flow. These are indirect estimates, however, and it is possible that rapid absorption of bile acids in the upper jejunum²⁴ after the medium chain triglyceride meal has accentuated the difference in bile acid concentrations. The main differences in the test meals other than the lipid composition were that the medium chain triglyceride meal was of higher osmolality. This would tend to slow gastric emptying, but as Hunt and Knox²⁵ have shown that gastric emptying is impaired by long chain triglyceride, it seems likely that these two effects have cancelled each other out and the two meals have entered the jejunum at approximately the same rate.

The phenol red concentration curves in the jejunum indicate that the transit and dilution of the meals was similar. Also the jejunal sodium concentrations were not significantly different for the two meals. It would seem, therefore, that differences in gastric emptying and/or transit cannot explain our results. The use of phenol red as a marker of meal transit and dilution, however, is subject to the same limitations as the use of any single phase marker. Lipid soluble markers may not be appropriate as they have been validated only for long chain triglyceride and could be more difficult to interpret when mixtures of long chain triglyceride and medium chain triglyceride enter the duodenum, becoming richer in long chain triglyceride after the rapid absorption of the medium chain triglyceride. Our overall conclusions contrasting the effects on normal gut function of long chain triglyceride- and medium chain triglyceride-containing diets appear to be supported by the observations of Hofmann and Poley⁵ on patients with ileal resection that lower

hepatic bile synthesis and faecal bile acid excretion occur in subjects taking medium chain triglyceride compared with long chain triglyceride diets. Although they found that the small intestine absorbs more than 95% of the amount of bile acids entering the upper jejunum after the ingestion of either meal, we have shown the bile acid colonic input to be significantly higher after the long chain triglyceride meal. This could also explain the increased ileal flow of fluid and sodium into the colon after the long chain triglyceride meal because conjugated dihydroxy bile acids impair the absorption of salt and water by the human small intestine.^{9 10}

This increased flow of water and bile acids into the colon after a long chain triglyceride meal is likely to produce diarrhoea in patients with either small or large gut disease. In small gut disease, the amounts of fluid and bile acids entering the colon are likely to be greater if small gut absorption is impaired. In colonic disease, the capacity of the colon to absorb water is already impaired and could be reversed to secretion if concentrations of luminal bile acids were increased. These phenomena may account for the observation that restriction of dietary fat (long chain triglyceride) intake is often of great benefit to patients with diarrhoea after small bowel resections of various types.

Finally, dietary fat chain length may be of relevance to the incidence of colonic cancer. A high incidence of colon cancer has been related epidemiologically to a high intake of dietary fat²⁶ and high faecal bile acid excretion^{27 28} and possibly to an increased colonic input and bacterial degradation of bile acids after cholecystectomy.^{29 30} Our findings suggest that in studies relating fat intake to bile acid excretion or turnover, the chain length, as well as the total amount, of dietary fat is also worthy of attention.

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