Effect of medium- and long-chain triglycerides on lower esophageal sphincter pressure: role of CCK

M. LEDEBOER, A. A. M. MASCLEE, I. BIEMOND, AND C. B. H. W. LAMERS
Department of Gastroenterology-Hepatology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

Ledeboer, M., A. A. M. Masclee, I. Biemond, and C. B. H. W. Lamers. Effect of medium- and long-chain triglycerides on lower esophageal sphincter pressure: role of CCK. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G1160–G1165, 1998.—Fat meals are known to decrease lower esophageal sphincter (LES) pressure, possibly through postprandial CCK release. Dietary fat consists mainly of long-chain triglycerides (LCT), which potently stimulate CCK release. Medium-chain triglycerides (MCT), which do not induce CCK release. We reported that loxiglumide prevented the decrease in LES pressure that occurs after ingestion of a mixed meal.

Dietary fat mainly consists of long-chain triglycerides (LCT), which are known to potently release CCK. In this study we have investigated the effect of intraduodenal administration of LCT on CCK release and on LES pressure. The studies were performed with and without the infusion of loxiglumide. In addition, gallbladder volumes were measured, because gallbladder contraction is mainly regulated by CCK. In contrast to LCT, medium-chain triglycerides (MCT) do not induce CCK release or gallbladder contraction. It is, however, not known whether MCT affects LES pressure. Therefore, the effect of an equimolar amount of intraduodenal MCT on LES pressure, CCK release, and gallbladder motility was studied without and with concomitant infusion of loxiglumide.

METHODS

Subjects. Six healthy volunteers (4 male, 2 female; mean age 26 yr, range 19–39 yr) participated in the study. The subjects were studied after an overnight fast of at least 10 h. None of the subjects had a history of gastrointestinal disease, gallstones, or surgery, and none of the subjects were taking any medication. The presence of gallstones was excluded by ultrasonography. Informed consent was obtained from each individual, and the protocol was approved by the ethics committee of the Leiden University Medical Center.

Study protocol. The subjects participated in five experiments performed on separate occasions in random order with an interval of at least 7 days. At 8:00 AM the subjects were intubated with a single-lumen polyurethane feeding tube (Flocare Ch 10; Nutricia, Zoetermeer, The Netherlands) that was positioned under fluoroscopic control in the horizontal part of the duodenum. The manometry catheter to record LES pressure was inserted into the duodenum and positioned under fluoroscopic control in the horizontal part of the duodenum. Blood samples for determination of plasma CCK levels were obtained 30, 15, and 0 min before the start of the experiment (time 0). LES pressure was recorded continuously for a 30-min basal period from −30 to 0 min. At time(t) = −15 min, intravenous infusion of saline or loxiglumide (CR-1505; Rotta Research Laboratories, Monza, Italy; loading dose: 30 mg·kg⁻¹·h⁻¹ for 10 min (= 5 mg/kg), followed by continuous infusion of 10 mg·kg⁻¹·h⁻¹) was started. LES pressure was
recorded continuously, and gallbladder images and blood 
samples were obtained at 15-min intervals during continuous 
intraduodenal administration of either LCT (corn oil, fatty 
acids composition 93% C16-C18), MCT (Ceres-MCT dietary oil; 
Van den Bergh en Jurgens, Rotterdam, The Netherlands; 
fatty acid composition 98%, C6-C10), or saline (control; 20 
ml/h) at an infusion rate of 20 mmol/l (MCT 15 g/90 min; LCT 
30 g/90 min) with concomitant intravenous infusion of either 
saline (intraduodenal LCT, MCT, or control) or loxiglumide 
intraduodenal LCT or MCT) for 90 min. 

Manometric recording technique and analysis. Esophageal 
pressure recordings were obtained using a polyvinyl assem-

bly of 5-mm outer diameter, incorporating a 6-cm-long sleeve 
sensor and seven side holes, one located at the distal margin 
to monitor intragastric pressure and six located 0, 3, 6, 9, 15, 
24, or 30 cm proximal to the upper margin of the sleeve sensor 
(Dent Sleeve) (9). The manometric assembly was passed trans-
asally into the esophagus, and the sleeve sensor was 
positioned so that it permitted continuous monitoring of LES 
pressure. Simultaneously, pressures were recorded from the 
gastric fundus, LES (sleeve), esophageal body (side holes 3, 9, 
and 15 cm above the upper margin of the sleeve sensor), and 
pharynx (24 or 30 cm above the sleeve sensor). The side holes 
and sleeve sensor were connected to a pressure transducer 
(Medex, Hilliard, OH) and perfused with gas-free distilled 
water by a low-compliance pneumatic infusion pump 
(Arndorfer Medical Supplies, Greendale, WI) at a rate of 0.5 
ml/min. The side hole located in the pharynx, used to monitor 
swallows, was filled with water but not perfused to avoid 
excessive swallowing. The outputs from pressure transducers 
were processed by an eight-channel polygraph (Synetics 
Medical, Stockholm, Sweden) displayed on a monitor and 
stored on a personal computer.

The pressure tracings were analyzed visually to calculate LES 
pressure. Each manometric tracing was coded. Analysis 
of the tracings was performed in a blinded fashion by 
investigators who were unaware of the code. Mean end-

exploratory LES pressure was determined at 10-min intervals 
and referenced to end-expiratory intragastric pressure, 
defined as zero.

Measurement of gallbladder volume. Gallbladder volumes 
measured with real-time ultrasonography (Technicare 3; 
5-MHz transducer) were calculated by the sum-of-cylinders 
method, using a computerized system (11, 16). In this method 
the longitudinal image of the gallbladder is divided into 
sections of equal length, with diameters perpendicular to the 
longitudinal axis of the gallbladder image. The uncorrected 
volume is the sum of the volumes of these separate cylinders. 
To correct for the displacement of the longitudinal image 
of the gallbladder from the central axis, a correction factor 
was calculated from the longitudinal and transverse scans of the 
gallbladder. Gallbladder volume is calculated by multiplying 
the uncorrected volume with the square of the correction 
factor. The mean of the two measurements was used for 
further analysis. The assumptions and the mathematical 
formula used to calculate gallbladder volume have been 
described and validated previously (11, 16).

Assay of CCK. Blood samples were collected in tubes 
containing EDTA and kept on ice during the experiment. 
Plasma CCK was measured by a sensitive and specific radioimmunoassay, using antibody T204 (21, 22). This anti-
body binds to all carboxy-terminal CCK peptides containing 
the sulfated tyrosyl region. The detection limit of the assay 
was 0.3 pmol/l plasma. The intra-assay variation ranged from 
4.6% to 11.5%, and the interassay variation ranged from 
11.3% to 26.1% (22).

Statistical analysis. Results are expressed as means ± SE. 
LES pressures are expressed in millimeters of mercury, 
gallbladder volumes are expressed in cubic centimeters, and 
plasma CCK levels are expressed in picomoles per liter. 
Differences in LES pressures, gallbladder volumes, and CCK 
levels within or between the experiments were analyzed for 
statistical significance by multiple ANOVA. When this indi-
cated a probability of <0.05 for the null hypothesis, Student-
Newman-Keuls analyses were performed to determine which 
values differed significantly between or within the experiments. The significance level was set at P < 0.05.

RESULTS

LES pressure. Basal LES pressures were not signifi-
cantly different among the five experiments (Figs. 1 
and 2). During intravenous infusion of saline, LES 
pressure 0 min before intraduodenal administration of 
LCT, MCT, and saline was 29 ± 3, 30 ± 2, and 27 ± 3 
mmHg, respectively. During intravenous infusion of 
loxiglumide LES pressure was 27 ± 3 (LCT) and 27 ± 2 
mmHg (MCT) at 0 min. During intraduodenal adminis-
tration of both LCT and MCT with concomitant intrave-
nous saline, a rapid and significant (P < 0.05) reduction 
in LES pressure was observed, starting at t = 10 min 
to 20 ± 3 and 19 ± 3 mmHg, respectively; Fig. 1). LES 
pressure remained significantly reduced during all 90 
min of intraduodenal administration of both LCT and 
MCT. The reduction in LES pressure between intraduo-

nental LCT and MCT was not significantly different. 
Intravenous infusion of loxiglumide prevented the re-
duction in LES pressure during intraduodenal adminis-
tration of LCT (Fig. 2A). However, intravenous infusion of 
loxiglumide did not affect the reduction in LES pressure 
during intraduodenal administration of MCT 
(19 ± 2 mmHg at t = 10 min; Fig. 2B). No significant 
alterations in LES pressure were observed during 
intraduodenal administration of saline (Fig. 1).

Plasma CCK concentrations. Basal plasma CCK con-
centrations (t = −15 min) were not significantly differ-

Fig. 1. Lower esophageal sphincter pressures (LESP; means ± SE; 
mmHg) during fasting and during intravenous infusion of saline with 
 simultaneous intraduodenal administration of long-chain triglycer-
ides (LCT; □), medium-chain triglycerides (MCT; ○), or saline (▲) in 
a dose of 20 mmol/l for 90 min. *Significant (P < 0.05) difference 
compared with basal levels and saline (control).
ent among the five experiments: 2.6 ± 0.3 (LCT), 2.4 ± 0.3 (MCT), and 2.5 ± 0.3 pmol/l (saline) before intravenous infusion of saline (Fig. 3A), and 2.6 ± 0.2 (LCT) and 2.5 ± 0.2 pmol/l (MCT) before intravenous infusion of loxiglumide (Fig. 3B). No significant changes in plasma CCK levels were observed during intravenous infusion of either saline or loxiglumide from $t = -15$ to $t = 0$ min. Intraduodenal administration of LCT during concomitant intravenous saline resulted in a significant ($P < 0.05$) increase in plasma CCK over basal levels starting from 15 min to a maximum of 4.3 ± 0.6 pmol/l at 75 min (Fig. 3A). Administration of LCT also induced significant ($P < 0.05$) increases in plasma CCK levels from 15 min until 90 min compared with control (saline). No significant alteration in plasma CCK levels over basal was observed during MCT administration. Infusion of loxiglumide did not affect the plasma CCK increase in response to intraduodenal administration of LCT. The significant increase in plasma CCK started at 60 min and reached a peak value of 4.7 ± 0.5 pmol/l at 90 min (Fig. 3B). During intraduodenal administration of MCT and loxiglumide no significant alterations in plasma CCK levels were observed.

Gallbladder volumes. Fasting gallbladder volumes at $t = -15$ min were not significantly different between the five experiments: 28.3 ± 4.5 cm$^3$ before LCT, 28.8 ± 3.5 cm$^3$ before MCT, 27.7 ± 3.8 cm$^3$ before saline (Fig. 4A), 28.1 ± 1.7 cm$^3$ before LCT and loxiglumide, and 29.4 ± 3.4 cm$^3$ before MCT and loxiglumide (Fig. 4B). No significant changes in gallbladder volumes were observed during intravenous infusion of saline or loxiglumide from $t = -15$ to $t = 0$ min. During intraduodenal administration of LCT with concomitant intravenous infusion of saline a significant ($P < 0.05$) reduction in gallbladder volume was observed, starting at 60 min and reaching a residual volume of 10.8 ± 3.4 cm$^3$ (61 ± 12% contraction) at $t = 75$ min (Fig. 4A). Intraduodenal administration of MCT with concomitant intravenous infusion of saline did not result in

Fig. 2. LESP (means ± SE; mmHg) after fasting and during intravenous infusion of loxiglumide in a dose of 10 mg·kg$^{-1}$·h$^{-1}$ (□) or saline (■) with simultaneous intraduodenal infusion of LCT (A) or MCT (B) in a dose of 20 mmol/l for 90 min. *Significant ($P < 0.05$) difference compared with basal.

Fig. 3. A: plasma CCK concentrations (means ± SE; pmol/l) after fasting and during intravenous infusion of either saline (A) or loxiglumide (10 mg·kg$^{-1}$·h$^{-1}$; B), with simultaneous intraduodenal administration of LCT (■), MCT (□), or saline (▲) in a dose of 20 mmol/l for 90 min. *Significant ($P < 0.05$) difference compared with basal levels and with saline (control).
gallbladder contraction but, on the contrary, resulted in a small but significant (P < 0.05) increase in gallbladder volume, reaching a maximum of 37.8 ± 4.1 cm³ (135 ± 14%) at t = 90 min. No significant alterations in gallbladder volume were observed during intraduodenal administration of saline (control).

During intravenous infusion of loxiglumide and intraduodenal administration of both LCT and MCT a significant (P < 0.05) increase in gallbladder volume was observed, starting at 30 min and reaching peak values of 50.9 ± 3.4 cm³ (181 ± 12%) and 52.8 ± 3.0 cm³ (180 ± 10%) at t = 90 min, respectively (Fig. 4B). No significant differences in gallbladder volume were observed between the two experiments.

**DISCUSSION**

In this study we have shown that intraduodenal administration of LCT or MCT at a dose of 20 mmol/h significantly decreases LES pressure. Loxiglumide, a potent and specific receptor antagonist of the CCK-A receptor, prevented the reduction in LES pressure induced by intraduodenal administration of LCT, but not during administration of MCT. By measuring CCK release during fat administration and the effect of CCK receptor blockade, we were able to determine the role of CCK in the LES pressure reduction induced by LCT and MCT. It appears that the effect of LCT on LES pressure is dependent on endogenous CCK release, whereas the effect of MCT is not mediated by CCK.

The effect of CCK on LES pressure has been questioned in previous studies (7, 12, 26, 33, 35). Infusion of CCK in humans decreases LES pressure, but this effect may occur only at supraphysiological doses, as suggested by Brazer et al. (7) and by Katschinski et al. (25).

In a recent study we demonstrated that infusion of CCK in a dose of 1.0 IU·kg⁻¹·h⁻¹, leading to plasma CCK levels similar to those induced by a fatty meal, decreases LES pressure significantly, by ~40% (26).

Infusion of CCK in a lower dose of 0.5 IU·kg⁻¹·h⁻¹ increased plasma CCK levels to 6.1 ± 0.4 pmol/l but did not affect LES pressure. In the present study, although plasma CCK levels reached during intraduodenal infusion of LCT were lower (at maximum 4.3 ± 0.6 pmol/l), LES pressure decreased significantly. The effect of LCT on LES pressure was completely antagonized during loxiglumide infusion. These results emphasize the role of endogenous CCK in fat-induced reduction of LES pressure. Furthermore, our findings are in agreement with those of Katschinski et al. (25), who demonstrated that duodenal perfusion of a mixed liquid meal significantly reduced LES pressure at relatively low plasma CCK levels, whereas exogenous CCK reduced LES pressure only at higher plasma CCK levels. In the study of Katschinski et al. (25), loxiglumide antagonized the reduction in LES pressure during intraduodenal administration of the mixed meal. In the present study we have demonstrated that intraduodenal LCT decreases LES pressure and that this effect is prevented by blockade of CCK receptors.

It is remarkable that LCT fat induces a decrease in LES pressure at plasma CCK levels well beneath those at which exogenous CCK results in a significant reduction in LES pressure. Several factors may account for the observed discrepancy in the effect between exogenous and endogenous CCK on LES pressure. First, different molecular forms of CCK exist, which may vary in biological activity (6, 22, 27). However, the radioimmunoassay used is highly specific for bioactive CCK peptides. Second, the LES itself or its neurons may differ in sensitivity to various molecular forms of CCK. In our study on the effect of exogenous CCK on LES pressure, we used CCK-33 (26). This molecular form of CCK did not affect LES pressure at physiological plasma levels comparable to those seen after ingestion of a regular meal. Similarly, in studies with CCK-8 a reduction was not observed in LES pressure at plasma CCK levels in the postprandial range (7, 25). Third, intraduodenal LCT may influence the effect of endogenous CCK by acting through vagal afferent pathways,
as has been reported previously for the effect of duodenal distension by nutrients on gastric motility (15). Fourth, it has been suggested that CCK decreases LES pressure through a nonadrenergic, noncholinergic neural mechanism, using peptides such as vasointestinal polypeptide or peptide histidine-isoleucine as neurotransmitters (4, 5). It is not known whether these neurotransmitters are more sensitive to endogenous than to exogenous CCK.

A direct effect of circulating long-chain fatty acids (LCFA) on LES pressure, after absorption from the intestinal lumen, cannot be excluded. Recent studies have shown that circulating fat (LCFA) influences gastric emptying and small intestinal motility (8, 13). LCT are absorbed as LCFA after dispersion by bile salts and hydrolysis by pancreatic lipase, which are both secreted in response to CCK release. CCK receptor blockade with loxiglumide inhibits pancreatic enzyme secretion and gallbladder contraction in response to food stimulation by 60–100% (14). Basal pancreatic enzyme secretion and biliary output are nearly abolished during loxiglumide infusion (23). Therefore, during CCK receptor blockade, digestion and absorption of LCFA may be impaired, and a presumed direct effect of circulating LCFA on LES pressure may be lacking. A direct effect of circulating medium-chain fatty acids (MCFA) may account for the observed reduction in LES pressure after MCT. Hydrolysis of MCT is very rapid and is caused by nonpancreatic lipases (especially gastric lipase), and dispersion occurs in the absence of bile salts (1, 31). Small amounts of MCT are able to enter the intestinal cell without prior hydrolysis. Thus absorption of MCT or MCFA from the intestinal lumen is largely independent of pancreatic enzyme or biliary secretion. Infusion of loxiglumide will not inhibit digestion and absorption of MCT. Therefore, a direct effect of circulating MCT or MCFA on LES pressure may be possible. A direct effect of MCT or MCFA on LES is supported by the finding that loxiglumide does not prevent the MCT-induced reduction in LES pressure. The effects of fatty acids in the postabsorptive or circulatory phase of digestion on LES pressure have not previously been studied in detail. In one study no significant change in LES pressure after intravenous infusion of a lipid emulsion was found. However, LES pressures in that study were not measured with a sleeve catheter, and a possible effect could have been masked by the long interval between fat administration and the onset of LES recording (8).

Other mechanisms involved in the MCT-induced reduction in LES pressure remain speculative. An effect of MCT on LES pressure mediated by CCK is highly unlikely. During intraduodenal administration of MCT no release of CCK was detected, and infusion of loxiglumide did not antagonize the MCT-induced reduction in LES pressure. Other intestinal hormones could be responsible for the observed effect of MCT on LES pressure. However, peptides such as pancreatic polypeptide and gastric inhibitory peptide are not released during MCT ingestion, whereas others such as gastrin and motilin do not reduce LES pressure (19, 24).

During intraduodenal administration of MCT, a significant increase in gallbladder volume to 135% of fasting volume was observed. This increase in gallbladder volume after ingestion of MCT has been reported previously, but its mechanism is unclear (19). MCT accelerates intestinal transit and induces diarrhea (36). Although MCT does not stimulate secretion of proximal gut hormones, recent studies suggest that MCT may activate the ileal brake and peptide YY secretion (28, 36). The relaxation of the gallbladder during loxiglumide infusion and concomitant intraduodenal administration of LCT or MCT reflects the role of CCK in maintaining basal gallbladder tone and mediating postprandial gallbladder contraction, as reported earlier (10, 30). Our results on gallbladder motility confirm the effect of loxiglumide as a specific CCK receptor blocker in vivo.

Postprandial levels of endogenous CCK have been reported to be higher during loxiglumide infusion (14, 20, 25). The augmented CCK response is explained by a negative feedback between biliary acid concentrations in the intestinal lumen and CCK-secreting cells. Decreased intraluminal bile acid concentrations increase CCK secretion. In our study, however, an increase in plasma CCK levels during loxiglumide was observed only after 60 min of LCT administration. The reason for this delayed increase in plasma CCK is probably related to the administration of undigested LCT. Intraduodenally administered LCT has to be dispersed by bile salts and hydrolyzed by pancreatic lipase into monoglycerides and LCFA to release CCK. These split products of lipolysis are potent stimuli of CCK release. During infusion of loxiglumide in the dose we used, both biliary output and pancreatic enzyme secretion are markedly reduced. This reduced pancreatic-cilliary secretion impairs digestion of LCT and results in a delayed increase in plasma CCK.

In conclusion, LCT and MCT, administered in equimolar amounts intraduodenally, both significantly reduce LES pressure. The effect of LCT on LES pressure is suggested to be mediated by endogenous CCK because 1) intraduodenal LCT increases plasma CCK levels and 2) CCK receptor blockade with loxiglumide prevents the reduction in LES pressure induced by intraduodenal LCT. The effect of MCT on LES pressure is not likely to be dependent on endogenous CCK because 1) intraduodenal MCT does not release CCK and 2) loxiglumide does not prevent the MCT-induced reduction in LES pressure.

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Address for reprint requests: A. A. M. Masclee, Dept. of Gastroenterology-Hepatology, Leiden Univ. Medical Center, Bldg. 1, C4-P, PO Box 9600, 2300 RC Leiden, The Netherlands.

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