# Effect of predigested fat on intestinal stimulation of plasma cholecystokinin and gall bladder motility in coeliac disease

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**Abstract**

**Cholecystokinin (CCK) release and gall bladder emptying in response to a fatty meal are completely abolished in coeliac disease. To determine the effect of lipid digestion on CCK release and gall bladder motility, six patients with untreated coeliac disease and a flat jejunal mucosa were studied on two separate days. After an overnight fast, the plasma CCK concentration and gall bladder volume were measured before and at regular intervals after the intraduodenal instillation of 60 ml corn oil (triglycerides) incubated with 40 ml saline or with 40 ml bile and pancreatic juice. The mean (SEM) concentration of free fatty acids in the aqueous phase of corn oil after incubation with bile and pancreatic juice (predigested corn oil) was 78 (35) mM compared with 0·1 (0·1) mM in the aqueous phase of corn oil incubated with saline (undigested corn oil). Integrated plasma CCK in response to predigested corn oil was significantly greater than that in response to undigested corn oil (101 (18) pM. 80 min *v* −2 (9) pM.80 min; $p<0·005$). Similarly, integrated gall bladder contraction in response to predigested corn oil was significantly larger than that after undigested corn oil (817 (210) ml. 80 min *v* −225 (243) ml. 80 min; $p<0·05$). In contrast to undigested corn oil, corn oil that has been predigested with bile and pancreatic juice induces plasma CCK secretion and gall bladder contraction in patients with untreated coeliac disease, presumably by generating and rendering soluble lipolytic products.**

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Cholecystokinin (CCK) is a major factor in mediating the gall bladder contraction in response to intestinal fat.[1–4] The cells that produce CCK are located in the mucosa of the upper small intestine.[5] In patients with a flat jejunal mucosa as a result of untreated coeliac disease, plasma CCK and gall bladder responses to oral or intestinal fat are abolished,[6 7] and intestinal concentrations of CCK have been reported to be lower in patients with coeliac disease than in healthy subjects.[8] The finding of an increased number of CCK producing cells in duodenal biopsy specimens from patients with coeliac disease seems to contradict the absence of gall bladder emptying and plasma CCK release in response to the appropriate stimulus in this disorder.[9 10]

Little is known of the mechanisms by which fatty nutrients stimulate CCK producing cells. In dogs with gastric and pancreatic fistulas, fat did not stimulate pancreatic protein secretion unless the triglycerides were mixed and incubated with bile and lipase active pancreatic juice.[11] The results of this study suggested that intraluminal stimulation of pancreatic enzyme secretion, and possibly CCK release, depends on conditions for optimal lipolysis and solubilisation of the fatty nutrients. The aim of this study was therefore to determine if fatty nutrients do stimulate plasma CCK release and gall bladder contraction when conditions for optimal lipolysis and solubilisation are met in patients with coeliac disease.

## Methods

Six patients (three men and three women aged 23–53 years) with coeliac disease and a flat jejunal mucosa were studied on two separate occasions before gluten withdrawal. Each of these patients had two to five jejunal biopsy specimens taken from the first part of the jejunum within three weeks of the study. In each case, histological examination of the biopsy specimen showed a flat jejunal mucosa. All subjects gave informed consent before entering the study. The study was approved by the local ethics committee.

After an overnight fast, the tip of a duodenal tube was positioned under fluoroscopic control in the third part of the duodenum. On one occasion, 60 ml of undigested corn oil (Genfarma bv, Maarssen, The Netherlands) incubated with 40 ml saline, and on the other occasion 60 ml of corn oil predigested with 40 ml of the patient's own bile and pancreatic juice, were infused intraduodenally over a 10 minute period. Corn oil consists of triglycerides, and the proportions of individual fatty acids are as follows: linoleic acid 57–46%, oleic acid 27–37%, palmitic acid 10–13%, and small amounts of stearic acid, linolenic acid, arachidic acid, and myristic acid. The total amount of free fatty acids in 60 ml corn oil is less than 0·3 mmol.

Pancreato-biliary secretion was obtained in each patient after intravenous infusion of 1 Ivy Dog Unit/kg CCK (Ferring Pharmaceuticals, Malmö, Sweden), with subsequent aspiration of the duodenal juice. Forty ml of this bile and pancreatic juice were subsequently incubated

with 60 ml of corn oil at 37°C for 24 hours in a shaking water bath followed by heating for five minutes at 100°C to inactivate enzyme activity.

Blood samples were drawn before and at 10 minute intervals after intraduodenal instillation of the corn oil meal. They were collected on ice in glass tubes containing 2 g/l EDTA, rapidly centrifuged, and the plasma was stored at −20°C before analysis of CCK. Each time a blood sample was drawn, two longitudinal and two transverse images of the gall bladder were obtained by real time ultrasonography, as described previously.[12] In two series of experiments using bile and pancreatic juice obtained from the subjects, the amount and composition of free fatty acids in the aqueous phase of 1200 μl corn oil plus 800 μl pancreato-biliary secretion and in 1200 μl corn oil plus 800 μl saline was measured each time before and after predigestion followed by heating for five minutes at 100°C to inactivate enzyme activity.

In order to exclude the possibility that pancreato-biliary juice itself induced plasma CCK release and gall bladder contraction, two men aged 67 and 49 years with a flat jejunal mucosa because of coeliac disease were studied after an overnight fast. Plasma CCK and gall bladder volumes were measured before and after intraduodenal infusion of 60 ml saline mixed with 40 ml of the patient's own bile-pancreatic juice, obtained and heated according to the same protocol described for the predigestion experiment.

Plasma CCK concentrations were measured by a sensitive and specific radioimmunoassay using antibody T204.[13] The antibody binds to all carboxy-terminal CCK peptides containing the sulphated tyrosine region. The antibody shows less than 2% cross reactivity with sulphated gastrins and does not bind to unsulphated forms of gastrin. It does not cross react with structurally unrelated peptides, such as insulin, glucagon, secretin, pancreatic polypeptide, bombesin, and neurotensin. One ml of the plasma samples was extracted by adding 2 ml of 96% (v/v) ethanol. After mixing and centrifugation, the supernatant was evaporated to dryness under nitrogen at 37°C. The dried supernatant was reconstituted in assay buffer to the original sample volume. The detection limit of the assay was between 0·5 and 1·0 pmol/l of plasma. The intra-assay variation ranged from 4·6–11·5%.

Gall bladder volume was calculated by the sum of cylinders method using a computer system.[12] The variation of volume measurements ranged from 6·0–22·4%. Both plasma CCK levels and gall bladder volumes were measured in duplicate and the mean of two measurements was used for further analysis of results.

The concentration of free fatty acids was determined in the aqueous phase of the lipid mixtures. After centrifugation for five minutes at 15 000 *g*, the aqueous phase was aspirated by a needle through the top layer. Twenty μl of the aqueous phase were mixed with 200 μl heptadecanoic acid dissolved in ethanol (200 μM) as internal standard. Subsequently, 200 μl of distilled water and 20 μl of 37·5% HCl were added. The fatty acids were extracted with 3 ml n-hexane, twice. The combined hexane layers were dried down under $N_2$ at 37°C. To the residue, dissolved in 1 ml methanol, 0·1 ml of 37·5% HCL and 1 ml dimethoxypropane were added. The mixture was incubated in the dark for one hour and brought to dryness under $N_2$ at room temperature followed by dissolution of the residue in 0·4 ml hexane. This solution was analysed by gas chromatography on a 30 m×0·25 mm id, DB-23 column (J & W Scientific, Folsom, CA, USA), with a film thickness of 0·25 μm and a cyanopropyl/polysiloxane phase. Analysis was performed on a Varian 3400 GC (programmed temperature vaporiser system; injector temperature was raised within 48 seconds from 60°C to 220°C; Varian, Harbor City, CA, USA) equipped with a flame ionisation detector (detector temperature 260°C) and a Varian 4400 integrator. Helium was used as the carrier gas at a flow rate of 3 ml/min. The injected sample volume was 0·8 μl on the column. The temperature was programmed from 60–150°C at a rate of 30°C/min. The peak quantitation was based on peak area comparison with the internal standard.

Results were expressed as mean (SEM). Integrated plasma CCK was determined by calculating the area under the plasma concentration time curve after subtraction of the basal value. Integrated gall bladder contraction was determined by calculating the area under the gall bladder contraction time curve. Statistical analysis was carried out by two way analysis of variance and by Student's *t* test for paired data.

TABLE I *Free fatty acid (FFA) concentration (mean (SEM) in the aqueous phase of lipid mixtures before and after incubation for 24 hours at 37°C*

| | *FFA (mmol/l)* | |
|---|---|---|
| *Mixture* | *Before incubation* | *After incubation* |
| Corn oil with bile and pancreatic juice | 10·5 (4·1) | 78·0 (35·1) |
| Corn oil with saline | 0·1 (0·1) | 0·1 (0·1) |

## Results

The predigestion procedure increased the amount of free fatty acids in the aqueous phase of the corn oil meal by 67·5 (31·2) mM (Table I). The relative contributions of individual fatty acids to this increase were linoleic acid 51 (1)%, oleic acid 24 (1)%, palmitic acid 18 (1)%, and other fatty acids 7 (1)%, reflecting the fatty acid composition of corn oil. On the other hand, the concentration of free fatty acids in the aqueous phase of the corn oil meal with saline (0·1 (0·1) mM) was not affected by the incubation procedure (Table I).

Intraduodenal administration of undigested fat did not induce any statistically significant change in the basal plasma CCK level 2·3 (0·3) pM. On the other hand, predigested corn oil evoked an immediate and statistically significant increase in the plasma CCK concentration from a basal value of 2·3 (0·2) pM to a

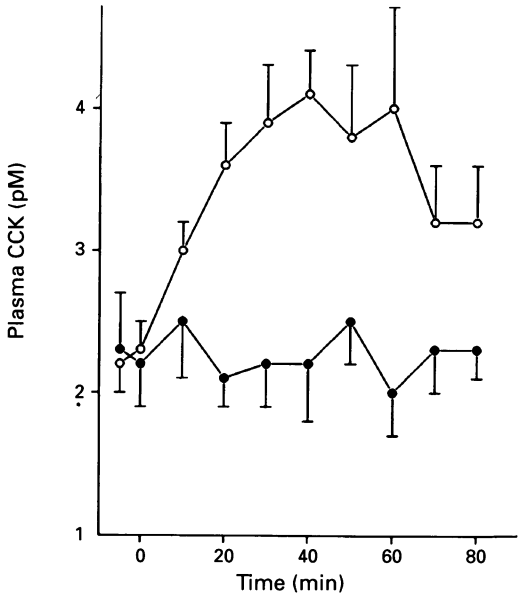


*Figure 1: Plasma cholecystokinin (CCK) in response to intraduodenal fat without (closed circles) or with (open circles) predigestion in six patients with untreated coeliac disease.*

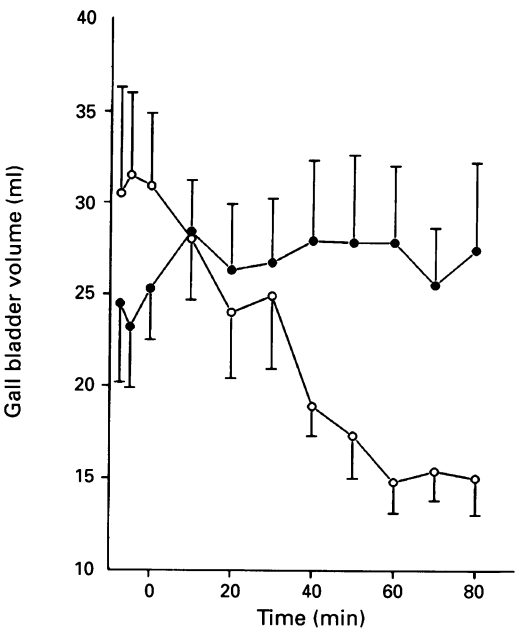


*Figure 2: Gall bladder volume in response to intraduodenal fat without (closed circles) or with (open circles) predigestion in six patients with untreated coeliac disease.*

peak level of 4·1 (0·3) pM at 40 minutes ($p<0·0005$; Fig 1). Integrated plasma CCK secretion in response to the predigested fat (101 (18) pM.80 min) was significantly ($p<0·005$) larger than that after undigested fat (−2 (9) pM. 80 min).

No statistically significant change in the basal gall bladder volume (24·3 (3·4) ml) was observed in response to the intraduodenal administration of undigested corn oil. When the corn oil was predigested with bile and pancreatic juice, however, a highly significant ($p<0·0001$) reduction in gall bladder volume from a basal volume of 31·0 (4·6) ml to 14·8 (1·7) ml at 60 minutes was induced (Fig 2). Similar to integrated CCK response, integrated gall bladder contraction in response to predigested triglycerides (817 (210) ml. 80 min) was significantly ($p<0·05$) larger than that after undigested triglycerides (−225 (243) ml. 80 min).

Intraduodenal administration of pancreatobiliary juice without corn oil neither increased plasma CCK levels nor reduced gall bladder volumes (Table II).

TABLE II *Plasma cholecystokinin (CCK) and gall bladder responses to intraduodenal infusion (0–10 minutes) of 40 ml pancreato-biliary juice and 60 ml saline in two patients with a flat jejunal mucosa as a result of coeliac disease*

| | *Time (min)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *−10* | *0* | *10* | *20* | *30* | *40* | *50* | *60* |
| Plasma CCK (pM): | | | | | | | | |
| Patient 1 | 2·3 | 2·9 | 2·5 | 2·2 | 2·7 | 2·3 | 2·8 | 2·8 |
| Patient 2 | 2·5 | 2·3 | 1·7 | 2·0 | 2·2 | 1·9 | 2·3 | 2·3 |
| Gall bladder volume (ml): | | | | | | | | |
| Patient 1 | 18 | 17 | 20 | 18 | 24 | 21 | 22 | 24 |
| Patient 2 | 25 | 21 | 23 | 32 | 30 | 27 | 28 | 28 |

## Discussion

This study shows that intraduodenal administration of corn oil predigested with bile and pancreatic juice stimulates plasma CCK release and gall bladder contraction in coeliac patients with a flat jejunal mucosa, whereas undigested corn oil does not.

Intraduodenal administration of corn oil is a potent stimulant of plasma CCK release and gall bladder motility in healthy volunteers.[7 14] The present study confirms that plasma CCK release and gall bladder emptying in response to oral or intestinal fat is completely abolished in patients with coeliac disease and a flat jejunal mucosa.[6 7] Absence of CCK responsiveness in coeliacs may be related to reduced amounts of CCK in the intestinal mucosa.[8] However, an increased number of CCK producing cells in the crypts of the intestinal mucosa has been shown by immunocytochemical studies.[9 10] In normal subjects CCK cells are scattered evenly in the crypts and villi of the duodenum and jejunum, with a few cells also present more distally in the small intestine.[5 9 15] Absence of stimulation in coeliac patients may therefore be related to either the presence of normal functioning CCK producing cells in the elongated crypts which cannot be reached by stimulating substances, or an impaired release of the endocrine secretory products despite appropriate stimulation. The finding that predigestion of fat with bile and pancreatic juice converts corn oil from a non-stimulant into a stimulant for the release of CCK indicates that these CCK cells in the crypts of the intestinal mucosa of coeliac patients can be stimulated to secrete CCK.

Several factors may have accounted for the stimulating properties of the predigested corn oil. In theory bile-pancreatic juice itself may contain a factor which stimulates the CCK cell. However, intraduodenal infusion of the pancreato-biliary juice alone does not induce plasma CCK release and gall bladder contraction in coeliac patients as demonstrated in the present study. This finding agrees with the observation in healthy volunteers that pancreatic enzymes[16–18] and bile acids[19 20] do not stimulate but rather inhibit plasma CCK

secretion. Furthermore, pancreatic enzymes inactivated by boiling do not affect CCK and gall bladder responses to intraduodenal corn oil in patients with pancreatic insufficiency.[21] Therefore, the stimulating property of the predigested corn oil is probably related to the generation and solubilisation of lipolytic products.

This hypothesis is supported by the finding that products of fat digestion – for example, long chain fatty acids and monoglycerides – stimulate pancreatic enzyme secretion[11 22] and plasma CCK release.[23 24] Indeed, the concentration of total free fatty acids in the aqueous phase of the predigested fatty meal in the present study was comparable with fatty acid concentrations in these previous reports,[11 22 23] and indicated significant lipolysis of corn oil triglycerides. The suggestion that lipolytic activity has a role in plasma CCK and gall bladder responses to fatty nutrients is further supported by impaired responses to fatty meals in the absence or during inhibition of intraluminal lipase activity.[21 25 26]

Micellar solubilisation of fatty acids and monoglycerides may be another factor which accounted for the stimulating properties of the predigested corn oil, because in dogs bile had to be included in a predigested fatty meal in order to evoke pancreatic enzyme secretion.[11] Micelles are quantitatively the most important mechanism for delivery of lipolytic products to the aqueous barrier of the epithelial and endocrine cells of the intestinal mucosa.[27] When the only CCK producing cells are located deeply in the elongated crypts of the intestinal mucosa of coeliac patients, this transport and subsequent delivery of stimulating substances to the CCK cells may be crucial. Because the degree of steatorrhoea in coeliac patients correlated best with decreases in intraluminal bile acid concentrations and micellar lipid content, rather than lipase activity, it is even suggested that bile acids may well be the more important factor when a fatty meal is turned into a stimulus in these patients.[28] Further work will be required to delineate more precisely the role for each of these factors in the release of CCK.

In contrast to coeliac patients with a flat jejunal mucosa, in coeliac patients with a virtually normal mucosa after gluten withdrawal and in normal subjects intraduodenal instillation of undigested corn oil has a profound stimulatory effect on plasma CCK release and gall bladder contraction.[6 7] In these subjects the presence of villi in the intestinal mucosa that contain CCK producing cells makes intraluminal conditions that stimulate CCK less critical on one hand, and on the other an increased basal or early postprandial release of bile and pancreatic juice may improve digestion and solubilisation of fatty nutrients.

The results of the present study may have implications for the treatment of coeliac patients with a poor clinical and histological response to gluten withdrawal from the diet. As has been suggested previously, feeding of bile acids and pancreatic enzymes might contribute to a better digestion and absorption of fatty nutrients and thereby improve steatorrhoea and nutritional status.[28]

In conclusion, the results of the present study point to an important role of fat digestion in intestinal stimulation of plasma CCK release and gall bladder contraction in patients with coeliac disease.

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