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Liver and biliary

Effects of a pharmacological dose of cholecystokinin on bile acid kinetics and biliary cholesterol saturation in man

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SUMMARY In order to study the mechanisms influencing bile acid pool size and cholesterol saturation index of fasting gall bladder bile, eight obese volunteers were placed on a low calorie diet for six weeks, and given intramuscular injections of a pharmacological dose of cholecystokinin octapeptide (CCK-OP, 5 μg) at mealtimes for half that period (alternating order). During CCK-OP administration, postprandial emptying of the gall bladder (mean±SEM) increased from 58±11% to 82±5% ($p<0.005$), and small intestinal transit time decreased from 205±27 to 178±26 minutes (NS). Bile acid pool size decreased from 4·6±0·3 to 3·1±0·3 mmol ($p<0.001$), while fractional turnover rate for chenodeoxycholic acid increased from 0·23±0·02 to 0·36±0·03 per day ($p<0.005$), suggesting an increase in recycling frequency of the pool. Synthesis rate was unchanged (0·43±0·08 *vs* 0·44±0·07 mmol/day), suggesting a new steady state. The cholesterol saturation index of fasting gall bladder bile increased in all subjects from 1·3±0·1 to 1·6±0·1 ($p<0.005$). Fasting gall bladder volume was reduced from 29±4 to 20±7 ml ($p<0.01$). Fractional turnover rate on the two regimens correlated with gall bladder emptying ($n=16$, $r=0.61$, $p<0.01$), but not with small intestinal transit time ($r=0.07$, NS). Bile acid pool size correlated with fractional turnover rate ($r=-0.73$, $p<0.005$) and with cholesterol saturation index ($r=-0.56$, $p<0.025$). These findings suggest that CCK influences bile acid kinetics and cholesterol saturation index of fasting gall bladder bile in man; and that these effects of CCK are mainly mediated *via* alterations in gall bladder emptying rather than through alterations in small intestinal transit rate.

Cholesterol gall stone disease is frequently associated with a reduced bile acid pool size and an increased cholesterol saturation index of fasting gall bladder bile; but the physiological mechanisms controlling bile acid pool size and saturation index of fasting gall bladder bile in man are not clearly understood. In the steady state situation, bile acid pool size depends on both synthesis rate and fractional turnover rate. Low-Beer and Pomare have reported an inverse relationship between the size and fractional turnover rate of the bile acid pool in man.[1] Fractional turnover rate is itself dependent on absorption efficiency and on the recycling frequency of the bile acid pool. Northfield and Hofmann[2] have observed an inverse relationship between the size and recycling frequency of the bile acid pool in healthy subjects and in cholesterol gall stone patients, with normal bile acid synthesis and absorption efficiency. They have suggested that bile acid pool size may be mainly determined by its recycling frequency.[2]

The recycling frequency of the bile acid pool is itself likely to be enhanced by increased gall bladder emptying and/or by rapid small intestinal transit rate, as these are the two slow phases in the enterohepatic circulation of bile acids. Hepner[3] reduced gall bladder contraction in human subjects by using a 95% carbohydrate diet, and found a slight increase in the size of the primary bile acid pools, mainly because of a reduction in fractional turnover rate. Duane[4] has shown that artificially induced alterations in small intestinal transit rate influence bile acid pool size, mainly by altering the synthesis rates of the primary bile acids. Duane and Hanson[5] have also determined both gall bladder emptying rate and small intestinal transit time in 11 normal

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volunteers, and have shown that they both correlated with bile acid pool size in the steady state situation. Gall bladder emptying and small intestinal motility are both stimulated by cholecystokinin (CCK).[6–10] The C-terminal octapeptide of CCK (CCK-OP) has similar actions on the gall bladder[11] and on small intestinal transit.[12]

In the hope of throwing more light on these interrelationships, we have determined the effect of CCK-OP on gall bladder emptying and on small intestinal transit time in a group of eight obese volunteers on a controlled low caloric intake. We then related these effects to changes in bile acid pool size, synthesis rate, and fractional turnover rate in an attempt to assess the main regulatory mechanisms involved in bile acid kinetics in man. In order to determine whether CCK-OP administration provides a model mimicking the pathophysiology of cholesterol gall stone formation in man, we also related these effects to saturation index of fasting gall bladder bile.

Methods

SUBJECTS

EXPERIMENTAL DESIGN

Eight obese subjects were studied as inpatients for a period of six weeks on a low calorie diet. The age, weight, and other personal details of the subjects are in Table 1. Ultrasound examination in all eight subjects revealed that three had gall stones. All subjects gave written informed consent before participating in the study, and all the studies were approved by the local hospital ethical committee.

During half the six week period, subjects had an intramuscular injection of 5 μg CCK-OP (Squibb) 10 minutes after the start of each meal. During the other three weeks they did not receive these injections. These two regimens (CCK regimen and control regimen) were given in alternating order. During both of these three week regimens, measurements were carried out during the third week in order to allow two weeks for the subjects to reach a steady state situation with regard to bile acid kinetics. All measurements were carried out after a 12 hour fast.

The diet consisted of three equal meals of 200 calories each per day given at regular times (0800, 1200, and 1800 hours). These contained constant proportions of protein, fat, and carbohydrate (20%, 40%, and 40% respectively).

GALL BLADDER VOLUME AND EMPTYING

Gall bladder volume was determined by ultrasonography (Unirad EDP 1000 static scanner).[13]

For measurement of gall bladder emptying, the subjects had an intravenous injection of 1 mCi of ^{99m}Tc HIDA (Technetium labelled diethyl phenylcarbamomethyl iminodiacetate; The Radiochemical Centre, Amersham, Bucks, England). Ninety minutes later, Tc HIDA radioactivity over the gall bladder area was determined using a collimated gamma camera computer system with area of interest facility (Technicare, Sigma 410S/MCS-560). A 200 calorie meal with or without CCK-OP was then taken by the subject and 30 minutes later a second gall bladder radioactivity scan was carried out. Thirty minutes after the stimulus was found to be the optimum time for measuring gall bladder emptying from our previous studies.[14] We validated the measurement of gall bladder emptying by gamma camera in response to a 200 calorie meal alone and with CCK-OP in five normal subjects before this study, and found that CCK-OP when given with the meal caused a higher percentage gall bladder emptying in comparison with the meal alone in all five subjects. Gall bladder emptying was expressed as the per cent radioactivity remaining over the gall bladder area at 30 minutes.

SMALL INTESTINAL TRANSIT TIME

A modified hydrogen breath test using a sensitive semiconductor system was used, thus allowing the

Table 1 *Details about subjects studied*

Subject	*Sex*	*Age (yr)*	*Height (cm)*	*Weight (kg)*	*% IBW**	*Gall stones*
1	F	60	159	115	182	+
2	F	52	170	120	169	–
3	F	46	165	140	215	–
4	F	51	168	109	158	+
5	F	57	155	89	148	–
6	M	33	185	117	141	–
7	M	52	175	89	115	–
8	F	62	147	89	155	+
Mean±SEM		52±3	166±4	109±7	160±11	

*% IBW indicates percentage of ideal body weight for that height and weight.

use of a solid test meal[15] as a more physiological stimulus than the conventional lactulose.[16] The technique used was similar to that of Read and colleagues.[15] Briefly, it involves measurement of the time interval between oral administration of a solid meal containing baked beans as a source of a non-absorbable carbohydrate (raffinose), and detection of a significant rise in hydrogen in breath samples analysed at 10 minute intervals by the hydrogen sensitive semiconductor system. The rise in hydrogen concentration is caused by fermentation of the carbohydrate by colonic bacteria, and therefore indicates the arrival of the meal in the caecum. We found that[17] the hydrogen breath test for small intestinal transit gave reproduceable results (coefficient of variation 8%), these results correlated well with measurements made using abdominal scanning of a gamma labelled isotope incorporated in the solid phase of the meal in eight subjects ($r=0{\cdot}99$, $p<0{\cdot}001$).

BILE ACID KINETICS

An intravenous injection of 5 μCi of ^{14}C-chenodeoxycholic acid (The Radiochemical Centre, Amersham, Bucks, England) was given immediately before the last meal of the day. On the next morning, and on the subsequent three mornings, a fasting gall bladder bile sample was obtained by nasoduodenal intubation, using a double lumen polyvinyl chloride tube, and gall bladder contraction induced by intravenous infusion of 115 units CCK (Pancreozymin, Boots Ltd.) in 100 ml saline over 20 minutes. An additional sample of bile was obtained on the morning of the day on which the patients received the intravenous injection of ^{14}C chenodeoxycholic acid on the second of the two regimens, in order to check that there was no significant residual radioactivity present from the injection given during the first regimen.

Bile samples were analysed for bile acid,[18] phospholipid[19] and cholesterol content.[20] Bile acid composition was determined by enzyme assay after separation by thin layer chromatography (with chloroform/ethanol/acetic acid/water 12:8:4:1 by volume as solvent system). The specific activity of the ^{14}C chenodeoxycholic acid spot after thin layer chromatographic separation was determined by combination of enzyme assay and liquid scintillation counting. The bile acid pool size, synthesis rate, and fractional turnover rate for chenodeoxycholic acid were determined according to the method of Lindstedt.[21] Cholesterol saturation index of gall bladder bile was determined according to the criteria of Hegardt and Dam[22] and Holzbach *et al*[23] using the polynomial equation of Thomas and Hofmann.[24]

STATISTICAL ANALYSIS AND COMPARISONS

The mean±SEM was calculated for each of the above variables. The effect of CCK-OP was compared in each subject using paired Student's *t* test, and paired Wilcoxon's test when data were not normally distributed. The coefficient of linear correlation was used to relate changes in gall bladder emptying and small intestinal transit time to changes in bile acid kinetics and in cholesterol saturation index.

Results

WEIGHT

The mean weight reduction in all subjects was 12±0·5 kg over the six week period, and this was divided equally between the two regimens (5·9±0·7 kg weight loss on diet alone, and 6·1±0·7 kg on diet plus CCK-OP injections).

GALL BLADDER EMPTYING AND SMALL INTESTINAL TRANSIT TIME (Table 2)

Percentage gall bladder emptying (mean±SEM) was 58±11% on diet alone, and increased to 82±5%

Table 2 *Effect of CCK-OP on gall bladder emptying and small intestinal transit time*

Subject	*Gall bladder emptying (%)*		*Small intestinal transit (min)*	
	Control	*CCK-OP*	*Control*	*CCK-OP*
1	78	80	190	80
2	21	54	200	130
3	21	69	200	200
4	16	85	170	320
5	81	95	180	120
6	82	95	240	200
7	81	91	240	190
8	84	87	220	180
Mean±SEM	58±(11·3)	82±(4·7)	205±(27)	178±(26)
Significance	$p<0{\cdot}005$		NS	

during CCK-OP ($p<0.005$). The subjects comprised two groups regarding gall bladder emptying. Three subjects had a low percentage emptying on the meal alone, and a considerable increase on the CCK-OP regimen; the other five had a marked emptying on the meal alone, and were therefore relatively less affected by CCK-OP. These two populations did not differ according to sex ratio or incidence of gall stones (Table 2).

The small intestinal transit time tended to be more rapid on CCK-OP (178±26 min) than on the control regimen (205±27 min) but this difference did not reach statistical significance. There was no relationship between the gall bladder emptying and small intestinal transit time before and after CCK ($n=16$, $r=0.11$, NS), indicating that these two variables are independent.

BILE ACID KINETICS (Tables 3 and 4)

In the first three patients, the radioactivity of ^{14}C chenodeoxycholic acid in bile was assessed on the day before the second injection of the radioisotope. In all three there was less than 1% radioactivity remaining from the initial dose. Total bile acid pool size on the CCK-OP regimen decreased from 4·6±0·3 mmol to 3·1±0·3 mmol ($p<0.001$). The fractional turnover rate for chenodeoxycholic acid increased from 0·23±0·02 to 0·36±0·03 ($p<0.005$). Synthesis rate, on the other hand, was unchanged (0·43±0·08 mmol/day *vs* 0·44±0·07 mmol/day).

The greatest alteration during the CCK-OP regimen was in the pool size for the two primary bile acids. The pool size for chenodeoxycholic acid fell from 1·76±0·17 to 1·19±1·13 mmol ($p<0.05$) and that for cholic acid from 1·66±0·07 to 1·00±0·11 mmol ($p<0.05$). By contrast, the decrease in pool size for deoxycholic acid was only from 1·15±0·06 to 0·89±0·11 mmol, (NS). The relative fraction of deoxycholic in the pool was increased from 25±5% to 27±4% (NS). Individual values for the fraction of deoxycholate, measured during both the CCK-OP regimen and the control regimen, correlated with saturation index during the corresponding regimen ($r=0.55$, $p<0.05$).

Table 3 *Effect of CCK-OP on bile acid kinetics*

	Total bile acid pool size (mmol)		*Fractional turnover rate (Chenodeoxycholic acid)*		*Synthesis rate (μmol/d) (Chenodeoxycholic acid)*	
Subject	*Control*	*CCK-OP*	*Control*	*CCK-OP*	*Control*	*CCK-OP*
1	4·4	3·5	0·19	0·29	319	418
2	4·3	3·2	0·14	0·23	220	232
3	4·2	3·7	0·24	0·41	394	533
4	4·0	1·7	0·21	0·38	210	213
5	4·5	2·9	0·25	0·38	428	441
6	4·8	3·4	0·20	0·32	348	358
7	4·9	2·4	0·29	0·43	595	447
8	5·4	3·8	0·33	0·46	888	860
Mean±SEM	4·6±(0·33)	3·1±(0·34)	0·23±(0·02)	0·36±(0·03)	425±80	438±70
Significance	$p<0.001$		$p<0.005$		NS	

Table 4 *Effect of CCK-OP on the size of total and indivdual bile acid pools (mmol)*

	Total pool		*Chenodeoxycholic acid*		*Cholic acid*		*Deoxycholic acid*	
Subject	*Control*	*CCK*	*Control*	*CCK*	*Control*	*CCK*	*Control*	*CCK*
1	4·4	3·5	1·68	1·44	1·57	1·13	1·15	0·92
2	4·3	3·2	1·57	1·01	1·69	0·85	1·05	1·34
3	4·2	3·7	1·64	1·30	1·43	1·04	1·13	1·37
4	4·0	1·7	1·0	0·56	1·56	0·69	1·44	0·45
5	4·5	2·9	1·71	1·16	1·63	0·97	1·16	0·78
6	4·8	3·4	1·74	1·12	1·81	1·62	1·24	0·66
7	4·9	2·4	2·05	1·04	2·02	0·69	0·83	0·68
8	5·4	3·8	2·69	1·87	1·55	0·99	1·16	0·94
Mean±SEM	4·6±0·33	3·1±0·37	1·76±0·17	1·19±0·13	1·66±0·07	1·0±0·11	1·15±0·06	0·89±0·11
Significance	$p<0.001$		$p<0.05$		$p<0.05$		NS	

Table 5 *Effect of CCK-OP on saturation index and gall bladder volume*

Subject	*Saturation index* *Control*	*CCK-OP*	*Gall bladder volume (ml)* *Control*	*CCK-OP*
1	0·82	1·30	17	11
2	1·62	1·97	21	19
3	1·40	1·85	23	21
4	1·82	2·07	19	15
5	1·46	1·58	44	32
6	1·32	1·51	38	29
7	0·84	1·05	36	14
8	1·21	1·50	36	18
Mean±SEM	1·31±(0·12)	1·60±(0·12)	29·4±(3·6)	19·9±(7·3)
Significance	$p<0·005$		$p<0·01$	

GALL BLADDER VOLUME AND CHOLESTEROL SATURATION INDEX (Table 5)

Saturation index of fasting gall bladder bile increased in all subjects during CCK-OP from 1·31±0·12 to 1·60±0·12 ($p<0·005$). Gall bladder volume decreased in all subjects during CCK-OP from 29·4±3·6 to 19·9±7·3 ml, ($p<0·01$).

INTER-RELATIONSHIPS

A. Steady state relationships

There was a significant correlation between gall bladder emptying and fractional turnover rate when all data points both before and during CCK were included ($n=16$, $r=0·61$, $p<0·01$). By contrast, there was no correlation between small intestinal transit time and fractional turnover rate ($n=16$, $r=0·07$, NS).

There was a significant correlation overall between fractional turnover rate and bile acid pool size before and after CCK ($n=16$, $r=-0·73$, $p<0·005$); and between bile acid pool size and saturation index of fasting gall bladder bile before and after CCK ($n=16$, $r=0·56$, $p<0·025$). There was also a significant correlation between fasting gall bladder volume and bile acid pool size ($n=16$, $r=0·59$, $p<0·02$).

B. Dynamic relationships

There was a significant correlation between the change in gall bladder emptying caused by CCK and the change in fractional turnover rate due to CCK ($n=8$, $r=0·72$, $p<0·025$). By contrast there was no significant correlation between change in small intestinal transit time and change in fractional turnover rate ($n=8$, $r=-0·58$, NS).

There was no significant correlation between change in fractional turnover rate and change in bile acid pool size ($n=8$, $r=0·36$, NS), but there was a significant correlation between change in bile acid pool size and change in saturation index of fasting gall bladder bile ($n=8$, $r=0·63$, $p<0·05$).

Discussion

We chose obese subjects for the study because there was a clinical indication for admitting them to hospital for six weeks, and because the low calorie diet they received was likely to give a low background level of postprandial CCK release for comparison with the pharmacological dose of CCK given during the study. Three of the obese subjects had gall stones on ultrasound examination, and the other five did not. Although the subjects separated into two groups according to initial gall bladder emptying, this separation bore no relationship to the presence or absence of gall stones. Three subjects had a small degree of gall bladder emptying in response to a standard meal (16–21%), whereas five subjects had marked emptying (78–84%). One gall stone patient had a small degree of emptying (16%) and two had a marked degree (78% and 84%). There was no difference in the response of other measurements to CCK injections between the gall stone and control subjects, and all subjects acted as their own controls.

In hospital we were able to achieve a constant eating pattern for the subjects by giving them equicaloric meals containing 20% protein, 40% fat and 40 carbohydrate. In order to further minimise biological and personal variations, the subjects acted as their own controls. The order in which the subjects had the low caloric diet either alone or with CCK-OP injections was alternated. The first subject had the control regimen followed by CCK-OP, the second subject had them in the opposite order and so on alternately. The reason for alternating the regimens was the fact that weight reduction alone is known to influence biliary cholesterol saturation index in obese subjects.[25] The subjects were kept for three weeks on each regimen (equivalent to four to five biological half lives of the bile acid pool), in

order to allow time for a new steady state to be reached, as was later confirmed by the finding that synthesis rate was the same on both regimens; and also to ensure that negligible radioactivity was carried over from the first set of measurements. We checked the specific activity of ^{14}C-chenodeoxycholic acid immediately before the second injection, and found that this was less than 1% of that obtained on the first day after the original dose of isotope. We used only ^{14}C-chenodeoxycholic acid for measuring bile acid kinetics not combined with an isotope of cholic acid, because we were already using another isotope (^{99m}Tc HIDA) for gall bladder emptying and it was thought not ethically justifiable to use a third isotope.

Intramuscular administration of CCK-OP at mealtimes led as expected to an augmentation of gall bladder emptying in all the subjects from a mean value of 58% to a mean value of 82% emptying ($p<0{\cdot}005$). The effect of CCK-OP in causing gall bladder emptying in the fasting state has been reported both in man[11] and in animals.[26] Small intestinal transit time tended to be shorter on the CCK-OP regimen in the present study than on diet alone. This trend narrowly failed to reach statistical significance, because one subject (no 4 in Table 2) had a much longer transit time on the CCK-OP regimen than on the control regimen. Although CCK-OP is known to increase intestinal motility, we know of no previous data defining the effect of CCK or CCK-OP on small intestinal transit time using the hydrogen breath test in man.

An increase in recycling frequency of the bile acid pool provides the most likely explanation for the increased fractional turnover rate and decreased size of the bile acid pool, because an increase in gall bladder emptying or an increase in small intestinal transit rate are both likely to enhance recycling frequency. This possibility is supported by the finding of an inverse relationship between the size and recycling frequency of the bile acid pool in a group of healthy controls and gall stone subjects.[2] A similar relationship was found in the present study between fractional turnover rate and bile acid pool size. In this study, an alteration in gall bladder emptying is likely to have been the more important factor causing the increased fractional turnover rate because there was a significant increase in gall bladder emptying, whereas the increase in small intestinal transit rate was of smaller magnitude and did not reach statistical significance with the small number of subjects studied (mainly because one subject had a large change in the opposite direction). Furthermore, fractional turnover rate was significantly correlated with gall bladder emptying, but not with small intestinal transit time in the steady state situation; and the increase in fractional turnover rate due to CCK-OP was significantly correlated with the increase in gall bladder emptying but not with the decrease in small intestinal transit time. In two subjects (nos 1 and 8 in Table 2), there was little change in gall bladder emptying during CCK-OP, but in these individuals there was a decrease in small intestinal transit time which may have contributed to the alterations in bile acid kinetics that were observed.

The reduction in gall bladder volume during the CCK regimen appears to reflect the reduction in bile acid pool size, as there was a significant correlation between the two measurements. We have previously shown that there is a significant correlation in healthy non-obese subjects without gall stones between fasting gall bladder volume and bile acid pool size,[27] and have also shown that artificial depletion of the bile acid pool significantly reduces gall bladder volume.[28]

Administration of CCK-OP resulted in a significant increase in cholesterol saturation index of fasting gall bladder bile, and we attribute this to the reduction in bile acid pool size because we have previously shown that artificial depletion of the bile acid pool results in an increase in cholesterol saturation index. These changes were associated with a significant reduction in bile acid and phospholipid mass within the gall bladder, without a significant change in cholesterol mass. In the present study, there was a significant correlation between bile acid pool size and cholesterol saturation index during the CCK-OP and control regimens.

Our overall interpretation of the effects of CCK-OP in the present study is that it caused an increase in gall bladder emptying, thus increasing recycling frequency of the bile acid pool. Initially, this would result in an increased bile acid return to the liver, thus temporarily reducing synthesis rate as a result of the well known negative feedback mechanism.[29] By the time the patients were restudied, a new steady state had been reached with a small pool recycling more frequently, thus leading to a normal bile acid return to the liver and a reversion of synthesis rate to the initial value. An increase in cholesterol saturation index of fasting gall bladder bile resulted from the reduced bile acid pool size. If this interpretation is correct, a hormonal effect (CCK-OP) has caused a motility effect (increased gall bladder emptying), which in turn has influenced a homeostatic mechanism (that controlling bile acid pool size), and thus led to a physicochemical effect (an increase in cholesterol saturation of gall bladder bile). Although the present study has shown that a pharmacological dose of CCK-OP can cause these effects, it does not answer the question of whether

physiological blood concentrations of CCK can cause them; but recent evidence indicates that physiological CCK concentrations do stimulate gall bladder contraction in a dose-related manner.[30] That these mechanisms may be involved in health and disease is suggested by the finding that cholesterol gall stone patients, who are known to have a reduced bile acid pool size[31] and increased saturation index of fasting gall bladder bile,[32] have also been reported to have increased gall bladder emptying,[14] and increased gall bladder sensitivity to infused CCK[33]; and by the finding that patients with coeliac disease, who have an abnormally large bile acid pool,[34] have decreased serum CCK concentrations,[35] sluggish gall bladder empyting,[35] and slow small intestinal transit rate[36] by comparison with healthy controls.

Preliminary results of this study were reported to the Medical Research Society in 1982, and published in abstract form (*Clin Sci* 1982; **63:** 57).

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