hepatitis than in those with HBsAg-negative hepatocellular carcinoma (55% *vs* 16%, respectively; $p < 0.001$); the prevalence of anti-HBc was similar in the two groups (9% *vs* 16%, respectively; NS).

Discussion

Sequential development of cirrhosis and hepatocellular carcinoma has been observed in patients with non-A, non-B hepatitis acquired after blood transfusion.[15-19] Such a direct link between blood-borne non-A, non-B hepatitis and hepatocellular carcinoma has not been found in Italy, but indirect evidence correlates community-acquired non-A, non-B hepatitis with many instances of hepatocellular carcinoma in patients with cirrhosis of unknown cause.[8-10] Our observation of a high prevalence of anti-HCV in patients with chronic community-acquired hepatitis and hepatocellular carcinoma unequivocally links this form of hepatitis to community-acquired HCV infection, and may indicate pathogenic mechanisms.

HCV is now thought to be the major cause of blood-borne non-A, non-B hepatitis.[11-13] Even though the biological meaning of anti-HCV is unclear, the presence of such antibodies indicates ongoing viral infection, and anti-HCV has been detected in the blood of infected donors and patients with hepatitis who transmitted non-A, non-B hepatitis to chimpanzees.[12] The background infection rate of HCV among the general population may be as high as that of HBV: in the US, 2 of 412 (0·5%) symptom-free blood donors with normal liver function and who were anti-HBc-negative had circulating anti-HCV.[12] In Italy, this prevalence may be 4–5 times higher:[20] this high seropositivity for HCV in the general population could account for the high prevalence of anti-HCV found in patients with community-acquired chronic hepatitis. This high prevalence of HCV-related chronic liver disease might also indicate the cause of hepatocellular carcinoma in patients with no other known predisposing factor: 65% of our patients with hepatocellular carcinoma had circulating anti-HCV. Furthermore, the co-occurrence of both anti-HCV and anti-HBc in patients with hepatocellular carcinoma was higher than that in patients with non-A, non-B chronic hepatitis. Indirect evidence for this association between HBV and HCV had already been reported by Okuda et al,[21] who retrospectively analysed data for 113 patients with hepatocellular carcinoma who did not have a history of alcohol abuse. Although co-occurrence of anti-HBc and anti-HCV might simply reflect a similar distribution of HBV and HCV,[22] we found that anti-HBc and anti-HCV occur together more frequently in patients with hepatocellular carcinoma than in patients with non-A, non-B chronic liver disease—and previous evidence indicates that patients infected with more than one virus are likely to develop more serious liver disease than those infected with a single agent.[23]

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SATURATION OF FAT AND CHOLECYSTOKININ RELEASE: IMPLICATIONS FOR PANCREATIC CARCINOGENESIS

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Summary In a study to determine the effect of saturation of fats on their ability to stimulate cholecystokinin (CCK) release six normal volunteers ate five test meals containing different fats with intervals of 1 week. Plasma CCK levels were measured by a specific radioimmunoassay and the gallbladder volume was calculated from ultrasound measurements. The sodium salt of the monounsaturated fatty acid oleic acid (3·5 g) produced a significantly greater integrated CCK response than that of the saturated fatty acid stearic acid (mean [SEM] 103 [41] *vs* 8 [41] pmol.l^{-1}.min). The gallbladder contracted to 42 (3)% of its initial volume after oleate but remained at 89 (8)% of its initial volume after stearate. Integrated CCK responses to dietary triglycerides (30 g) also differed significantly according to the degree of saturation—277 (58) pmol.l^{-1}.min after corn oil (predominantly diunsaturated), 143 (14) pmol.l^{-1}.min after olive oil (predominantly monounsaturated), and 44 (12) pmol.l^{-1}.min after suet

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(predominantly saturated). The finding that unsaturated fats are stronger stimulants of CCK release than saturated fats may explain the promotion of pancreatic carcinogenesis in rats by unsaturated but not saturated fats and may support the role of CCK in this effect.

Introduction

DURING the past 50 years the incidence of pancreatic cancer has doubled in the United Kingdom and trebled in the United States.[1] The cause of such a rapid change is almost certainly environmental and possibly dietary. Several epidemiological studies have shown a relation between pancreatic cancer and a diet high in fat, both saturated and unsaturated.[2,3]

In both human beings and rats oral fat stimulates a rise in plasma cholecystokinin (CCK) immunoreactivity.[4,5] In rats exogenous CCK promotes pancreatic growth and neoplasia[6] and pancreatic carcinogenesis is also promoted by a diet high in unsaturated fat.[7] Surprisingly, saturated fats do not have this effect.[7]

It is important to find out whether similar effects occur in man, particularly in view of the dietary trend towards unsaturated fat.[8] We have therefore compared the CCK-releasing effects of saturated and unsaturated fat in man.

Subjects and Methods

The study protocol was approved by the hospital ethics committee. Six healthy volunteers aged 20–41 years, one woman and five men, took part in the study after giving informed consent. They consumed each of five meals with different fat contents (see table) after fasting overnight and with at least a week between meals. Two meals consisted of 3·5 g pure fatty acid in 10 ml 'Hycal' (Beecham) with 150 ml water to drink. The other three meals contained 30 g triglyceride incorporated into 25 g instant mashed potato and 50 ml water with 150 ml water to drink. Fats of similar chain length but varying degrees of saturation were selected.[9]

Plasma CCK peptides were extracted from venous blood samples with C18 'SepPak' cartridges (Waters, Harrow).[10] Plasma CCK was measured by a specific radioimmunoassay based on antiserum A2, raised by immunising a rabbit with natural porcine CCK 33. Antiserum A2 ($1/6 \times 10^4$) was incubated with standard CCK 8 or plasma samples plus CCK 8 tracer labelled with iodine-125 (1000 cpm, Amersham) in 0·05 mol/l sodium phosphate buffer pH 7·4 at 4°C for 3 days. Free and bound tracer were separated by the addition of 6% (weight/volume) charcoal ('Norit PN5', BDH, Poole). The concentrations of pure peptides which produced half maximum inhibition of binding of tracer to A2 were 2·0 pmol/l for CCK 8, 2·4 pmol/l for CCK 33, and 2·2 nmol/l for gastrin 17. The coefficients of variation within and between assays were 8·2% and 12·8%, respectively.

Images of the gallbladder were obtained by real-time ultrasonography before and after the fatty acid meals by means of an 'Acuson 128' scanner. Gallbladder volume was calculated by the sum of cylinders method.[11]

Other regulatory peptides were measured in venous blood by standard methods with antisera NT58 for neurotensin[12] and Y21 for peptide YY.[13]

COMPOSITION OF FATS IN TEST MEALS

	Percentage of			
—	Palmitate (16:0)	Stearate (18:0)	Oleate (18:1)	Linoleate (18:2)
Pure fatty acids				
Stearic acid	0	100	0	0
Oleic acid	0	0	100	0
Triglycerides				
Corn oil	13	2	29	48
Olive oil	11	2	69	11
Beef suet	22	23	28	1

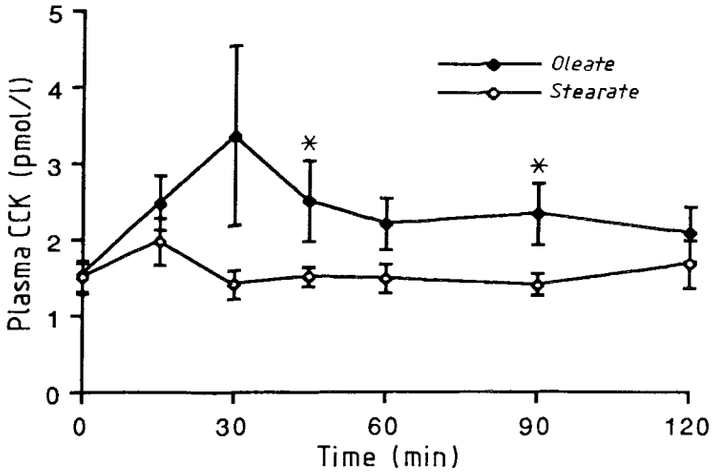

Fig 1—Mean plasma CCK concentrations before and after 3·5 g oleate or 3·5 g stearate.

Vertical bars = SEM. *p < 0·05.

Results are expressed as mean and standard error of the mean (SEM). Plasma peptide concentrations are expressed as 2 h integrated responses calculated as the area under the curve minus the baseline. Statistical analysis was by Student's paired *t* test.

Results

The mean baseline plasma CCK concentration was 1·81 (0·09) pmol/l. The meal containing sodium oleate (monounsaturated) produced an integrated plasma CCK response of 103 (41) pmol.l^{-1}.min, compared with a response of only 8 (14) pmol.l^{-1}.min to the meal containing the saturated fatty acid salt sodium stearate (fig 1; $p < 0·02$). The contraction of the gallbladder 30 min after ingestion of the test meal was significantly greater for oleate than for stearate (fig 2; $p < 0·002$).

Corn oil (predominantly diunsaturated) was the greatest stimulant of CCK release (fig 3); the integrated response (277 [58] pmol.l^{-1}.min) was significantly greater than the responses to olive oil (predominantly monounsaturated; 143 [14] pmol.l^{-1}.min, $p < 0·05$) or to suet (predominantly saturated; 44 [12] pmol.l^{-1}.min; $p < 0·005$). The difference in response between olive oil and suet was also significant ($p < 0·005$).

Neither oleate nor stearate stimulated release of neurotensin (integrated responses −65 [263] and −171 [400] pmol.l^{-1}.min, respectively) or peptide YY (−75 [186]

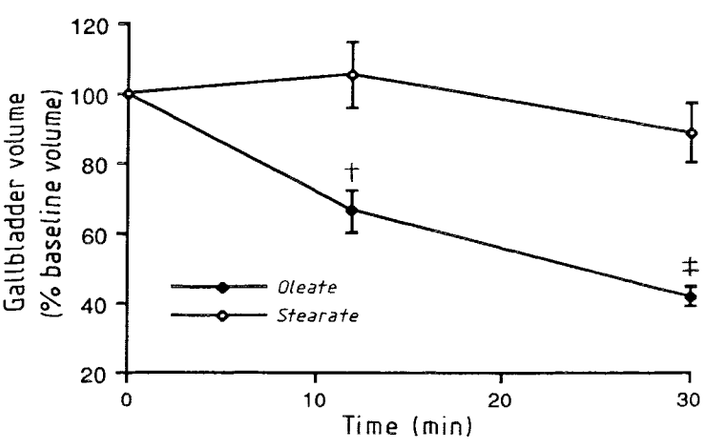

Fig 2—Mean gallbladder volume (% baseline volume) before and after 3·5 g oleate or stearate.

† < 0·005; ‡p < 0·002.

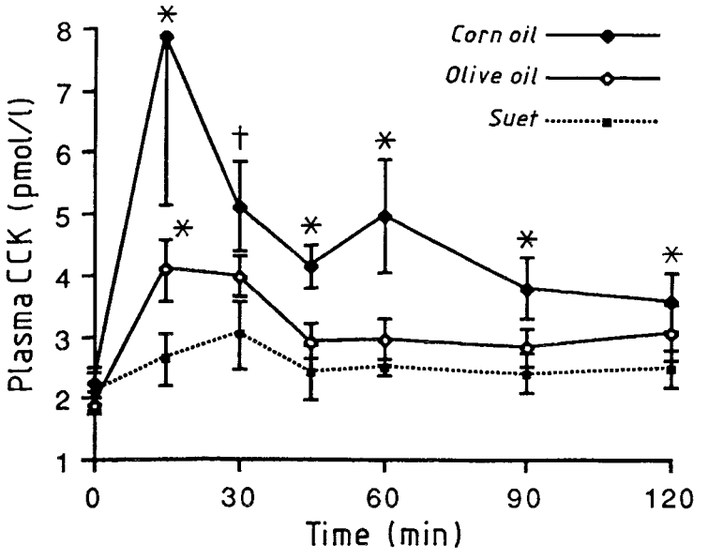

Fig 3—Mean plasma CCK concentrations before and after 30 g corn oil, olive oil, or beef suet.

*p < 0·05, †p < 0·01 compared with values after suet.

and 25 [216] pmol.l^{-1}.min, respectively). The triglycerides did stimulate neurotensin release but there was no significant difference in the plasma integrated responses (corn oil 1283 [375] pmol.l^{-1}.min, olive oil 978 [236] pmol.l^{-1}.min, suet 832 [512] pmol.l^{-1}.min). There was a similar pattern for peptide YY release (corn oil 381 [162] pmol.l^{-1}.min, olive oil 238 [198] pmol.l^{-1}.min, suet 289 [181] pmol.l^{-1}.min).

Discussion

We have shown for the first time that unsaturated fats are substantially more potent stimulants of CCK release than saturated fats—both pure fatty acids and triglycerides. The CCK response is greater the more unsaturated the fat. Release of neurotensin and peptide YY was not differentially affected by fats of different saturation.

Our gallbladder contraction data show that the greater CCK release stimulated by unsaturated fat is associated with appropriate physiological effects. This knowledge could be used to therapeutic advantage in disorders such as bulimia nervosa[14] and coeliac disease,[15] in which low CCK release is thought to contribute to the symptoms by reducing satiety and pancreatic enzyme secretion, respectively. More importantly, differences in CCK release induced by dietary fat may be an aetiological factor in the pathogenesis of pancreatic cancer.

Several epidemiological studies have found a positive correlation between human pancreatic cancer and total fat intake.[2,3] However, differences in saturation of fat were not considered. Fats stimulate pancreatic growth and promote pancreatic carcinogenesis in rats. This fact has been shown in studies with chemical carcinogens[7] and from observations that the use of corn oil as a vehicle for gavage resulted in pancreatic hyperplasia.[16] However, both the growth-promoting and cancer-promoting effects are restricted to unsaturated fats.[7]

CCK has growth-promoting effects on the rat pancreas, and long-term administration leads to hypertrophy and hyperplasia in the gland.[6] Our finding that unsaturated fats are stronger stimulants of CCK release than saturated fats suggests that the cancer-promoting effect of unsaturated fats in rats[7] may be mediated by CCK. A similar growth-promoting effect of raw soya on the rat pancreas is inhibited by a specific CCK antagonist, confirming that it is mediated by CCK.[17] These findings raise the possibility that the increasing frequency of human pancreatic cancer over the past 50 years[1] might be related to increased CCK release in response to greater consumption of fat, with a rise in the proportion of unsaturated fats.[8]

Several questions must be answered before any link between fat, CCK, and pancreatic cancer can be established. By necessity, studies on pancreatic cancer have been conducted in animals, usually rats. It has not been established that CCK promotes pancreatic cancer in man. Further studies with CCK antagonists[17] are required to confirm that the effect of unsaturated fat on the pancreas in rats is mediated by CCK.

The epidemiological association between blood cholesterol, dietary saturated fat, and coronary heart disease has led to advice in developed countries that unsaturated rather than saturated fats should be eaten. The effects of this advice on other disorders, including pancreatic cancer, have not been adequately investigated. Most studies of diet in coronary artery disease have failed to show a fall in mortality.[18,19] Our results here emphasise the need to determine the long-term effects of increased ingestion of unsaturated fats before the public are advised to make this dietary change.

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