

Effect of intrajejunal acidity on lipid digestion and aqueous solubilisation of bile acids and lipids in health, using a new simple method of lipase inactivation

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SUMMARY We have investigated whether acid-mediated bile acid precipitation, pancreatic enzyme inactivation, and fatty acid partitioning occur in health when intraluminal pH falls below 5. In order to assess lipolysis and aqueous solubilisation of lipid, we first developed a new technique for inactivating lipase in jejunal aspirate (acid inactivation), and showed it to be more effective and simpler than the established technique (heat inactivation). We then studied 14 healthy subjects, aspirating jejunal content for three hours after a liquid meal, and pooling according to pH. Eighteen per cent of the total aspirate was collected at pH<5 compared with 56% at pH>6 ($p<0.01$). Forty eight per cent of the bile acids were precipitated at pH<5 compared with 18% at pH>6 ($p<0.01$), leading to a reduction in aqueous phase bile acid concentration at low pH (2.1 mmol/l at pH<5 vs 5.8 mmol/l at pH>6, $p<0.01$). Lipase activity was reduced at low pH (133 IU/l at pH<5 vs 182 IU/l at pH>6, $p<0.01$), leading to reduced lipolysis at low pH (14% at pH<5 vs 32% at pH>6, $p<0.01$). Aqueous phase lipid concentration was reduced at low pH (3.5 mmol/l at pH<5 vs 12.5 mmol/l at pH>6, $p<0.01$). This reduction was less dependent on bile acid precipitation than on lipase inactivation and fatty acid partitioning. We conclude that intraluminal acidity influences aqueous solubilisation of bile acids and lipid in health.

Maintenance of intraluminal pH near neutrality during digestion of food is important for several reasons. The *in vitro* activity of the three major pancreatic enzymes – lipase, trypsin, and amylase – decreases markedly with pH.^{1,2} Glycine conjugated bile acids precipitate out of aqueous solution below pH 5, whereas taurine conjugated bile acids precipitate only below pH 2.³ The partitioning of fatty acids into the aqueous phase *in vitro* also decreases markedly as the pH is reduced below 6.⁴

The *in vivo* effects of jejunal hyperacidity on fat digestion have been shown in a patient with Zollinger-Ellison syndrome.⁵ Greater precipitation of glycine conjugated bile acid occurred in jejunal samples of low pH, while lipase was 50% inactivated below pH 5. Bile acid precipitation and lipase inactivation together led to reduced solubilisation of

lipid in the aqueous phase.

Early studies of intraluminal digestion in health showed that the pH of postprandial samples aspirated from the duodenum was always above 5⁶⁻⁸ suggesting that bile acid precipitation and lipase inactivation would not occur. pH probe studies in health, however, have shown that duodenal pH falls below 5 for about one third of a 100 minute postprandial period.⁹ The physiological effect of this has not been studied previously. The purpose of the current study was to determine whether acid mediated bile acid precipitation and lipase inactivation occur in health, and if so, whether they affect lipid solubilisation. The study involved the comparison of pancreatic enzyme concentration, bile acid precipitation, lipolysis and lipid solubilisation between samples aspirated at different pHs. To facilitate this comparison, samples were pooled according to their pH in three pH pools: pH<5, pH 5-6, and pH>6.

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In order to investigate lipid digestion and solubilisation in intestinal contents it is essential to arrest lipolysis in samples immediately after aspiration, as lipolysis proceeds rapidly at room temperature.^{10 11} Most investigators have used the method of heat inactivation at 70°C first described by Hofmann and Borgström.¹¹ Although this method completely inactivates lipase, it has been found to lead to a 46% increase in fatty acid concentration as lipolysis is accelerated during the time taken for the sample to reach 70°C.¹² This would lead to an artefactual increase in lipolysis and aqueous phase lipid concentration. The method becomes cumbersome when large samples of varying volume are aspirated, as numerous small aliquots of equal volume collected at one to two minute intervals must be heated separately in order to achieve a rapid and constant rate of heat transfer to the samples.¹¹ We therefore decided to develop a new technique of lipase inactivation exploiting the fact that lipase is irreversibly inactivated at pH 3⁵ while bile acid precipitation⁵ and fatty acid protonation^{4 13} are reversible. In this method, aspirates destined for lipid analyses are adjusted to pH 3 immediately after collection by addition of acid, and restored to their original pH immediately before analysis by addition of an equivalent amount of alkali. Aspirates destined for enzyme and bile acid analyses are not treated. In this report we describe the validation of this method of inactivating lipase and its application to the study of healthy subjects.

Methods

SUBJECTS

The subjects were adults in good general health attending a gastroenterology clinic with non-specific abdominal pain. Routine tests including upper-intestinal endoscopy, oral cholecystography, barium studies, and a Lundh test of pancreatic function were performed where appropriate and were normal. These subjects were considered suitable for the study as they were not suffering from any disease known to affect the variables under investigation. All subjects gave permission for the study according to the declaration of Helsinki. The protocol was approved by the local hospital ethical committee on 4th May 1977. The experiment involved a radiation exposure of 100 mrad to the whole body and 30 mrad to the bone marrow. Permission was therefore refused to study asymptomatic medical staff or students, as exposure additional to that encountered in the course of their duties could not be justified. Women were studied within 10 days of menstruation. The new technique of lipase inactivation was validated in two experiments involving 14 subjects.

The technique was then applied to 14 different subjects studied in the main investigation (11 men and three women; average age 32 years, average weight 68 kg).

EXPERIMENTAL METHODS: VALIDATION OF TECHNIQUE FOR LIPASE INACTIVATION

This experiment was performed to compare the effects of the conventional heat inactivation technique and of our acid inactivation technique on lipolysis and bile acid precipitation. Six subjects were involved in the first part of the experiment, and eight in the second.

Intubation and meal

After an overnight fast, a double lumen weighted tube (internal diameter 4 mm) was passed through the nose so that the tip lay approximately 6 inches beyond the duodenojejunal flexure under fluoroscopic control; its position was confirmed at the end of the test. The resting contents of the duodenum were aspirated and discarded, and the patient drank a Lundh type liquid test meal containing 40 g dextrose, 15 g skimmed milk powder (Casilan) and 2.5 g polyethylene glycol 4000 (PEG), dissolved in 230 ml water and thoroughly mixed with 18 g corn oil.

Sampling (Fig. 1)

Whenever the flow of fluid from the tube was sufficiently rapid that at least 20 ml could be collected within a few seconds, this sample was used for validation of the technique. After mixing, a 1 ml aliquot (A) was extracted within 20 seconds for analysis of fatty acid concentration. This sample was considered to be the most accurate estimate of the intraluminal concentration before artefactual lipolysis. A 7 ml aliquot (B) in an Erlenmeyer flask was swirled by hand in a 70°C water bath for one minute and then left in the water bath for a further nine minutes exactly as described by Hofmann and Borgström.¹¹ An 11 ml aliquot (C) was immediately adjusted to pH<3 using 1N-HCl and kept at 0°C in crushed ice. A 1 ml aliquot (D) was stored untreated after immediate measurement of its pH. All aspirate not used for this purpose was pooled and analysed for trypsin and lipase as a test of pancreatic function.

Laboratory procedures (Fig. 1)

On arrival in the laboratory three hours later, 1 ml of B was extracted for fatty acid and 5 ml ultracentrifuged overnight at 100 000 g, 37°C; the aqueous phase was removed in its entirety, mixed and analysed for bile acid concentration. Aliquot C was restored to its original pH (as recorded for D) by gradual addition of 1N-NaOH during thorough

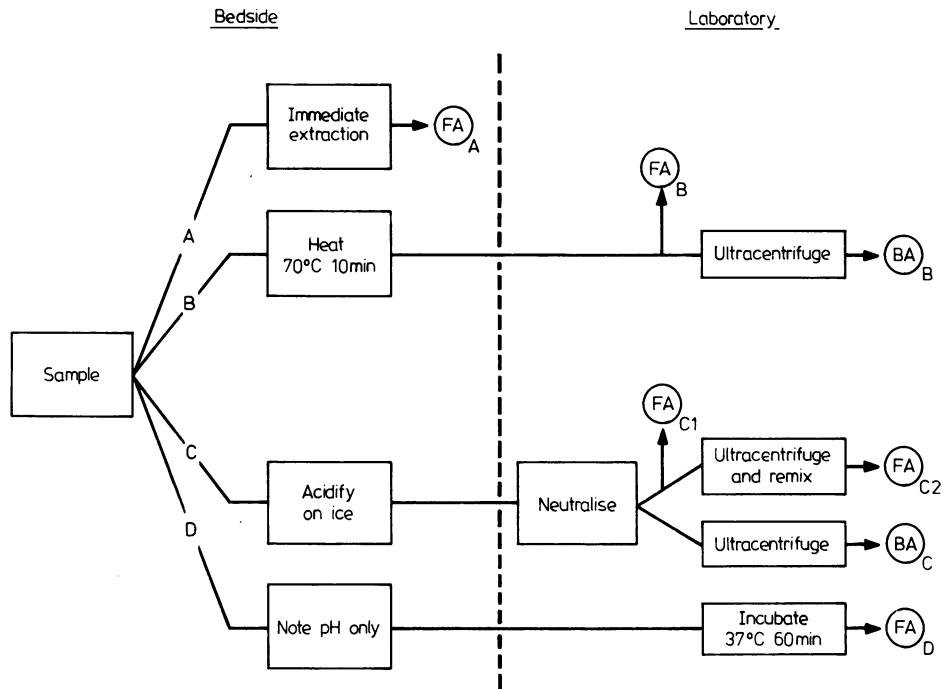


Fig. 1 Processing of samples of duodenal aspirate in validation experiment: acid inactivation of lipase.

mixing and its pH rechecked after final mixing. One millilitre was extracted for fatty acid (C₁) and two 5 ml aliquots in separate tubes were loaded into an ultracentrifuge precooled to 0°C. Immediately before starting the motor, the temperature control was reset to 37°C. The aliquots were centrifuged at 100 000 g overnight, the temperature rising to 37°C over the first two to three hours. The aqueous phase was removed from one tube, mixed, and analysed for bile acid concentration. The other tube was mixed thoroughly to recombine oil phase, aqueous phase and precipitate, and extracted for fatty acid (C₂). D was incubated at 37°C for one hour and then extracted for fatty acid.

A separate experiment was then performed to check that cooling samples did not irreversibly alter the partitioning of lipids after rewarming. In eight different subjects, postprandial chyme was collected at 37°C and stirred at this temperature for three hours, in order to ensure that lipolysis had reach equilibrium. A 10 ml sample of this fluid was then acidified as in C, and divided in two. Half was cooled to 0°C until being restored to its original pH and ultracentrifuged overnight at 100 000 g, 37°C. The other half was held at 37°C, then stored and ultracentrifuged in the same machine. Aqueous phases were separated and analysed for fatty acid,

and for total saponifiable lipid. All fatty acid estimations were performed together on the day following collection (using 0.01 M tetra-n-butyl ammonium hydroxide). The results were corrected for dilution by the volumes of acid and alkali added in initial treatment.

EXPERIMENTAL METHODS: INVESTIGATION OF HEALTHY SUBJECTS

Intubation and meal were as described above.

Sampling (Fig. 2)

As much jejunal content as possible was collected continuously by siphonage and syringe aspiration into ice cooled 10 ml measuring cylinders. The second lumen of the tube was used as an air vent. Each time sufficient sample was obtained (c. 3 ml), its pH was measured immediately with a glass electrode. It was then mixed and divided in two. Half was assigned to the appropriate one of three pH pools (pH<5, pH 5-6, pH>6) and the pools maintained at a pH below 3 by the addition, after each sample, of about 0.1 ml 1N-HCl per 3 ml of sample. The other half was assigned to one of three similar pH pools which were not acidified. All pools were kept at 0°C in crushed ice. At the end of each hour, the pH of each untreated pool was noted, and

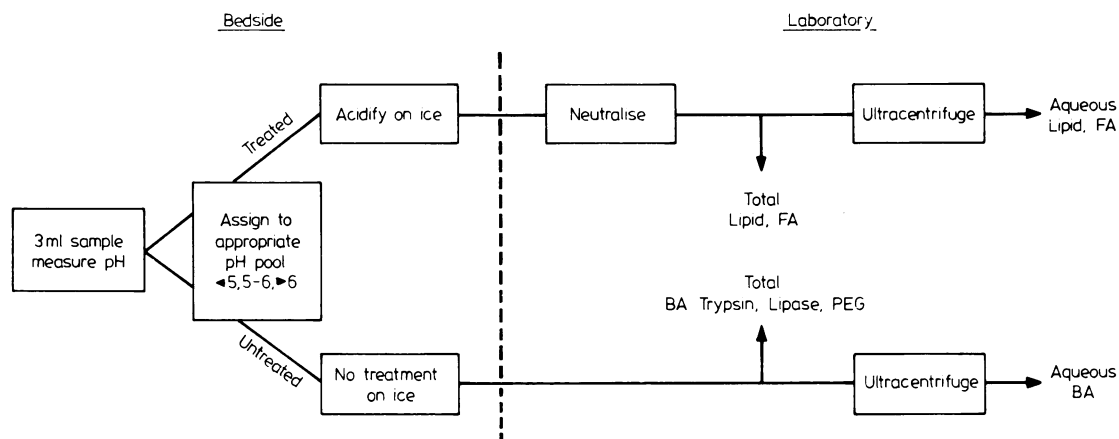


Fig. 2 Processing of samples of duodenal aspirate in main experiment: investigation of healthy subjects.

new pools started. The test was concluded three hours after the administration of the meal. There could thus be a maximum of three treated and three untreated pools for each of three hours – 18 in total.

Laboratory procedures (Fig. 2)

Treated pools: on the afternoon of collection, each treated pool was restored to its original pH (the pH recorded for the corresponding untreated pool) with 1N-NaOH. An aliquot was extracted immediately in ethanol-toluene for titration of total fatty acid concentration.¹⁴ A 5 ml aliquot was loaded into an ultracentrifuge precooled to 0°C, and centrifuged overnight after resetting the temperature control to 37°C as described above. The aqueous phase was removed in its entirety, mixed, and an aliquot extracted and titrated for aqueous phase fatty acid concentration.¹⁴ Total and aqueous phase saponifiable lipids were extracted and titrated similarly after alkaline hydrolysis in 5% ethanolic KOH. All titrations were performed together on the day after collection and the results corrected for dilution as described above. Both fatty acid and total lipid concentrations are expressed in terms of mmol of fatty acid titrated. **Untreated pools:** on the afternoon of collection, 5 ml aliquots of each pool were ultracentrifuged similarly. The aqueous phase was removed in its entirety, mixed and stored with the uncentrifuged pools at -20°C. Total and aqueous phase bile acid concentrations were later determined enzymatically.¹⁵ Trypsin,¹⁶ lipase,¹⁷ and PEG¹⁸ concentrations were measured in the uncentrifuged pools.

MATHEMATICAL ANALYSIS

For each subject comparison between the pH<5,

pH 5-6, and pH>6 pools was made after mathematically pooling the separate hourly pools for each pH. The three hourly pools were compared after similarly pooling the separate pH pools for each hour. The 'overall mean' was calculated by mathematically pooling all the separate pools. This method takes into account the widely differing volumes of each pool; individual pools of small volume contribute proportionately little to the pH means, hourly means and overall mean. The pHs recorded for each pool were converted to hydrogen-ion concentration in order to calculate the pH of the hourly pools.

Derivations were calculated as follows, before pooling:

$$\text{Lipolysis (\%)} = (\text{total fatty acid/total lipid}) \times 100$$

$$\text{Aqueous phase 'glyceride' (mmol/l)} = \text{aqueous phase saponifiable lipid} - \text{aqueous phase fatty acid}$$

$$\text{Bile acid precipitation (\%)} = (\text{total} - \text{aqueous phase bile acid/total bile acid}) \times 100$$

$$\text{Fatty acid solubilisation in aqueous phase (\%)} = (\text{aqueous phase fatty acid/total fatty acid}) \times 100.$$

The calculation of bile acid precipitation and fatty acid solubilisation assumes the oil phase and precipitate to have negligible volume: where this was not so, values less than 0% or greater than 100% may therefore occur. The formula for pooling was as follows:

$$\text{Pooled value} = \Sigma (CV)/\Sigma V.$$

where for each pool to be pooled,

C = concentration or derivation, and V = volume.

STATISTICAL ANALYSIS

pH pools

The hypothesis that values for the pH<5 and pH>6 pools were equal was tested using the Wilcoxon signed rank test.¹⁹ The pH 5–6 pool was not examined statistically. Similarity between all three pH pools was tested using the Friedmann two way analysis of variance by ranks.¹⁹

Hourly pools

We had no hypothesis to predict the behaviour of the hourly pools. In order to avoid the error of 'data snooping',¹⁹ pH values in the three hourly pools were compared using the Friedmann two way analysis of variance by ranks; application of Wilcoxon's critical range method for multiple comparisons¹⁹ showed a significant difference in pH between first and second hour. We then examined differences between the first and second hour for other values using the Wilcoxon signed rank test.

Results

VALIDATION OF TECHNIQUE

Seventeen samples were obtained from the six subjects. Conventional heat inactivation (B) caused a 12±4% (mean ± SEM) rise in fatty acid over the aliquot extracted immediately (A), while acid inactivation (C₁) caused only a 3±2% rise, indicating that acid inactivation caused significantly less artefactual lipolysis than heat activation (*p*<0.01). Fatty acid in C₂ was 2±4% lower than in C₁ (NS) indicating that lipolysis did not resume after neutralisation and ultracentrifugation overnight at 37°C. Aqueous phase bile acid was 5±4% higher after heat inactivation than acid inactivation (NS)

suggesting that our technique gives results comparable with those of the conventional method. Fatty acid in D was increased by at least 20% in all cases, confirming that there was capacity for further lipolysis if permitted.

In the second experiment eight samples were obtained from eight subjects. The aqueous phase fatty acid concentrations in the samples cooled to 0°C were similar to those in the samples kept at 37°C (2.60±0.80 vs 2.69±0.63 mmol/l, NS), as were the aqueous phase total lipid concentrations (3.97±1.10 mM/l vs 4.11±0.87, NS). This suggests that the cooling of samples to 0°C did not irreversibly alter the distribution of fatty acid and lipid between aqueous and oil phases.

INVESTIGATION OF HEALTHY SUBJECTS
COMPARISON OF PH POOLS (Table 1)*pH*

Eighteen per cent of the total PEG aspirated was recovered at the critical pH<5, significantly less than at pH>6. The proportions of lipid aspirated at each pH paralleled those of PEG. This suggests that the pH effects discussed below apply equally to the lipid and aqueous portions of the meal. Polyethylene glycol and total lipid concentrations were similar at each pH suggesting that gastric contents and pancreato-biliary fluid had mixed completely before entering the jejunum, and that the differences in concentration of other substances at each pH were not caused by differences in dilution.

Pancreatic enzymes

The overall mean trypsin for each patient (range 12–23 IU/ml) was within the accepted normal range.²⁰ (A normal range for lipase using this

Table 1 Healthy subjects – comparison of pH pools

	pH<5	pH 5–6	pH>6	Significance* pH<5 vs pH>6
PEG recovery (% of total recovered)	17.7±4.8	26.6±4.3	55.7±7.3	<i>p</i> <0.01
Lipid recovery (% of total recovered)	18.6±5.3	24.5±4.2	57.0±6.9	<i>p</i> <0.01
PEG concentration (g/l)	2.5±0.4	2.9±0.3	2.7±0.2	NS†
Trypsin (IU/l)	14.7±1.5	17.2±0.8	19.1±1.5	<i>p</i> <0.05
Lipase (U/l)	133±9	165±7	182±11	<i>p</i> <0.01
Total lipid (mmol/l)	36.4±9.2	34.5±6.4	34.6±3.6	NS†
Total fatty acid (mmol/l)	2.6±0.6	4.8±0.6	10.6±1.9	<i>p</i> <0.01
Lipolysis (%)	13.5±3.9	19.6±2.9	32.4±3.2	<i>p</i> <0.01
Total bile acid (mmol/l)	4.1±0.4	4.6±0.4	6.9±0.7	<i>p</i> <0.01
Aqueous phase bile acid (mmol/l)	2.1±0.3	3.5±0.4	5.8±0.8	<i>p</i> <0.01
Bile acid precipitation (%)	47.9±3.5	22.7±4.5	17.6±3.7	<i>p</i> <0.01
Aqueous phase lipid (mmol/l)	3.5±0.4	7.2±1.4	12.5±3.1	<i>p</i> <0.01
Aqueous phase fatty acid (mmol/l)	0.4±0.2	1.9±0.3	7.1±1.8	<i>p</i> <0.01
Proportion fatty acid in aqueous phase (%)	13.2±6.0	41.0±7.5	65.0±10.4	<i>p</i> <0.01
Aqueous phase glycerides (mmol/l)	3.1±0.4	5.3±1.4	5.4±1.4	NS†

* Wilcoxon test. NS = Not significant. † No difference between any pools (Friedmann test).

technique has not been previously described in the literature). The activities of trypsin and lipase were significantly lower at pH<5 than pH>6, but still within the normal range. The pH 5-6 concentration was intermediate.

Lipid digestion

Total lipid concentration was similar at each pH, but total fatty acid concentration and lipolysis were significantly lower at pH<5 than at pH>6, pH 5-6 being intermediate.

Bile acids

Total bile acid concentration was slightly but significantly lower at pH<5 than at pH>6. Half of the available bile acid was precipitated at pH<5, more than twice that at pH>6. This was accompanied by a marked reduction in aqueous phase bile acid concentration at pH<5, the concentration at pH 5-6 again being intermediate.

Lipid solubilisation

Both aqueous phase lipid and fatty acid concentrations were markedly lower at pH<5 than at pH>6. At pH<5, only 13% of the available fatty acid was solubilised, one fifth of the value at pH>6.

COMPARISON OF HOURLY POOLS (Table 2)

Meal recovery

An average of 30% of the PEG in the meal was recovered during the three hour study. Only 17% of the total PEG and 25% of the total lipid aspirated were recovered during the third hour, while only very small samples could be obtained at the end of the third hour. In a pilot study of six healthy subjects

in which jejunal aspirate after an identical test meal was pooled at 15 minute intervals (unpublished), PEG concentration fell almost to zero by the end of the third hour (Fig. 3). These findings indicate that the whole of the meal was sampled during the three-hour study, and suggest that our extrapolation from PEG recovery to recovery of the aqueous portion of the meal is valid.

Bile acids

pH decreased significantly from the first to the second hour. This was accompanied by a significant increase in bile acid precipitation and corresponding decrease in aqueous phase bile acid concentration.

Enzymes

Concentrations of trypsin and lipase remained constant over the three hours, normal concentrations being maintained throughout the test.

Lipid digestion and solubilisation

Lipolysis and fatty acid concentration also remained constant from hour to hour. The proportion of fatty acid in the aqueous phase fell from the first to the second hour, corresponding to the increase in bile acid precipitation.

Discussion

VALIDATION OF THE METHODS

Our validation experiments have shown that addition of acid to intestinal aspirate, and subsequent neutralisation before analysis, arrests all lipolysis without altering bile acid distribution. Cooling of the acidified samples was essential for

Table 2 Healthy subjects - comparison of hourly pools

	1st hour	2nd hour	3rd hour	Significance* 1st hour vs 2nd hour
PEG recovery (%)	48.7±6.7	34.1±4.9	17.2±6.1	p<0.05‡
Lipid recovery (%)	32.8±3.6	42.2±4.3	25.0±4.1	NS†
PEG concentration (g/l)	3.4±0.4	2.6±0.3	1.3±0.3	p<0.01‡
pH	6.2±0.2	5.3±0.2	5.4±0.2	p<0.05
Total bile acid (mmol/l)	7.1±1.1	4.9±0.5	5.9±0.6	p<0.05
Aqueous phase bile acid (mmol/l)	6.1±1.1	3.5±0.4	4.2±0.6	p<0.01
Bile acid precipitation (%)	17.6±4.8	29.5±3.0	31.4±4.5	p<0.05
Trypsin (IU/l)	16.8±2.0	19.6±1.2	20.6±2.6	NS†
Lipase (U/l)	171±13	172±10	175±11	NS†
Total lipid (mmol/l)	32.1±5.1	44.9±5.9	33.8±4.9	NS†
Total fatty acid (mmol/l)	7.5±3.2	10.1±3.4	10.9±1.3	NS
Lipolysis (%)	24.9±4.2	26.0±4.2	31.1±2.5	NS†
Aqueous phase lipid (mmol/l)	13.7±5.3	8.7±3.6	8.0±1.9	NS†
Aqueous phase fatty acid (mmol/l)	7.2±3.5	4.7±2.8	4.5±0.9	NS†
Proportion fatty acid in aqueous phase (%)	74.7±9.8	36.5±8.5	42.7±6.4	p<0.01

* Wilcoxon test. NS = Not significant. † No difference between any pools (Friedmann test).

‡ 1st vs 3rd hour also significant (Friedmann + Wilcoxon critical range test).

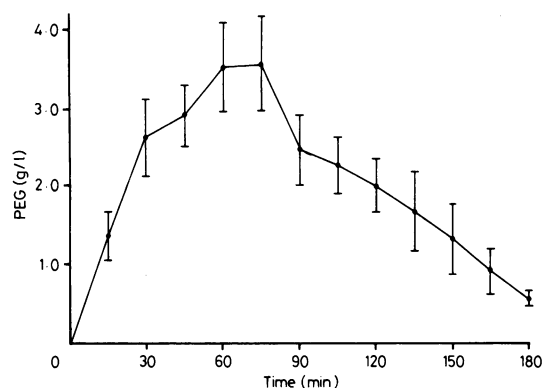


Fig. 3 Relationship between postprandial PEG concentration in duodenal aspirate and time in pilot study of six healthy subjects: PEG concentrations fell almost to zero by the end of the third hour.

this inactivation, but did not irreversibly alter the ultimate distribution of lipid between aqueous and oil phases at 37°C. This technique of acid inactivation is less cumbersome than heat inactivation and reduces the artefactual lipolysis inherent in heat inactivation.¹² Several investigators have simplified the original method of heat inactivation by pooling samples over 30⁵ 21 or 45²² minutes before heating but this allows lipolysis to proceed at room temperature. In another study, aspirate was collected continuously into a vessel maintained at 70°C for at least 20 minutes,²³ but this simplification may lead to increased phospholipolysis, as phospholipase activity is maintained at this temperature.²⁴ In contrast, our method appears to arrest phospholipolysis, as the negligible rise in fatty acid concentration reflects both lipolysis and phospholipolysis; phospholipolysis is known to be inhibited at pH 3.²⁴ Our results for lipolysis and lipid solubilisation in the upper jejunum are slightly lower than those found by others in health,^{5 23 25 26} probably reflecting the improved efficacy of our method for lipase inactivation.

The use of a transyloric tube to aspirate postprandial jejunal content does not alter gastric acid secretion or the rate of gastric emptying.²⁷ The use of a liquid meal rather than a more physiological solid meal is convenient; solid meals elicit greater acid secretion,²⁸ and would therefore tend to increase the effects we have observed. The use of PEG as a non-absorbable marker of dilution for both the aqueous and lipid phases of intestinal content has been validated^{6 8 29} but criticised.³⁰ In our study, the proportions of PEG at each pH paralleled those of lipid, suggesting that it was an adequate lipid marker for comparing pH pools. The

total recovery of PEG over the three hour study period (mean 30%) compares favourably with that obtained by other investigators.⁶

APPLICATION TO HEALTHY SUBJECTS

Several investigators have reported postprandial jejunal pH falling intermittently below 5 in health, but have not quantitated the amount of meal so affected. A study using a pH probe in the descending duodenum in healthy subjects reported pHs below 5 for 30% of a 100 minute postprandial period.⁹ Our findings of only 18% of the meal aspirated at pH<5 probably reflects our more distal aspiration site, and also the fact that a minimum requirement of 3 ml of aspirate to measure pH would tend to obscure extremes of pH in either direction.

The gradient of pancreatic enzyme activity with pH probably reflects intraluminal inactivation, as differences in dilution have been excluded by the similar PEG concentrations at each pH. This interpretation is supported by the decreased lipolysis at low pH, showing that the decrease in lipase activity exerted a functional effect on lipolysis.

We have shown a marked gradient of bile acid precipitation with pH. The figure for pH 5-6 is close to that for pH>6, as would be expected from the *in vitro* observation that the critical pHs of precipitation for glycine conjugates are slightly below 5.³ Bile acid precipitation resulted in a marked gradient of aqueous phase bile acid concentration with pH; at pH<5 only half the available bile acid was in aqueous solution and able to contribute to micelle formation. The finding of 18% bile acid precipitation at pH>6, well above the critical pH, suggests that binding of bile acid to food residue also reduces aqueous bile acid concentration. We have demonstrated a similar degree of binding to the protein content of the Lundh meal *in vitro*.³¹ Glycine conjugated bile acid remaining in solution at pH<5 is likely to be reabsorbed in the jejunum by passive non-ionic diffusion³² further reducing intraluminal bile acid concentration. The reduction in total bile acid concentration at pH<5 is unlikely to be caused by greater dilution by gastric acid, as PEG and total lipid concentrations did not vary with pH. It is more likely to be because of precipitation of bile acid onto pieces of debris not recovered up the tube. The figure of 48% bile acid precipitation at pH<5 may therefore be an underestimate.

Analysis of the experiment in relation to time, rather than pH, supports our findings, as the results expressed as hourly pools were calculated quite independently of the pH of individual samples. The fall in pH in the second hour from 6.2 to 5.3 was associated with a significant increase in bile acid

precipitation and fall in aqueous phase bile acid concentration, leading to a significant reduction in the proportion of fatty acid solubilised. We did not detect inactivation of trypsin or lipase accompanying this slight fall in pH, and lipolysis remained constant.

Three pH dependent factors may reduce the digestion and solubilisation of non-polar dietary triglyceride at pH<5: (i) lipase inactivation reducing lipolysis; (ii) bile acid precipitation reducing solubilisation of the polar products of lipolysis (fatty acid and monoglyceride) into the aqueous phase; (iii) partitioning of protonated fatty acid into the oil phase at pH<6, even in the presence of an adequate bile acid concentration.⁴ This possibility was not evaluated in studies showing a correlation between pH and lipid solubilisation in Zollinger-Ellison syndrome⁵ and pancreatic insufficiency.³³ Our results can be used to separate all three pH effects. The marked pH gradients we found for aqueous phase lipid (total saponifiable) and fatty acid concentrations reflect the combined effect of all three factors. The gradient for the proportion of total fatty acid in the aqueous phase eliminates the effect of lipase inactivation and reflects only bile acid precipitation and fatty acid partitioning. To separate the contribution of these two factors *in vivo* is problematic since reduction of pH below 6.0 affects both simultaneously. Analysis of covariance, by removing variation between individuals, reveals a linear relationship between the proportion of fatty acid in the aqueous phase and bile acid precipitation (Fig. 4: $F = 27.7$, $p < 0.01$). The intercept of the

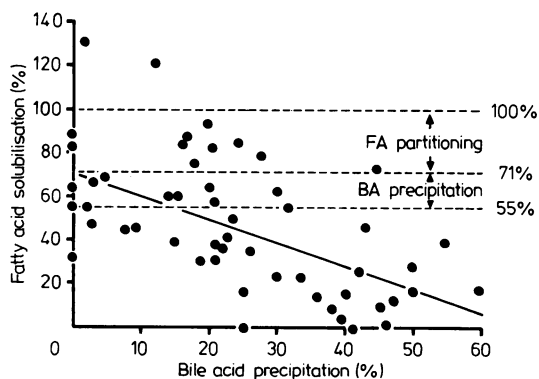


Fig. 4 Relationship between fatty acid solubilisation (adjusted for variation between individuals) and bile acid precipitation ($F=27.7$, $p<0.01$). This demonstrates relative contribution of fatty acid partitioning and bile acid precipitation in limiting fatty acid solubilisation (overall mean fatty acid solubilisation observed = $55 \pm 7\%$, fatty acid solubilisation at zero bile acid precipitation = $71 \pm 10\%$).

regression line indicates that in the absence of bile acid precipitation $71.2 \pm 10\%$ of the fatty acid partitions into the aqueous phase. In the presence of bile acid precipitation the overall mean proportion of fatty acid actually in the aqueous phase was 54.7% . Therefore, of the 45.3% of fatty acid not solubilised 28.8% ($=100-71.2$) could be attributed to partitioning, and only 16.5% ($=71.2-54.7$) to bile acid precipitation. A similar process of elimination can be applied to aqueous phase glyceride, the other component of total lipid, which also showed a pH gradient (Table 1). As glycerides are not susceptible to pH-dependent partitioning⁴ this gradient must be attributable to bile acid precipitation or lipase inactivation. The mean fall in aqueous phase glyceride concentration from pH>6 to pH<5 (2.0 ± 1.6 mM/l) can be attributed entirely to lipase inactivation, as it is less than half the mean fall in total fatty acid concentration from pH>6 to pH<5 (6.7 ± 1.3 mM/l) and lipolysis releases two moles of fatty acid for each mole of monoglyceride. Thus the fatty acid and glyceride data both concur that aqueous solubilisation of lipid at low pH in health is limited less by bile acid precipitation than by lipase inactivation and fatty acid partitioning.

We conclude that even in health a significant proportion of a meal passing down the upper jejunum is exposed to an intraluminal pH below 5 which allows lipase inactivation, fatty acid partitioning and bile acid precipitation to interfere with solubilisation of dietary fat. Our studies were limited to the upper jejunum, but the classical studies of Borgström and colleagues⁶ indicated that the absorption of dietary fat begins in the duodenum and is completed in the proximal 100 cm of the jejunum, a conclusion supported by a recent authoritative review.³⁴ Thus we were studying that part of the small intestine of most importance for digestion, solubilisation and absorption of dietary fat. In health, there is a large reserve function for these processes further down the small intestine. These studies form a baseline for comparison with diseased states (i) where this reserve function is reduced, as in the steatorrhoea that follows surgical resection of the ileum, (ii) where bile acid secretion may be reduced so that bile acid precipitation has a critical effect on aqueous phase bile acid concentration, again as in ileectomy steatorrhoea, (iii) where intraluminal pH may fall more often as in pancreatic exocrine insufficiency.³³

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