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Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery

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Abstract

The major end products of fermentation, short chain fatty acids (acetate, propionate, butyrate) were measured in portal and peripheral venous blood after the caecal instillation of lactulose at surgery in patients undergoing elective cholecystectomy. Blood samples for short chain fatty acid measurement were taken before and at 15 minute intervals up to 60 minutes after caecal instillation of either 20 ml sterile saline or 6·7 g or 10 g lactulose. Fasting concentrations (n=28) were (μmol/l, mean (SD)); portal acetate 128·0 (70·8), propionate 34·4 (23·3), butyrate 17·6 (18·4); peripheral acetate 67·0 (23·0), propionate 3·7 (1·2), butyrate traces only. After lactulose there was a rapid rise in portal short chain fatty acids with peak concentrations being reached in 15 to 45 minutes. Mean peak concentrations (μmol/l (SD)) after 10 g lactulose were acetate 240·9 (142·2), propionate 39·0 (17·8) and butyrate 26·9 (17·6). The changes in acetate concentrations seen in portal blood were reflected in peripheral blood acetate measurements. In contrast with portal blood, only small amounts of propionate and traces of butyrate were found in peripheral blood.

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The principal end products of fermentation are the short chain fatty acids acetate, propionate and butyrate and the gases carbon dioxide, methane and hydrogen.[1] In ruminants and other herbivores short chain fatty acids contribute substantially to basal energy requirements,[2] while in man there have been few studies investigating the fate of these acids.[3]

There are many lines of evidence now which suggest that fermentation occurs in the human colon and that absorption of the resultant short chain fatty acids is rapid and contributes significantly to portal and peripheral blood short chain fatty acid concentrations.[1 3] The major short chain fatty acid, acetate, is also an important metabolic fuel and in species such as the rat, falling portal blood levels trigger active liver production of acetate.[4 5]

Studies of the fermentation process in man and the fate of its short chain fatty acid end products have been limited by the inaccessibility of both the large bowel and the portal venous system. We have therefore attempted to establish in the present study not only that fermentation occurs in the human caecum but that short chain fatty acids are rapidly absorbed into the portal venous circulation and beyond by measuring short chain fatty acids in portal and peripheral blood after the caecal instillation of the fermentable carbohydrate lactulose in patients undergoing elective cholecystectomy.

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Methods

PATIENTS

Twenty eight patients (23 female, five male) in normal health, average age 47·6 (15·7) years (range 23–74) undergoing elective cholecystectomy for gall stones were randomly allocated to three groups. In two groups lactulose was injected into the caecum at surgery and in the third group sterile saline. Patients were maintained on polysaccharide free food for 24 hours before operation and none had taken antibiotics in the three months before surgery.

SAMPLING

Caecal injections and portal vein samplings were done by a senior surgeon and no patient suffered any ill effects as a result of these procedures. No operation was prolonged to allow additional portal blood samples to be taken. Peripheral venous blood samples were taken for up to two hours. All samples were collected into heparinised tubes, mixed and the plasma separated by centrifugation for 10 minutes at 1600 *g*.

Short chain fatty acid measurement

Short chain fatty acid concentrations were measured in duplicate plasma samples by a modification of the method described by Pomare *et al*[3] which utilises a vacuum transfer technique followed by gas chromatography. Plasma was first deproteinised with an equal volume of 0·36 mol/l perchloric acid, centrifuged at 1600 g for 15 minutes and the supernatant retained and stored at −20°C until analysed. At a later date an aliquot of the thawed supernatant (equivalent to 1 ml plasma) was mixed with 50 μl 2·7 mmol/l isobutyric acid (internal standard) and vacuum distilled. When the distillation process was completed (60–90 minutes) the distillate was thawed at room temperature, mixed and refrozen in liquid nitrogen before being freeze dried. Immediately before gas chromatographic analysis 100 μl of 3·33 mol/l phosphoric acid was added to the dried sample and 1 μl injected onto the column.

CHROMATOGRAPHY

A Varian 3700 gas chromatograph (Varian Instrument Group, Sunnyvale, California 94089,

USA) with a flame ionisation detector was fitted with a 2 m×2 mm internal diameter glass column packed with phosphoric acid treated Tenax GC, 80–100 mesh. The columns were conditioned at 150°C for two to three days before use. The analyses were performed isothermally at 140°C. The injector and detector temperatures were 200°C and the carrier gas nitrogen at a rate of 30 ml/min. Short chain fatty acid concentrations were calculated relative to the internal standard by the peak height ratio method and all results given as the mean (1) standard deviation.

STATISTICAL ANALYSIS

Data were analysed using the Kruskal-Wallis one way analysis of variance and the Mann-Whitney U Test.[6]

ETHICS

Permission for this study was obtained from the Wellington Hospital Board Research Ethical Committee.

Results

FASTING SHORT CHAIN FATTY ACID CONCENTRATIONS

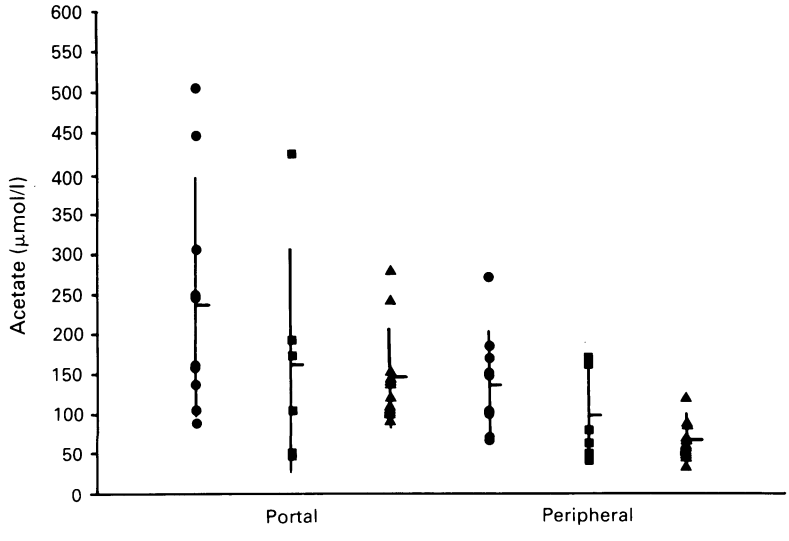
The fasting short chain fatty acid concentrations in both portal and peripheral venous plasma are shown in Table I. There was a wide inter-individual variation in fasting short chain fatty acid concentrations in portal and peripheral blood. There was also a marked difference between portal and peripheral fasting short chain fatty acid concentrations, particularly propionate (peripheral approximately 10% portal) and butyrate (virtually undetected in peripheral plasma). There were no statistically significant differences between the three study groups for fasting portal or fasting peripheral short chain fatty acid concentrations of acetate, propionate and butyrate.

TABLE I *Fasting short chain fatty acid concentrations in plasma* (μmol/l)*

	Acetate	Propionate	Butyrate
Portal	128·0 (70·8) [27·9–375·3]	34·4 (23·3) [2·6–110·9]	17·6 (18·4) [0–86·0]
Peripheral	67·0 (23·0) [21·5–113·8]	3·7 (1·2) [1·5–6·5]	–

*mean (1 SD) [range], n=28.

Individual peak portal and peripheral acetate concentrations (μmol/l) following: ● 10 g lactulose (n=10), ■ 6·7 g lactulose (n=6), ▲ 20 ml sterile saline (n=12). Horizontal line indicates mean, vertical bars indicate (1 SD).



PORTAL SHORT CHAIN FATTY ACID CONCENTRATIONS

Peak portal acetate concentrations are shown in the Figure. Ten grams lactulose produced peak portal concentrations (μmol/l, mean (SD)) of 240·9 (142·2), 39·0 (17·8), and 26·9 (17·6) respectively for acetate, propionate and butyrate; 6·7 g lactulose produced peak portal concentrations (μmol/l, mean (SD)) of 166·4 (140·7), 30·9 (18·4), and 22·4 (23·5) respectively for acetate, propionate and butyrate. This compares with peak portal concentrations (μmol/l, mean (SD)) of 144·0 (58·5), 37·6 (21·1), and 23·1 (17·0) respectively for acetate, propionate and butyrate after sterile saline. There were no statistically significant differences between peak portal short chain fatty acid concentrations after 10 g or 6·7 g lactulose or sterile saline. With regard to peak portal acetate concentrations, however, 6·7 g lactulose was associated with a 15·6% increase and 10 g lactulose with a 67·3% increase above the peak portal acetate concentrations after sterile saline. The difference between fasting portal concentrations and peak portal concentrations of acetate (mean (SD), 111·1 (116·8) μmol/l) was statistically significant after 10 g lactulose compared with sterile saline (mean (SD), 12·9 (77·1) μmol/l) ($p<0·05$).

The results of portal short chain fatty acid measurements after the caecal injection of lactulose and sterile saline are shown in Table II. Lactulose produced a rapid rise in portal acetate with peak concentrations reached at 15 to 45 minutes. The 6·7 g dose of lactulose appeared to be completely fermented in 30 to 45 minutes with portal short chain fatty acid concentrations back to or below fasting levels in all but one subject at 45 minutes. After 10 g lactulose, portal short chain fatty acid concentrations were still raised at 45 minutes in all subjects. Peak portal short chain fatty acid concentrations were measured at similar times which indicated that both doses of lactulose were rapidly fermented, however, the fermentation of the larger 10 g dose of lactulose appeared more prolonged.

TABLE II *Short chain fatty acid concentrations in portal plasma* (μmol/l)*

Time		Acetate	Propionate	Butyrate
10 g Lactulose				
Fasting	(n=10)	129·7 (92·5)	25·6 (14·4)	11·2 (7·6)
15 min	(n=9)	164·2 (142·9)	27·7 (19·0)	17·1 (17·4)
30 min	(n=9)	170·8 (91·9)	27·5 (6·4)	17·3 (12·3)
45 min	(n=6)	197·2 (150·7)	25·7 (16·4)	15·4 (12·7)
6·7 Lactulose				
Fasting	(n=6)	120·1 (47·8)	39·2 (12·6)	16·6 (8·7)
15 min	(n=6)	146·0 (141·8)	26·6 (14·8)	13·6 (11·4)
30 min	(n=5)	139·5 (78·6)	25·2 (20·7)	17·4 (17·2)
45 min	(n=4)	96·2 (69·8)	22·3 (15·1)	19·9 (26·3)
60 min	(n=3)	110·4 (82·5)	20·3 (16·7)	19·6 (21·9)
Sterile Saline				
Fasting	(n=12)	130·6 (64·8)	39·2 (31·4)	23·3 (26·1)
15 min	(n=12)	118·2 (56·6)	30·2 (18·3)	17·6 (16·2)
30 min	(n=12)	116·3 (53·5)	32·2 (21·2)	19·8 (15·7)
45 min	(n=6)	112·3 (32·6)	26·5 (20·7)	19·0 (18·0)

*Mean (1 SD)

TABLE III *Short chain fatty acid concentrations in peripheral plasma* (μmol/l)*

Time		Acetate	Propionate
10 g Lactulose			
Fasting	(n=10)	75·2 (17·4)	3·5 (1·2)
15 min	(n=9	101·5 (78·2)	3·1 (0·9)
30 min	(n=9)	113·8 (63·5)	2·9 (0·5)
45 min	(n=6)	106·0 (67·6)	3·6 (1·7)
60 min	(n=6)	129·7 (73·8)	3·4 (1·9)
120 min	(n=6)	125·6 (44·2)	3·8 (1·8)
6·7 g Lactulose			
Fasting	(n=6)	60·8 (31·6)	3·7 (1·4)
15 min	(n=6)	70·2 (47·0)	3·2 (1·4)
30 min	(n=5)	77·9 (34·4)	3·3 (1·3)
45 min	(n=5)	80·7 (54·4)	3·4 (1·6)
60 min	(n=5)	78·2 (46·8)	3·3 (1·6)
120 min	(n=6)	67·4 (48·1)	3·4 (1·6)
Sterile Saline			
Fasting	(n=12)	63·2 (22·3)	3·9 (1·1)
15 min	(n=12)	55·1 (19·2)	4·0 (1·7)
30 min	(n=12)	55·0 (14·7)	3·8 (1·5)
45 min	(n=9)	61·3 (25·5)	3·8 (1·5)
60 min	(n=8)	48·8 (16·6)	3·7 (1·9)
120 min	(n=9)	49·9 (12·0)	3·6 (1·7)

*mean (1 SD)

PERIPHERAL SHORT CHAIN FATTY ACID CONCENTRATIONS

Peak peripheral acetate concentrations are shown in the Figure. Ten grams lactulose produced peak peripheral concentrations (μmol/l, mean (SD)) of 132·4 (65·0) and 4·1 (1·6) respectively for acetate and propionate; 6·7 g lactulose produced peak peripheral concentrations (μmol/l, mean (SD)) of 94·7 (56·6), and 4·0 (1·2) respectively for acetate and propionate. This compares with peak peripheral concentrations (μmol/l, mean (SD)) of 68·3 (21·4) and 4·3 (1·8) respectively for acetate and propionate after sterile saline. No quantifiable amount of butyrate was found in any of the peripheral samples. The difference between peak peripheral acetate concentrations after 10 g lactulose and sterile saline was statistically significant ($p<0·05$), with 10 g lactulose producing a 93·8% higher peak peripheral acetate concentration than sterile saline. 6·7 g lactulose was associated with a 38·6% higher peak peripheral acetate concentration than sterile saline. The difference between fasting and peak peripheral concentrations of acetate (mean (SD), 64·0 (67·7) μmol/l) was statistically significant after 10 g lactulose compared with sterile saline (mean (SD), 7·0 (13·2) μmol/l) ($p<0·05$).

The results of peripheral short chain fatty acid measurements after caecal injection of lactulose and sterile saline are shown in Table III. The changes seen in peripheral acetate concentrations produced by lactulose (either 10 g or 6·7 g), were smaller and slightly less rapid when compared with the changes in portal acetate concentrations. The pattern of change for acetate, however, was similar in both portal and peripheral blood. There were no significant changes in propionate concentrations after either dose of lactulose or sterile saline. Peak peripheral acetate concentrations were reached more rapidly after 6·7 g lactulose but the acetate concentrations remained higher for longer after 10 g lactulose.

Discussion

In the fasting state the origins of short chain fatty acids in portal and peripheral blood are several. In portal blood short chain fatty acids are principally derived from gut fermentation of colonic residues consisting of undigested food, mucus and proteins.[4 7-18] This is in contrast with peripheral blood where acetate, the only readily measurable short chain fatty acid, is mainly endogenous in origin.[4 10 11] There is little or no endogenous release of propionate or butyrate into the peripheral circulation.[10] There have been few studies in man which have followed the fate of short chain fatty acids, the major end products of the fermentation process.[3 19 23] This is understandable given the difficulty of gaining access to both the human colon and portal vein in vivo. The present study reports the largest number of fasting portal short chain fatty acid measurements made in man. It is also the only in vivo study measuring portal short chain fatty acid concentrations after the direct instillation of fermentable carbohydrate into the human caecum. Fasting portal short chain fatty acid concentrations measured in the present study are similar to those measured by Dankert *et al*[21] in five patients also undergoing gall bladder surgery. Fasting peripheral short chain fatty acid concentrations measured in the present study are similar to those measured by Pomare *et al*[3] and Tollinger *et al*[22] but are slightly higher than those measured by Dankert *et al*[21] who used serum rather than plasma and a different technique for the measurement of short chain fatty acids. The wide interindividual variation in fasting portal and peripheral short chain fatty acid concentrations found in the present study was also found by others.[3 21 22]

In the fed state the major source of portal and peripheral short chain fatty acids is gut fermentation of dietary carbohydrate.[10 23 24] In the present study we have measured the change in portal and peripheral plasma short chain fatty acid concentrations after the caecal instillation of the disaccharide lactulose (O-β-D-galactopyranosyl-1-4-D-fructofuranose), which escapes digestion in the small intestine but is broken down in the colon.[25] Portal plasma short chain fatty acid concentrations, principally acetate, increased rapidly with peak concentrations after lactulose being reached in 15 to 45 minutes indicating rapid colonic fermentation of the lactulose and absorption of the resultant short chain fatty acids. Portal plasma acetate and propionate concentrations measured in the present study are less than those found in sudden death victims by Cummings *et al*[23] but portal plasma butyrate concentrations are comparable. The doses of lactulose given in our study, however, were small and the quantity of undigested food present in the gut of the sudden death victims was considerable (average 291 g). Peripheral plasma acetate concentrations also increased after caecal lactulose instillation but the increases were smaller and occurred more slowly than those measured in portal plasma. Peripheral plasma propionate and butyrate concentrations remained unchanged. The peripheral plasma acetate concentrations followed 10 g lactulose measured in the present study are comparable with those measured by Pomare *et al*.[3]

There were large differences between portal and peripheral short chain fatty acid concentrations, especially for propionate and butyrate,

measured in the present study. In the fasting and fed state peripheral acetate concentrations were approximately 45% lower than portal acetate concentrations and peripheral concentrations of propionate and butyrate was 87–100% lower than portal concentrations. These differences have also been shown in the fasting state by Dankert *et al*[21] and in the fed state by Cummings *et al*[23] The propionate and butyrate in portal blood would therefore appear to be virtually completely extracted by the liver[10 23 26 27] unlike acetate which is also delivered systemically to peripheral tissues where it is extensively metabolised especially in the fasting state.[3 4 10 18–20 23 28 29]

In each subject (with one exception), the pattern of change in acetate concentration measured in peripheral blood mirrored that measured in portal blood. A slight time delay in the peripheral changes was seen in some subjects. Therefore, although the magnitude of the changes in acetate concentrations is larger in portal blood, peripheral blood measurements provide a reasonable reflection of the pattern of change. The same cannot be said for propionate and butyrate as the peripheral concentrations remained unchanged despite considerable increases in portal concentrations after lactulose.

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