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Breath hydrogen excretion or plasma acetate levels during the lactulose tolerance test?

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Summary

Since both acetate and hydrogen are produced by colonic bacterial fermentation, the clinical utility of the measurement of either parameter in nine subjects for the lactulose tolerance test was tested. The fasting plasma acetate concentration (mean ± s.d., mmol/l) of 0.11 ± 0.06 increased to peak levels between 150 min (0.23 ± 0.12) and 180 min (0.23 ± 0.09), both $P < 0.01$, after ingesting 20 g lactulose. In one subject with previous gastrectomy and intestinal hurry, the peak was at 30 min. Mean post-lactulose acetate levels (0.21 ± 0.09) were higher than fasting levels ($P < 0.03$). Breath hydrogen excretion exhibited a similar trend. Indeed, a significant correlation (r_s 0.39, $P < 0.01$) was demonstrated between the acetate and hydrogen values. It is therefore concluded that patients for the lactulose breath test show fairly similar changes in plasma acetate and breath hydrogen excretion after lactulose ingestion. Either measurement could thus be used in assessing colonic fermentation in humans.

Résumé

Vu que l'acétate et l'hydrogène sont produits par la fermentation bactérienne du côlon, nous avons éprouvé l'utilité clinique du mesurage des deux paramètres dans neuf sujets qui sont venus pour l'essai de tolérance du lactulose. Le titre d'acétate du plasma — diète absolue (moyen ± s.d., mmol/l) de 0.11 ± 0.06 a augmenté à la pointe entre 150 min (0.23 ± 0.12) et 180 min (0.23 ± 0.09), les deux $P < 0.01$, après avoir ingéré 20 g du lactulose. Dans un sujet qui a eu une gastrectomie et qui avait de la diarrhée, la pointe a été atteint après 30 min. Les niveaux moyens de l'acétate post-lactulose (0.21 ± 0.09) étaient plus élevés que les niveaux diètes ($P < 0.03$). L'excrétion hydrogène soufflante a montré une tendance similaire. En fait, une corrélation significative (r_s 0.39, $P < 0.01$) a été démonstrée entre les valeurs de l'acétate et de l'hydrogène. On conclut donc, que les patients pour l'essai lactulose soufflante montrent des changements dans l'acétate plasmatique et l'excrétion hydrogéne soufflante après l'ingestion du lactulose qui sont à peu près identiques. Les deux mesures peuvent être ainsi utilisés pour l'évaluation de la fermentation bactérienne du côlon dans les humains.

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Introduction

Pomare, Branch and Cummings [1] measured the acetate and breath hydrogen responses to increasing oral lactulose doses in five normal subjects, and noted a parallel increase in both variables. The same procedure was therefore re-explored in patients with chronic diarrhoea who were to have a lactulose tolerance test in the course of routine clinical investigations. The aim was to assess whether plasma acetate measurements could be used interchangeably with measurements of breath hydrogen excretion in conditions where breath hydrogen estimation was considered routine.

Subjects and methods

Nine (six male and three female) clinically stable patients on follow-up at the Gastroenterology Unit of the John Radcliffe Hospital, Oxford were studied. Their age-range, clinical

features and drug treatment are shown in Table 1. None was on antibiotics or any other medications known to alter gut flora. All were scheduled for routine lactulose tolerance tests and were overnight fasted. Blood (collected in heparinized tubes) and breath samples were taken 30 min after cannula insertion (fasting) and again every 30 min after starting to drink (over 2 min) 30 ml Duphalac (Duphar, Southampton, U.K.), containing per 5 ml: 0.3 g lactose, 3.35 g lactulose and 0.55 g galactose. End-expiratory breath samples were collected into sealed 20 ml plastic syringes through a modified Haldane–Priestley tube at the end of a prolonged expiration [2].

Analysis for breath H_2, usually within 6 h of sample collection, was by gas chromatography (column temperature 50°C), calibrated with 20 ml of 100 ppm H_2–N_2 mixture with argon gas as carrier. Plasma acetate was measured by an enzymatic spectrophotometric method [3].

The results (means ± s.d.) were compared by paired Student's *t*-tests and ANOVA. Spearman rank correlations (r_s) were estimated for individual subjects and for the whole group. Statistical calculations were done by microcomputer using an OXSTAT package. The level of statistical significance was $P < 0.05$.

Results

There was a rise of varying extent in plasma acetate levels in all nine subjects after ingestion of lactulose. In four, the rise came within the first 30 min. The peak plasma acetate level (mmol/l) was achieved in eight of the subjects between 150 min (0.23 ± 0.12) and 180 min (0.23 ± 0.09), both values being significantly different ($P < 0.01$) from the fasting values (0.11 ± 0.06; Fig. 1). Subject 2, while demonstrating peak acetate values at 30 min, also showed the highest percentage post-lactulose acetate increase; she had lost gastric reservoir function from previous gastrectomy and showed clinical evidence of intestinal hurry in loose motions containing partially digested food

Table 1. Clinical characteristics of the subjects

Case no.	Age (years)	Sex	Clinical features	Symptom duration (years)	Drugs used in treatment
1	34	M	Lactose intolerance	1	—
2	60	M	Post-gastrectomy, intermittent diarrhoea, malabsorption	4	—
3	46	M	Crohn's disease, previous right hemicolectomy and ileal resection	17	Prednisolone; Salazopryn
4	41	M	Persistent non-infective diarrhoea? irritable bowel syndrome	2	Cortisone acetate
5	81	F	Profuse diarrhoea of unknown actiology	5	Lomotil
6	73	M	Diabetic diarrhoea ?autonomic neuropathy	3	Chlorpropamide; Metformin
7	41	F	Chronic intermittent diarrhoea	2	—
8	70	F	Chronic intermittent diarrhoea	2	—
9	66	M	Chronic intermittent diarrhoea	5	—
Mean	56.9			4.6	
s.d.	16.8			4.9	

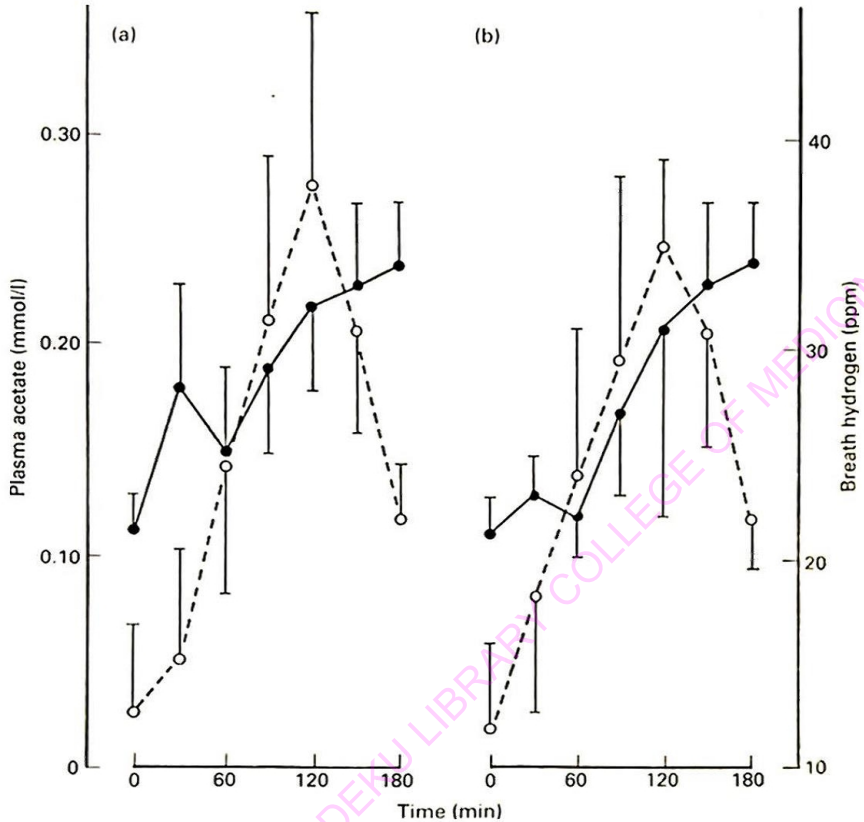

Fig. 1. Breath hydrogen excretion (○) and plasma acetate levels (●) after ingestion of 20 g lactulose. Mean values ± s.d. are shown (a) for all subjects ($n = 9$) and (b) excluding subject 2. All time points from 90 min are significantly higher than the time 0 value.

material. Subject 3, with previous extensive ileal and colonic resection for Crohn's disease, had the smallest fasting acetate concentration and about the slowest post-lactulose acetate increase. In all nine patients, the mean post-lactulose plasma acetate concentrations (0.21 ± 0.09) were significantly greater ($P < 0.05$) than fasting values.

The changes in post-lactulose breath hydrogen excretion initially paralleled the observed changes in plasma acetate (Fig. 1), although the breath hydrogen values had started falling by 120 min when the plasma acetate concentration was still rising. The fasting breath hydrogen (ppm) of 12.7 ± 11.7 increased to 17.4 ± 14.7 (P: NS) by 30 min and levels reached peak values by 120 min (37.9 ± 17.1), significantly different from the fasting values ($P < 0.01$; Fig. 1). Again, subject 3, with previous extensive gut resection had the smallest fasting (5.0 ppm) and mean post-lactulose (13.3 ppm) breath hydrogen excretion, as well as the slowest rise. Although subject 2 had one of the highest rises in breath hydrogen excretion, this peak was achieved by 120 min, much slower than the peak observed with plasma acetate (30 min).

On analysis, the fasting and post-lactulose acetate and breath hydrogen values of the subjects exhibited a significant relationship (r_s 0.39, $P < 0.01$; Fig. 2). Also, when the data were analysed using ANOVA for the regression in individual subjects and in all the subjects pooled together, the relationship was again significant ($P < 0.001$ for the pooled regression

line), although the responses of individual subjects differed ($P < 0.01$; Fig. 2).

Discussion

The lactulose breath test is a simple, non-invasive method of investigating cases of carbohydrate malabsorption and assessing mouth to caecum transit times [4, 5]. In this test, hydrogen is produced as a result of the colonic fermentation of lactulose, a semi-synthetic, indigestible disaccharide. Such hydrogen is absorbed and excreted in the breath in amounts proportional to its intestinal production [4]. The factors that influence the quantitative relationship between fermentation and breath hydrogen excretion include changes in colonic bacterial flora, variations in small intestinal motility and emotional stress [5,6]. These factors probably contribute to the observation that the lactulose hydrogen breath test is frequently not reproducible between individuals [5]. There is therefore a need for a reproducibly measurable blood metabolite with changing blood levels closely parallel to the rising breath hydrogen excretion profile during colonic fermentation.

The other absorbed products of colonic bacterial fermentation are the volatile fatty acids — acetate, propionate and butyrate [7,8]. Of these, only acetate reaches the peripheral (as distinct from the portal) circulation to any extent [1,9]. It may therefore be hypothesized that post-lactulose plasma acetate levels should change in a similar fashion to breath hydrogen. This was indeed confirmed in this study. It was also clear, as in one of the subjects, that the earlier the exposure of lactulose to the colon, e.g. when the reservoir function of the stomach is lost post-gastrectomy, the earlier the rise in plasma acetate. Also, with loss of major protions of the colon, especially the caecum where most acetate production occurs [10], the production of these fermentation products is reduced. The substantial rise of both hydrogen excretion and plasma acetate after 90 min confirms that most fermentation occurs in the distal gastrointestinal tract.

In general, the plasma acetate and breath hydrogen values do correlate (Fig. 2) and the possibility exists that one or both could be used

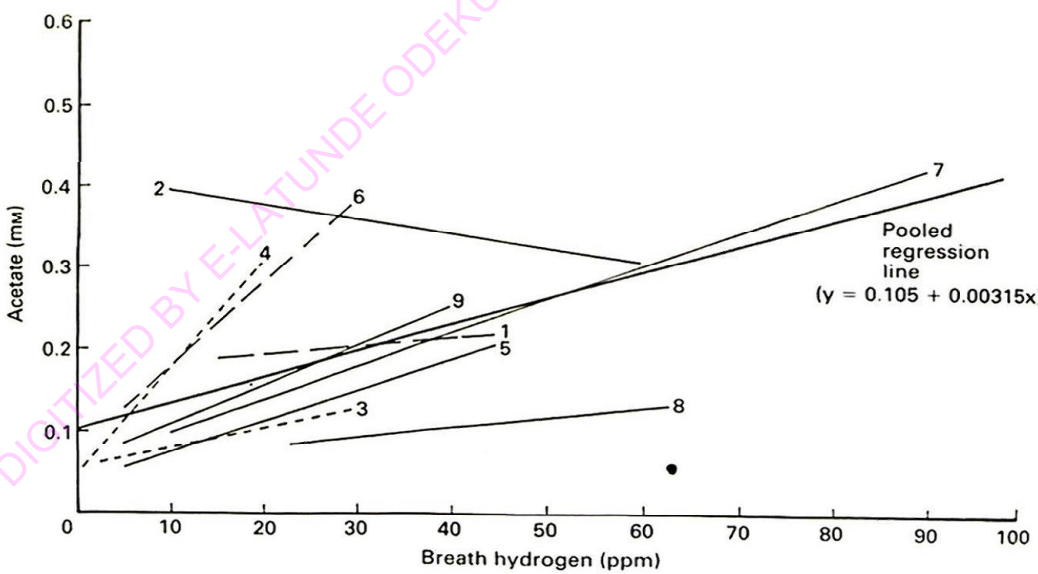

Fig. 2. Fasting and post-lactulose acetate and breath hydrogen, showing individual slopes for the nine subjects, numbered as in Table 1, and pooled regression line. $P < 0.001$ for significance of common slope; $P < 0.01$ for differences between individual slopes.

for the investigation of carbohydrate malabsorptive disorders. However, the response as measured via the two different metabolic products can differ between subjects (Fig. 2): while in six of the nine subjects the acetate and hydrogen values for an individual at the six sampling times correlated significantly ($r_s > 0.7$, $P < 0.01$), in three there was no such association ($r_s < 0.5$, P: NS). It may be notable that the three patients without a significant correlation included the post-gastrectomy and hemicolectomy patients, as well as one with lactose intolerance. Thus, none of the patients with non-specific diarrhoea (suspected malabsorption) failed to show a significant correlation.

This study has clinical implications. The measurement of plasma acetate is a relatively easily performed biochemical test and, as an alternative to breath hydrogen estimation, could be useful in the investigation of malabsorptive disorders. Furthermore, and probably more commonly, plasma acetate could be measured in individuals who suffer severe flatulence after specific meals, especially legumes, in attempting to evaluate the respective contributions to their discomfort, of fermentation and/or an intestinal disorder, and institute appropriate therapy.

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