Lactose malabsorption and colonic fermentations alter host metabolism in rats

Objectifs

Des données complémentaires sont nécessaires pour préciser *in vivo* les conséquences d'une ingestion aigue de lactose dans l'environnement colique et le métabolisme de l'hôte.

L'objectif de cette étude est d'évaluer les conséquences métaboliques d'une ingestion aigue de lactose, chez le rat, en relation avec l'activité du microbiote intestinal et ses interactions avec l'hôte malabsorbeur.

Matériel et méthodes

L'étude a été réalisée en deux parties. Dans chacune de ces parties, nous avons utilisé des rats mâles Wistar (350-400g) répartis en 2 groupes (n = 6) recevant 5 g de régime équilibré contenant soit 25 % de saccharose (groupe S25 contrôle) soit 25 % de lactose (groupe L25 expérimental).

Dans la première partie de l'étude, nous avons placé les rats en chambre calorimétrique, sous cycle jour/ nuit artificiel (lumière de 8h à 20h). Leurs paramètres métaboliques ont été enregistrés pendant les 24 heures suivant l'ingestion des 5 g de régimes tests L25 et S25.

Dans la deuxième partie de l'étude, nous avons effectué des abattages séquentiels (6 rats L25 et 6 rats S25) à différents temps (t = 0h, 2h, 4h, 6h, 8h et 13h) après l'ingestion des 5 g de régime test. Pendant les abattages, nous avons prélevé du sang porte, les contenus intestinaux et la muqueuse colique. Les acides gras à chaines courtes et les acides organiques ont été dosés respectivement pas chromatographie en phase gazeuse et par kits enzymatiques, dans le sang porte et dans les contenus intestinaux. La quantification des transporteurs d'acides gras à chaines courtes (sMCT1 et MCT1) a été réalisée par PCR en temps réel sur la muqueuse colique.

Principaux résultats

Nous observons une augmentation significative des concentrations en acides gras à chaines courtes et en lactate dans le caecum et dans le sang porte des rats du groupe L25 en comparaison à ceux du groupe S25. Ces concentrations atteignent respectivement leur

maxima à 6 et 8 heures après l'ingestion de lactose. L'expression du transporteur d'acides gras à chaine courte sMCT1 est également significativement plus élevée à 6 heures et 8 heures après l'ingestion de lactose, chez les rats du groupe L25 en comparaison aux rats du groupe S25.

En parallèle, les enregistrements de calorimétrie révèlent une montée significativement moindre du QR et de la Gox, ainsi qu'une inhibition moindre de la Lox, sur les 5 heures suivant l'ingestion du régime L25 en comparaison au régime S25. Ces résultats soulignent l'absorption et l'utilisation métabolique moindre du lactose par l'hôte. Cependant, au-delà de 6 heures, la tendance s'inverse et le QR et la Gox du groupe L25 deviennent significativement supérieurs à ceux de S25.

Conclusions

Cette étude montre qu'à partir d'une certaine dose de lactose malabsorbé, des changements métaboliques se produisent. Ces changements impliquent des fermentations caecales et coliques du lactose qui conduisent à la production d'acides gras à chaines courtes, à l'activation de leurs transporteurs coliques, à leur absorption colique et à leur utilisation métabolique par l'hôte.

Ces résultats soulèvent la question d'une réorientation métabolique du microbiote colique et de l'hôte malabsorbeur, lors d'une ingestion chronique de lactose. Ils ouvrent également la voie à de nouvelles techniques pour le diagnostic de la malabsorption du lactose. En effet, les études calorimétriques permettraient de mettre en évidence la malabsorption du lactose et de suivre l'adaptation métabolique consécutive du microbiote intestinal de l'hôte. Des études cliniques sont nécessaires pour valider ces résultats sur l'Homme.

Lactose malabsorption and colonic fermentations alter host metabolism in rats

Virginie Alexandre[1,2], Patrick C. Even[1], Christiane Larue-Achagiotis[1], Jean-Marc Blouin[1], François Blachier[1], Robert Benamouzig[3], Daniel Tomé[1] and Anne-Marie Davila[1]*

[1]*AgroParisTech, UMR 914, CRNH-IdF, Nutrition Physiology and Ingestive Behavior Department, 16 rue Claude Bernard, 75005 Paris, France*

[2]*Vivatech, 75008 Paris, France*

[3]*Gastroenterology Unit, CRNH IdF, AP-HP, University Paris 13, Hôpital Avicenne, Bobigny, France*

(Submitted 1 June 2012 – Final revision received 8 November 2012 – Accepted 11 November 2012)

Abstract

Lactose malabsorption is associated with rapid production of high levels of osmotic compounds, such as organic acids and SCFA in the colon, suspected to contribute to the onset of lactose intolerance. Adult rats are lactase deficient and the present study was conducted to evaluate *in vivo* the metabolic consequences of acute lactose ingestion, including host–microbiota interactions. Rats received diets of 25% sucrose (S25 control group) or 25% lactose (L25 experimental group). SCFA and lactic acid were quantified in intestinal contents and portal blood. Expression of SCFA transporter genes was quantified in the colonic mucosa. Carbohydrate oxidation (Cox) and lipid oxidation (Lox) were computed by indirect calorimetry. Measurements were performed over a maximum of 13 h. Time, diet and time × diet variables had significant effects on SCFA concentration in the caecum ($P<0·001$, $P=0·004$ and $P=0·007$, respectively) and the portal blood ($P<0·001$, $P=0·04$ and $P<0·001$, respectively). Concomitantly, expression of sodium monocarboxylate significantly increased in the colonic mucosa of the L25 group ($P=0·003$ at $t=6$ h and $P<0·05$ at $t=8$ h). During 5 h after the meal, the L25 group's changes in metabolic parameters (Cox, Lox) were significantly lower than those of the S25 group ($P=0·02$). However, after 5 h, L25 Cox became greater than S25 ($P=0·004$). Thus, enhanced production and absorption of SCFA support the metabolic changes observed in calorimetry. These results underline the consequences of acute lactose malabsorption and measured compensations occurring in the host's metabolism, presumably through the microbiota fermentations and microbiota–host interactions.

Key words: Lactose malabsorption: Colonic fermentation: Host–microbiota interactions: Indirect calorimetry

Lactose malabsorption affects 20% of the population worldwide. It results from a decline in lactase expression, naturally occurring in mammals after weaning. This decline is within a specific time period for each animal species: 15 d for rabbits, 1 month for rats and to or until decades for human subjects[1,2]. A low intestinal lactase activity leads to the presence of undigested lactose in the large intestine, where it is then fermented by the microbiota. Lactose intolerance is defined as the onset of non-specific gastrointestinal symptoms such as bloating, rumbling, abdominal pain, nausea and diarrhoea. Although the factors responsible for lactose intolerance are not clearly understood, the intervention of osmotically active compounds is suspected[3]. Few treatments are available and many lactose-intolerant people are left with no other choice than to practise an exclusion diet that could lead to mineral and vitamin deficiencies.

The colonic metabolism of lactose has been reported to be associated with an increased production of bacterial metabolites, including SCFA (acetate, propionate and butyrate) and gases (hydrogen, carbon dioxide and methane)[4–7] as well as, under specific circumstances, organic acids (such as lactate, succinate and formate) and ethanol[8,9]. In physiological conditions (luminal pH 4·5–7), SCFA are specifically transported by monocarboxylate (MCT1) and sodium MCT1 (sMCT1) transporters present in the colonocyte brush border membranes[10,11]. Once absorbed, SCFA are carried in the portal vein to the liver where they can be oxidised. Butyrate is the preferred energy substrate of the mucosal cells[12,13]. In standard conditions, SCFA are at the forefront of the mammals' colonic health, and represent approximately 65% of the colonic anions (70–130 mmol/l)[10–12]. However, their concentrations are 3-fold higher as a consequence of lactose

Abbreviations: Cox, carbohydrate oxidation; L25, diets containing 25% lactose; Lox, lipid oxidation; MCT1, monocarboxylate; S25, diets containing 25% sucrose; sMCT1, sodium monocarboxylate.

British Journal of Nutrition

malabsorption[9,14]. Such metabolic modifications may be expected in sugar malabsorption. However, most experiments on lactose malabsorption have been conducted *ex vivo* or *in vitro*. Studying the *in vivo* kinetics of bacterial fermentation, colonic absorption and subsequent host metabolism is necessary in order to be able to determine the onset of lactose intolerance. Such data could also pave the way to new strategies to improve the clinical management of lactose intolerance.

Accordingly, the aim of the present study was to investigate, *in vivo*, the metabolic consequences of malabsorption after an acute ingestion of lactose in rats, and the consequences of host–microbiota interactions. For this purpose, the present study was carried out using the rat model in which the incomplete absorption of lactose and the subsequent overproduction by the gut microbiota of lactose-derived SCFA could be detected by indirect calorimetry. Rats were given a single meal of either 25% lactose or 25% sucrose. SCFA concentrations were quantified in the large-intestinal contents and portal blood, expressions of MCT1 and sMCT1 measured in the colonic mucosa and oxidation of carbohydrate (Cox) and lipid (Lox) recorded by indirect calorimetry. These measurements were performed over a maximal 13 h period.

Experimental methods

Animals and diets

The experiments were carried out following the European directive 2010/63/UE relative to the protection of animals used for scientific purposes, and were approved by the ethical committee of the National Institute of Agronomic Research (INRA) (approval reference 11/012). A total of eighty-four male Wistar rats (350–400 g) were housed under an artificial 12 h light–12 dark cycle (lights on at 08.00 hours). Being more than 12 weeks old at the onset of the studies, they were naturally lactase deficient. Rats were adapted for 8 d to a standard diet of the American Institute of Nutrition (AIN) of 74% carbohydrate (63% starch, 11% sucrose), 14% protein and 12% fat as energy. This standard diet was composed of dairy proteins (140 g/kg), starch (622 g/kg), sucrose (100 g/kg), cellulose (50 g/kg), soyabean oil (40 g/kg), mineral mix AIN-93M (35 g/kg), vitamin mix AIN-93V (10 g/kg) and choline (2·3 g/kg)[15]. The experimental calibrated test meals were 5 g (362 kJ) of the standard diet modified to contain 25% (w/w) of either sucrose (control group, termed S25) or lactose (experimental group, termed L25) added at the expense of starch. The rats were randomly assigned to one of the following groups: L25 or S25. The test meals were administered after an overnight fast. $n_{i,j,k}$ = seventy-two rats were serially killed for sample collection, where: i(diet) = 1,2 (1 = L25, 2 = S25), j(time point from diet ingestion) = 1,..,6 (1 = 0, 2 = 2 h, 3 = 4 h, 4 = 6 h, 5 = 8 h, 6 = 13 h) and k(rat) = 1,...,6. $n_{i,k}$ = twelve rats were assigned to the indirect calorimetry study.

Blood, intestinal contents and colonic mucosa sampling and SCFA extraction

Portal blood (2 ml) was sampled directly from the hepatic vein using a heparinised syringe. Blood samples were centrifuged for 4 min at 4°C and 600 ***g***. A volume of 315 µl of the plasma fraction was kept in 35 µl of 5% H_3PO_4 for derivatisation and GC quantification. The rest of the plasma fraction was stored at −80°C until analysis. The colonic tissues were washed in ice-cold 0·9% (w/v) NaCl (pH 7·0) and the mucosa was scraped off using a glass slide before immediate freezing in liquid N_2 and storage at −80°C until quantitative real-time-PCR analyses. The caecal and proximal colonic contents were simply sampled in sterile tubes. SCFA were extracted by vigorous mixing with deionised water. The volume of deionised water added was 0·9-fold of the weight for caecal samples and 1·8-fold for colonic ones. As with the plasma fraction, 315 µl of the supernatant were mixed with 35 µl of 5% H_3PO_4 for subsequent derivatisation and GC quantification. The rest of the caecal and colonic fractions were kept for enzymatic reactions and spectrophotometric quantification. Samples were stored at −80°C until further analysis.

SCFA quantification

The SCFA were measured after derivatisation (esterification) with a modified Kristensen technique[16], followed by GC. This method is based on SCFA esterification by 2-chloroethyl-chloroformate in aqueous and acid media, using isocaproic acid as the internal standard. More precisely, 350 µl samples (315 µl sample + 35 µl 5% H_3PO_4), prepared as mentioned earlier, were mixed with 20 µl of 37% HCl and 100 µl of 1% isocaproate solution (w/v). Then, 750 µl of acetonitrile, 750 µl of 2-chloroethanol and 100 µl of pyridine were added. After careful homogenisation, the mixtures were centrifuged for 10 min at 3000 rpm and 4°C. The reaction was initiated by adding 50 µl of 2-chloroethyl chloroformate to 1·5 ml of the supernatant. Subsequently, 4 ml of distilled water were added and the esters were extracted with 500 µl of cyclohexane. They were then analysed with a GC (Varian CP3800; Varian Medical Systems) equipped with a capillary column (30 m, 0·32 mm inner diameter, Restek Rtx 502.2; Restek) and fitted with a flame ionisation detector and a split/splitless injector. Helium was used as the carrier gas (1 ml/min) and 6 µl of each sample were injected. The column was maintained at 75°C for 6 s, then the temperature was increased to 170°C at a rate of 7°C/min. This temperature was maintained for 10 min, before being increased to 220°C (50°C/min) and maintained for 3 min. The injector and the detector were utilised at 200 and 300°C, respectively. The amount of SCFA was determined with reference to an internal standard (isocaproate) and external standards (200 mM-acetate, 80 mM-propionate, 80 mM-butyrate, 10 mM-isobutyrate, 10 mM-isovalerate, 8 mM-valerate and 8 mM-caproate).

Lactate enzymatic assay

D/L-Lactate concentrations were determined with a commercial kit (Megazyme International Ireland Limited). Samples, prepared as mentioned earlier, were homogenised and centrifuged for 5 min at 4°C and 14 000 ***g***. The supernatant was collected for a second round of centrifugation for 15 min at

British Journal of Nutrition

4°C and 14 000 **g**. A volume of 500 µl of the supernatant was applied to a spin column of a centrifuge nanosep filter (30 kD; Pall Corporation) and centrifuged at 4°C and 14 000 **g**, until filtration of the whole volume. D/L-Lactate was then quantified in the final ultra-filtrates, according to the manufacturer's recommendations.

Gene expression of sodium monocarboxylate and monocarboxylate

The colonic expression of genes solute carrier family 16, member 1 (*SLC16A1*) and solute carrier family 5 (iodide transporter), member 8 (*SLC5A8*), which, respectively, encode for the MCT1 and sMCT1 transporters, was investigated using quantitative RT-PCR. The colonic mucosa was scraped and total RNA isolated using TRIzol reagent, according to the manufacturer's protocol (Ambion/Applied Biosystems, Life Technologies Corporation). Sample quality was assessed by 260/280 nm absorption ratio determination (between 1·8 and 2·0) and checked on a 1% agarose gel. For quantitative RT-PCR analyses, 1 µg total RNA was first reverse transcribed using the High Capacity cDNA Archive kit (Applied Biosystems). Reverse transcribed RNA was amplified on a thermal cycler (7300 Real-Time PCR System, Applied Biosystems) using the SYBR green (Power SYBR Green, Applied Biosystems) fluorescence method and specific oligonucleotides. The primers used were as follows: rat RPL13A – forward 5′-GGATCCCTCCACCCTATGACA-3′, reverse 5′-CTGGTACTTCCACCCGACCTC-3′; rat MCT1 – forward 5′-GCAACGACCAGTGAAGTGT-3′, reverse 5′-AAGCCCAAGACCTCCAAT-3′; and rat SMCT1 – forward 5′-CTGGGCTTGTTTTCTTTGG-3′, reverse 5′-CGTTGTGCGTGCTGTTAG-3′. Results were analysed with 7300 SDS system software (Applied Biosystems). Quantification of RNA was carried out by comparison of the number of cycles required to reach reference and target threshold values (δ–δC_t method).

Indirect calorimetry

Cox and Lox were computed using the Weir equation from continuous measurements of V_{O2} and carbon dioxide production (V_{CO2}) by indirect calorimetry[17]. The metabolic cage was equipped with force transducers placed below the metabolic chamber. This made possible the removal of the part of the respiratory exchange that was directly related to the energy expended on spontaneous activity. Thus, it was possible to compute meal-induced changes in Cox and Lox, free of short-term changes due to the cost of activity[18]. The rats were housed in the metabolic cage at 18.00 hours, with free access to water but no access to food. Temperature in the cage was regulated at 26°C to limit energy expenditure for thermoregulation. Basal Cox and Lox were measured between 06.00 and 10.00 hours the next day. The test meals were given at 10.00 hours and the changes in Cox and Lox *vs.* baseline were measured during 8 and 13 h. At the end of the experiment, rats were euthanised by an intra-peritoneal injection of pentobarbital (0·1 ml/100 g body weight) and the intestinal contents, the intestinal mucosa and the portal blood samples were collected.

Statistical analyses

Results are presented as means with their standard errors. Analyses were performed with either an ANOVA using SAS software (version 9.1, SAS Institute, Inc.) or a Student's *t* test for the unpaired data.

Results

SCFA and lactic acid quantification in intestinal contents and portal blood

Lactic acid and SCFA were quantified in intestinal contents and portal blood in L25 (25% lactose diet) and S25 (25% sucrose diet) groups (Fig. 1 and Table 1). An increase of SCFA concentration was observed in intestinal contents

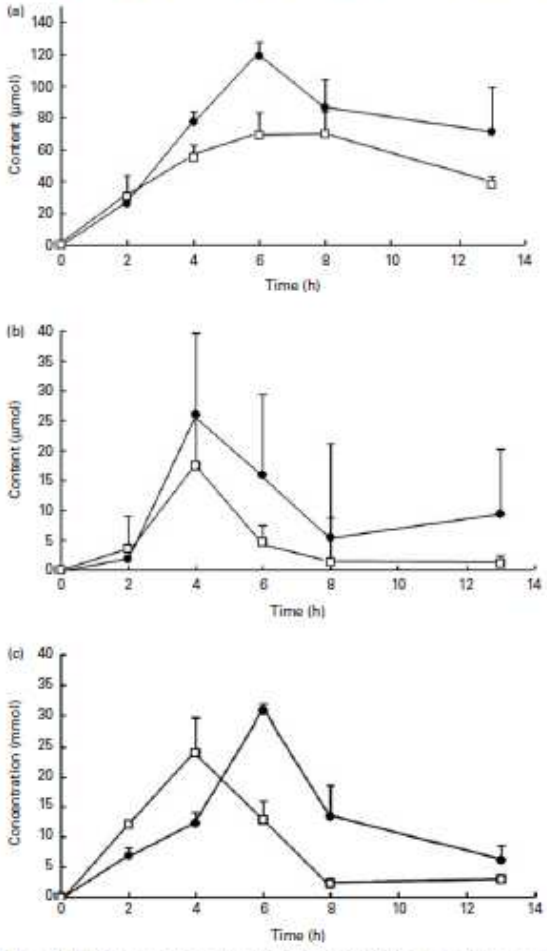

Fig. 1. Total SCFA variations after L25 (●) and S25 (□) diets in (a) caecum, (b) proximal colon, (c) portal blood. S25: diet containing 25% sucrose; L25: diet containing 25% lactose. Time ((a) $P<0·001$, (b) $P=0·24$, (c) $P<0·001$), diet ((a) $P=0·004$, (b) $P=0·06$, (c) $P=0·04$) and time × diet ((a) $P=0·007$; (b) $P=0·63$; (c) $P<0·001$) effects were evaluated by ANOVA. A P value over 0·05 is considered as not significant. Comparisons between S25 and L25 were performed at each time point by a Student's *t* test for unpaired data and are presented in Supplementary material (available online). $n_{ijk}=72$, where i(diet) = 1,2, j(time point from diet ingestion) = 1,...,6 and k(rat) = 1,...,6.

British Journal of Nutrition

Table 1. Acetate, propionate, butyrate and lactate in the caecum, proximal colon and portal blood after L25 and S25 diets*

(Mean values with their standard errors)

	S25	L25	SEM	*P*
Caecum				
Acetate	0·52	0·76	0·02	0·001
Propionate	0·11	0·14	0·01	0·17
Butyrate	0·04	0·05	0·003	0·54
Lactate	0·23	3·96	0·16	0·001
Proximal colon				
Acetate	0·12	0·18	0·01	0·21
Propionate	0·01	0·03	0·001	0·01
Butyrate	0·005	0·04	0·01	0·54
Lactate	ND	1·29	0·06	–
Portal blood				
Acetate	59·6	95·6	3·07	0·01
Propionate	47·7	56·8	3·35	0·47
Butyrate	16·8	22·6	1·67	0·29
Lactate	0·07	0·08	0·003	0·56

S25, containing 25 % sucrose; L25, containing 25 % lactose; ND, not detected.

* Data are expressed as means of AUC from the plot of contents (μmol) *v.* time (h) ± pooled SEM for caecum and colon, and concentration (mmol/l) *v.* time (h) ± pooled SEM for portal blood. Data are compared by a Student's *t* test for unpaired data. A *P* value over 0·05 is considered as not significant. $n_{i,j,k} = 72$, where *i*(diet) = 1,2, *j*(time point from diet ingestion) = 1,...,6 and *k*(rat) = 1,...,6.

(caecum and colon) for both groups, with a peak occurring between 4 and 8 h after the ingestion of the test meal. The higher amounts of SCFA and lactic acid were mainly encountered in the caecum. Total SCFA were 140 % higher in the caecum and 80 % higher in the colon in the L25 group compared with the S25 group. Time, diet and time × diet variables presented significant effects on caecal concentrations of SCFA ($P<0·001$, $P=0·004$ and $P=0·007$, respectively) and lactate ($P=0·004$, $P<0·001$ and $P=0·001$, respectively). Significant differences, presented in the Supplementary material (available online), were especially noted at 6 h ($P=0·004$) after the ingestion of the test meal. No significant difference was noticed in the proximal colon between the L25 and S25 groups, except for propionate concentrations ($P<0·05$). A moderate increase of SCFA was also noticed after the S25 meal. Finally, quantifications in the portal blood also revealed an increase of total SCFA over time in the S25 and L25 groups, with peaks occurring at 4 and 6 h after the diets, respectively. Time, diet and time × diet variables presented significant effects on SCFA concentrations in the portal blood ($P<0·001$, $P=0·04$ and $P<0·001$, respectively). Significant differences, presented in the Supplementary material (available online), were especially noted at 4 h ($P=0·009$) and 6 h ($P<0·001$) after the ingestion of the test meal. Total SCFA concentrations exhibited a 2-fold higher peak in the L25 group compared with the S25 group, probably driven by a significant increase of acetate concentrations ($P=0·01$) (Table 1).

Expression of sodium monocarboxylate and monocarboxylate genes in the colonic mucosa

The expression of SCFA transporters sMCT1 and MCT1 was analysed in the colonic mucosa in order to determine their absorption. Quantitative RT-PCR analyses revealed that diet presented a significant effect on the expression of sMCT1 ($P=0·02$) (Fig. 2). The differences, presented in the Supplementary material (available online), were significant for sMCT1 at 6 h ($P=0·03$) and 8 h ($P<0·05$).

Indirect calorimetry after a sucrose- or lactose-containing test meal

Calorimetric records following ingestion of the test meals revealed that Cox and Lox, respectively, presented a significantly lower increase and lower inhibition after ingestion of an L25 meal compared with an S25 meal (Fig. 3). Time, diet and time × diet variables presented significant effects on Lox ($P<0·001$, $P<0·001$, $P=0·004$, respectively) and Cox ($P<0·001$, $P<0·001$, $P<0·001$, respectively) kinetics over the 13 h post-ingestion. During the 8 h period after the test meals, the difference in Cox between the L25 and S25 groups was 6·6 kJ (Table 2). Interestingly, the differential amount (L25–S25) of total SCFA accumulated in the caecum resulted in a differential energy of 7 kJ (difference of 2443 μmol, with an average of $M = 74$ g/mol). After intersection of the curves, a recovery of energy occurred for the L25 group, but remained partial

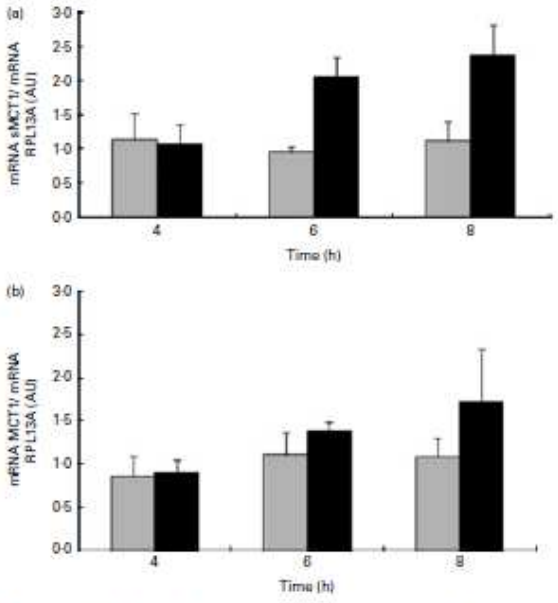

Fig. 2. (a) Sodium monocarboxylate (sMCT1) expression after L25 (□) and S25 (■) diets; (b) monocarboxylate (MCT1) expression after L25 and S25 diets. S25, diets containing 25 % sucrose; L25, diets containing 25 % lactose; Time ((a) $P = 0·24$, (b) $P=0·34$), diet ((a) $P=0·02$, (b) $P=0·29$) and time × diet ((a) $P=0·19$, (b) $P=0·72$) effects were evaluated by ANOVA. A *P* value over 0·05 is considered as not significant. Comparisons between S25 and L25 were performed at each time point by a Student's *t* test for unpaired data and are presented in Supplementary material (available online). $n_{i,j,k} = 36$, where *i*(diet) = 1,2, *j*(time point from diet ingestion) = 3,4,5 and *k*(rat) = 1,...,6. RPL13a, ribosomal protein L13a; AU, arbitrary units.

British Journal of Nutrition

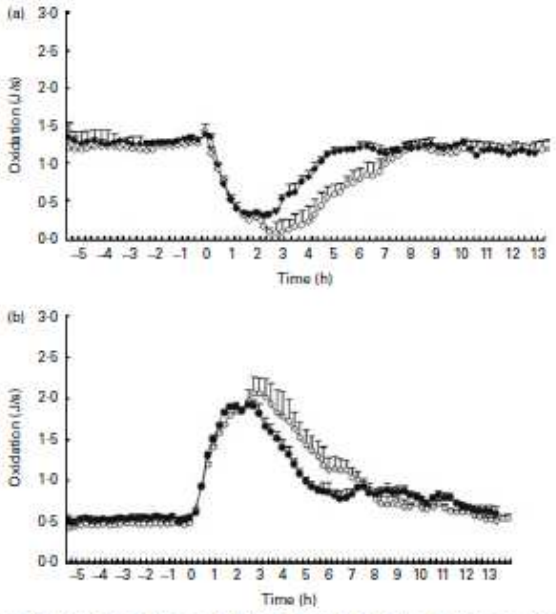

Fig. 3. (a) Lipid oxidation and (b) carbohydrate oxidation kinetics over 13 h after L25 (●) and S25 (○) diet. S25: diet containing 25 % sucrose; L25: diet containing 25 % lactose. Time ((a) $P < 0{\cdot}001$, (b) $P < 0{\cdot}001$), diet ((a) $P < 0{\cdot}001$, (b) $P < 0{\cdot}001$) and time × diet effects ((a) $P = 0{\cdot}004$; (b) $P < 0{\cdot}001$) are evaluated by ANOVA. A P value over 0·05 is considered as not significant. $n_{i,k} = 12$, where i(diet) = 1,2 and k(rat) = 1,...,6.

with a difference of less than 2·5 kJ between the 8 and 13 h. As the decrease in Cox was compensated by a lower inhibition of Lox, overall postprandial thermogenesis (Cox + Lox) was not significantly different between the L25 and S25 groups.

Discussion

The present study contributes to a better knowledge of the *in vivo* kinetics of the metabolic consequences of acute lactose malabsorption in rats. Lactose malabsorption appears to be mainly associated with modifications in the production of SCFA by the gut microbiota and their subsequent absorption and metabolic utilisation by the host. The rat model provides control of both internal parameters, such as genetic background (allowing similar physiological responses), and external parameters, such as the 13 h kinetics in the calorimetric chamber plus samplings of the portal vein, the intestinal contents and the colonic mucosa.

Lactose malabsorption led to microbial production (quantified in intestinal contents) and colonic absorption (indicated by quantification in portal blood and sMCT1 expression in the colonic mucosa) of SCFA. Finally, the consequent metabolic changes occurring in the lactose malabsorber host were supported by the calorimetric parameters.

In the mammalian colon, a close similarity in the molar ratio of acetate, propionate and butyrate is noted among species. The SCFA profiles are not fixed in human subjects, but, in standard conditions, the molar ratio has been reported to be about 60:20:20 (acetate:propionate:butyrate) in colonic contents and about 85:10:5 in blood, with some variation depending upon intestinal segment (left or right) and blood sample origin (portal, hepatic or peripheral)[19]. Indeed, these molar ratios may be altered by consumption of fermentable fibres or modified foods. Recent studies also underline the role of dietary proteins in the SCFA production, either in quantity or quality[20,21]. Such questions are of interest for the medical community, as SCFA, and especially butyrate, have demonstrated to have a beneficial effect on the host. The main health benefits attributed to butyrate are its role in the reduction of colonic inflammation and permeability[22] and in the protection against colorectal cancer[23]. SCFA concentration in the large bowel is the result of SCFA production by the intestinal microbiota, of *in situ* metabolisation by colonocytes and of colonic absorption. Therefore, high SCFA concentrations in the caecum may be explained either by an active production and/or by a low uptake. Furthermore, once absorbed, SCFA are carried through portal blood to the liver, where most of them are metabolised, accounting for the consecutive lower concentrations in the peripheral blood[19]. In the present study, after ingestion of lactose, lactic acid and total SCFA were significantly higher in the caecum and the portal blood. One may assume that these differences account for lactose ingestion specifically, as L25 and S25 groups had both the same protein intake. Interestingly, major differences were noticed 6 h after lactose ingestion. The bioavailability of substrate and fermentation kinetics of the microbiota may explain the delayed caecal production of intermediary and end metabolites after the ingestion of lactose[24]. The substantial absorption of colonic SCFA, usually between 6·1 and 12·6 μmol/cm^2 per h[25], explains the gradual metabolite concentration decrease and pH increase (from 5·6 to 6·6) from the caecum to the distal colon[19]. These findings could account for the absence of significant differences in the proximal colon after S25 or L25 diets. Similar kinetics in bell curves were observed for SCFA variations in the caecum, the proximal colon and the portal blood. However, the peaks were observed at 6 h after

Table 2. Meal-induced changes (kJ) in carbohydrate oxidation (Cox) and lipid oxidation (Lox) during the periods 0–8 h and 8–13 h, after single L25 and S25 meals*

(Mean values with their standard errors)

	S25		L25		
	Mean	SEM	Mean	SEM	*P*
0–8 h					
Cox	40·6	2·5	34·0	1·8	0·049
Lox	16·7	1·8	25·1	1·0	0·009
8–13 h					
Cox	11·5	0·4	13·9	0·8	0·004
Lox	21·0	2·0	21·3	0·3	0·88

S25, containing 25 % sucrose; L25, containing 25 % lactose.
* Data are expressed as means of Cox or Lox plots (kJ) ± SEM and are compared by a Student's t test for unpaired data. A P value over 0·05 is considered as not significant. $n_{i,k} = 12$, where i(diet) = 1,2 and k(rat) = 1,...,6.

the L25 meal in the caecum and the portal blood, and at 4 h after the meal in the proximal colon.

The decrease after 4 h of SCFA colonic concentrations is concomitant to the increase of mRNA for sMCT1 and MCT1 transporters in the colonic mucosa. This observation could suggest that, before 6 h, the activity of these transporters was disrupted (pH drop) or exceeded (unusually high concentrations of SCFA), leading to SCFA accumulation until the biosynthesis of a new pool of transporters. The pattern of the SCFA concentrations in portal blood is consistent with this hypothesis. We did not follow-up the transcription of sMCT1 and MCT1 in the caecal mucosa, but *in vitro* experiments proved that SCFA abundance resulted in MCT1 and sMCT1 mRNA rises, probably in order to maximise intracellular availability of SCFA[26–29]. An increase in passive uptake through the paracellular route may be also implied in the increased SCFA concentrations in the portal blood, as a fructo-oligosaccharide-induced increase in intestinal permeability was demonstrated in rats[30].

At last, indirect calorimetry underlines the smaller increase of Cox, the smaller inhibition of Lox and the fairly stable overall thermogenic response after ingestion of an L25 meal compared with an S25 meal. Indirect calorimetry confirms the lower absorption and subsequent metabolic utilisation of lactose in rats, with a reversed trend between the L25 and S25 groups, beyond 6 h after the test meals. Finally, with the L25 diet, the excess energy available in caecal contents (as SCFA) is in the same range (6–7 kJ) as the energy underexpended for Cox during 8 h after the ingestion of the diet. Furthermore, the recovery of this amount of energy may also explain the absence of differences when comparing the thermogenesis after lactose and sucrose diets. These results can be interpreted as the oxidation of the SCFA produced by the microbial fermentation of lactose, whose metabolites are then absorbed and oxidised by the host. Thus, the use of new metabolites, largely produced and accumulated under lactose ingestion, may be considered as capable of providing this delayed energy. Such an idea has already been suggested in a calorimetric study in rats fed with various dietary fibres, in which Cox remained higher for a longer period after the ingestion of the fibres than after the ingestion of the control diet[31]. Among the accumulated metabolites, caecal and colonic SCFA are presumably playing a major metabolic role. Indeed, several studies confirm their capacity to be easily absorbed across the colonic mucosa, resulting in a salvage of carbohydrate energy that otherwise would have been lost in faeces[32–34].

Overall, the results provided by the measurements of the luminal and the blood SCFA, transporter gene expression and by indirect calorimetry method showed that up to a given load of undigested lactose, metabolic changes occur from 6 h after lactose ingestion. They involve caecal and colonic fermentation of lactose, leading to the caecal production of SCFA, subsequent activation of SCFA-specific transporters and colonic absorption of the SCFA that are metabolised in the host's tissues, accounting for the metabolic compensations from 6 h. The present study emphasises the adaptation of both the colonic microbiota and the host metabolisms, but also supports the suggested nutritional strategies to optimise the microflora and host adaptation in order to alleviate the symptoms of lactose intolerance. These results raise the question of the metabolic reorientation of the colonic microbiota and the host over chronic lactose ingestion by lactose malabsorbers. They also pave the way to the use of indirect calorimetry as a non-invasive tool to detect intestinal lactose malabsorption and to explain, at least partly, the subsequent metabolic adaptation through microbial fermentations. Clinical trials are needed to confirm the validity of this methodological approach in human subjects.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114512005557

Acknowledgements

We (V. A. and A.-M. D.) want to express our thanks to Patrick Even, Christiane Larue-Achagiotis and Jean-Marc Blouin for their expert technical assistance and to François Blachier, Robert Benamouzig and Daniel Tomé for their precious help in the redaction of this article. We gratefully acknowledge Corine Delteil and Sarah Bock for their assistance during the microbiological analyses, Nachiket Nadkarni for his helpful English advice, Tristan Mary-Huard and Pierre Barbillon for their help for the statistical analyses. We also thank Vivatech for financial support. No conflict of interest has been reported.

References

1. Wang Y, Harvey CB, Hollox EJ, *et al.* (1998) The genetically programmed down-regulation of lactase in children. *Gastroenterology* **114**, 1230–1236.
2. Sebastio G, Villa M, Sartorio R, *et al.* (1989) Control of lactase in human adult-type hypolactasia and in weaning rabbits and rats. *Am J Hum Genet* **45**, 489–497.
3. Marteau P & Flourie B (2001) Tolerance to low-digestible carbohydrates: symptomatology and methods. *Br J Nutr* **85**, Suppl. 1, S17–S21.
4. Cummings JH & Macfarlane GT (1991) The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* **70**, 443–459.
5. Arola H & Tamm A (1994) Metabolism of lactose in the human body. *Scand J Gastroenterol Suppl* **202**, 21–25.
6. Beards E, Tuohy K & Gibson G (2010) Bacterial, SCFA and gas profiles of a range of food ingredients following *in vitro* fermentation by human colonic microbiota. *Anaerobe* **16**, 420–425.
7. Hawksworth G, Drasar BS & Hill MJ (1971) Intestinal bacteria and the hydrolysis of glycosidic bonds. *J Med Microbiol* **4**, 451–459.
8. Cummings JH & Macfarlane GT (1997) Role of intestinal bacteria in nutrient metabolism. *JPEN J Parenter Enteral Nutr* **21**, 357–365.
9. He T, Priebe MG, Harmsen HJ, *et al.* (2006) Colonic fermentation may play a role in lactose intolerance in humans. *J Nutr* **136**, 58–63.
10. Papillon E, Bonaz B & Fournet J (1999) Short chain fatty acids: effects on gastrointestinal function and therapeutic

potential in gastroenterology. *Gastroenterologie clinique et biologique* **23**, 761–769.
11. Soergel KH (1994) Colonic fermentation: metabolic and clinical implications. *Clin Investig* **72**, 742–748.
12. Bugaut M (1987) Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. *Comp Biochem Physiol B* **86**, 439–472.
13. Hamer HM, Jonkers D, Venema K, *et al.* (2008) Review Article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* **27**, 104–119.
14. Hertzler SR & Savaiano DA (1996) Colonic adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. *Am J Clin Nutr* **64**, 232–236.
15. Reeves PG, Rossow KL & Lindlauf J (1993) Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. *J Nutr* **123**, 1923–1931.
16. Kristensen NB, Pierzynowski SG & Danfaer A (2000) Net portal appearance of volatile fatty acids in sheep intraruminally infused with mixtures of acetate, propionate, isobutyrate, butyrate, and valerate. *J Anim Sci* **78**, 1372–1379.
17. Cunningham JJ (1990) Calculation of energy expenditure from indirect calorimetry: assessment of the Weir equation. *Nutrition* **6**, 222–223.
18. Pinheiro Volp AC, Esteves de Oliveira FC, Duarte Moreira Alves R, *et al.* (2011) Energy expenditure: components and evaluation methods. *Nutr Hosp* **26**, 430–440.
19. Cummings JH, Pomare EW, Branch WJ, *et al.* (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221–1227.
20. Morita T, Kasaoka S & Kiriyama S (2004) Physiological functions of resistant proteins: proteins and peptides regulating large bowel fermentation of indigestible polysaccharide. *J AOAC Int* **87**, 792–796.
21. Andriamihaja M, Davila AM, Eklou-Lawson M, *et al.* (2010) Colon luminal content and epithelial cell morphology are markedly modified in rats fed with a high-protein diet. *Am J Physiol* **299**, G1030–G1037.
22. Ferreira TM, Leonel AJ, Melo MA, *et al.* (2012) Oral supplementation of butyrate reduces mucositis and intestinal permeability associated with 5-fluorouracil administration. *Lipids* **47**, 669–678.
23. Fung KY, Cosgrove L, Lockett T, *et al.* (2012) A review of the potential mechanisms for the lowering of colorectal oncogenesis by butyrate. *Br J Nutr* **108**, 820–831.
24. Wong JM, de Souza R, Kendall CW, *et al.* (2006) Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* **40**, 235–243.
25. Cummings JH (1981) Short chain fatty acids in the human colon. *Gut* **22**, 763–779.
26. Kawamata K, Hayashi H & Suzuki Y (2007) Propionate absorption associated with bicarbonate secretion *in vitro* in the mouse cecum. *Pflugers Archiv* **454**, 253–262.
27. Kirat D, Inoue H, Iwano H, *et al.* (2006) Monocarboxylate transporter 1 gene expression in the ovine gastrointestinal tract. *Vet J* **171**, 462–467.
28. Kirat D & Kato S (2006) Monocarboxylate transporter 1 (MCT1) mediates transport of short-chain fatty acids in bovine caecum. *Exp Physiol* **91**, 835–844.
29. Cuff MA, Lambert DW & Shirazi-Beechey SP (2002) Substrate-induced regulation of the human colonic monocarboxylate transporter, MCT1. *J Physiol* **539**, 361–371.
30. Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, *et al.* (2005) Dietary fructooligosaccharides increase intestinal permeability in rats. *J Nutr* **135**, 837–842.
31. Aust L, Dongowski G, Frenz U, *et al.* (2001) Estimation of available energy of dietary fibres by indirect calorimetry in rats. *Eur J Nutr* **40**, 23–29.
32. Jeppesen PB & Mortensen PB (1999) Colonic digestion and absorption of energy from carbohydrates and medium-chain fat in small bowel failure. *JPEN J Parenter Enteral Nutr* **23**, S101–S105.
33. Mortensen PB & Clausen MR (1996) Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. *Scand J Gastroenterol Suppl* **216**, 132–148.
34. Pomare EW, Branch WJ & Cummings JH (1985) Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. *J Clin Invest* **75**, 1448–1454.

II. Publication 2: Agreement between indirect calorimetry and traditional tests of lactose malabsorption

Objectifs

Les tests utilisés pour le diagnostic de la malabsorption du lactose manquent souvent de fiabilité. L'étude présentée dans la publication 1 montre que la mesure du QR, chez le rat, peut mettre en évidence la malabsorption du lactose dans les heures qui suivent son ingestion.
Cette étude clinique exploratoire vise à déterminer l'intérêt de suivre le QR (mais aussi la Gox et la Lox) après l'ingestion de lactose dans le but d'identifier une malabsorption chez l'Homme, ainsi que d'évaluer ses corrélations avec les méthodes indirectes traditionnellement utilisées.

Matériel et méthodes

Après recueil des consentements, une série consécutive de sujets a été sélectionnée selon leur statut absorbeur (n=10) ou malabsorbeur (n=10). Ce statut a été déterminé, après une nuit de jeûne, par leur pic glycémique pendant l'heure suivant l'ingestion de lactose (25g) : un pic inférieur à 1.0 mmol/L est spécifique d'un malabsorbeur alors qu'un pic supérieur à 1.5 mmol/L est spécifique d'un absorbeur.
Nous avons suivi leur expiration d'hydrogène et leur cinétique de QR, respectivement pendant 3 et 4h après une nuit de jeûne suivi de l'ingestion de lactose (25g). Les sujets ont également évalué, à la fin du test, l'intensité et la localisation de leurs symptômes gastro-intestinaux.

Principaux résultats

Les différences de QR ($p = 0.002$), de Gox ($p = 0.009$) et de Lox ($p = 0.006$) sont significatives entre les absorbeurs et les malabsorbeurs sur les 100 minutes suivant l'ingestion de lactose. Les différences de QR, Gox et Lox sont également significatives sur la plus courte fenêtre 30-50 minutes (respectivement $p = 0.001$, $p = 0.002$, $p = 0.001$). Le score total des symptômes ($p = 0.04$), notamment les flatulences ($p = 0.05$), et l'intensité de la douleur dans l'hypogastre ($p = 0.02$) sont significativement plus élevées chez les malabsorbeurs que chez les absorbeurs.

Par ailleurs, le QR est bien corrélé aux marqueurs traditionnels de la malabsorption et de l'intolérance : $R = 0.74$ ($p < 0.001$) avec la glycémie, $R = -0.51$ ($p < 0.01$) avec le test respiratoire à l'hydrogène et le score total des symptômes $R = -0.51$ ($p = 0.05$).

Conclusions

Les cinétiques des paramètres métaboliques (QR, Gox et Lox) sont significativement différentes entre des sujets absorbeurs et des sujets malabsorbeurs, sur les 4 heures suivant l'ingestion de lactose. Un suivi de ces paramètres sur la fenêtre 30-50 min après ingestion de lactose révèle des mesures également significativement différentes.

La calorimétrie indirecte semble donc un outil simple et non-invasif pour détecter la malabsorption du lactose. Des études complémentaires sont nécessaires pour préciser la place de ce nouveau test diagnostique dans de plus larges populations.

Agreement between indirect calorimetry and traditional tests of lactose malabsorption

Virginie Alexandre[1, 2], Anne-Marie Davila[1, *], Michel Bouchoucha[3], Claire Bertin[2], Patrick Even[1], Christine Lamberto[4], Daniel Tomé[1], Robert Benamouzig[3]

[1] AgroParisTech, Nutrition Physiology and Ingestion Behavior, 75005 Paris, France
[2] Vivatech, 8 rue Christophe Colomb, 75008 Paris, France
[3] Gastroenterology Department, Avicenne Hospital, 93000 Bobigny, France
[4] Functional Explorations Department, Avicenne Hospital, 93000 Bobigny, France

*** Corresponding author**
Anne-Marie Davila
AgroParisTech, Nutrition Physiology and Ingestion Behavior Department,
16 rue Claude Bernard, 75005 Paris, France.
E-mail: davila@agroparistech.fr
Tel: + 33 1 44 08 86 76
Fax: + 33 1 44 08 18 58

Electronic word count (excluding abstract, references, tables, figures): 3876

We thank Vivatech for financial support. No conflict of interest has been reported.

ABBREVIATIONS

A: Absorber

Cox: Carbohydrate Oxidation

HBT: Hydrogen Breath Test

Lox: Lipid Oxidation

M: Malabsorber

RQ: Respiratory Quotient

ABSTRACT

Background

Lactose malabsorption occurs frequently and the variably consecutive intolerance may seriously impair quality of life. An adequate diagnostic must be based on a reliable and convenient method but such a test is not yet applied in routine. A recent animal study showed that the respiratory quotient changed significantly after ingestion of sucrose and lactose in naturally lactase-deficient rats.

Aims

This exploratory study evaluated the relevance of monitoring respiratory quotient after lactose ingestion, in order to detect malabsorption.

Methods

10 lactose absorbers and 10 lactose malabsorbers were identified and classified by a lactose tolerance test (25g). After an overnight fast, a second lactose challenge was performed to monitor hydrogen excretions and respiratory quotient kinetics over 4 hours. Participants also completed questionnaires to score and localise their gastro-intestinal symptoms.

Results

Respiratory quotient kinetics were different between absorbers and malabsorbers during the 100 minutes after lactose ingestion ($p<0.01$), and even during the shorter initial 30-50 minute period. Respiratory quotient was significantly positively correlated to the peak of glycaemia ($R=0.74$) and negatively to hydrogen excretions ($R=-0.51$) and the symptoms score ($R=-0.46$).

Conclusions

Indirect calorimetry could improve the reliability of lactose malabsorption diagnosis. Studies on larger populations are needed to confirm its validity and to propose a simplified measurement.

KEYWORDS

Lactose malabsorption, diagnostic, respiratory quotient

INTRODUCTION

Hypolactasia is a widespread intestinal problem that affects almost 75% of the population worldwide, with large variations depending on ethnic background. Despite recent identification of the genetic locus, lactose intolerance remains poorly diagnosed by the medical community, mainly due to the heterogeneity of terminologies and the questionable reliability of the diagnostic tools.

Hypolactasia, which refers to the deficiency or absence of lactase secretion, may be detected in duodenal or jejunal biopsies and by genetic tests. Biopsy is the only direct method but is an invasive technique. The genetic test, identifying some mutations in the gene encoding lactase, has a reported sensitivity of 93-100% and specificity of 95-100%, but remains poorly available.

Several other techniques are available to detect malabsorption, but they present limitations. These tests require the ingestion of a lactose load and include the dynamic study of glycaemia, the analysis of stool pH, urine galactose, the HBT or the measurement of ^{13}C-glucose in serum or $^{13}CO_2$ in exhaled air. Glycaemia analysis presents sensitivity and specificity both reported to be from 70 to 95 % [1], with no reliability in diabetics or in patients with bacterial overgrowth. The stool pH is easily analysed but its reliability may be disturbed by intestinal motility and water reabsorption. Urine galactose presents variable sensitivity (77-96%) and specificity 88-100% [2]. The HBT, quantifying the amount of hydrogen created from the colonic fermentation of the undigested carbohydrates, has been considered a gold standard for over 30 years. Its sensitivity and specificity range from 80-100% and 70-100% respectively [3]. However, around 20% of patients present an intestinal bacterial overgrowth leading to false positive results or a methano-excretion leading to false negative results [4]. Since 2008, it has been suggested to detect both hydrogen and methane in the expired air to reduce the number of false negatives [5]. The monitoring of ^{13}C-glucose in serum or $^{13}CO_2$ in exhaled air following ingestion of labelled ^{13}C-lactose was developed in 2000. According to these studies, the proportion of lactose malabsorbers is almost 50% higher than described in the literature [6-8]. However, this technique remains rarely used.

Subjective techniques can highlight lactose intolerance, corresponding to the expression of symptoms. The available tests are usually self-completed questionnaires to score the main gastro-intestinal symptoms, such as rumbling, bloating, cramping, nausea and diarrhoea. The sensitivity and specificity of these questionnaires are only 75% and 67% respectively due to

the variability in appreciating one's own symptoms [9], with an overestimation observed in 30% of the subjects [10].

Thus, none of the available techniques allows a precise and absolute diagnosis. In this context, it is useful to evaluate new tools or new combinations in order to optimise the diagnosis of lactose malabsorption.

A recent animal study showed that the kinetics of Respiratory Quotient (RQ), Carbohydrate Oxidation (Cox) and Lipid Oxidation (Lox), after ingestion of sucrose and lactose, depended on the capacity of the host to absorb sugars (Alexandre et al., 2012, British Journal of Nutrition, In Press). Therefore, we aimed to monitor, on selected absorbers and malabsorbers, the RQ, Cox and Lox during after a lactose load (25g) and to evaluate their efficacy in diagnosing lactose malabsorption. Concomitantly, blood sugar levels were measured using a portable glycaemia lector, hydrogen breath excretion was quantified using a portable hydrogen detector, and RQ, Cox and Lox kinetics were monitored using a ventilated-hood system. Symptoms were also evaluated before the ingestion of lactose and after the 4 hours, by subjectively scoring the five main gastro-intestinal symptoms (diarrhoea, pain, nausea, rumbling and bloating).

MATERIALS AND METHODS

Participants

The study was carried-out on 20 healthy volunteers (10 first lactose absorbers and 10 first lactose malabsorbers) identified by the one-hour glycaemia after lactose ingestion. No participant had gastrointestinal or pulmonary disease, had taken antibiotics or other drugs affecting intestinal function for 8 days prior to the study, or had practiced intense physical activity for two days prior to the study. Additionally, they had followed the nutritional recommendations for meals during the two days before the study [11,12].

Experimental protocol

The study took place in the Gastroenterology Department of Avicenne Hospital (Bobigny, France). After being identified as lactose absorbers or malabsorbers, the participants ingested 25 g of lactose diluted in 250 ml of water after an overnight fast. Their respiratory parameters were measured using a ventilated-hood system (canopy) for four hours and their hydrogen excretions were recorded over three hours. At the end of the test the participants also completed a symptoms questionnaire to score and localise their gastrointestinal symptoms.

Lactose tolerance test

Samples of capillary blood to test glucose concentration were taken at 0, 15, 30, 45 and 60 minutes, using a *Precision XceedPro* (Abbott, Rungis, France). For each point, measures were performed twice on the same sample and the mean value was recorded. A glycaemia rise equal to or higher than 1.5 mmol/ l was considered to be lactose absorption; a plasma glucose rise equal to or lower than 1.0 mmol/ l was considered to be lactose malabsorption [1]. All the included participants presented glycaemia peaks above or equal to 1.5 mmol/ l for the lactose absorbers and below or equal to 1.0 mmol/ l for the lactose malabsorbers, confirming their classifications [13], [2].

Hydrogen Breath Test (HBT)

The exhaled hydrogen was measured in parts per million using a *GastroLyzer* (Bedfont Scientific Ltd, Maidstone, Kent, England). A hydrogen excretion 20 ppm higher than baseline in at least two subsequent measurements was associated to lactose malabsorption [14,15].

RQ, Cox and Lox kinetics

RQ, Cox and Lox were computed from $VCO_{2\ exhaled}$ and $VO_{2\ consumed}$, recorded every minute, using a canopy *Deltatrac II* (Datex Ohmeda, Limonest, France). In such a system, the subject inspires the atmospheric air through a hole in the capsule and expires via a non return valve into a measurement unit [16-18]. When participants came for the measurements, they were laid down in supination with the canopy overhead. They had at least 30 minutes to become habituated. Once parameters were stable, we recorded 10 minutes of baseline. Then, the participants ingested the lactose and the measurements were launched over 4 hours.

Symptom evaluation

Each participant rated the intensity of the five main gastrointestinal symptoms (nausea, bloating, diarrhoea, borborygmi and abdominal pain), before and after the lactose challenge, on a 10-cm visual analogue scale ranging from 0 (no symptom) to 10 (maximum symptoms) [9]. Symptoms were associated to lactose malabsorption for a mean delta above or equal to 7.5/10 [9].

Pain topology was also evaluated. Participants reported, on a diagram, the pain intensity that they experienced after the lactose challenge, using a 10 point Likert scale ranging from 0 (none) to 9 (extreme): right hypochondrium (segment 1), epigastrium (segment 2), left hypochondrium (segment 3), right lumbar (segment 4), periumbilical (segment 5), left lumbar (segment 6), right iliac (segment 7), hypogastrium (segment 8) and left iliac (segment 9) (Figure 4).

Ethics

The study was conducted according to the Declaration of Helsinki, and approved by the Ethical Committee of Saint-Germain-en-Laye (Paris XI). Written informed consent was given by all participants before inclusion.

Statistical analysis

Results are presented as means ± SEM. Analyses were performed with either an ANOVA on repeated measures or a Student's t-test for unpaired data. The areas under the curves for RQ, glycaemia, HBT and symptoms were correlated by computing the Pearson's coefficients. The software used was SAS (version 9.1). The significance level of all statistical analyses was set at $p < 0.05$.

RESULTS

Participants characteristics

The study was carried-out on the 10 lactose absorbers (mean age: 29 ± 4 years old, men ratio: 40%) and the 10 lactose malabsorbers (mean age: 39 ± 4 years old, men ratio: 20%). No statistical difference was observed for the patients' baseline characteristics, even if a slightly higher mean age was noticed in the malabsorber group (Table 1).

Glycaemia

Peak and evolution of glycaemia over one hour after the lactose ingestion is reported in Figure 1. The average of the peak is 1.9 ± 0.2 mmol/ L blood in the absorbers group while it is 0.6 ± 0.1 mmol/ L blood in the malabsorbers group. The differences were highly significant ($p < 0.001$) since the status of the participants was based on this value. Time and time*status presented significant effects on glycaemia ($p < 0.001$ for both) and differences between the two groups became significant from 45 min.

Hydrogen Breath Test

Peak and evolution of expired hydrogen over three hours after the lactose ingestion is reported in Figure 2. An 8-ppm increase was noticed in the absorber group while a significant higher 40-ppm increase was noticed in the malabsorber group ($p = 0.0002$), with a peak reached from 150 min after the lactose ingestion. However, one lactose absorber (A6) presented a hydrogen peak of 23 ppm whereas one lactose malabsorber (M1) presented a hydrogen peak of 10 ppm. Time and time*status presented significant effects on glycaemia ($p < 0.001$ for both) and differences between the two groups became significant from 135 min.

RQ, Cox and Lox monitoring

The kinetics of RQ, Cox and Lox during 4 hours after the ingestion of lactose are reported in Figure 3. Time, status and time*status variables presented significant effects on RQ ($p < 0.001$, $p<0.001$, $p<0.001$ respectively), Cox ($p<0.001$, $p<0.001$, $p=0.004$ respectively) and Lox ($p<0.001$, $p=0.002$, $p<0.001$ respectively). RQ and Lox raised over the time, with a maximum reached around 60 min post-ingestion in both groups, but presented lower increases in the malabsorber group during 100 min post-ingestion ($p = 0.002$ and $p = 0.009$ respectively). Lox decreased over the time, with a minimum reached around 60 min post-ingestion in both groups, but presented a lower inhibition in the malabsorber group during 100 min post-ingestion ($p = 0.006$). The differences between the two groups were also significant over the short period of 30-50 minutes after the lactose load ($p = 0.001$ for RQ, $p = 0.002$ for Cox, $p = 0.001$ for Lox).

Symptoms evaluation

Symptom scores and localisation are presented in Figures 4. In the malabsorber group, the total score of symptoms was significantly higher ($p = 0.04$), with an increase in bloating ($p < 0.05$) and in the hypogastrium pain (segment 8) ($p = 0.02$). Indeed, during the test, five participants experienced symptoms with moderate to high intensity and two participants experienced symptoms with low intensity. No other symptom or segment appeared different between the two groups.

Correlations between parameters

The correlations between parameters are presented in Table 2. The correlations between RQ and traditional techniques are good indicators of RQ reliability in diagnosing lactose malabsorption. 1-hour RQ was positively correlated to glycaemia ($R = 0.74$, $p = 0.0002$) and negatively correlated to HBT ($R = -0.51$, $p = 0.03$) and to the total score of symptoms ($R = -0.46$, $p < 0.05$). Other parameters were also significantly correlated such as 1-hour Cox versus glycaemia ($R = 0.9$, $p = 0.003$) and HBT ($R = -0.99$, $p = 0.04$), 3-hour Cox versus hypogastrium pain ($R = -0.48$, $p = 0.04$), and 4-hour Cox versus hypogastrium pain ($R = -0.49$, $p = 0.03$).

Individual analyses

Results of all the completed outcomes, for each participant, are presented in supplementary data (S1). The following cut-offs were used according to the literature: a 1.5 mmol/ L peak for glycaemia [13], a 20 ppm peak for HBT [14] and a score of 8/10 for total symptoms [9]. A 0.05 peak was arbitrarily proposed for the 1 hour-RQ. In the absorber group, one participant

presented divergent results with RQ and glycaemia suggesting lactose absorption while HBT and score of total symptoms suggested malabsorption. In the malabsorber group, five participants were asymptomatic but four of them presented HBT, RQ and glycaemia indicating a malabsorption.

DISCUSSION

The objective of this exploratory study, performed on 10 absorbers and 10 malabsorbers of lactose, was to evaluate the reliability of monitoring RQ after a lactose ingestion to highlight malabsorption.

RQ, as well as Cox and Lox computed from RQ and energy expenditure, presented significant differences between absorbers and malabsorbers during 100 minutes after ingestion of the lactose load, confirming in humans results previously reported in rats. Indeed, normal lactose absorption leads to a switch in the metabolic pathways from lipid utilization in the fasted state to glucose utilization in the post-prandial state. On the opposite, lactose malabsorption leads to a moderate switch or even to no switch at all from lipid to carbohydrate utilizations. Furthermore, monitoring RQ over a short period after ingestion seems to be sufficient to detect malabsorbers. Indeed, differences in RQ changes were observed between absorbers and malabsorbers within 45 minutes after the lactose load ($p = 0.01$), supposing a putative diagnostic usefulness.

RQ changes, in particular the 1 hour-RQ changes were significantly correlated with traditional techniques, such as glycaemia, hydrogen expiration and symptoms. Besides, similar correlations were noticed between RQ and symptoms, and HBT and symptoms, suggesting that RQ could also be used to detect lactose intolerance. However, these two last correlations, RQ versus HBT and RQ versus total score of symptoms are low and clinical relevance may be questioned despite low *p*-values.

The total score of symptoms was significantly higher at the end of the test in the malabsorber group compared to the absorber group. The significant symptom reported by the malabsorber group was bloating, which confirms that bloating is the most sensitive symptom of lactose intolerance [19]. Besides, the major localisation of symptoms in the hypogastrium corresponds to the rectosigmoid and is linked to the significant increase of bloating.

The significant differences in the hydrogen expiration confirmed the correct allocation of participants in the two subgroups but raises questions for participants A6 and M1. The status

of participant A6 was confirmed by his glycaemia (1.8 mmol/L) and RQ (0.11) delta versus baseline. Such analysis led us to believe that patient A6 may not have fully respected the recommended residue-free diet, or may have had an undiagnosed intestinal bacterial overgrowth. The status of participant M1 was a bit more uncertain since different status could be determined according his glycaemia (0.3 mmol/L) and RQ (0.08) delta versus baseline. We also noticed that the RQ kinetics were not similar to those of other absorbers and presented abrupt peaks. Such irregular kinetics are more specific to hyperventilation than enhanced glucose oxidation [20,21]. Thus, considering that RQ peaks of participant M1 were an artefact, the kinetics would fit with their low glycaemia peak. Additionally, methano-production, occurring in 20% of the population, would account for the absence of both a hydrogen peak and gastro-intestinal symptoms. A second battery of tests, including the evaluation of methane expiration, would be necessary to confirm or rebut our diagnostic.

In conclusion, these two participants illustrated well the questionable reliability of HBT and our exploratory clinical study suggests that monitoring RQ may optimise the diagnostic of lactose malabsorption. However, we have to concede that the main limitation of RQ is the risk of hyperventilation, disturbing the kinetics. The baseline step should not be neglected and should last at least 30 minutes before the beginning of recording, which raises the problem of duration. The initial selection of lactose absorbers and malabsorbers participants must also be more restrictive, in order to prevent from wrong classification. To do so, an option is to combine the glycaemia tests and the HBT as inclusive criteria.

Future work will involve confirming the reliability of RQ for detecting lactose malabsorption in larger and more variable populations, such as participants presenting a glycaemia peak in the twilight zone, diabetics, patients with an intestinal resection, patients with a short transit time. Complementary studies would help to determine standard responses in each population, to identify the specific time of response in order to reduce the time window of RQ measurements, and to define a degree of malabsorption depending on the amplitude of the RQ changes. At last, complementary studies are essential to optimize the design of the protocol by defining specific points of measurements that would reduce the time under the canopy and discomfort for the patient, but would also allow simultaneous evaluation of 2 or 3 patients (as actually possible with the HBT).

ACKNOWLEDGEMENTS

We gratefully acknowledge Marie-Claude Amard and Philippe Gomez for their technical assistances, and Nachiket Nadkarni for his helpful English advice. We also thank Claire Bertin from Vivatech for financial support. No conflict of interest has been reported.

REFERENCES

[1 Jarvela IE: Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). Scand J Clin Lab Invest 2005;65:535-539.

2 Arola H: Diagnosis of hypolactasia and lactose malabsorption. Scand J Gastroenterol Suppl 1994;202:26-35.

3 Rosado JL, Solomons NW: Sensitivity and specificity of the hydrogen breath-analysis test for detecting malabsorption of physiological doses of lactose. Clin Chem 1983;29:545-548.

4 Pyleris E, Giamarellos-Bourboulis EJ, Tzivras D, Koussoulas V, Barbatzas C, Pimentel M: The prevalence of overgrowth by aerobic bacteria in the small intestine by small bowel culture: Relationship with irritable bowel syndrome. Digestive diseases and sciences;57:1321-1329.

5 Hovde O, Farup PG: A comparison of diagnostic tests for lactose malabsorption--which one is the best? BMC Gastroenterol 2009;9:82.

6 Koetse HA, Stellaard F, Bijleveld CM, Elzinga H, Boverhof R, van der Meer R, Vonk RJ, Sauer PJ: Non-invasive detection of low-intestinal lactase activity in children by use of a combined 13co2/h2 breath test. Scand J Gastroenterol 1999;34:35-40.

7 Vonk RJ, Lin Y, Koetse HA, Huang C, Zeng G, Elzinga H, Antoine J, Stellaard F: Lactose (mal)digestion evaluated by the 13c-lactose digestion test. Eur J Clin Invest 2000;30:140-146.

8 Vonk RJ, Stellaard F, Priebe MG, Koetse HA, Hagedoorn RE, De Bruijn S, Elzinga H, Lenoir-Wijnkoop I, Antoine JM: The 13c/2h-glucose test for determination of small intestinal lactase activity. Eur J Clin Invest 2001;31:226-233.

9 Casellas F, Varela E, Aparici A, Casaus M, Rodriguez P: Development, validation, and applicability of a symptoms questionnaire for lactose malabsorption screening. Digestive diseases and sciences 2009;54:1059-1065.

10 Casellas F, Aparici A, Casaus M, Rodriguez P, Malagelada JR: Subjective perception of lactose intolerance does not always indicate lactose malabsorption. Clin Gastroenterol Hepatol;8:581-586.

11 Kerlin P, Wong L: Breath hydrogen testing in bacterial overgrowth of the small intestine. Gastroenterology 1988;95:982-988.

12 Romagnuolo J, Schiller D, Bailey RJ: Using breath tests wisely in a gastroenterology practice: An evidence-based review of indications and pitfalls in interpretation. The American journal of gastroenterology 2002;97:1113-1126.

13 Fielding JF, Baynes S, Fottrell PJ: The lactose tolerance test and intestinal lactase activity. Irish journal of medical science 1983;152:196-198.

14 Casellas F, Malagelada JR: Applicability of short hydrogen breath test for screening of lactose malabsorption. Digestive diseases and sciences 2003;48:1333-1338.

15 Corazza GR, Sorge M, Strocchi A, Lattanzi MC, Benati G, Gasbarrini G: Methodology of the h2 breath test. Ii. Importance of the test duration in the diagnosis of carbohydrate malabsorption. Ital J Gastroenterol 1990;22:303-305.

16 Levine JA: Measurement of energy expenditure. Public Health Nutr 2005;8:1123-1132.

17 Levine JA, Schleusner SJ, Jensen MD: Energy expenditure of nonexercise activity. The American journal of clinical nutrition 2000;72:1451-1454.

18 Weissman C, Damask MC, Askanazi J, Rosenbaum SH, Kinney JM: Evaluation of a non-invasive method for the measurement of metabolic rate in humans. Clin Sci (Lond) 1985;69:135-141.

19 Beyerlein L, Pohl D, Delco F, Stutz B, Fried M, Tutuian R: Correlation between symptoms developed after the oral ingestion of 50 g lactose and results of hydrogen breath testing for lactose intolerance. Aliment Pharmacol Ther 2008;27:659-665.

20 Brandi LS, Bertolini R, Calafa M: Indirect calorimetry in critically ill patients: Clinical applications and practical advice. Nutrition 1997;13:349-358.

21 Lev S, Cohen J, Singer P: Indirect calorimetry measurements in the ventilated critically ill patient: Facts and controversies--the heat is on. Crit Care Clin;26:e1-9.

FIGURE LEGENDS

Figure 1: Glycaemia peak **(A)** and evolution **(B)** over one hour after the lactose ingestion. (▮), (●—): Lactose malabsorbers; (▯), (○—): Lactose absorbers. Data are presented as means ± SEM and are compared by a Student's test for unpaired data. (*): $p < 0.05$. Time, status and time*status effects are evaluated by an ANOVA. A p-value over 0.05 is considered as not significant. n = 20.

Figure 2: Expired hydrogen peak **(A)** and evolution **(B)** over three hours after the lactose ingestion. (▮) , (●—): Lactose malabsorbers; (▯),(○—): Lactose absorbers. Data are presented as means ± SEM and are compared by a Student's test for unpaired data. (*): $p < 0.05$. Time, status and time*status effects are evaluated by an ANOVA. A p-value over 0.05 is considered as not significant. ppm: part per million. n = 20.

Figure 3: RQ **(A)**, Cox **(B)** and Lox **(C)** kinetics over four hours after the lactose challenge. (●—): Lactose malabsorbers; (○—): Lactose absorbers. Data are presented as means ± SEM. Time, status and time*status effects are evaluated by an ANOVA. A p-value over 0.05 is considered as not significant. RQ, Cox and Lox changes are also compared over 100 minutes after the lactose challenge by a Student's test for unpaired data. (*): $p < 0.05$. n = 20.

Figure 4: Symptoms score amplitude **(A)** and abdominal pain topology **(B)**, at the end of the lactose challenge. (▯): Lactose absorbers; (▮): Lactose malabsorbers. Data are presented as means ± SEM and are compared by a Student's test for unpaired data. (*): $p < 0.05$. n = 20.