

## Amino acid uptake from a probiotic milk in lactose intolerant subjects

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This trial was designed to assess the effect of live probiotic consumption on leucine assimilation from fresh and pasteurised yoghurt in volunteers with different lactose digestibility. Thirty-three volunteers (mean age 32, s.d. 7 years) participated in this parallel single-blind study (16 of them with moderate lactose intolerance). Breath samples were taken before and at 15 min intervals over 3-h after the ingestion of fresh and pasteurised yoghurt extrinsically labelled with (1-<sup>13</sup>C)leucine. The <sup>13</sup>C enrichment in breath was measured by isotopic rate mass spectrometry and mathematically converted to a percentage of assimilated leucine (100-%<sup>13</sup>C-dose in breath) and the assimilation kinetic constant (min<sup>-1</sup>). The <sup>13</sup>C-leucine assimilation was statistically higher after the fresh yoghurt intake than after the pasteurised product intake ( $P = 0.032$ ) while the kinetic constant of assimilation was slower in intolerance status ( $P = 0.014$ ) although a product-related effect ( $P = 0.445$ ) was not found. In conclusion, fresh yoghurt intake resulted in higher short-term leucine assimilation, while lactose intolerance appears to negatively affect the assimilation rate of leucine from dairy products. These findings offer new insight on acute *in vivo* amino acid assimilation in the presence of probiotics and moderate lactose intolerance.

**Nutrient availability: Stable isotopes: Probiotic: Lactose intolerance**

Differences in the nutritional utilisation and health benefits of fermented milk compared with other dairy products have been attributed to the probiotic content of fermented milk, since live bacteria appear to increase nutrient digestibility<sup>1</sup> and immunocompetence<sup>2,3</sup>. These properties could be relevant in the context of dietary habits of people at a risk of some nutritional imbalance<sup>4</sup>. One such example are those with lactose intolerance, where milk and dairy product consumption is affected (decreased or avoided) since lactase-deficiency often produces adverse gastrointestinal symptoms after lactose intake<sup>5</sup>. Thus, research in the field of nutrition to optimise the assimilation of milk-derived components is of interest in subjects with this kind of pathological condition. Indeed, chronic consumption of yoghurt containing live bacterial cultures has been shown to ameliorate maldigestion in lactase-deficient subjects, who showed higher lactose absorption from fresh yoghurt than from a pasteurised product<sup>6</sup>. This observation has been related to the intake of fermented dairy products which induces changes in the equilibrium and metabolism of the intestinal microflora and may have beneficial effects on the host, mainly due to intraluminal processes in which the live probiotics seem to be involved<sup>6</sup>.

Taking into account these considerations, the maintenance of live probiotic microorganisms in these products would be crucial for the health benefits claimed for fermented milks, and storage of the products at approximately 4°C is required to maintain the probiotic load. The shelf life of yoghurt can be increased by pasteurisation but, by using this technology, the probiotic bacteria initially present are killed. In this case, the *in vitro* nutritional quality of the pasteurised yoghurt could be comparable to that of fresh yoghurt in some

instances, but the *in vivo* assimilation processes are likely to be changed.

Based on the potential differences in nutrient bioavailability from fresh and pasteurised dairy products, the aim of this nutritional intervention trial was to assess the effect of live probiotic cultures on the assimilation of nutrients, defined as the process of digestion and absorption in the gastrointestinal tract, with regard to amino acids.

### Methods

#### Subjects

Subject recruitment was devised to select a group of volunteers, homogeneous in respect of lifestyle and socio-economic characteristics. This sample was sourced from the volunteer database of the Department of Nutrition of the University of Navarra (UNAV), and through replies to newspaper and radio advertisements. Thus, 204 potential volunteers were contacted and eventually, forty volunteers (50% of them with mild to moderate lactose intolerance) were enrolled to participate in the trial. These volunteers were selected and monitored by a physician in the Department of Physiology and Nutrition of the UNAV and all were in apparent good health, as assessed by medical history, physical examination and routine blood analyses at baseline. The general inclusion criteria were: no history of metabolic, immune and/or gastrointestinal disease (apart from lactose intolerance), no current medication, no treated disease, no severe obesity (body mass index > 35 kg/m<sup>2</sup>), and no specific dietary regimen (apart from avoidance of dairy products in the case of those with lactose

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intolerance). Before starting the experimental period, 7 volunteers dropped out (lactose intolerant:  $n = 4$  and lactose tolerant:  $n = 3$ ). All participants gave their written informed consent to be involved in this experimental trial, which was previously approved by the local Ethics Committee at the UNAV (Ref. 3/2004).

Before beginning the intervention, a hydrogen breath test was carried out to confirm the degree of lactose intolerance of the volunteers, since only those who were lactose-tolerant and moderately lactose intolerant were included in the trial. The breath test was performed in the morning, after ingestion of 25 g lactose dissolved in 250 ml water. The hydrogen content in the breath was measured at baseline, with subjects in the fasting state, and after the lactose load at 15-minute intervals for four hours. The result of the test was considered positive when a 20 ppm increment in breath hydrogen and/or adverse gastrointestinal symptoms were detected during the test period<sup>1</sup>. Volunteers with severe symptoms were not enrolled in the trial for ethical reasons.

#### Trial design

For the ten days prior to the nutritional intervention (the wash-out period), volunteers did not consume fermented milk products. After that, the volunteers received three units per day (125 g per unit) of the assigned product (Table 1). The first arm of the study involved the fresh yoghurt intervention and the second arm, the pasteurised yoghurt intervention. Both treatments were administered in a single-blinded design, starting with the fresh product to maximize the total probiotic load in the cups of live yoghurt during the three days before the assimilation study during the acute intake measurements. The composition of both dairy products was similar from a nutritional point of view, but differed in micro-organism loads (Table 1).

The assimilation study started at 8:00 a.m. and was performed at rest after an overnight fast (12 h). The test breakfast given consisted of fresh or pasteurised yoghurt containing 2 mg/kg body weight of stable ( $1\text{-}^{13}\text{C}$ )leucine. Blood samples were taken before and after the product intake to monitor postprandial changes in serum concentration of glucose and insulin. Glucose was measured by a colorimetric assay (ABX, Germany), using a COBAS Mira autoanalyzer (Roche, Switzerland) and insulin by a commercially available RIA (DPC, USA). Other biochemical measurements

(circulating phosphorus, magnesium, calcium and phosphatase activity) were carried out following standard protocols.

Breath samples were collected before the ingestion and at 15-minute intervals for 180 minutes to measure the  $^{13}\text{CO}_2$  enrichment in breath. The  $^{13}\text{CO}_2$  enrichment was measured by isotopic rate mass spectrometry (Finnigan, Germany), and mathematically converted to a percentage of assimilated leucine (100-% $^{13}\text{C}$ -dose in breath), detecting the time of the maximal oxidation rate or  $T_{\text{max}}$  and the kinetic constant of leucine assimilation ( $\text{min}^{-1}$ ), that was calculated as the slope of the first portion of the oxidation curve<sup>7</sup>.

#### Statistical analysis

Kolmogorov-Smirnov and the Shapiro-Wilk tests were applied to explore the normal distribution of the variables. Comparison between parametric variables was evaluated using the Student *t*-test and analysis of variance (ANOVA), while Mann-Whitney U and the Wilcoxon matched pair non-parametric tests were performed when needed. Results were expressed with the mean  $\pm$  standard deviation and considered statistically significant if two-sided *P*-values were  $< 0.05$ . All statistical analyses were carried out using SPSS version 13.0 for Windows XP (Microsoft, USA).

#### Results

At baseline, the lactose intolerance group and the control volunteers showed comparable nutritional status, as assessed by the biochemical analyses performed in blood (data not shown). Interestingly, the lactose intolerant volunteers had statistically lower plasma phosphorus ( $3.8 \pm 0.5$  mg/dl vs  $3.3 \pm 0.5$  mg/dl;  $P = 0.011$ ) and marginally lower magnesium concentrations ( $2.0 \pm 0.1$  mg/dl vs  $1.9 \pm 0.1$  mg/dl;  $P = 0.058$ ) than the control group. These results could be due to the common low consumption of milk and dairy products in lactose intolerant people. However, these values were in the normal healthy range and no differences were detected between groups for circulating calcium ( $9.7 \pm 0.3$  mg/dl vs  $9.4 \pm 0.3$  mg/dl;  $P = 0.212$ ) and alkaline phosphatase activity ( $58 \pm 15$  UI/l vs  $57 \pm 17$  UI/l;  $P = 0.912$ ). Therefore, the nutritional status of both groups was considered comparable at baseline.

Lactose intolerant volunteers showed an apparently slower carbohydrate uptake from the fresh yoghurt than from the pasteurised product, since one hour after the ingestion an increased glycaemia was still detected only after the fresh product intake and in the lactose intolerant group ( $P = 0.031$ ). In agreement with this, the circulating insulin was more markedly increased at 60 minutes after the ingestion of the fresh product, accompanying the carbohydrate absorption in this group ( $P = 0.002$ ). The observed product-related effect was not detected in the control group of lactose tolerant volunteers ( $P = 0.604$ ).

With respect to the leucine assimilation test, no statistical differences were detected in the time of the maximal oxidation rate or  $T_{\text{max}}$  (Fig. 1) depending on the product ingested in the control group ( $P = 0.651$ ) as well as in the lactose intolerant subjects ( $P = 0.272$ ). Also, no differences between both calculated assimilation kinetic constants, depending on the ingested product were seen (Table 2). However, the  $^{13}\text{C}$ -leucine assimilation measured as the non excreted % $^{13}\text{C}$ -dose at

**Table 1.** Description of the products according with the promoter supplied values

Product description	Fresh yoghurt	Pasteurised yoghurt
Weight per unit (g)	125	125
<i>L. bulgaricus</i> (CFU/g)	$> 10^8$	$< 10$
<i>L. thermophilus</i> (CFU/g)	$> 10^8$	$< 10$
Lactic acid and lactate (mg/kg)	8-832	8-755
Saccharose (%)	8.5	8.1
Lactose (%)	5.1	5.4
Calcium (mg/kg)	1.111	1.154
Total carbohydrates (%)	14.94	15.14
Total lipids (%)	1.99	1.91
Total proteins (%)	3.05	2.83

180 min after the test meal ingestion (Table 2), was significantly higher after the intake of the fresh product than after the pasteurised product in both experimental groups ( $P = 0.035$  and  $P = 0.002$  for tolerant and intolerant groups, respectively).

Finally, statistically higher  $^{13}\text{C}$ -leucine assimilation ( $P < 0.001$ ) was detected after the fresh yoghurt intake (Fig. 1) with no differences detected in the calculated assimilation kinetic constant ( $P = 0.348$ ) between the two ingested products. As a result, the kinetic constant of the assimilation process was significantly slower in the intolerant group ( $P = 0.014$ ), with no changes related to the ingested product ( $P = 0.445$ ). However, a clear product-related effect was found ( $P = 0.032$ ) in the percentage of assimilated leucine, expressed as the  $^{13}\text{C}$ -leucine that was retained during 180 minutes after the product ingestion (Fig. 1).

## Discussion

Lactose intolerance is a relevant factor influencing dietary habits, since lactase-deficiency often produces adverse gastrointestinal symptoms after lactose intake and, as a result, lactose intolerant people are at particular risk of some nutritional deficits due to their need to avoid a wide range of foods that contain lactose<sup>8</sup>. It has been proposed that probiotics increase the digestibility of food in these patients<sup>9</sup>. Hence, the purpose of the present trial was to compare acute leucine assimilation, with regard to the presence of live probiotic in fermented milk, evaluating the potential involvement of lactose intolerance in this process.

Tolerance of fresh yoghurt in lactose intolerant people has been described, in which an increased hydrolysis of the carbohydrate and a slowed absorption are some of the hypothesised mechanisms involved<sup>10</sup>. In agreement with this, the results observed here point toward slower glucose assimilation with fresh yoghurt intake compared with the pasteurised product. Thus, these results might indirectly indicate the fact that nutrient assimilation from fresh yoghurt is easier than from the pasteurised one, especially in people with dysfunction in the absorption process since the observed effect was evident in lactose intolerant subjects. Moreover, the peak in plasma glucose that was detected in the intolerant group after the fresh yoghurt intake could be related to a delayed breakdown of lactose that has been described as a mechanism to explain the tolerance of fresh yoghurt in people with this dysfunction<sup>11</sup>. Therefore, these results suggest the potential role of a live probiotic load in improving short-term nutrient assimilation.

In order to test if this finding extended to amino acids,  $^{13}\text{C}$ -leucine was used as a tracer to measure its assimilation depending on whether the probiotic load was alive or not in the fermented milk ingested. Labelling nutrients with  $^{13}\text{C}$  is a valuable tool to trace their metabolic fate<sup>6</sup>. Thus, the utilisation of lysine from wheat in comparison to milk has been studied by  $^{13}\text{C}$ -leucine infusion in the postabsorptive and postprandial states after ingestion of a test meal<sup>12</sup>. In fact, test meals prepared with products labelled with  $^{13}\text{C}$ -tracer, such as eggs ( $^{13}\text{C}$ -leucine)<sup>13</sup>, are suitable for the study of assimilation and requirements of nutrients.

The  $^{13}\text{C}$ -breath test is based on the oxidation of the labelled tracer (amino acid) that appears once the assimilation process (digestion and absorption) occurs. The breath test measures the limiting step in  $^{13}\text{CO}_2$  breath enrichment, because of the quick elimination of the  $^{13}\text{CO}_2$ , produced depending on the amino acid absorption<sup>14</sup>. We performed the (1- $^{13}\text{C}$ )leucine breath test to study the amino acid assimilation from fresh yoghurt vs the pasteurised product, since the leucine is immediately absorbed in the proximal intestine after gastric emptying occurs. After that, the (1- $^{13}\text{C}$ )leucine is specifically decarboxylated by a dehydrogenase complex, producing  $^{13}\text{CO}_2$ . Therefore, the  $^{13}\text{C}$ -enrichment in breath depends on dehydrogenase activity that is modulated by individual factors, such as protein turnover, or amino acid assimilation<sup>12,15</sup>. The results of the breath test could be modified by the gastric emptying<sup>16</sup>, although this rate was assumed to be constant since no statistical differences ( $P = 0.651$  for the control group and  $P = 0.272$  for lactose intolerant subjects) were detected in the time of the maximal oxidation rate or  $T_{\text{max}}$  (Fig. 1).

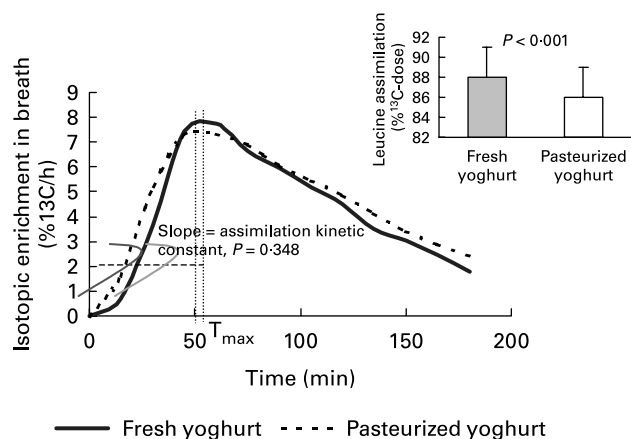
Based on this, the [1- $^{13}\text{C}$ ] leucine breath test could detect differences in short-term amino acid assimilation depending on the ingested product, fresh or pasteurised yoghurt, in which the tracer was incorporated. Dehydrogenase activity and protein turnover were considered constant since the test was performed within the same subjects. Thus, no differences between both calculated assimilation kinetic constant were detected depending on the ingested product (Table 2). However, after the fresh yoghurt intake the  $^{13}\text{C}$ -leucine assimilation, measured as the non excreted % $^{13}\text{C}$ -dose at 180 min after the test meal ingestion, was statistically higher than after the pasteurised product intake in both experimental groups (Table 2). These data suggest a slightly higher acute bioavailability of leucine from the fresh yoghurt as compared with the pasteurised yoghurt after fermentation.

Since the trend towards a higher leucine assimilation after the fresh product ingestion was detected in both groups, the

**Table 2.** Parameters describing the assimilation process of (1- $^{13}\text{C}$ )leucine measured by the breath test during 180 minutes after the ingestion of fresh yoghurt and pasteurised yoghurt extrinsically labelled with the stable isotope, in volunteers with and without lactose intolerance

	Lactose tolerants ( $n = 17$ )		Lactose intolerants ( $n = 16$ )	
	Fresh yoghurt Mean (sd)	Pasteurised yoghurt Mean (sd)	Fresh yoghurt Mean (sd)	Pasteurised yoghurt Mean (sd)
$^{13}\text{C}$ -leucine assimilation				
Assimilated $^{13}\text{C}$ -leucine (% $^{13}\text{C}$ -dose)	87 (3)	86 (3)*	88 (2)	86 (3)*
Kinetic constant of assimilation ( $\text{min}^{-1}$ )	0.348 (0.11)	0.380 (0.12)	0.274 (0.13)	0.292 (0.14)

\*  $P < 0.05$  as compared to fresh yoghurt Wilcoxon matched test.



**Fig. 1.**  $[1-^{13}\text{C}]$  leucine oxidation and assimilation after the ingestion of fresh yoghurt and pasteurised yoghurt extrinsically labelled with the stable isotope in all volunteers (with and without lactose intolerance) \*Wilcoxon matched test.

statistical analysis of data was performed including volunteers with and without lactose intolerance. In this way, no differences in the calculated assimilation kinetic constant were found, depending on the ingested product, while the highest  $^{13}\text{C}$ -leucine assimilation was detected after the fresh yoghurt intake (Fig. 1). Thus, this research showed that this short-term assimilation process was improved when fresh yoghurt is consumed, especially in those with moderate lactose intolerance, which is in accordance with data concerning calcium assimilation<sup>17</sup>.

In conclusion, fresh yoghurt intake resulted in higher acute leucine assimilation than during intake of the pasteurised product. Considering that the new health claims on probiotics require further scientific studies to determine *in vivo* the potential differences in nutritional value between fresh and heated yoghurt<sup>18</sup>, the outcome of this trial provides new insights on the acute *in vivo* leucine assimilation from yoghurt, depending on whether lactic acid bacteria are alive or not in the product in volunteers with or without lactose intolerance symptoms.

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