Original Research

Acute Calcium Assimilation from Fresh or Pasteurized Yoghurt Depending on the Lactose Digestibility Status

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Key words: calcium assimilation, stable isotopes, yoghurt, lactose maldigestion

Objective: The major aim of this trial was to evaluate the potential interaction of fresh or pasteurized yoghurt intake with lactose intolerance on calcium assimilation by means of the stable isotope ^{43}Ca as a tracer.

Methods: Forty volunteers (age: 32 ± 7 years) participated in this parallel simple blind study (20 of them with moderate lactose intolerance). The protocol included the intake of a test meal consisting on ^{43}Ca-labelled fresh or pasteurized yoghurt. Volunteers, in whom the calcium status was assessed, collected the 24-h urine before and after the test meal to measure the stable isotope output. The intake-related ^{43}Ca enrichment in urine was measured by isotopic rate mass spectrometry.

Results: In lactose tolerant and intolerant volunteers taken together, the fresh yoghurt consumption resulted in a statistically higher circulating calcium levels ($p = 0.028$) and urinary ^{43}Ca output ($p = 0.017$) than after the pasteurized yoghurt intake. The lactose maldigestion status resulted in higher urinary ^{43}Ca excretion ($p = 0.013$) after the fermented milk consumption, regardless of the nature of ingested product ($p = 0.887$).

Conclusions: This novel and non-aggressive protocol allowed the *in vivo* comparison of calcium utilization from two different dairy sources, revealing a higher acute calcium assimilation from fresh as compared to the pasteurized yoghurt, in both lactose digesting and maldigesting subjects.

INTRODUCTION

The nutritional value of calcium is mainly attributed to the key role played on bone growth and homeostasis [1]. However, calcium is additionally involved in numerous biological processes such as Ca-mediated signal transmission [2], body weight regulation [2, 3] or insulin function [4].

The metabolic involvement of this mineral makes of interest to establish daily requirements [5], and to assess calcium bioavailability from different food sources [6, 7], as well as in different biological and physiological states, like pregnancy [8], childhood [9], menopause [10] or poor lactose hydrolysis capacity (lactose maldigestion), including lactose intolerance (accompanied by clinical symptoms after lactose intake) [11].

Dairy products, specifically milk, yoghurt and cheese, provide most of calcium in the typical Western diet [12]. Indeed, a number of studies have been published using different methods to evaluate calcium bioavailability from these sources [6, 13–14]. In fact, calcium absorbability, or the availability of calcium for absorption by the intestines, is the first step towards bioavailability, which also depends on incorporation of absorbed calcium into bone, urinary excretion and faecal loss of endogenous calcium, physiological factors, particularly hormones, and certain types of food [15].

Single meal studies have been performed in humans to measure calcium absorption using tracer methods, by including a radioisotope in the test meal. In this way, radioactivity is monitored in the whole body [16] or from blood samples [17] to assess calcium assimilation, defined as the process of digestion and absorption in the gastrointestinal tract [18].

The hazards related to radioactivity led to devise methods applying stable isotopes as tracers to evaluate calcium utilization from different foods, following different approaches [19–21]. Among them, stable isotope single-tracer protocols have been developed to perform comparative analyses of *in vivo* calcium availability from different foods [17].

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Based on these assumptions, the aim of the present study was to evaluate the impact of moderate lactose maldigestion on calcium assimilation (digestion plus absorption in the gastrointestinal tract), comparing the effect of live yoghurt or pasteurized yoghurt intake by using the stable isotope ^{43}Ca as tracer.

MATERIAL AND METHODS

Subjects

The recruitment pattern was devised to include a homogeneous group of volunteers concerning lifestyle and socio-economic characteristics. This process was carried out from the volunteer database of the Department of Physiology and Nutrition of the University of Navarra and through newspaper and radio advertisements. Volunteers were selected and monitored by a physician at the Department of Physiology and Nutrition of the University of Navarra. All were in apparent good health, as assessed by medical history, physical examination and routine blood analyses at baseline. General inclusion criteria were no history of metabolic, immune and/or gastrointestinal disease, no current medication, no severe obesity (body mass index >35 kg/m^2).

Forty volunteers (50% of them with mild to moderate lactose maldigestion) were enrolled to participate in this trial after this biochemical characterisation (Table 1). Before the inclusion, a hydrogen breath test was carried out to confirm the lactose maldigestion degree of volunteers, since only lactose tolerant and volunteers with moderate lactose maldigestion were allowed to be included in the trial. The breath test was performed by ingestion of 25g lactose dissolved in 250 ml water [22] after a night fast (dinner at 20:00h–21:00h), beginning between 8:00h and 9:00h. The hydrogen content in breath was measured by an H_2-specific electrochemical sensor (EC60 Gastrolyzer, Bendfont, UK), at baseline and after the lactose intake, at 15-minute intervals during four hours. The result was considered as positive when a 20ppm increment in breath hydrogen and/or symptoms were detected during the test period [23]. Volunteers with severe lactose maldigestion symptoms or discomfort were not enrolled in the trial based on ethical reasons. Dietary milk and dairy product intake was asked during the medical history in order to assure that the included volunteers were able to consume three yoghurts per day during three days without discomfort and a diary to register adverse effects was given to volunteers. The physician revised the diary during the visits at the Metabolic Unit to assure that no adverse effects were reported. Enrolled participants ($n = 40$) gave their written informed consent to be involved in this experimental trial, which was previously approved by the local Ethics Committee at the University of Navarra (Ref. 3/2004). Finally, the effect of lactose malabsorption on calcium assimilation from fresh and pasteurized yoghurt was analyzed by using the experimental data obtained from thirty-two volunteers, since eight participants did not complete the trial.

Trial Design and Measurements

Ten days before the nutritional intervention (adaptation period), volunteers were not allowed to consume fermented milk products. After that time, the volunteers received three cups per day of the assigned product (125g per unit). The first part of the study involved the fresh yoghurt intervention (starter ferments content: *L. bulgaricus* $>10^8$ CFU/g) and *L. thermophilus* $>10^8$ CFU/g) and the second part, the pasteurized yoghurt intervention (starter ferments content: *L. bulgaricus* <10 CFU/g and *L. thermophilus* <10 CFU/g). Both treatments were administered in a simple-blind pattern (Fig. 1), beginning with the fresh product intervention.

At inclusion, serum levels of glucose, insulin and lipid profile were measured by automatized analyses (ABX, USA) using a COBAS MIRA equipment (Roche, Switzerland), as biochemical markers of nutritional status. In order to evaluate

Table 1. Groups Description and Biochemical Parameters at Baseline

Descriptive parameters	LACTOSE TOLERANTS	LACTOSE MALABSORBERS	Laboratoy reference range	Comparison between groups (p value)
Gender distribution (women/men)	9/7	9/7	-	-
Age (years old)	32 ± 7	34 ± 9	-	0.409
Body mass index (kg/m^2)	23.5 ± 2.5	24.9 ± 4.7	-	0.275
pl-Glucose (mg/dL)	87 ± 13	85 ± 8	80–110	0.548
pl-Insulin (microU/mL)	7.6 ± 3.8	6.5 ± 3.6	< 25	0.484
srm-Tryacilglycerol (mg/dL)	75 ± 24	98 ± 59	< 170	0.154
srm-Cholesterol (mg/dL)	204 ± 40	197 ± 28	< 240	0.549
srm-HDL-c (mg/dL)	59 ± 11	57 ± 16	> 40	0.632
srm-LDL-c (mg/dL)	130 ± 51	120 ± 29	< 160	0.455
srm-Calcium (mg/dL)	9.7 ± 0.3	9.4 ± 0.3	8.1–10.4	0.212
srm-Phosphorus (mg/dL)	3.8 ± 0.5	3.3 ± 0.5	2.7–4.5	0.011
srm-Magnesium (mg/dL)	2.0 ± 0.1	1.9 ± 0.1	1.7–2.5	0.058
srm-Alkaline phosphatase (UI/L)	58 ± 15	57 ± 17	42–128	0.912

Product description	FRESH YOGHOURT	PASTEURIZED YOGHURT
Weight per unit (g)	125	125
L. bulgaricus (CFU/g)	$>10^8$	<10
L. thermophilus (CFU/g)	$>10^8$	<10
Lactic acid and lactate (mg/kg)	8.8	8.8
Sucrose (%)	8.5	8.1
Lactose (%)	5.1	5.4
Calcium (mg/kg)	1.1	1.1
Total carbohydrates (%)	14.9	15.1
Total lipids (%)	1.9	1.9
Total proteins (%)	3.1	2.8

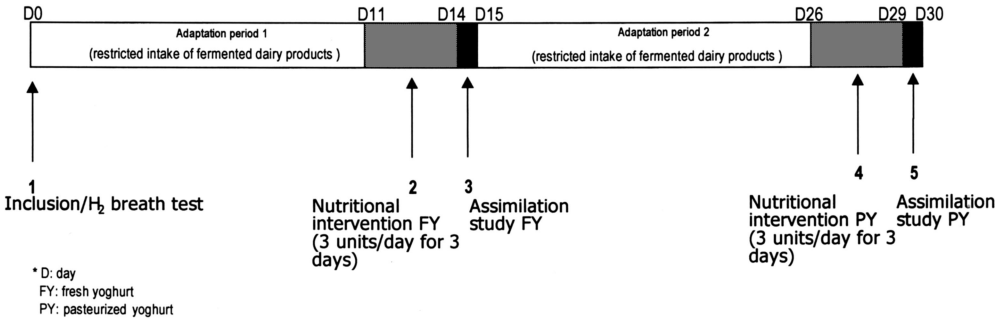

Fig. 1. Product composition and trial design.

the general status of calcium metabolism, blood levels of calcium, magnesium and phosphorus were measured by colorimetric methods (Beckman, USA), as well as the alkaline phosphatase activity (Beckman, USA) and the 24h-urinary calcium output (Beckman, USA).

The calcium assimilation study started at 8:00 a.m. and was performed after an overnight fast. The protocol included the ingestion of the assigned test meal, fresh or pasteurized yoghurt, which contained 0.013mg/kg body weight of $^{43}CaCO_3$ extrinsically incorporated by addition of the tracer to the yoghurt and homogenisation two hours before the intake as described elsewhere [24]. Blood was taken before and at 60 minutes after the ingestion of the test meal [10] to measure circulating calcium. The total calcium absorbed one hour after the ingestion was calculated by the trapezoidal area procedure: [(blood calcium (mg/dl) at fasting state + blood calcium (mg/dl) at postprandial time)/2] × sampling period (h).

Volunteers collected the 24-h urine sample before and after the labelled product ingestion to measure the ^{43}Ca-enrichment in urine in relation to baseline excretion values (24h-urine sample recovered before the product ingestion). The ^{43}Ca enrichment in urine was measured by isotopic rate mass spectrometry (Finnigan, Germany), and was mathematically transformed into the percentage of excreted tracer (%^{43}Ca). Eight volunteers separately performed the whole experimental trial without ^{43}Ca in the ingested product to take into account the natural isotope content in urine.

Statistical Analysis

The Kolmogorov-Smirnov and the Shapiro-Wilk tests were applied to explore normality of the variables. Comparison between parametric variables was evaluated using the Student *t*-test, while non-parametric variables were analyzed by the Mann-Whitney U test and the Wilcoxon matched pair test. In agreement with the experimental design, a factorial 2×2 analysis of the variance was performed to examine potential interactions between the product intake and the lactose maldigestion status based on its known robustness. Results were expressed as the mean ± standard deviation and considered statistically significant if two-sided *P*-values were <0.05. All statistical analyses were carried out using the SPSS 11.0 version for Windows 98 (SPSS Inc., Chicago, USA).

RESULTS

Baseline Measurements

At baseline, the lactose maldigesting group and the control volunteers showed comparable nutritional status, as assessed by the biochemical analyses performed in blood (Table 1). However, the lactose maldigesters had plasma phosphorus statistically lower ($p = 0.011$) and magnesium levels marginally lower ($p = 0.058$) than the control group, but all within the normal laboratory healthy reference range. No differences were detected between groups for circulating calcium ($p = 0.212$) and alkaline phosphatase activity ($p = 0.912$). Therefore, both groups were considered as nutritionally comparable at baseline.

Serum Calcium after Product Intake

The experimental design to evaluate the *in vivo* calcium assimilation included the measurement of circulating calcium

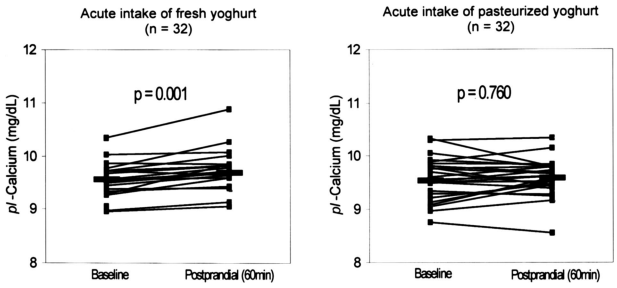

Fig. 2. Product-related effect (mean value is indicated with a thick line) for the change in blood calcium at 60-minute after the intake of fresh or pasteurized yoghurt found in subjects with (n = 16) and without (n = 16) lactose maldigestion.

changes in blood at 60 minutes after the yoghurt intake. In the lactose maldigesters, plasma levels of calcium statistically increased after the fresh yoghurt ingestion (9.6 ± 0.3 mg/dL *vs* 9.9 ± 0.4 mg/dL; p = 0.003) with no changes after the pasteurized product intake (9.4 ± 0.5 mg/dL *vs* 9.4 ± 0.4 mg/dL; p = 0.854). With respect to lactose digesting volunteers, circulating calcium marginally increased (9.4 ± 0.3 mg/dL *vs* 9.6 ± 0.4 mg/dL; p = 0.094) after the fresh yoghurt consumption, while no statistical changes were detected after the pasteurized yoghurt intake (9.6 ± 0.3 mg/dL *vs* 9.7 ± 0.3 mg/dL; p = 0.559). Taken together in both groups of subjects accordingly with the 2×2 factorial design, with and without lactose maldigestion, circulating calcium markedly increased (p = 0.001) one hour after the fresh yoghurt intake, while no changes (p = 0.760) were detected after the pasteurized consumption at the same postprandial time (Fig. 2).

Furthermore, according to the experimental design, the analysis of the data (Table 2) evidenced the statistically (p = 0.028) significant product-related effect, while no effect (p = 0.367) was found concerning lactose maldigestion nor product-lactose maldigestion interaction (p = 0.893).

Urinary ^{43}Ca Urine Enrichment after Product Intake

Daily calcium excretion in urine was measured to assure the steady state for included volunteers. No differences were detected in total urinary calcium before the intake of products (before fresh yoghurt intake: 156 ± 78 mg/24h *vs* or before pasteurized yoghurt intake 150 ± 56 mg/24h; p = 0.715). Similarly, no changes in urinary calcium content were found after the experimental product intake (156 ± 60 mg/24h *vs* 169 ± 62 mg/24h; p = 0.349). Furthermore, the experiment carried out in eight volunteers with no labelled products showed that the ^{43}Ca enrichment in urine was negligible (0.2 ± 0.9% *vs* 0.3 ± 0.9%; p = 0.203) after the unlabelled fresh or pasteurized yoghurts intake (fresh yoghurt: .0.17 ± 0.91% pasteurized yoghurt: 0.31 ± 0.89%; p = 0.203). This result assured that the chosen tracer dose was enough to detect changes related to ^{43}Ca assimilation. In contrast, taken together lactose tolerant and intolerant volunteers, the fresh yoghurt consumption involved a statistically higher urinary ^{43}Ca-enrichment (2.97 ± 1.02% vs 2.53 ± 0.82%; p = 0.017).

The analysis of the variance marginally showed significant differences (p = 0.095) in urinary ^{43}Ca enrichment depending on product intake (Table 2). After the labelled product consumption, the lactose maldigestion status involved different (p = 0.020) urinary ^{43}Ca enrichment, regardless (p = 0.861) of the fresh or pasteurized yoghurt intake (Table 2), so volunteers with moderate lactose maldigestion showed a high (p = 0.021) urinary ^{43}Ca enrichment (Fig. 3).

DISCUSSION

Lactose maldigestion is a relevant factor influencing milk and dairy product consumption, since lactase-deficiency often produces gastrointestinal symptoms after lactose intake [25]. Unless other calcium enriched foods are regularly consumed, milk and other dairy products are the commonest sources, and limiting consumption of these foods can increase the risk of inadequate intake of this mineral [26, 27]. As a result, most of lactose intolerant or maldigester people are at particular risk of calcium intake below recommended levels [25]. Moreover, reduction in calcium absorption has been described in lactose intolerant people, who improved the mineral assimilation by the intake of dairy products containing lactose [27]. Hence, the

Table 2. Data from the Calcium Assimilation Study Concerning Serum Calcium Changes and Urinary Calcium Excretion in Lactose Tolerant and Intolerant Volunteers after Fresh or Pasteurized Yoghurt Intake

Calcium assimilation (changes after intake)	LACTOSE TOLERANTS (n = 12)		LACTOSE MALABSORBERS (n = 12)		Analysis of the variance (p-value)		
	Fresh yoghurt	Pasteurized yoghurt	Fresh yoghurt	Pasteurized yoghurt	Product-related effect	Lactose maldigestion-related effect	Interaction: product × lactose maldigestion
srm-calcium (mg/dL × h)	2.04 ± 2.18	0.15 ± 2.58*	1.49 ± 2.95	0.35 ± 2.18	p = 0.028	p = 0.367	p = 0.893
Urinary calcium change (%)	8.7 ± 48	5.5 ± 39	14.6 ± 51	16.1 ± 36	p = 0.969	p = 0.482	p = 0.801
Urinary ^{43}Ca-enrichment (%dose)	2.6 ± 0.5	2.2 ± 0.8	3.3 ± 1.2	2.8 ± 0.7$^{\#}$	p = 0.095	p = 0.020	p = 0.861

Comparison between products: * $p < 0.05$; $^{\#}$ $p < 0.10$.

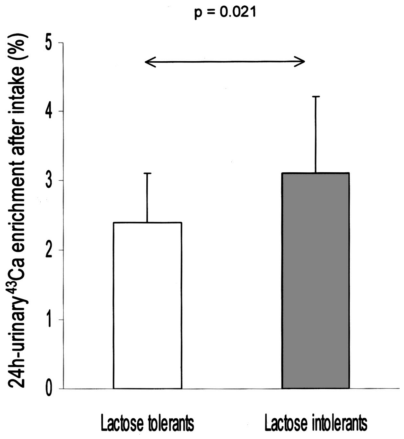

Fig. 3. Effect of lactose maldigestion in ^{43}Ca enrichment (mean and standard deviation) assessed in 24-hour urine after the ingestion of fresh or pasteurized yoghurt in 24 volunteers, twelve with lactose tolerance and twelve with moderate lactose maldigestion (n = 12).

purpose of the present trial was to compare the calcium assimilation after fresh or pasteurized yoghurt intake, evaluating the potential involvement of lactose maldigestion in this process.

As previously described, lactose maldigesting subjects could benefit from improved intake of calcium-rich non-dairy foods or specific dairy products if they are well tolerated, as yoghurt [29]. However, calcium utilization depends upon individual factors, such as the lactase-deficiency degree as well as on food characteristics [30]. Thus, several studies have shown that yoghurt is better tolerated than milk [11], because some lactase activity from yoghurt bacteria could participate in lactose digestion, as well as the delayed oro-cecal transit time [23, 31]. Therefore, yoghurt could allow lactose maldigesting people to comfortably consume a dairy food naturally rich in calcium.

Under our experimental conditions, the acute calcium uptake from the fresh yoghurt was higher than from the pasteurized, as evidenced by the increase in circulating plasma levels of calcium one hour after intake [31]. The heat treatment of yoghurt to increase the shelf live of the product could diminish the effect on lactose digestibility due apparently to enzymatic inactivation [32], together with the proposed effect on oro-caecal transit time, described as shorter for the pasteurized yoghurt [23]. This finding could be partially explained because calcium assimilation seems to be enhanced by lactose absorption [11]. Moreover, changes in the structure of the yoghurt related to the pasteurization process could also modify the calcium disposal. In fact, some proteins are denaturated and aggregated during the heating process, and a non-specifically binding to calcium has been described, decreasing the availability of the mineral to be absorbed [33].

Urinary calcium output can be interpreted as an indirect marker of retention, since calcium homeostasis in adults involves that the entry of this mineral from the gut equals the urinary calcium excretion on a daily basis, since other excretion routes are considered unchanged in normal subjects [34]. Based on this statement, results obtained from circulating plasma levels after intake could be supported by urinary analyses, if a steady state of calcium turnover was reached [34]. In order to maintain a comparable dynamic equilibrium, volunteers followed a adaptation period, in which the fermented dairy product consumption was avoided before the experimental intervention [35]. With respect to bone metabolism, calcium is controlled with a slow turnover, so it could be considered under strict balance with the exception of children/childhood, pregnant or ageing people, who were not included in the study. Thus, bone turnover was considered constant during the experimental period. Moreover, the study involved intra-subject comparisons (pair tests) and the experiments were carried out within 30 days. Hence, total calcium excretion in urine was considered constant and a marker of the balanced status in calcium metabolism during the intervention period.

After the acute intake of both fresh and pasteurized products, the urinary calcium output was higher in lactose maldigesting subjects as compared to controls. This observation could reflect the habitual low intake of calcium from milk products in subjects with lactose maldigestion and a lower percent absorption demands in this people [25].

We completed the assimilation study of calcium by using the single labelling method [16]. In order to reduce time-consuming procedures, aggressiveness and economic cost, we carried out a stable isotope single-tracer protocol to perform comparative analyses *in vivo* of calcium availability from different foods, following the principles of single meal studies by including a isotope in the test meal [17]. The ^{43}Ca was the selected tracer based on non hazardous and non radioactive features and the possibility to quantify the enrichment in urine with a high degree of accuracy [24]. After the calcium balance homogenization of volunteers, the acute ingestion of the single-labelled yoghurt was carried out, considering changes on ^{43}Ca enrichment in urine after the test yoghurt intake as an indirect marker of calcium assimilation. The tracer dose was fixed based on previous works [24]. The natural isotope content of volunteers, as well as the isotope enrichment due to the tested products could affect the assay. However, no changes in ^{43}Ca-urine content were detected after the non-labelled product intake, suggesting that the adjusted tracer dose could be able to detect changes in ^{43}Ca enrichment in urine depending on tracer assimilation [17].

The trend of a higher calcium excretion after the fresh yoghurt intake as compared to the pasteurized fermented milk, confirmed the observed increase in blood calcium levels, suggesting a higher acute bioavailability, which may be of importance in lactose intolerance as has been found for short-term leucine assimilation [36].

CONCLUSION

The comparative study concerning these two products showed that calcium assimilation was improved in the fresh as

compared to the pasteurized fermented milk intake, in both lactose digesting and maldigesting subjects. Therefore, yoghurt containing live ferments can be considered as an important calcium source, since it could involve an optimised calcium assimilation, although further investigations are needed to confirm these results and their possible implications for human health.

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