

A regular curd consumption improves gastrointestinal status assessed by a randomized controlled nutritional intervention

*Santiago Navas-Carretero, Itziar Abete, Marta Cuervo, M. Ángeles Zulet, J. Alfredo Martínez**

Department of Nutrition, Food Science, Physiology and Toxicology. University of Navarra, 31008 Pamplona, Navarra. Spain

*** Corresponding Author:** J Alfredo Martínez. Department of Nutrition, Food Science, Physiology and Toxicology. University of Navarra. C/ Irunlarrea s/n, 31008 Pamplona, Navarra, Spain. Phone: +34 948 42 56 00. ext. 6424 E-mail: jalfmtz@unav.es

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ABSTRACT

This study evaluated the influence of curd consumption (a dairy product in which most whey proteins are discarded) on nutritional status markers and on gastrointestinal symptoms through an open label randomised nutritional intervention. A total of 20 males and 20 females were involved in the study. Body weight and plasma levels of different health markers were measured at baseline and the end of the study.

Gastrointestinal symptoms and satiety were assessed by self-reported subjective questionnaires. There were no relevant changes in body weight and composition, neither in all screened plasma determinations after the intervention. Satiety score analyses revealed no differences between the two experimental groups. The regular consumption of curd improved abdominal pain (19%) and deposition scores (16%) when compared with those participants non-consuming curd, which may indicate a better tolerability of this product. Curd intake within a balanced diet improved some subjective markers of gastrointestinal status, which may be explained by the nutritional composition of curds.

Introduction

A systematic review of the socioeconomic differences in food habits revealed that cheese and milk are highly consumed all around Europe (Sanchez-Villegas et al., 2003). The inclusion of functional dairy foods in a conventional diet have been shown to improve the nutrient profile (Berasategi et al., 2010).

Additionally, derived/fermented dairy products such as yogurt, kefir, kumis, cheese, curd, buttermilk, etc. are highly appreciated not only by their nutritional value, but also because they may potentially provide specific healthy benefits to alleviate malnutrition status (Ferry, 2011), overcome lactose intolerance (Brown-Esters et al., 2011, Parra, D. and Martinez, 2007) or to treat gastrointestinal disturbances (Alvaro et al., 2007), allergies (Boyle and Tang, 2006), infections (Zanini et al., 2007), etc. Indeed, the consumption of different fermented kind of milks have been found to be a useful and healthy approach to ensure covering the nutritional requirements in different age groups as a source of indispensable nutrients (Ferry, 2011, Inano and Pringle, 1975, Lohse et al., 2011) or by providing functional ingredients with a healthy impact (Jetter and Cassady, 2006, Tester et al., 2011).

In this context, curds are dairy products prepared by coagulating milk preferably with rennet or alternatively with other non-animal sources of acidic substances like lemon, citric acids, vinegar, etc (Agboola et al., 2004, Johnson and Lucey, 2006). Rennet is an enzymatic mixture with a protease activity mainly attributed to rennin or chymosin (EC.3.4.23.4) obtained from the inner mucosa of the abomasums of unweaned calves, which also contains pepsin, lipases, etc. (Garg and Johri, 1994). The role of rennet is to tangle milk casein (proteins commonly from lactating cows or sheep) into solid masses or curds, and separate them from the whey proteins (Garg and Johri, 1994).

Different curd products such as cottage cheese, quark, paneer, can be found in the street market depending on the region or culture (Gaucheron, 2011). Thus, in England, USA and Canada, a popular curd dessert is known as junket, or in Spain is named “cuajada”, while in Sweden, curds are ingredient of traditional “ostkaka”, or in Turkey curds are called kés, being consumed for breakfast. Different dairy products obtained by curdling milk using yeast as ferment, are widely consumed in India. The time and temperature taken for coagulation also varies depending on the technological processing as well as the texture and structure (Agboola et al., 2004, Garg and Johri, 1994, Johnson and Lucey, 2006).

The aim of this research was focused on analyzing the potential benefits of consuming a traditionally prepared Spanish curd from sheep milk (cuajada) within a controlled balanced diet on health issues, different biochemical markers or the improvement of tolerability and subjective gastrointestinal symptoms such as abdominal pain and stool features.

Subjects and Methods

The current controlled randomized nutritional intervention was approved by the Research Ethics Committee of the University of Navarra (ref. 101/2009). Written informed consent was obtained from all the volunteers prior their participation in the study.

Subjects

For the intervention, 40 adult men and women fitting inclusion criteria were recruited and randomly allocated in either the control (n=20) or the curd group (n=20).

Participants were all over 20 years old, without chronic diseases and with LDL-cholesterol levels between 100 and 190 mg/dL, with a body mass index (BMI) between 20 and 30 kg/m².

Exclusion criteria were to present LDL-cholesterol levels out of the range (below 100 mg/dL or above 190 mg/dL), to have a BMI out of the range (20-30 kg/m²), to suffer from any disease related to metabolism or being under pharmacological treatment, inability to adhere to the follow-up, known food allergies, including lactose intolerance and dislikes for milk, or being pregnant, lactating or menopausal women.

Experimental design

For the present trial, a randomized, open label, prospective 6-week nutritional intervention with two parallel groups, the Control group and the Curd group, was designed. Previous to the 6-week intervention, volunteers followed a two-week wash out controlled period.

Both intervention groups consumed a nutritionally balanced, personalized isocaloric diet, following the guidelines of the Spanish Community Nutrition Society and the

Spanish Federation of Nutrition, Food and Dietetics Societies (Cuervo and Federación Española De Sociedades De Nutrición Alimentación Y Dietética (Fesnad), 2010), in which 50% of total energy intake (TEI) came from carbohydrates, 20% of TEI came from proteins and 30% from lipids. Prior to the beginning of the nutritional intervention, all the participants followed a balanced isocaloric diet during two weeks, to ensure baseline homogeneity concerning gastrointestinal and health aspects (table 1).

The control group followed the prescribed isocaloric diet, adjusting to the advised portion sizes and with the specific interdiction of consuming fermented dairy products. The participants were allowed to consume other dairy products as well as to increase semi-skimmed milk daily intake instead of the curd consumed by the experimental group.

The second experimental group (Curd) was prescribed with the isocaloric diet in which the daily consumption of a 130g curd was included. This product was advised to be consumed as dessert of lunch or dinner. The participants could not consume any other fermented or enzymatically processed dairy product. Curds were provided by the company SAT Ultzamakoak (Navarra, Spain).

Nutrient intake was assessed by a validated 72-hours food intake record at baseline and during the last study week (Navas-Carretero et al., 2011b), based on Spanish food composition tables (Moreiras et al., 1998). Although a specific questionnaire or daily registry of milk consumption was not used for the intervention, in order to assess differences in milk and dairy products consumption during the study the 72-hours food intake records were used.

Experimental product

The experimental product was elaborated following standardized procedures by the dairy product company SAT Ultzamakoak (Navarra, Spain). This process consists on curdling pasteurized sheep milk at a temperature of 55°C with the specific rennet (SAT Ultzamakoak, Navarra, Spain) during 10 minutes prior to the subsequent botling and refrigeration until consumption. The manufactured curds had a commercial expiry date of 15 days and were freshly elaborated each 15 days for the study.

Nutritional composition of the curd employed in the study was analyzed by the official Lecumberri Dairy Institute (Navarra, Spain). The average range values for labeling are detailed in table 2.

Anthropometry and body composition

At baseline and the end of the study height, weight, waist circumference and blood pressure were measured. Body composition and bone mineral density were measured by bioimpedance, with a Tanita SC-330-S equipment (TANITA corp, Japan) following validated procedures (De Eguilaz et al., 2010). Systolic and diastolic blood pressure were measured using a manual sphygmomanometer (Riester, Germany), three times with 2-5 minutes intervals between them, according to validated procedures (White et al., 1990).

Biochemical and health markers

At baseline and at the end of the nutritional intervention, lipid, glucose and cardiovascular risk biomarkers were analyzed. Serum total cholesterol (Horiba Medical, Madrid, Spain), HDL-cholesterol (Horiba Medical, Madrid, Spain), triglycerides (Horiba Medical, Madrid, Spain), glucose (Horiba Medical, Madrid, Spain), free fatty acids (Wako Diagnostics, Neuss, Germany), uric acid (Horiba Medical, Madrid, Spain), transaminases (AST and ALT, Horiba Medical, Madrid, Spain) and homocysteine

(Demeditec Diagnostics, Kiel-Wellsee, Germany) were analyzed with an autoanalyzer ABX Pentra C200 (Horiba Medical, Madrid, Spain). LDL-cholesterol was calculated through the Friedewald formula (Friedewald et al., 1972).

Serum insulin and C-reactive protein were measured by ELISA Kits (Mercodia, Uppsala, Sweden; and Immunodiagnosics, MA, USA, respectively) in a Triturus analyzer (Grifols SA, Barcelona, Spain). The index of insulin resistance, HOMA-IR, was calculated as follows (Matthews et al., 1985): $HOMA-IR = [Fasting\ glucose\ (mmol/L) \times Fasting\ insulin\ (\mu U/mL)] / 22.5$

Satiety evaluation and gastrointestinal symptoms

Satiety was examined through Visual analogue scales, previously validated (Navas-Carretero et al., 2011a), which were filled by the volunteers at baseline and at the end of the intervention, just before, 60, 120 and 180 minutes after lunch.

Gastrointestinal symptoms were monitored through subjective, 15-days recall questionnaires previously validated (Labayen et al., 2001), in which volunteers must fill multiple choice questions about abdominal pain (frequency, intensity and duration) and deposition (frequency and fluidity). Total score, as well as pain (sum of frequency, intensity and duration), and deposition (sum of frequency and fluidity) scores as well as each question were analysed to assess differences between baseline and the end of the nutritional intervention.

Statistical analysis

Assuming a maximum difference between groups of 1.0 ± 0.5 points in gastrointestinal score, for an α value of 0.05 (5%) and an statistical power higher than 80%, the number of participants needed was estimated at 34 volunteers. Assuming an expected 20% drop-

out during the trial, the minimum sample size required was established at 40 volunteers, 20 for each experimental group.

Differences between baseline and endpoint were analysed through paired t-tests and univariate ANOVA, once normality of data was tested. Gastrointestinal symptoms questionnaires were also analysed with the Mann-Whitney test to evaluate differences between dietary treatments. All statistical analysis were done with the SPSS 15.0 software for Windows (IBM Ibérica, Spain).

Results

Adherence to the study

The flow diagram about the study development is depicted on figure 1. A total of forty-five subjects volunteered to participate in the nutritional intervention, from which 5 were excluded for not fitting inclusion criteria, three of them had higher BMI than 30 kg/m² and two of them were taking unaccepted medication.

Thus, the remaining 40 participants were randomly allocated on either control diet or curd diet group. Drop outs were similar in both groups (ns), with a mean drop out rate of 27.5%, which was slightly higher than expected (25%).

Volunteers' total intake on dairy products did not change significantly between the beginning and the end of the study, being the consumption of milk and dairy products for the control group 285±154 g/day and for the curd group 298±128 g/day. When analyzing only milk consumption, no changes were observed in relation to semi skimmed and skimmed milk in both groups, while in the control group, a significant decrease on whole fat milk consumption was observed (from 247±37 g/day to 111±64 g/day, p=0.013). In relation to dairy products both groups reduced significantly yoghurt consumption (91±75 g/day vs 12± 40 g/day p=0.008 for the control group and 65±80 vs 0.00±0.00 p=0.007 for the curd group).

Anthropometry

The evaluation of baseline and endpoint anthropometrical characteristics (weight, BMI, fat mass, fat-free mass, waist circumference, tricipital skinfold, systolic and diastolic blood pressure and bone mineral density) revealed no differences between baseline and

endpoint, nor between intervention groups at the beginning, during or at the end of the nutritional intervention (table 3).

Biochemical parameters

The assessment of baseline and endpoint glucose and lipid metabolism and cardiovascular risk markers showed no differences between baseline and endpoint, nor between intervention groups (tables 4 and 5)

Gastrointestinal symptoms and satiety score

The analysis of the subjective questionnaires on gastrointestinal symptoms related to pain and deposition revealed some differences between the curd and the control diet groups. While the scores on pain, deposition and the global score remained without significant changes between baseline and endpoint in the control diet, participants in the curd diet group experienced an improvement in both abdominal pain ($p = 0.050$) and deposition ($p = 0.042$), and consequently in total score ($p = 0.016$) during the nutritional intervention (figure 2). When each question was analyzed by Mann-Whitney tests, these differences between groups were focused on two questions, about duration of the abdominal pain ($p = 0.025$) and deposition frequency ($p = 0.032$), both improved significantly in the curd diet group (figure 3).

According to the satiety scores no differences were observed between groups in relation to hunger, satiety, satisfaction or the desire to eat (figure 4). Participants from both groups presented similar feelings before and after eating, showing an increase on hunger and desire to eat feelings from one hour after meal reaching baseline levels three hours after consuming the meal. As expected, satiety and satisfaction showed the opposite evolution, increasing after eating and gradually decreasing to reach baseline levels three hours later.

Discussion

Milk and dairy products are a staple food in many countries by supplying relevant amounts of high quality protein, lactose, calcium, vitamin D, etc (Inano and Pringle, 1975, Lohse et al., 2011). Furthermore, in vegetarian patterns or in low income societies, foods or ingredients derived from the milk of different mammals (cow, sheep, buffalo, camel, etc) are important nutritional sources, even of energy in the case of some mature cheeses (Naska et al., 2007, Ranganathan et al., 2005), while that in other more affluent groups, fortified or enriched dairy products are becoming popular and widespread consumed (Jetter and Cassady, 2006, Tester et al., 2011). Indeed, the functional food market is becoming highly appreciated by customers, who are increasingly aware of these healthy products and their nutritional claims (Wills et al., 2012, Gallagher et al., 2011).

Although scientific research has mainly focused on investigating the potentially beneficial effects of probiotics in milk and fermented dairy products on health (Parra, D. and Martinez, 2007, Parra, M. D. et al., 2007, Labayen et al., 2001, Agerholm-Larsen et al., 2000a, Agerholm-Larsen et al., 2000b, Simons et al., 2006, Chen et al., 2010, Torii et al., 2011), the literature investigating the tolerability and feasibility of other dairy products, such as curd is still scarce (Wilt et al., 2010).

In this context, differences in acute calcium and amino acid uptake from fresh or pasteurized yogurt have been found despite that they are assumed to have the same nutritional value (Parra, D. and Martinez, 2007, Parra, M. D. et al., 2007). Furthermore, low-fat dairy consumption appears to reduce hypertension risk (Alonso et al., 2005), while subjects with lactose intolerance can benefit from consuming hydrolyzed milk supplements (Shaukat et al., 2010). Interestingly, milk proteins have physiological

functions contributing to the regulation of body weight, satiety, food intake and glycaemia (Anderson et al., 2011), while feeding curd could be interesting to treat diarrhoea (Karnawat, 1987). The satiety evaluation in our two experimental dietary groups provided no relevant changes concerning hunger, satiety, satisfaction or desire to eat estimations, which showed the expected trends before and after meal consumption, but with no differences among them.

Curds are dairy products obtained through the action of rennet from the gut of some mammals calves, that generate a solid mass and a liquid containing whey proteins, different oligopeptides and amino acids that are discarded, being this removal a relevant difference when compared to plain milk or yogurt (Garg and Johri, 1994, Irigoyen et al., 2002). As it has been mentioned, despite that this procedure to obtain curd has been applied in different Asian, American and European regions, the number of studies concerning research of these dairy products and the conduction of randomised human intervention trials involving the intake of curds are scarce in relation with those involving yogurt/fermented milk products (Astiasarán Anchía and Martínez Hernández, 2003).

Curd, which is obtained by coagulating milk with a enzymatic complex with rennin (rennet) has a nutritional composition that mainly depends on the mammal's species, being the sheep milk the most appreciated and traditional source for preparing "cuajada" in the Mediterranean area (Astiasarán Anchía and Martínez Hernández, 2003).

Furthermore, this Spanish curd usually has a higher fat content than other curds from diverse origins (Prandini et al., 2009), and could explain some of the differences in pain relief and fecal deposition that we found when including this curd in the diet, as they may be considered to improve tolerability (Wilt et al., 2010). In addition, the differences on milk consumption did not differ between both groups, which allows to assume that

the subjective improvement may be due to the inclusion of this curd, rather than to differences in lactose intake between groups, which were not observed.

However, the current study shows an important limitation, which otherwise is not easy to avoid, and is the open label design, which may have played a role in these subtle changes observed, due to a placebo effect (Agerholm-Larsen et al., 2000a, Gonzalez-Espinoza et al., 2005, Mani et al., 2011), which should be considered. In fact, a recent study investigating the possible benefits of probiotics on gastrointestinal symptoms of patients with irritable bowel disease showed the possibility of existence of an important placebo effect (Tarrerias et al., 2011). Therefore, in our study, where subjects were healthy, the placebo effect may have played a role. Nevertheless, the characteristics of the product assayed makes it almost impossible to carry out a double blind design, as the authors have investigated the inclusion of an specific product in the diet, and the outcomes must be taken into account.

The current research investigated the differences of curd intake within a nutritionally balanced diet and was compared with subjects who consumed milk and non fermented dairy products instead of this dairy product. As it was foreseeable, no important effects in body anthropometry, blood measurements or health markers were found, once curd was consumed instead of milk. Interestingly, the gastrointestinal symptoms measured by two specific pain and deposition scores, revealed a statistically significant improvement in both measurements after the regular intake of the rennet-coagulated milk. The results must be examined carefully, as they have been self-reported by the volunteers, with the 15 days recall gastrointestinal questionnaire, although the questionnaire employed has been previously validated in research (Labayen et al., 2001). This outcome could be speculated to be due to the removal of whey proteins or peptide products in the curd, as has been hypothesized after the consumption of other products

without this component (Diepvens et al., 2008, Juvonen et al., 2011). Also, the curdling process may affect the texture and consistency with an impact on gastrointestinal status.

The role of dairy products on cholesterol management has been also investigated (Agerholm-Larsen et al., 2000a) with contrasting outcomes (Anderson et al., 2011, Simons et al., 2006), although the intake of “cuajada” produced no relevant changes in biochemical variables or anthropometrical determinations. These findings could be attributed to the fact that the participants were advised to follow a diet similar to their habitual dietary pattern, being the main change consuming curd instead of milk or dairy products, and assuming that the overall nutrient composition was similar in both intervention groups.

These outcomes could be attributed not only to changes in transit time commonly observed with higher fat loads (Labayen et al., 2001) but also to the different protein profile, since whey proteins and peptides are removed in the technological preparative process (Emmons et al., 2003). The effects of subtle changes in sphingolipids (Ohlsson et al., 2010), lactose (Szilagyi, 2002), or proteins (Merritt et al., 1990, Verwimp et al., 1995, Vivatvakin et al., 2010) content in “cuajada” could be also involved in the observed gastrointestinal benefits after curd intake.

These findings are complemented by evidences showing that a diet containing whey proteins modulates gut nitrogen metabolism in rats with mucositis (Boukhattala et al., 2010) and could be involved in anti inflammatory properties (Kanwar and Kanwar, 2009). Indeed, casein, beta-casomorphins, whey proteins or their peptide hydrolysates have demonstrated effects on gastrointestinal motility (Daniel et al., 1990), gastric secretion and enterogastrone response (Calbet and Holst, 2004) or anorexigenic hormones (Diepvens et al., 2008). Furthermore, milk protein consumption has been

related to gastrointestinal comfort in children (Vivatvakin et al., 2010) and diverse symptomatology (Merritt et al., 1990, Verwimp et al., 1995). Despite these evidences, the consumption of curd instead of other dairy products produced no changes in satiety score measurements, although the different fat content and the protein differences associated to curd preparation could contribute to explain the changes in the subjective assessment concerning pain and deposition responses included in the gastrointestinal symptoms questionnaire. Interestingly, the structure modification of a milk-protein based model food has been found to affect postprandial intestinal peptide release and fullness in healthy human (Juvonen et al., 2011). The composition and metabolism of the intestinal microbiota in consumers and non-consumers of yogurt has been associated with the amount of ingested fermented-milk (Alvaro et al., 2007). Actually, a significant linear increase in fecal consistency (looser stools) has been reported with increasing probiotic dose (Larsen et al., 2006), while prebiotic occurrence has been associated with increased motility and looser stools (Vivatvakin et al., 2010, Szilagy, 2002). Thus, it appears that not only the probiotic load is important since short-term nutrient assimilation is affected by the texture of the dairy product (Parra, M. D. et al., 2007).

Conclusions

Summing up, the current intervention trial provides scientific evidence that a daily consumption of a traditional Spanish curd (cuajada) has interesting and convenient benefits concerning gastrointestinal tolerability by improving abdominal pain and deposition markers. This improvement could be associated to the characteristic nutritional composition of this dairy dessert, prepared from fat-rich sheep milk in which subtle changes in protein or derived peptides, lactose, sphingomyelin and fat profile could be putatively involved.

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Declaration of interest

The authors declare not to have any conflict of interest.

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