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ORIGINAL CONTRIBUTION

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The effect of *Lactobacillus helveticus* fermented milk on acute changes in calcium metabolism in postmenopausal women

Summary *Background* Milk fermented with *Lactobacillus helveticus* (*L. helveticus*) has been shown to lower blood pressure and to increase bone mineral content in spontaneously hypertensive rats. The effect of *L.helveticus* may be due to better calcium availability. *Aim of the study* In the present study the effect of milk fermented with *L. helveticus* on acute changes in calcium metabolism and bone resorption in postmenopausal women was studied. *Methods* The study was performed as a randomised double-blind crossover study of 20 postmenopausal women (mean age 65, range 50–78). The study was carried out in two parts. Firstly, *L. helveticus* fermented milk was compared to a control milk. Secondly, juice containing peptides formed with *L. helveticus* bacteria was compared to a control juice. The acute effect on calcium metabolism was measured during the study day by serum ionised calcium (iCa), parathyroid hormone (PTH), calcium (Ca), phosphate (P), and urinary calcium. A direct marker of bone turnover, carboxyterminal telopeptide of type I collagen (ICTP), was measured from the serum. *Results L. helveticus* fermented milk reduced serum PTH (405.3 ± 37 ng/l vs. 454.9 ± 37, p = 0.012) and increased serum calcium (19.1± 0.2 mmol/l vs. 18.8 ± 0.2, p = 0.031) compared to the control milk. *L. helveticus*-derived peptides had no significant acute effect on calcium metabolism, in fact, ionised calcium was lower and PTH higher after the juice containing peptides compared to the control juice. *Conclusions* Fermentation of milk with *Lactobacillus helveticus* had a positive acute effect on calcium metabolism. This effect was not explained by the small peptides formed by *L. helveticus*.

Key words calcium metabolism – *L. helveticus* – bioactive peptides – postmenopausal women

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Introduction

Calcium is needed throughout life to ensure bone growth during adolescence and to support bone mass when age-related bone loss occurs [1]. In western countries the main source of calcium in the diet is milk products, accounting for up to 75 % of calcium intake [2, 3]. However, the use of milk products has been declining over the past decade [4], thus increasing the risk groups of low-calcium intake [5]. In the prevention of osteoporosis it is vital to increase calcium intake by augmenting the intake of milk products, by calcium fortification or with calcium supplements. These methods are not always successful because of dietary preferences and lack of compliance [1]. A further possibility is to improve calcium availability. Better calcium availability would compensate for the lower use of milk products and ensure an adequate supply of calcium to the bone.

Milk-derived caseinophosphopeptides (CPP), formed in the gastrointestinal tract or during fermentation enhance calcium absorption by preventing the for-

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mation of insoluble calcium salts in the intestine [6, 7]. Although the effect of CPP has been widely studied, the findings have not been conclusive, either in animal studies [8–13] or in human studies [14–17]. Other bioactive peptides are formed during milk fermentation, depending on the bacteria used. Fermentation with *L. helveticus* bacteria increases the production of proline-containing short peptides, such as isoleycyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) [18, 19]. These IPP and VPP peptides are known to have an angiotensin converting enzyme (ACE) inhibitory activity [20, 21]. ACE has a role in regulating blood pressure by converting angiotensin I (Ang I) to angiotensin II (Ang II), which contracts the blood vessels. A recent human study showed a decrease in blood pressure with subjects receiving *L. helveticus* fermented milk [22]. In a rat study, milk fermented with *L. helveticus* lowered blood pressure more than the same amount of the IPP and VPP peptides dissolved in water [21]. Therefore, in addition to peptides IPP and VPP, a factor capable of lowering blood pressure was postulated as being present in the *L. helveticus* fermented milk. As calcium is known to affect blood pressure [23], better calcium bioavailability has been postulated to explain this finding. In another rat study, the rats fed with *L. helveticus* fermented milk had increased bone mineral content (BMC) compared to the rats fed with sour milk [Narva M, Sipola M, Kärkkäinen M, Lamberg-Allardt C, Korpela R, unpublished observations, 2002]. It is therefore of importance to investigate the effect of *L. helveticus* fermented milk on calcium and bone metabolism.

The aim of the present study was to determine the effect of milk fermented with *L. helveticus* on acute changes in calcium metabolism and bone resorption in postmenopausal women. To analyse the mechanism of *L. helveticus* further, the effect of small peptides formed by the bacteria were also studied.

Subjects and methods

Twenty postmenopausal women (mean age 65, range 50–78, mean BMI 26, range 22–31) volunteered for the study. The exclusion criteria included medication affecting calcium and bone metabolism, hormone replacement therapy and the use of ACE inhibitors. The inclusion criteria required at least one postmenopausal year. None of the subjects took any vitamin or mineral supplements during the study. Written consent was obtained from the subjects. Prior to the study a routine medical examination and laboratory tests (erythrocytes, haemoglobin, hematocrit, MCV, MCH, MCHC, leucocytes, ionised calcium, potassium, sodium, glucose, liver and renal function tests) were performed to ensure that the subjects were in good health.

Study products

The products used were 1) Milk fermented with *Lactobacillus helveticus* LBK-16H bacteria, supplemented with the solid content of the same milk to achieve a peptide concentration of 14.5 mg/100 g of IPP and VPP together (Evolus®, Valio Ltd., Helsinki, Finland), 2) a control milk which is a normal sour milk fermented with a *Lactococcus sp*. mixed culture (Neopiimä, Valio Ltd., Helsinki, Finland), 3) orange juice to which a peptide fraction from *L. helveticus* fermented milk whey with the same amount of IPP and VPP as in the first product, was added, 4) orange juice to which calcium was added in the form of calcium lactate gluconate. All the products contained 500 mg of calcium/portion. The peptide content and mineral content of the products are shown in Table 1.

Study procedure

The study was a double-blind randomised crossover study with two separate parts. The study design can be seen in Fig. 1. There were two study days in both parts,

Table 1 The portion size, pH, and peptide and mineral contents of the study products

	L.helveticus milk	Control milk	Peptide juice	Control juice
Portion size, ml	220	420	400	220
pH	3.85	4.41	4.71	3.85
Intake mg/portion				
Calcium	570	550	420	480
Phosphate	370	420	320	30
Ca/P ration	1.5	1.3	1.3	16
Sodium	94	168	48	2
IPP[1]	16	–	16	–
VPP[2]	16	–	18	–

[1] *IPP* isoleucyl-prolyl-proline; [2] *VPP* valyl-prolyl-proline

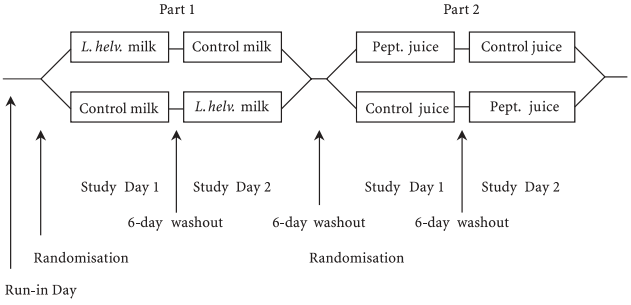

Fig. 1 The study design of the double-blind randomised crossover study with *Lactobacillus helveticus* fermented milk (*L.helv*. milk) compared to the control milk, and a juice containing peptides formed with *L.helveticus* bacteria (pept. juice) compared to a control juice

and a one-week washout period between the two parts. The day prior to the study day was a run-in day when the subjects consumed milk products (600 mg calcium) to control the amount of calcium that might affect the morning urine calcium values. At the beginning of the study day, fasting blood and urinary samples were collected before the administration of the study product with a light breakfast (Table 2). Blood and urinary samples were collected during the 8 hours following the administration of the study product. The effects of the interventions were evaluated by measuring the serum PTH (PTH), ionised calcium (iCa), calcium (Ca), phosphate (P) and carboxyterminal telopeptide of type I collagen (ICTP) at 0, 1, 2, 4, 6 and 8 hours, and urinary calcium (U-Ca) and creatinine (U-Crea) at 0, 2, 4, 6 and 8 hours. During the study day the subjects were given standardised meals with low calcium levels (Table 2). The study protocol was approved at the Ethical Committee of the Joint Authority for the Hospital District of Helsinki and Uusimaa (HUS).

Analytical methods

The analyses of the study products were performed at the laboratories of Valio Ltd. (R & D, Helsinki, Finland). The peptide content was analysed by HPLC [24] from the *L. helveticus* milk and the peptide juice. The mineral composition of all the study products was determined by atom absorption spectrometry (AAS).

The laboratory tests of erythrocytes, haemoglobin, hematocrit, MCV, MCH, MCHC, leucocytes, ionised calcium, potassium, sodium and glucose, and the liver and renal function tests were analysed at United Laboratories Ltd. (Helsinki, Finland).

Serum was separated from the blood samples immediately after the samples were taken. The serum ionised calcium concentration was measured within 90 min with an ion selective analyser (ISE cCa 2+/pH Analyser 634, Halstead, UK), adjusted to pH 7.4. The samples were stored at –20 °C until analysed for serum intact PTH concentration by an immunoradiometric assay using Allegro kits (Nichols Institute, San Juan Capistrano, CA, USA), and for serum phosphate and serum total calcium by routine laboratory methods. The serum ICTP was analysed using radioimmunoassay [25].

The urine samples were stored at –20 °C until the calcium and creatinine concentration analyses, which were carried out by routine laboratory methods. From urinary calcium the molar ratio to creatinine was measured.

The three-day food record was analysed with the Nutrica programme (Social Insurance Institution of Finland, 2000), which includes 600 food items and 30 nutrients.

Table 2 Study meals and nutrient intake during the study days

Meal	Food item	Quantity
Breakfast	white bread	40 g
	margarine	10 g
	cucumber	50 g
	tea	150 g
Lunch	minced meat in gravy	150 g
	potatoes	100 g
	tomatoes	60 g
	white bread	40 g
	margarine	10 g
	fruit puree	200 g
Snack	sweet roll	70 g
	tea	150 g

Average energy, vitamin and mineral content: energy, 3770 kJ; minerals and vitamins: calcium 90 mg, magnesium 95 mg, sodium 780 mg, vitamin D 2.5 µg

Statistical analysis

The sample size calculations were based on the PTH. It was estimated that the clinically significant difference between the treatments would be 0.25 pmol/l [26], and at the power of 90 % the number of subjects needed in a crossover study was calculated to be 16. The standard deviation was estimated to be 0.30 [26]. To take into consideration possible dropouts, 20 subjects were recruited. The randomisation was carried out with random permuted blocks with block size of four subjects.

The area under curve (AUC), AUC of changes from baseline (0 h), maximal change and change at two hours were used as summary statistics to measure the effect of the study products. The AUC of changes from baseline (0 h) and the maximal changes were determined in order to control for the differences in the baseline values.

The summary statistics were analysed using the conventional ANOVA for crossover analysis. ANOVA for repeated measures was applied to study treatment difference, period effect and the interaction between treatment and period (carry-over effect).

A value of $p < 0.05$ was considered significant. SPSS (Version 10.0) was used for the statistical analysis.

Results

Background information on the subjects

The medical examination and laboratory tests showed that all the subjects were healthy and had no medical reason for not participating in the study. Vitamin D values (18–77 nmol/l) were within the normal range. The fasting serum creatinine values (64–126 µmol/l) indi-

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cated normal renal function in all the subjects. The mean intake of calcium assessed with a three-day food record was 1180 ± 400 mg/d.

The first part of the study

In the first part of the study the effect of the *L. helveticus* fermented milk was compared to the control milk. At the baseline, the values did not differ between the interventions. The changes in serum calcium and PTH are shown in Fig. 2. The AUC of serum PTH was lower and of calcium higher after the *L. helveticus* milk compared to the control milk (p = 0.012, p = 0.031 respectively) (Table 3). PTH decreased more after the *L. helveticus* milk compared to the control milk (AUC of the changes –74.0 vs. 35.9, p = 0.053). A decreasing trend in the maximal change in serum PTH (p = 0.070) and an increasing trend in serum calcium (p = 0.051) could be seen after the *L. helveticus* milk compared to the control milk (Table 3). At 2 hours after the *L. helveticus* milk the serum PTH decreased (–14.3 vs. –8.1, p = 0.016) and calcium increased (0.04 vs. –0.01, p = 0.031) compared to the control milk. In serum calcium and PTH the period effects and carry-over effects were non-significant. No significant differences occurred in serum ionised calcium, phosphate, ICTP or urinary calcium between the two interventions (Table 3).

The second part of the study

In the second part, the effect of the peptide juice containing the peptide concentrate formed by *L. helveticus* bacteria was compared to the control juice without peptides (Fig. 3). There were no differences in the baseline values of the serum and urine variables between the interventions. After the peptide juice, the AUC of PTH was higher and the AUC of urea calcium lower compared to the control juice (p = 0.018, p = 0.031 respectively) (Table 4). PTH decreased less after the peptide juice compared to the control juice (AUC of the changes –21.0 vs. –90.0, p = 0.021). Serum ionised calcium and phosphate decreased more after the peptide juice compared to the

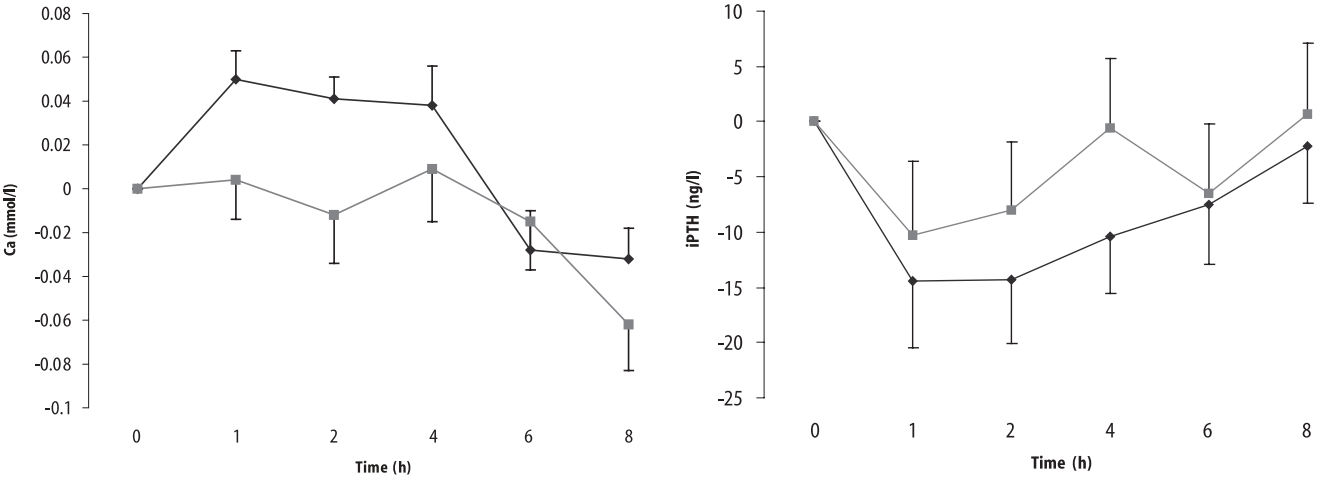

Fig. 2 The acute changes in serum calcium (± SEM) and serum intact PTH (± SEM) after milk fermented with *Lactobacillus helveticus* (- ◆ -) and after a control milk (- ■ -), in postmenopausal women (n = 20)

Table 3 Serum ionised calcium (iCa), total calcium (Ca), intact PTH (iPTH), phosphate (P), ICTP and urinary calcium, after *L. helveticus* fermented milk and control milk. AUC and maximal change are given as mean ± SEM (n = 20)

	L. helveticus milk		Control milk	
	AUC	Maximal change	AUC	Maximal change
Serum				
Ca (mmol/l)	19.10±0.24[1]	0.09±0.01	18.84±0.23	0.05±0.02
P (mmol/l)	8.91±0.20	–0.09±0.02	8.74±0.21	–0.09±0.02
iCa (mmol/l)	10.01±0.10	0.03±0.005	9.98±0.09	0.03±0.004
PTH (ng/l)	405.3±37.1[1]	–20.8±5.3	454.9±36.8	–15.4±6.4
ICTP (µg/l)	29.7±2.2	–1.09±0.28	27.9±1.6	–1.15±0.20
Urine				
U-Ca (mmol/l)	2.8±0.4	0.19±0.05	2.5±0.4	0.17±0.05

[1] In comparisons of results of *L. helveticus* milk to control milk, p < 0.05

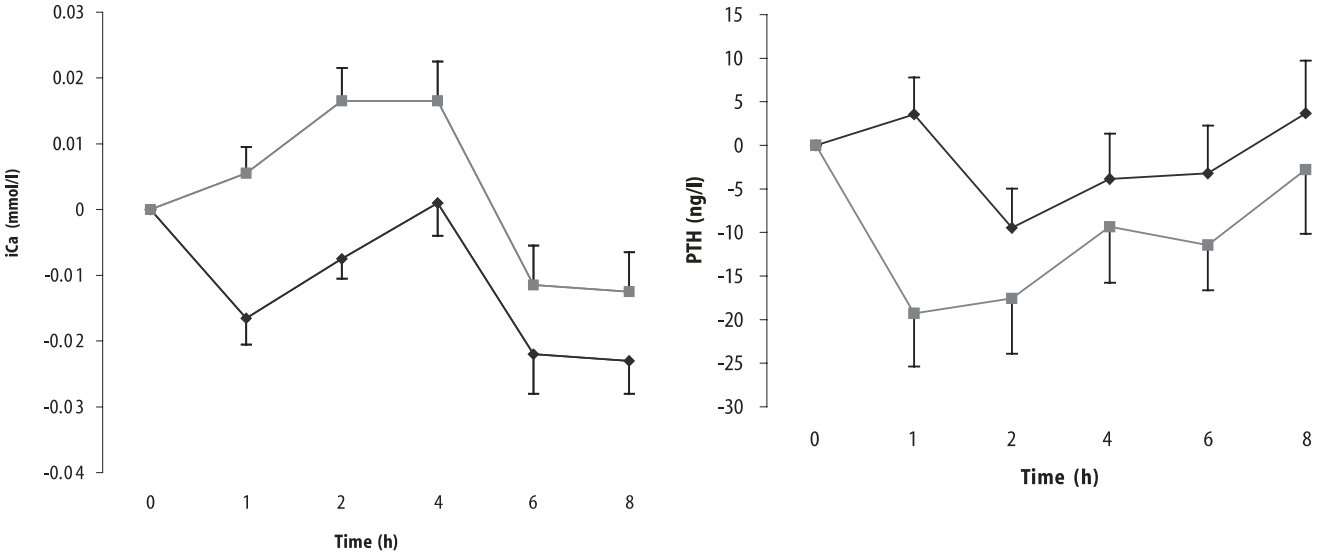

Fig. 3 The acute changes in serum ionised calcium (± SEM) and serum intact PTH (± SEM) after peptide juice containing peptides formed with *Lactobacillus helveticus* bacteria (- ■ -) and after control juice (- ◆ -), in postmenopausal women (n = 20)

Table 4 Serum ionised calcium (iCa), total calcium (Ca), intact PTH (iPTH), phosphate (P), ICTP and urinary calcium, after peptide juice and control juice. AUC and maximal change are given as mean ± SEM (n = 20)

	Peptide juice		Control juice	
	AUC	Maximal change	AUC	Maximal change
Serum				
Ca (mmol/l)	18.90±0.21	0.10±0.02	19.01±0.22	0.07±0.01
P (mmol/l)	8.33±0.24	−0.12±0.02	8.43±0.20	−0.11±0.01
iCa (mmol/l)	9.99±0.10	0.005±0.004[1]	10.05±0.08	0.026±0.005
PTH (ng/l)	470.6±43.3[1]	−13.0±4.7[1]	424.0±38.5	−23.7±5.9
ICTP (µg/l)	30.4±2.5	−1.10±0.10	31.0±2.7	−1.08±0.18
Urine				
U-Ca (mmol/l)	2.6±0.3[1]	0.15±0.04	2.9±0.4	0.21±0.07

[1] In comparisons of results of peptide juice to control juice, $p < 0.05$

control juice (AUC of the changes –0.09 vs. 0.03, p = 0.007 and –0.39 vs. –0.12, p = 0.025 respectively). The maximal changes of serum PTH and ionised calcium differed significantly after the two interventions (Table 4). At 2 hours the serum PTH decreased less (–9.5 vs. –17.6, p = 0.045) and ionised calcium decreased more (–0.01 vs. 0.02; p = 0.001) after the peptide juice compared to the control juice. In serum PTH, ionised calcium and phosphate, the period effects and carry-over effects were non-significant. In serum calcium and ICTP, no significant differences were found between the peptide juice and the control juice.

Discussion

In the present study the effect of milk fermented with *L. helveticus* on acute changes in calcium metabolism and bone resorption after the menopause was examined by measuring the variables in serum and urine in the course of one day. First the effect of *L. helveticus* milk was determined. The possible mechanism of *L. helveticus* milk was also examined by measuring the acute effect of the small peptides formed by *L. helveticus* bacteria on calcium metabolism.

Parathyroid hormone plays an important role in sustaining the calcium concentration in the blood. A high PTH concentration increase calcium release from bone and also bone resorption. An oral calcium load has been shown to suppress PTH concentrations [27] and also to decrease bone resorption [28]. In the present study the acute changes in calcium and bone metabolism were measured by changes in serum calcium, ionised calcium, parathyroid hormone, ICTP and urine calcium for eight hours following the ingestion of the study products. This method has been commonly used to measure the effect of calcium supplementation on calcium and bone metabolism acutely [27–30].

The responses to calcium intake may have been affected by other foods ingested during the study day, as well as the subjects' habitual calcium intake levels and vitamin D values, and the length of the menopause. All the subjects in the present study were postmenopausal, a time when calcium absorption decreases [31]. The effect of other nutrients on calcium metabolism was excluded by controlling calcium intake 12 hours prior to each study day (the run-in day) and by serving identical meals during the study days. Habitual calcium intake affects calcium absorption [32]; thus in the present study intra-individual comparison was used. The effect of habitual calcium intake could be seen in the present study when the women with low (< 1000 mg) habitual calcium intake were compared with women with high (> 1000 mg) calcium intake. After one randomly-chosen study product, the women who had a low habitual calcium intake had a greater increase in calcium and decrease in PTH than those with a high habitual calcium intake, suggesting higher calcium absorption efficiency with low habitual calcium intake. This notion makes it all the more important that such short-term studies should be intra-individually controlled. Vitamin D values were in the normal range in all the subjects at the beginning of the study, and during the study the use of vitamin supplements was forbidden. The study was carried out over a period of one month; thus the effect of vitamin D on the calcium absorption rate should have remained constant.

In the present study fermentation with *L. helveticus* bacteria had a positive effect on calcium metabolism by suppressing serum PTH and increasing serum calcium concentrations acutely compared to the normal fermented milk product. This finding supports the hypothesis propounded in previous studies with rats [21, Narva et al., unpublished observations, 2002]. In a long-term feeding trial with spontaneously hypertensive rats, *L. helveticus* fermented milk attenuated the development of hypertension [21]. This can be partly explained by the ACE-inhibiting peptides, IPP and VPP, produced in *L. helveticus* fermentation. However, the development of hypertension was hindered more with the *L. helveticus* milk than with purified IPP and VPP peptides dissolved in water. This additional effect on blood pressure can be explained by enhanced calcium availability. In another study – a thirteen-week experiment – the milk fermented with *L. helveticus* increased bone mineral content in rats [Narva M et al., unpublished observations, 2002]. This effect could be equally due to increased calcium availability but also to a direct effect on the bone.

The IPP and VPP peptides with proline residues are highly resistant to enzyme degradation [33] and they are shown to be absorbed through the gastrointestinal tract [24, 34]. In *in vitro* studies Ang II has decelerated osteoblast differentiation and mineralisation, and stimulated bone resorption [35, 36], one study showing the anabolic effect of Ang II in the bone formation process [37]. Ang II may act as a growth factor or as a vasoconstrictor in bone vasculature [38, 39]. However, in *in vivo* studies with rats the ACE inhibitors have not affected bone mineral density (BMD), bone mineral content (BMC) or histomorphic measurements [38–40]. In the present acute study neither the *L. helveticus* milk nor the peptide juice, both containing ACE inhibitory IPP and VPP, affected the bone biomarker, carboxyterminal telopeptide of type I collagen (ICTP). It is also possible that the effect on bone markers cannot be seen in such a short-term study, although biomarkers of bone resorption have been used previously in such similar acute studies [41–44]. A long-term intervention with *L. helveticus* milk containing IPP and VPP peptides might throw more light on their effect on bone mineral density.

It appears that the acute effect of *L. helveticus* on calcium metabolism is dependent on factors other than IPP and VPP peptides, since *L. helveticus* milk containing IPP and VPP peptides decreased PTH significantly, but the effect was not seen with the peptide juice, high in these tripeptides. Fermentation itself may increase calcium absorption by at least two mechanisms: by slowing down the gastrointestinal emptying rate, and by the formation of bioactive peptides, caseinophosphopeptides (CPP) [45]. These bioactive peptides bind calcium in a hydrophobic form, which inhibits the formation of insoluble caseinphosphates and increases the amount of soluble calcium [7]. Although studies on the effect of CPP on calcium bioavailability in man have not been conclusive [14–17], it is possible that a CPP formation could explain the differences between the study products in the response of calcium metabolism.

The response of calcium metabolism was lower after the peptide juice compared to the control juice. The products were both based on the same juice, but in the peptide juice a concentration of peptide fraction formed in *L. helveticus* fermentation had been added. This peptide fraction was produced from whey; thus there were no casein-derived CPPs that increase calcium solubility. There was also a great variation in the calcium:phosphate ratio of the products (Table 1). Phosphate ingestion has been shown to decrease serum calcium concentration and to increase serum PTH concentration within a few hours after administration [46]. In a previous study an oral administration of 500 mg of phosphate increased serum PTH concentration, and an administration of 1500 mg of phosphate both increased serum PTH and decreased calcium concentrations [46]. In the present study the phosphate content of the peptide juice was ten times higher than in the control juice. It can be postulated that the high level of phosphate in the peptide juice compared to the control juice could have concealed the effect of the peptide juice on acute calcium metabolism. The pH varied between the products – from 3.85 to 4.71 in the control juice and the peptide juice respec-

tively – thus the calcium solubility with lower pH may have affected the absorption of calcium.

The aim of the study was to examine the acute effect of *L. helveticus* fermented milk on calcium and bone metabolism in humans. The results suggest that milk fermented with *L. helveticus* may have a positive effect on calcium metabolism acutely and that the mechanism also relies on other factors than the small tripeptides IPP and VPP. Before drawing conclusions as to the possible role of *L.helveticus* fermented milk in osteoporosis prevention, long-term interventions are necessary to further enlighten the effect of *L. helveticus* on calcium absorption and bone metabolism.

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References

1. Cashman KD (2002) Calcium intake, calcium bioavailability and bone health. Br J Nutr 87:169–177
2. Charles P (1992) Calcium absorption and calcium bioavailability. J Intern Med 231:161–168
3. Fleming KH, Heimbach JT (1994) Consumption of calcium in the US: food sources and intake levels. J Nutr 124: 1426S–1430S
4. Osler M, Heitmann BL, Schroll M (1997) Ten year trends in the dietary habits of Danish men and women. Cohort and cross-sectional data. Eur J Clin Nutr 51: 535–541
5. Black RE, Williams SM, Jones IE, Goulding A (2002) Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. Am J Clin Nutr 276:675–680
6. Kitts DD, Yuan YV, Nagasawa T, Moriyama Y (1992) Effect of casein, casein phosphopeptides and calcium intake on ileal ^{45}Ca disappearance and temporal systolic blood pressure in spontaneously hypertensive rats. Br J Nutr 68:765–781
7. Lee YS, Noguchi T, Naito H (1980) Phosphopeptides and soluble calcium in the small intestine of rats given a casein diet. Br J Nutr 43:457–467
8. Bennett T, Desmond A, Harrington M, McDonagh D, FitzGerald R, Flynn A, Cashman K (2000) The effect of high intakes of casein phosphopeptide on calcium absorption in the rat. Br J Nutr 83: 673–680
9. Brommage R, Juillerat MA, Jost R (1991) Influence of casein phosphopeptides and lactulose on intestinal calcium absorption in adult female rats. Lait 71:173–180
10. Kopra N, Scholz-Ahrens KE, Barth CA (1992) Effect of casein phosphopeptides on utilization of calcium in vitamin D-replete and vitamin D-deficient rats. Milchwissenschaft 47:488–492
11. Lee YS, Noguchi T, Naito H (1983) Intestinal absorption of calcium in rats given diets containing casein or amino acid mixture: the role of casein phosphopeptides. Br J Nutr 49:67–76
12. Mykkänen HM, Wasserman RH (1980) Enhanced absorption of calcium by casein phosphopeptides in rachitic and normal chicks. J Nutr 110:2141–2148
13. Tsuchita H, Suzuki T, Kuwata T (2001) The effect of casein phosphopeptides on calcium absorption from calcium-fortified milk in growing rats. Br J Nutr 85:5–10
14. Hansen M, Sandström B, Jensen M, Sörensen SS (1997) Casein phosphopeptides improve zinc and calcium absorption from rice-based but not from whole-grain infant cereal. J Pediatr Gastrolog Nutr 24:56–62
15. Hansen M, Sandström B, Jensen M, Sörensen SS (1997) Effect of casein phosphopeptides on zinc and calcium absorption from bread meals. J Trace Elements Med Biol 11:143–149
16. Heaney RP, Saito Y, Orimo H (1994) Effect of caseinphosphopeptide on absorbability of co-ingested calcium in normal postmenopausal women. J Bone Miner Met 12:77–81
17. Narva M, Kärkkäinen M, Poussa T, Lamberg-Allardt C, Korpela R (2003) Caseinphosphopeptides in milk and fermented milk do not affect calcium metabolism acutely in postmenopausal women. J Am Coll Nutr 22:88–93
18. Matar C, Amiot J, Savoie L, Goulet J (1996) The effect of milk fermentation by Lactobacillus helveticus on the release of peptides during in vitro digestion. J Dairy Sci 79:971–979
19. Nakamura Y, Yamamoto N, Sakai K, Okubo A, Yamazaki S, Takano T (1995) Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. J Dairy Sci 78: 777–783
20. Nakamura Y, Yamamoto N, Sakai K, Takano T (1995) Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. J Dairy Sci 78:1253–1257
21. Sipola M, Finckenberg P, Santisteban J, Korpela R, Vapaatalo H, Nurminen ML (2001) Long-term intake of milk peptides attenuates development of hypertension in spontaneously hypertensive rats. J Phys Pharm 52:745–754
22. Seppo L, Jauhiainen T, Poussa T, Korpela R (2003) A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. Am J Clin Nutr 77:326–330
23. Pörsti I, Mäkynen H (1995) Dietary calcium intake: effects on central blood pressure control. Semin Nephrol 15: 550–563
24. Masuda O, Nakamura Y, Takano T (1996) Antihypertensive peptides are present in aorta after oral administration of sour milk containing these peptides to spontaneously hypertensive rats. J Nutr 126:3063–3068
25. Risteli J, Elomaa I, Niemi S, Novarmo A, Risteli L (1993) Radioimmunoassay for the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. Clin Chem 39: 635–640
26. Talbot JR, Guardo P, Seccia S, Gear L, Lubary D, Saad G, Roberts M, Fradinger E, Marino A, Zanchetta J (1999) Calcium bioavailability and parathyroid hormone acute changes after oral intake of dairy and nondairy products in healthy volunteers. Osteoporos Int 10: 137–142
27. Kärkkäinen M, Lamberg-Allardt C, Ahonen S, Välimäki M (2001) Does it make a difference how and when you take your calcium? Am J Clin Nutr 74: 335–342
28. Horowitz M, Wishart J, Goh D, Morris H, Need A, Nordin C (1994) Oral calcium suppresses biochemical markers of bone resorption in normal men. Am J Clin Nutr 60:965–968
29. Reid IR, Hannan SF, Schooler BA, Ibbertson HK (1986) The acute biochemical effects of four proprietary calcium preparations. Aust NZ J Med 16: 193–197

30. Guillemant J, Le H, Maria A, Guillemant S (2000) Acute effects of oral calcium load on parathyroid function and on bone resorption in young men. Am J Nephrol 20:48–52
31. Heaney RP, Recker RR (1986) Distribution of calcium absorption in middle-aged women. Am J Clin Nutr 43: 299–305
32. Heaney RP, Recker RR, Stegman MR, Moy AJ (1989) Calcium absorption in women: relationships to calcium intake, estrogen status, and age. J Bone Miner Res 4:469–475
33. Meisel H, Bockelmann W (1999) Bioactive peptides encrypted in milk proteins: proteolytic activation and tropho-functional properties. Antonie van Leeuwenhoek 76:207–215
34. Satake M, Enjoh M, Nakamura Y, Takano T, Kawamura Y, Arai S, Shimizu M (2002) Transepithelial transport of the bioactive tripeptide, Val-Pro-Pro, in human intestinal Caco-2 cell monolayers. Biosci Biotechnol Biochem 66: 378–384
35. Hagiwara H, Hiruma Y, Inoue A, Yamaguchi A, Hirose S (1998) Deceleration by angiotensin II of the differentiation and bone formation of rat calvarial osteoblastic cells. J Endocrinol 156: 543–550
36. Hatton R, Stimpel M, Chambers T (1997) Angiotensin II is generated from angiotensin I by bone cells and stimulates osteoclastic bone resorption in vitro. J Endocrinol 152:5–10
37. Lamparter S, Kling L, Schrader M, Ziegler R, Pfeilschifter J (1998) Effects of angiotensin II on bone cells in vitro. J Cell Physiol 175:89–98
38. Ma Y, Stimpel M, Liang H, Pun S, Jee W (1997) Impact of antihypertensive therapy on the skeleton: effects of moexipril and hydrochlorothiazide on osteopenia in spontaneously hypertensive ovariectomized rats. J Endochrinol 154: 467–474
39. Stimpel M, Jee W, Ma Y, Yamamoto N, Chen Y (1995) Impact of antihypertensive therapy on postmenopausal osteoporosis: effects of the angiotensin converting enzyme inhibitor moexipril, 17β-estradiol and their combination on the ovariectomy-induced cancellous bone loss in young rats. J Hypertens 13:1852–1856
40. Broulik PD, Tesar V, Zima T, Jirsa M (2001) Impact of antihypertensive therapy on the skeleton: effects of enalapril and AT1 receptor antagonist losartan in female rats. Physiol Res 50:353–358
41. Guillemant J, Le H, Maria A, Guillemant S (2000) Acute effects of oral calcium load on parathyroid function and on bone resorption in young men. Am J Nephrol 20:48–52
42. Green J, Booth C, Running R (2003) Acute effect of high-calcium milk with or without additional magnesium, or calcium phosphate on parathyriod hormone and biochemical markers of bone resorption. Eur J Clin Nutr 57:61–68
43. Rubinacci A, Divieti P, Polo RM, Zampino M, Resmini G, Tenni R (1996) Effect of an oral calcium load on urinary markers of collagen breakdown. J Endocrinol Invest 19:719–726
44. Scopacasa F, Need AG, Horowitz M, Wishart JM, Morris HA, Nordin BE (2000) Inhibition of bone resorption by divided-dose calcium supplementation in early postmenopausal women. Calcif Tissue Int 67:440–442
45. Mahé S, Marteau P, Huneau J, Thuillier F, Tomé D (1994) Intestinal nitrogen and electrolyte movements following fermented milk ingestion in man. Br J Nutr 71:169–180
46. Kärkkäinen MU, Lamberg-Allardt CJ (1996) An acute intake of phosphate increases parathyroid hormone secretion and inhibits bone formation in young women. J Bone Miner Res 11:1905–1912