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ORIGINAL CONTRIBUTION

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Short-term effect of bedtime consumption of fermented milk supplemented with calcium, inulin-type fructans and caseinphosphopeptides on bone metabolism in healthy, postmenopausal women

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Abstract *Background* Milk products are good sources of calcium and their consumption may reduce bone resorption and thus contribute to prevent bone loss. *Aim of the study* We tested the hypothesis that bedtime consumption of fermented milk supplemented with calcium inhibits the nocturnally enhanced bone resorption more markedly than fermented milk alone, and postulated that this effect was most pronounced when calcium absorption enhancers were added. *Methods* In a controlled, parallel, double-blind intervention study over 2 weeks we investigated the short-term effects of two fermented milks supplemented with calcium from milk minerals (f-milk + Ca, $n = 28$) or calcium from milk minerals, inulin-type fructans and caseinphosphopeptides (f-milk + Ca + ITF + CPP; $n = 29$) on calcium and bone metabolism in healthy, postmenopausal women, and compared them with the effect of a fermented control milk without supplements (f-milk, $n = 28$). At bedtime 175 ml/d of either test milk was consumed. Fasting blood samples and 48 h-urine were collected at baseline and at the end of the intervention. Urine was divided into a pooled daytime and nighttime fraction. Multifactorial ANOVA was performed. *Results* Fermented milk independent of a supplement ($n = 85$) reduced the nocturnal excretion of deoxypyridinoline, a marker of bone resorption, from 11.73 ± 0.54 before to 9.57 ± 0.54 μmol/mol creatinine at the end of the intervention ($P = 0.005$). No effect was seen in the daytime fraction. Differences between the three milks ($n = 28$ resp. 29) were not significant. Fermented milk reduced bone alkaline phosphatase, a marker of bone formation, from 25.03 ± 2.08 to 18.96 ± 2.08 U/l, with no difference between these groups either. Fermented milk increased the nocturnal but not daytime urinary excretion of calcium and phosphorus. The effects on calcium and phosphorus excretion were mainly due to the group supplemented with Ca + ITF + CPP. *Conclusion* Bedtime consumption of fermented milk reduced the nocturnal bone resorption by decelerating its turnover. Supplemented calcium from milk mineral had no additional effect unless the absorption enhancers ITF + CPP were added.

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A stimulated intestinal calcium absorption may be assumed, since urinary calcium excretion increased at a constant bone resorption.

Key words fermented milk – inulin-type fructans – caseinphosphopeptides – calcium supplementation – bone metabolism

Abbreviations BAP: Bone alkaline phosphatase, Ca: Calcium, CPP: Caseinphosphopeptides, DPD: Desoxypyridinoline, f-milk: Fermented milk, FOS: Fructooligosaccharide, ITF: Inulin-type fructans, PTH: Parathyroid hormone, TAP: Total alkaline phosphatase

Introduction

Osteoporosis prevention is of eminent interest because of the increasing global relevance of this bone disease. Estimates for 2006 worldwide concluded that 1 in 3 women and 1 in 10 men at an age of ≥55 years will have osteoporosis in their later life. In the United States, 10 million Americans have osteoporosis, with estimated costs of $17.9 billion annually while in the United Kingdom, 3 million persons are affected. For Europe the estimated costs are above €13.9 billion annually [22]. Besides exercise and vitamin D supply or its synthesis in the body sufficient intake of calcium is an important factor that helps to maintain bone mass throughout life. Inadequate intake of calcium accelerates loss of bone mass, and thus raises the risk for osteoporotic fractures after menopause [10, 45]. Due to the reduced intestinal calcium absorption capacity and renal function with increasing age and to the accelerated loss of bone mineral in females because of the postmenopausal oestrogen deficiency, when bone turnover increases, dietary calcium requirement increases [27].

Dairy products are main sources of calcium with a high availability and their calcium to phosphorus ratio is optimal. They belong to the few food items that contribute to the dietary vitamin D supply. Heaney [17] summarized in his review of about 140 papers that nearly all controlled intervention studies and approximately 75% of observational studies indicated an improvement of bone health by dietary calcium. Milk products are rich in calcium and this calcium has a high bioavailability [7, 17]. Thus milk products are considered to be optimal to build up bone tissue during growth and to attenuate loss of bone mineral throughout lifetime.

Parathyroid hormone (PTH) regulates calcium homeostasis, while its secretion is modulated by plasma calcium and calcium intake [27]. PTH stimulates osteoclast activity and thus bone resorption. Higher nocturnal serum PTH levels were observed in osteoporotic women [12], which contributes to the development of bone loss after menopause [32].

Bone resorption in human subjects shows a circadian rhythm with a peak in the early morning and a dip in the afternoon [4]. Blumsohn et al. [4] observed that the nighttime calcium supplementation markedly depressed this nocturnally increased excretion of deoxypyridinolin (DPD), a marker of bone resorption, and reversed the usually observed nocturnal rise of PTH; in contrast, the morning calcium supplementation had no effect. Based on these observations the milk supplement in our study was taken at bedtime.

Both, calcium rich foods or supplements and nutritional components that stimulate intestinal calcium absorption are attractive, low-cost options for osteoporosis prevention. In animal studies non-digestible inulin-type fructans (ITF) stimulated the intestinal absorption of minerals and raised bone mineral content; for review see [40, 43]. In humans, short-term studies also indicated enhanced intestinal calcium absorption by ITF [51]. In the meantime a stimulating effect on calcium absorption and bone mineral density was also proven for girls in the long-term [1]. For caseinphosphopeptides (CPP), resulting from tryptic hydrolysis of casein, there is conflicting literature; for review see [6, 44]. In rats CPP enhanced calcium absorption and/or improved calcium incorporation into bone in some studies [38, 44] but not in all [21, 44].

In the present controlled, parallel, double-blind intervention study, we investigated the effect of (a) fermented milk supplemented with calcium from milk minerals, or (b) fermented milk supplemented with calcium from milk minerals, ITF and CPP and compared them with a control group consuming (c) fermented milk without additives in healthy, postmenopausal women without receiving hormonal replacement therapy. The hypothesis was that bedtime consumption of fermented milk inhibits the nocturnally increased bone resorption optimally, that this effect is more pronounced when the milk was supplemented with calcium and most effective

when calcium absorption enhancers were added beyond it.

Materials and methods

Subjects

127 postmenopausal women were recruited by newspaper advertisements in Kiel and screened for inclusion and exclusion criteria by medical examination and records. All women gave written informed consent. The study design was reviewed and approved by the Human Ethics Committee of the Medical Faculty of the University of Kiel. 85 healthy women were enrolled in this study. They were between 48 and 67 years of age (58.7 ± 0.3) and had been postmenopausal for 10.5 ± 0.7 years. None had a history of osteoporotic fracture. Subjects with acute or chronic diseases known to affect bone or calcium metabolism were excluded. None was receiving hormone replacement therapy, vitamin D or calcium supplements, or other agents known to affect bone metabolism. The volunteers had a body-mass-index (BMI) of 26.81 ± 0.54 kg/m^2. Their dietary calcium intake was estimated twice, at the beginning and at the end of the study. For this a photo-illustrated, food-frequency questionnaire was applied, that assesses the dietary habits retrospectively with a focus on the consumption of milk and milk products. The subjects had been given instructions from a nutritionist how to handle the questionnaire, which was a modification of a questionnaire described in more detail before [11]. Gastrointestinal well-being was assessed by a questionnaire that scored gastrointestinal irritations at the beginning and end of the study. The questionnaire was a modification of that described by Cook et al. [8] and inquired about the intensity, duration, and frequency of abdominal pain as well as the frequency of bowel movement and any accompanying symptoms. Each questionnaire produced a score for GI symptoms between 1 (no symptoms) and 7 (most symptoms).

Study design and supplements

The clinical trial was a 14-day controlled, parallel, double-blind intervention study. The women were matched for age, time after menopause, BMI, and dietary calcium intake and were assigned at random to one of three test milks: 1. Control milk, which was a fermented milk with 1.5% fat (f-milk, $n = 28$), 2. the same fermented milk but supplemented with calcium (f-milk + Ca, $n = 28$) and 3. fermented milk supplemented with calcium, ITF, and CPP (f-milk + Ca + ITF + CPP, $n = 28$). The women were asked to incorporate a cup of 175 ml test-milk per day into their habitual diet and to drink it daily between 8 p.m. and 9 p.m. Thereafter, only the consumption of mineral water with less than 10 mg/l calcium was allowed, which was provided by the institute.

The three test-milks were prepared, packed and kindly provided by Campina (Heilbronn, Germany) and delivered twice weekly to the institute, where the women picked them up the same day. The control milk contained 210 mg calcium and 160 mg phosphorus per cup. The calcium supplemented milk contained Lactoval® (DMV International, the Netherlands), which is a calcium-rich mineral supplement derived from milk. Its concentration was 18 g/kg test milk and by this it provided additional 510 mg calcium and 320 mg phosphorus per cup. The third group received the fermented milk + calcium, which was supplemented with CPP and ITF. CPP derived from milk as well (CE90CPP, DMV International, Veghel, the Netherlands) and its content was 1 g/kg test milk or 0.175 g per cup. Synergy® (Orafti, Belgium), a combination of long-chain (inulin) and short-chain (oligofructose) inulin-type fructans, was given at a concentration of 10 g/kg test milk or 1.75 g per cup.

Fasting plasma and serum samples were collected from subjects in the early morning at baseline and at the end of the intervention. The women collected their complete urine samples for 48 h before and at the end of the intervention. The urinary collection period was divided into a day fraction (consisting of day one and day two fractions, each starting after spontaneous morning urine, and ending before the test milk consumption) and a night fraction (consisting of day one and day two fractions, each starting after the test milk consumption, and ending after spontaneous morning urine).

Clinical parameters

Biochemical markers of bone metabolism in plasma or serum (total alkaline phosphatase, TAP, bone alkaline phosphatase, BAP, a specific marker of bone formation, total calcium, and phosphorus) and urine (calcium, phosphorus, and creatinine) were analysed with an automatic analyser (KL 20i, Kone, Espoo, Finland). Serum ionized calcium was measured by a ion selective analyser (Microlyte, Labsystems, Espoo, Finland). Serum intact PTH was measured by ELISA (Sangui BioTech, Santa Ana, California). Urinary deoxypyridinoline (DPD), a specific marker of bone resorption was analysed by HPLC [2]. All urinary

Table 1 Baseline characteristics of postmenopausal women in the three experimental groups

	Fermented milk ($N = 28$)	Fermented milk + Ca ($N = 28$)	Fermented milk + Ca + ITF + CPP ($N = 29$)
Age (years)	58.6 ± 0.5	58.5 ± 0.8	58.8 ± 0.7
Years after menopause	10.4 ± 1.2	11.2 ± 1.2	10.4 ± 1.1
BMI (kg/m^2)	26.9 ± 1.0	26.7 ± 0.9	26.9 ± 0.9
Habitual Ca intake (mg/d)	920 ± 95	894 ± 99	905 ± 85

Least square means ± SEM by ANOVA; There were no significant differences between groups; *Ca* Calcium from milk mineral; *ITF* inulin-type fructans (Synergy®); *CPP* caseinphosphopeptides

parameters were calculated relative to creatinine excretion.

Statistical analysis

A multifactorial analysis of variance (MANOVA) was performed to gain mean values and their SEM, and to test the effect of the factors "intervention" (at baseline and at the end of intervention), and "test milk" (f-milk, f-milk + Ca, and f-milk + Ca + ITF + CPP). The changes by time within each group (delta values of concentrations at the end of the intervention minus concentration at baseline) were compared between groups by ANOVA followed by a two-sided test *(Student-Newman-Keuls test)*. A P-value < 0.05 was considered to be significant. The statistical package STATGRAPHICS plus 4.1 was used.

Results

The baseline characteristics of the subjects before intervention are shown in Table 1. There were no differences between groups. All 85 subjects completed the trial. The mean habitual calcium intake was 906.4 ± 53.2 mg/day. The changes between the first and the second food-frequency questionnaire with special reference to milk and milk products did not differ between the groups.

Effect of test-milks ($n = 28$ resp. 29)

After 2 weeks of intervention, the supplementation of the fermented milk with 510 mg of calcium and 320 mg of phosphorus from milk mineral (f-milk + Ca) did not significantly affect any of the clinical parameters compared with the control milk (f-milk, Table 2). When calcium absorption enhancers were added in addition (f-milk + Ca + ITF + CPP), the women excreted more calcium with the night urine with 104.3 ± 39.5 mg/g creatinine (change from baseline) as compared to women who consumed the f-milk + Ca with −2.3 ± 21.8 mg/g creatinine ($P < 0.05$), and tended to excrete more calcium than woman on f-milk with 36.7 ± 25.2 mg/g creatinine (Fig. 1). The change of nocturnal phosphorus excretion was also higher in the f-milk + Ca + ITF + CPP group with 243.0 ± 53.0 mg/g creatinine as compared to the f-milk + Ca group with 97.3 ± 40.5 mg/g creatinine ($P < 0.01$) and f- milk without supplements (−15.4 ± 53.0 mg/g creatinine, $P \leq 0.05$, Table 2). The

Table 2 Effect of intervention with supplemented fermented milk compared to a control milk on change from baseline of plasma and urine parameters of calcium and bone metabolism

	Fermented milk ($N = 28$)	Fermented milk + Ca ($N = 28$)	Fermented milk + Ca + ITF + CPP ($N = 29$)	P value[§]
Serum/plasma				
TAP (U/l)	−4.50 ± 5.90	−7.39 ± 5.98	−2.00 ± 4.88	0.79
BAP (U/l)	−4.41 ± 4.81	−11.03 ± 5.74	−2.88 ± 4.95	0.50
PTH (ng/l)	−12.56 ± 3.44	−8.21 ± 3.79	−8.67 ± 5.13	0.73
P (mg/l)	3.50 ± 0.64	0.83 ± 1.14	2.51 ± 0.71	0.16
Ca^{tot} (mg/l)	−8.24 ± 1.33	−9.54 ± 1.28	−8.81 ± 1.41	0.79
$Ca^{ion.}$(mg/l)	−0.07 ± 0.26	−0.03 ± 0.22	0.12 ± 0.18	0.81
Day urine				
Ca/Creat. (mg/g)	10.52 ± 22.05	4.15 ± 12.04	26.75 ± 17.34	0.64
P/Creat. (mg/g)	2.92 ± 39.74	−24.10 ± 51.29	38.81 ± 36.57	0.58
DPD/Creat. (μmol/mol)	−1.05 ± 1.11	−0.23 ± 0.87	−0.66 ± 0.77	0.83
Night urine				
P/Creat. (mg/g)	−15.36 ± 53.00[a]	97.27 ± 40.46[a]	242.96 ± 53.01[b]	0.002
DPD/Creat. (μmol/mol)	−3.72 ± 1.09	−1.20 ± 0.84	−0.75 ± 0.92	0.068

[§]By one-way ANOVA. Least square means ± SEM by ANOVA; values not sharing a superscript letter are significantly different; *BAP* bone alkaline phosphatase, Ca^{tot}, Ca^{ion} total resp. ionic calcium, *+Ca* calcium from milk mineral, *CPP* caseinphosphopeptides, *ITF* inulin-type fructans (Synergy®), *P* phosphorus, *PTH* parathyroid hormone, *TAP* total alkaline phosphatase

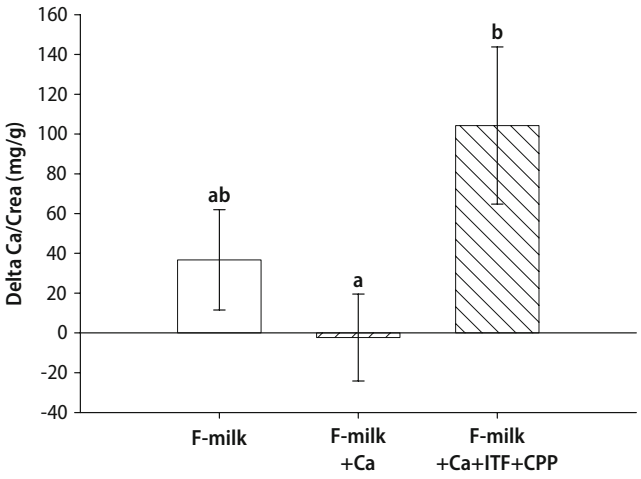

Fig. 1 Changes from baseline of calcium excretion in the night urine fraction (Delta mg/g creatinine) in postmenopausal women who consumed 175 ml of a fermented milk at bedtime for 2 weeks: Without supplement (*open bar* F-milk, $n = 28$), fermented milk supplemented with calcium from milk minerals (Lactoval®), (*striped bar* F-milk + Ca, $n = 28$), and fermented milk supplemented with calcium from milk minerals, inulin-type fructans and caseinphosphopeptides (*striped bar* F-milk + Ca + ITF + CPP, $n = 29$). Least square means ± SEM from ANOVA. Columns not sharing the same superscript letter are significantly different

changes of other parameters particularly of bone resorption, and of clinical parameters in the day urine did not differ significantly between the experimental groups (Table 2).

Table 3 Effect of intervention with fermented milk on change from baseline of urine parameters of calcium and bone metabolism

	Baseline ($N = 85$)	Final ($N = 85$)	*P* value
Day urine			
Ca/Creat (mg/g)	188.1 ± 10.4	202.1 ± 10.4	0.342
P/Creat (mg/g)	906.7 ± 27.5	913.0 ± 27.5	0.872
DPD/Creat (µmol/mol)	9.8 ± 0.43	9.2 ± 0.43	0.291
Night urine			
Ca/Creat (mg/g)	184.0 ± 13.8	230.9 ± 13.8	0.017
P/Creat (mg/g)	993.8 ± 27.7	1103.6 ± 27.7	0.006

Least square means ± SEM by two-way ANOVA performed for the factors "dietary group" and "intervention"; *DPD* deoxypyridinoline, *Creat* creatinine

Table 4 Effect of intervention with fermented milk on plasma parameters of bone metabolism

	Baseline ($N = 85$)	Final ($N = 85$)	*P* value
TAP (U/l)	111.3 ± 2.54	106.72 ± 2.54	0.201
PTH (ng/l)	47.9 ± 11.7	41.23 ± 11.67	0.689
P (mg/l)	36.0 ± 0.45	38.69 ± 0.45	0.000
Ca^{tot} (mg/l)	99.6 ± 0.64	90.73 ± 0.64	0.000
$Ca^{ion.}$(mg/l)	49.0 ± 0.14	49.05 ± 0.14	0.855

Least square means ± SEM by two-way ANOVA performed for the factors "dietary group" and "intervention"; Ca^{tot}, Ca^{ion}: total resp. ionic calcium, *P* phosphorus, *PTH* parathyroid hormone, *TAP* total alkaline phosphatase

Effects of an intervention with fermented milk ($n = 85$)

From the MANOVA including all participants no effect of the factor "test-milk", but an independent effect of the factor "intervention" was significant. Therefore we tested the effect of "fermented milk" in the pooled sample on several biochemical markers of calcium and bone metabolism by comparing values at the end of two weeks of intervention with baseline (Tables 3, 4). In the night urine the excretion of calcium and phosphorus was significantly higher (Table 3) and of DPD, a specific marker of bone resorption, significantly lower at the end of the intervention, compared with baseline (Fig. 2). In contrast, no effect was observed in the day urine fraction. Furthermore bedtime consumption of fermented milk, irrespective of the supplementation, significantly decreased serum activity of BAP (Fig. 2), the marker specific for bone formation. Plasma concentration of total calcium was decreased, the concentration of ionic calcium staying constant (Table 4). Plasma concentration of phosphorus was slightly but significantly increased by nighttime consumption of fermented milk.

Discussion

Effects of an intervention with fermented milk

Our data indicate that daily consumption of a fermented milk providing 210 mg calcium in the evening, regardless of a supplementation with calcium from milk mineral, or with Ca, ITF and CPP, decreased bone resorption by lowering bone turnover in healthy, postmenopausal women as indicated by lower plasma BAP activity and nocturnal urinary DPD excretion at the end of the two-week intervention period. This result is in accordance with studies showing that frequent milk consumption at all different ages was positively associated with bone mineral density or bone health [3, 20, 29], and that daily supplementation with approximately 700 ml milk decreased bone resorption and improved calcium balance in healthy, postmenopausal women [35]. It was repeatedly shown that calcium supplements from milk were more effective than from other sources [20, 26]. The reason for this can be awarded to other components in milk, such as a high availability of calcium, an optimal Ca:P-ratio of 1.3, other bone relevant minerals like magnesium and zinc [7], and encrypted bioactive peptides or proteins with a potential to improve calcium absorption and bone mineral density, like CPP and milk basic protein [50]; for review see [28, 44]. This,

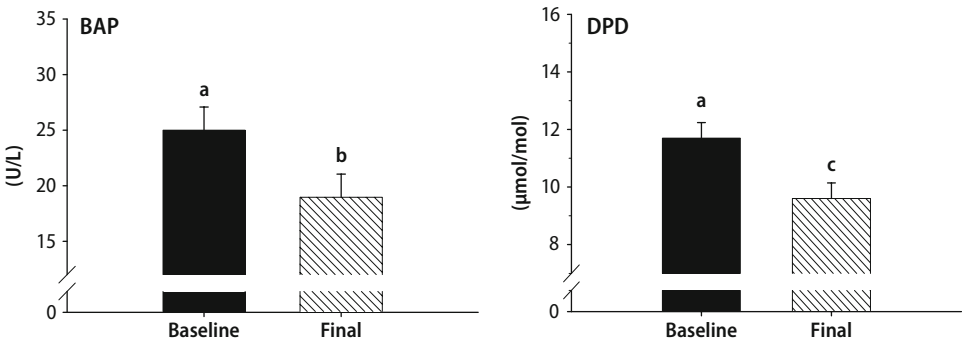

Fig. 2 Baseline and final concentrations of marker of bone formation (bone alkaline phosphatase, BAP) and bone resorption (deoxypyridinoline, DPD, in the night urine fraction) of postmenopausal women who consumed 175 ml of a fermented milk at bedtime irrespective of the supplement ($n = 85$). a,b: $P < 0.05$; a,c; $P < 0.01$

together with the point in time of milk consumption might explain why the supplementation with fermented milk and thus the additional calcium significantly depressed bone turnover in spite of the relatively high habitual calcium intake.

The reduced excretion of DPD (−18% compare to baseline, Fig. 2) in the night urine but not in the day urine confirms our initial hypothesis of a potent effect of a dairy supplement at bed-time. The decreased serum activity of BAP (−24% compared to baseline, Fig. 2) in our subjects after 2 weeks of intervention with fermented milk as a calcium source is in agreement also with long-term reports by others on calcium supplementation in humans [19, 36], and in animal models on a diet with a high calcium content [41]. The diminution of BAP in context with lower DPD after a rise in calcium intake is generally assumed to reflect a decrease in bone turnover. The decrease in total serum calcium concentration during the intervention period is difficult to interpret. It may be a secondary effect of the slightly lower plasma levels of PTH following the calcium load by the intervention with fermented milk. The slightly but significantly higher serum concentration of phosphorus is presumably the result of the accessory phosphorus provided by the fermented milk itself and above that by the milk mineral supplement in the other two groups.

Effect of test-milks

When fermented milk was supplemented with 510 mg calcium from milk mineral, the additional calcium load in this group (f-milk + Ca), which is about 50% of the recommendation, did not have any additional beneficial effect on calcium absorption and bone turnover above that of the fermented milk itself. The reason for this might be the smaller amount of supplemented calcium compared to the doses frequently used in other studies of 1,000–1,600 mg/d [4, 19, 24]. An other reason might be the relatively high habitual calcium intake of approximately 900 mg/d by the women in our study, since it was shown that calcium supplementation was more effective if habitual calcium intake was low [45]. Thus, the supplemented calcium raised the total calcium intake above a threshold level, where the solubility of luminal calcium is at its limit and no further absorption obviously occurs [25]. The trend for a higher urinary phosphorus excretion in the night urine of the group consuming fermented milk + Ca compared to the control group may be attributed to the additional phosphorus that was delivered with the milk mineral supplement.

As compared to fermented milk + Ca (f-milk + Ca) we have shown a significant increase in nocturnal calcium and phosphorus excretion in postmenopausal women when ITF + CPP were added. The rise in urinary calcium is supposed to be the result of a stimulated calcium absorption and not an increased bone resorption, because DPD excretion did not increase but slightly decreased. In accordance also others have found higher urinary calcium excretion after calcium supplementation, and interpreted this as more calcium absorbed, not as calcium resorbed from bone tissue, because the bone resorption marker was reduced [14]. The assumed stimulation of calcium absorption may be attributed to ITF or CPP, or both. Our data are in accordance with own observations in rats when bone calcium content rose only in case of additional oligofructose supplementation and not with dietary calcium supplementation alone [39]. Moreover, when diets contained oligofructose intestinal calcium absorption and calcium content of the bone was increased in spite of an increased urinary calcium excretion [39]. ITF have been repeatedly shown to enhance calcium absorption in rats and humans, and this stimulating effect is attributed to several luminal, physiological and biochemical factors. These include the increase of mineral solubility in the gut, the enlargement of the absorption surface, and the stimulation of active calcium absorption [31, for review see 5, 34, 40].

Supplementation of a fermented milk + Ca with ITF and CPP did not further inhibit bone resorption in our study although they obviously had raised intestinal calcium absorption. A significant stimulation of bone mineralization was observed in a long-

term intervention in young adults, when ITF with a higher degree of polymerization (DP) were given [1]. Our study is in contrast to expectations based on studies in rats when ITF increased bone mineral content, its density, and bone trabecular structure [37, 39, 47]. The effect of ITF on calcium absorption and bone mineral content depends on its dose and degree of polymerization. In adolescents a daily intake of 8 g of short-chain ITF (oligofructose) was not effective but of a product containing short-chain and long-chain oligomers was [15]. In most cases the effective doses ranged between 8 and 40 g/d [9, 15, 51]. Other confounders were the postmenopausal state in women [46] and the age in rats [33]. A non-significant stimulation of calcium absorption was reported when a single dose of 1.1 g of ITF was given [23]. The 1.75 g ITF/d in our study was chosen in order to avoid side effects like bloating or intestinal pain. However, the dose might have been too small and the duration too short to generate an effect on bone resorption large enough to reach significance.

There is less unanimity on the stimulating potential of CPP on calcium absorption; for review see [6] and [44]. In some studies CPP preparations or postulated encrypted CPP from casein stimulated calcium absorption [16, 49], due to their postulated capacity to keep calcium in a soluble form. In other experiments this was not the case [21, 30, 48]. When the acute effect of milk containing 1 g of CPP on calcium metabolism was investigated no significant effects on plasma intact PTH, ionized calcium, total calcium and phosphate, and 24-h calcium urinary calcium excretion was observed in postmenopausal women [30]. The effective dose of CPP was 1 g in the study by Hansen et al. [16] and thus much higher than in our study. The low dose in our study was chosen with respect to taste and acceptability. Thus, study conditions like habitual diet, food matrix [16], CPP/Ca ratio [13], vitamin D status [42, 44], and postmenopausal state [18] are important confounders on the effect of CPP on calcium metabolism.

In summary, in healthy, postmenopausal women who consumed a habitual diet with a calcium content of approximately 900 mg/d, bedtime consumption of a cup of 175 ml fermented milk reduced bone resorption due to a decelerated bone turnover, as indicated by a decline of nocturnal excretion of DPD and of plasma BAP activity. A supplement of 500 mg calcium from milk mineral without additional absorption enhancers had no further benefit, presumably due to a threshold amount of soluble luminal calcium. When the women consumed the calcium-enriched fermented milk together with ITF + CPP, a higher urinary calcium excretion indicated a rise of calcium absorption without negative effect on bone resorption. From our results we cannot ascribe this effect to one of the two absorption enhancers, since the aim of our study was to test a functional food. We cannot exclude that a higher dose of absorption enhancers or a prolongation of the study may have demonstrated additive effects on bone resorption and formation above the beneficial effect of fermented milk itself.

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