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ORIGINAL CONTRIBUTION

Effect of high in comparison to low dairy intake intervention on markers of bone and cartilage remodeling and phosphate metabolism in healthy adults with overweight

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Abstract

Background In the ageing population, issues with bone and joint health are highly prevalent. Both beneficial and potential risks of dairy products on bone and joint health are reported in epidemiological studies. Furthermore, the phosphorus (P) load from dairy could potentially lead to unfavorable changes in P metabolism.

Objective To investigate the effect of dairy intake on markers of bone and joint metabolism and P metabolism in an intervention study with high and low dairy intake.

Methods In a post hoc analysis of a randomized cross-over trial with overweight adults, the effect of a standardized high dairy intake [HDI (5–6 dairy portions per day) versus low dairy intake (LDI, ≤ 1 dairy portion/day)] for 6 weeks on markers of bone and joint health was assessed using enzyme-linked immunosorbent assays and electrochemiluminescence immunoassays. Markers indicative for cartilage breakdown, including urinary CTX-II, serum COMP and 4-hydroxyproline, and markers indicative for bone remodeling, such as serum CTX-I, PTH, 25(OH)D, osteocalcin, P1NP and FGF23, were investigated using linear mixed models. Furthermore, changes in P metabolism, including the main phosphate-regulating hormone FGF23 were explored.

Results This study was completed by 46 adults (57% female, age 59 ± 4 years, BMI 28 ± 2 kg/m^2). Following HDI, markers such as urinary CTX-II excretion, COMP, 25(OH)D, PTH and CTX-I were significantly lower after HDI, as compared to LDI. For example, CTX-II excretion was 1688 ng/24 h at HDI, while it was 2050 ng/24 h at LDI ($p < 0.001$). Concurrently, P intake was higher at HDI than at LDI (2090 vs 1313 mg/day, $p < 0.001$). While plasma P levels did not differ (1.03 vs 1.04 mmol/L in LDI, $p = 0.36$), urinary P excretion was higher at HDI than at LDI (31 vs 28 mmol/L, $p = 0.04$). FGF23 levels tended to be higher at HDI than at LDI (76.3 vs. 72.9 RU/mL, $p = 0.07$).

Conclusions HDI, as compared to LDI, reduced markers that are indicative for joint and bone resorption and bone turnover. No changes in P metabolism were observed.

Clinical trial registry This trial was registered at https://trialsearch.who.int/Trial2.aspx?TrialID=NTR4899 as NTR4899.

Keywords Dairy · Bone · Joint · Phosphorus

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Abbreviations

Ca	Calcium
COMP	Cartilage oligomeric matrix protein
CTX-I	C-terminal telopeptide of type I collagen
CTX-II	C-terminal telopeptide of type II collagen
ECLIA	Electrochemiluminescence immunoassay
FGF23	Fibroblast growth factor 23
HDI	High dairy intake
P	Phosphorus
PRAL	Potential renal acid load
PTH	Parathyroid hormone
LDI	Low dairy intake

Introduction

Ageing of the population is a globally observed trend, which is often accompanied with a decline in physical performance and difficulties with activities of daily living on the long term. Studies have shown that overweight and obesity can affect bone resorption and turnover, making the skeletal health of overweight individuals an area of interest [1].

Dairy products are naturally rich in nutrients, such as calcium (Ca), phosphorus (P), vitamin K2 and protein, that are relevant for bone and joint health [2–6]. Ca and P are crucial for the activity of osteoblasts and osteoclasts that facilitate bone regeneration and chondrocytes that facilitate joint health. Protein is relevant for maintenance of bone and joint matrices and for promotion of intestinal Ca absorption [7]. However, it has also been argued that the acid load that accompanies dairy intake results in increased bone resorption or osteoarthritis [8]. Additionally, a high dietary P load could be detrimental for cardiovascular health, especially in elderly in which the prevalence of cardiovascular disease has excessively risen up to 75–86% [9, 10].

Serum C-telopeptide of type I collagen (CTX-I) is an established marker for bone resorption, which is generated during breakdown of bone by osteoclasts [11]. Procollagen Type I N-terminal Propeptide (P1NP) is an established marker for bone formation [12]. As a precursor molecule for mature type I collagen, which is the most abundant collagen in bone, elevated levels of P1NP suggest active bone formation. Both markers are used for the assessment of fracture risk and monitoring therapy in clinical settings [13]. While CTX-I and P1NP relate to bone turnover, osteocalcin is a marker related to the functionality of osteoblasts, and it has been shown that circulating concentrations of it are more informative for risk of hip fractures in older males than P1NP and CTX-I [14]. In the field of joint health, urinary C-terminal crosslinking telopeptide of type II collagen (CTX-II) is an established marker, which relates to for cartilage degradation in the joints [15]. Elevated excretion of CTX-II suggests increased cartilage turnover, often a sign of degenerative joint diseases. Furthermore, serum cartilage oligomeric matrix protein (COMP), and serum 4-hydroxyproline are indicators of articular cartilage health [15]. The former is a structural protein of cartilage, whereas the latter, 4-hydroxyproline, indicates collagen breakdown. These markers are predictive for destruction of articular cartilage in e.g. osteoarthritis [15]. We hypothesize that an increased dairy intake, despite its accompanying acid load, leads to a decrease in these bone and joint markers.

The P intake in Western diets generally exceeds the European adequate intake (AI) of 550 mg/day P, but is well below the upper tolerable level of 4000 mg/day P in US guidelines [16, 17]. The relatively high intake is mainly the consequence of excessive use of inorganic P additives in (ultra)processed foods and unawareness of this by the general public, which is in part due to lack of requirements for declaring of total P content of food products on pack. High dietary P load might adversely affects P homeostasis, including increased levels of plasma and urine phosphate, the main phosphate-regulating hormone fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) [18].

The aim of the current post hoc study was to investigate the effect of high dairy intake (HDI) versus low dairy intake (LDI) on key markers of bone and joint health. Specifically, we sought to elucidate how varying levels of dairy intake, considering components such as Ca, P, protein and dietary acid load, are associated with these markers. Furthermore, our study delved into P metabolism, including levels of PTH and FGF23, under conditions of HDI and LDI.

Methods

Subjects

This is a post hoc study of a randomized, crossover intervention study (NTR4899). The original study was performed in 46 middle-aged, overweight individuals [age 45–65 years, BMI 25–30 (kg/m^2)]. Eligible subjects were low–medium dairy consumers (~1–3 portions/day) and were used to consuming 3 main meals/day. They were not involved in intensive sports activities more than twice a week and had a relatively stable weight (fluctuations < 3 kg in the past 3 months). Individuals with diabetes mellitus as defined by fasting glucose ≥ 7.0 mmol/L or glycated hemoglobin ≥ 6.5% (48 mmol/mol) and clinically relevant abnormalities in blood lipids (total cholesterol > 8 mmol/L, triglycerides > 6 mmol/L, LDL > 5.7 mmol/L), hematology [Hb < 8.7 mmol/L (men) or < 7.5 mmol/L (women)], or elevated markers for liver damage (alanine aminotransferase and aspartate aminotransferase > 45 U/L) or kidney damage (urinary albumin:creatinin ratio > 30 mg/mmol) were excluded. Participants with a history of gastrointestinal disorders or prior digestive tract surgery were also excluded. Additionally, those on lipid-lowering medications from the screening phase up to the conclusion of the study, as well as those who took antibiotics with the month prior to the screening, were excluded. However, consistent use of blood pressure lowering medication was permitted. Participants were also not allowed to take any nutritional supplements, including Ca, P and vitamin D, from the screening period until the study's end. Further information on the subjects and randomization is described by Eelderink et al. [19].

The study was conducted according to the principles of the Declaration of Helsinki. Approval was obtained from the Medical Ethics Committee of the University Medical Center Groningen, Groningen, The Netherlands (METc2014/298).

Experimental design

Each subject participated in two dietary interventions i.e. a high dairy intake (HDI) and a low dairy intake diet (LDI) for 6 weeks in a crossover design, with a washout period of 4 weeks. After inclusion, the subjects came to the hospital on 6 occasions during the 16 weeks study period: at the start of each diet (weeks 0 and 10), after 3 weeks for a short visit (weeks 3 and 13), and after 6 weeks of each intervention for a test day (weeks 6 and 16). Treatment orders were allocated to participants based on minimization (using the software Minim, Stephen Evans, Patrick Royston and Simon Day, UK), to ensure minimal differences in gender, age and BMI. During the HDI, dairy intake consisted of 5 portions per day for women and 6 portions per day for men, whereas during the LDI period the subjects consumed ≤ 1 dairy portion/day. The amount of dairy in both diets was within the range of habitual dairy intake of males or females in the Netherlands [20]. The prescribed dairy portions were 200 g semi-skimmed yoghurt, 30 g reduced fat cheese (30 + cheese made from semi-skimmed milk containing 30% fat based on dry weight, ~ 19 g fat/100 g cheese), and 250 mL semi-skimmed milk and/or buttermilk (FrieslandCampina). During the HDI, participants were instructed to consume 1–2 portions of cheese (maturity free of choice) and ≥ 2 portions of yoghurt each day, whereas the other 1–3 dairy portions could be chosen freely according to preference (mainly milk and/or buttermilk). Except for the prescribed dairy products, all basic and unsweetened, no other dairy products were allowed during the intervention periods, except for the occasional use of small amounts of dairy in, for instance, coffee or tea. To maintain a stable caloric intake during both intervention periods, the subjects' habitual diet was advised to be adjusted by instruction of a dietician. Compliance to the diet was checked and maintained by regular contact with the dietician and/or researcher and by dietary assessment.

Measurements

The subjects were asked to complete a 3-day food diary (for 2 weekdays and 1 weekend day) on five occasions: before the start of the study to estimate their habitual diet, and in the third and sixth weeks of both diets. Total potential renal acid load (PRAL) was calculated as follows: PRAL (mEq/day) = 0.49 × protein (g/day) + 0.037 × P (mg/day)—0.021 × potassium (mg/day)—0.026 × magnesium (mg/day)—0.013 × Ca (mg/day).

Fasting blood samples were taken for direct measurements (4.5-mL Lithium-Heparin PST II tube, BD Diagnostics) and for separation of plasma (4-mL EDTA tube and 4-mL Lithium-Heparin tube, BD Diagnostics). Measurements were performed on a Roche/Hitachi Modular automatic analyzer (Roche Diagnostics, Hitachi).

A day before their 6-week visit, participants were instructed to collect their urine over a 24-h period, storing it in a container and refrigerating it. The total volume was determined by weighing the urine and dividing it by 1.015. After spinning the samples at 2000×*g* for 10 min at room temperature, they were portioned into 2-mL tubes and frozen at − 80 °C. The 24-h urine samples were analyzed for urea, calcium, sodium, potassium, and magnesium levels, serving as indicators for dietary intake. These assessments were conducted using a Roche/Hitachi Modular automatic analyzer from Roche Diagnostics.

To determine bone resorption and turnover, serum levels of CTX-I, P1NP and osteocalcin were measured. To determine resorption of joint, urinary CTX-II, COMP and 4-hydroxyproline were measured. COMP was also measured before start of both diets. Besides joint and bone markers, calciotrophic and phosphaturic hormones were measured, such as 25-hydroxyvitamin D (25(OH)D) and PTH, and FGF23, respectively.

P1NP and PTH were measured with electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany) using an ECLIA Elecsys autoanalyzer (Cobas e 601). Osteocalcin was measured using chemiluminescence immunoassay (CLIA) (Immulite 2000xpi Siemens Healthcare, Erlangen Germany). Total 25(OH)D (i.e., 25(OH)D2 plus 25(OH)D3) in all serum samples was measured using a liquid chromatography–tandem mass spectrometry method. COMP, 4-hydroxyproline, CTX-I and C-terminal FGF23 were measured in plasma EDTA ELISA (QPS). The intra-assay and inter-assay coefficients of variation of the biochemical assays performed to measure the concentrations of the aforementioned biochemical indices were for 25(OH)D; less than 4%; for PTH 2.3% and 2.1%, respectively, for P1NP; 2.8% and 4.5%, respectively, for osteocalcin less than 15%, for 4-hydroxyproline less than 9.2%, for COMP less than 5.2%, for CTX-I less than 9.4%, for FGF23 less than 16% and 5%, respectively.

Statistical analyses

All analyses were performed using R Studio version 3.4.2 (Vienna, Austria). Normally distributed variables are reported as mean ± SD and non-normally distributed variables as median (IQR). Linear mixed model analyses were used to examine the significance of differences in the values of bone and joint markers after both intervention periods, with additional adjustment for period, sequence, season and

carryover (period*sequence) effects. Natural log transformation was performed in case of nonnormal distribution. Linear mixed models were also performed to assess the association between protein intake, Ca and P intake or PRAL, and bone or joint markers using the data obtained after 6 weeks both intervention periods.

Results

Baseline

Baseline characteristics of 46 subjects are shown in Table 1. At baseline, the PRAL of the diet was on average neutral to basic and the protein intake was 1.0 g/kg body weight per day or 15% of the total energy consumption. The total Ca intake was 1013 mg/day and P intake was 1465 mg/day. A flow chart is shown in Supplemental Fig. 1.

Intervention with HDI and LDI

Changes in dietary intake, markers related to Ca and P metabolism and markers related to bone and joint health after HDI versus LDI are presented in Table 2. HDI increased the total protein, Ca and P intake. Although plasma Ca and P levels did not change significantly, the urinary Ca and P excretion was higher after HDI. Furthermore, the predicted renal acid load was higher after HDI.

Concerning the bone and joint markers, we found that a HDI caused a decrease in markers of bone and cartilage breakdown, as well as in markers of bone turnover, as shown by significant decreases in CTX-II, COMP, vitamin D, PTH and CTX-1. C-reactive protein (CRP) and FGF23 levels tended to be higher after HDI as compared to LDI, although the difference did not reach statistical significance ($p = 0.06$ and $p = 0.07$, respectively).

Table 1 Baseline characteristics

Characteristics	
Gender (*n* % male)	20 (43)
Age (years)	59.0 ± 4.3
BMI (kg/m^2)	28 ± 1.9
PRAL (mEq/100 g diet)	− 3.0 ± 14
Energy intake (kcal)	2163 ± 496
Protein intake (energy %)	15 ± 2.5
Protein intake (g/kg BW/day)	1.0 ± 0.22
Fat intake (g/day)	87.0 ± 24.3
P intake (mg/day)	1465 ± 321
Ca intake (mg/day)	1013 ± 256

Values are mean ± SD or *n* (%), $n = 46$

BMI body mass index, *PRAL* potential renal acid load, *BW* body weight

Determinants of bone and joint markers by measures of dietary intake

Univariate associations of protein, Ca and P intake and PRAL with bone and joint markers are shown in Table 3. Higher intake of protein, Ca, P and PRAL were all associated with a higher urinary Ca and P excretion. A negative association for the same intake measurements with urinary CTX-II, COMP (except for the association with PRAL) and CTX-I was found. Furthermore, a higher PRAL was associated with lower plasma concentrations of P, vitamin D and osteocalcin. Results yielded similar upon sensitivity analyses for dairy components adjusted for total energy intake (Supplemental Table 1).

Discussion

This post hoc study, explored the effects of HDI versus LDI on pivotal markers of bone and joint health. We expanded our study to explore how elements of dairy, such as Ca, P, protein and dietary acid load, affect bone and joint markers. Additionally, we aimed to understand P metabolism in the context of HDI.

For bone turnover, HDI led to lower circulating concentrations of CTX-I than LDI, while circulating concentrations of P1NP and osteocalcin were not significantly different between HDI and LDI. According to a meta-analysis of 20 randomized controlled trials (RCTs) in adults, milk supplementation reduced the concentrations of several bone turnover markers (including P1NP and CTX-I), while circulating concentrations of osteocalcin were not affected [21]. This is in line with the results of the current study, although the current study found no reduction in P1NP. This discrepancy could be due to differences in the duration of the current study compared to the RCTs in the meta-analysis (6 weeks vs. 1–36 months, respectively), variation in the specific dairy products consumed and differences in the baseline nutritional status of participants. Additionally, although our study did not directly examine fractures and falls, the clinical benefits of increased consumption of milk, yogurt, and cheese (not fortified with vitamin D) was highlighted by a 2-year randomized controlled trial. In this trial, there was a significant 33% reduction in fracture risk, likely due to increased protein and Ca intake [22].

To our knowledge, the effect of intervention with low to medium fat dairy products on joint markers has not been reported before. However, a positive association between dairy intake and a lower presence of knee osteoarthritis was previously found in cross-sectional analyses [23].

Table 2 Effect of 6 weeks high versus low dairy intake on dietary measures, joint, calciotrophic and bone markers

	Intervention		Effect HDI as compared to LDI	*p* value
	HDI ($n=46$)	LDI ($n=46$)	Δ (95% CI)	
Dietary measures				
Energy intake (kcal/day)	2299 ± 584	2151 ± 462	− 150 (− 275 to − 25)	**0.02**
Protein intake (g/day)	110 ± 25	78 ± 18	− 31 (− 37 to − 25)	**< 0.001**
Protein intake (energy kcal %)	19.2 ± 2.2	14.8 ± 2.6	− 4.5 (− 5.2 to − 3.7)	**< 0.001**
Protein intake (g/kg BW/day)	1.3 ± 0.2	0.9 ± 0.2	− 0.4 (− 0.4 to − 0.3)	**< 0.001**
Fat intake (g/day)	87.3 ± 26.1	82.5 ± 22.2	− 4.8 (− 12.6 to 2.9)	0.22
P intake (mg/day)	2090 ± 410	1313 ± 316	− 778 (− 882 to − 675)	**< 0.001**
Ca intake (mg/day)	1955 ± 280	719 ± 157	− 1237 (− 1327 to − 1147)	**< 0.001**
Vitamin D intake (µg/day)	3.0 ± 1.4	3.9 ± 1.8	0.9 (0.4 to 1.4)	**0.001**
PRAL (mEq/100 g of diet)	8.3 ± 14.5	− 2.8 ± 14.2	− 11.1 (− 15.3 to − 7.0)	**< 0.001**
Ca and P homeostasis				
Plasma Ca (mmol/L)	2.33 ± 0.07	2.33 ± 0.08	0.00 (− 0.01 to 0.02)	0.55
Plasma P (mmol/L)	1.03 ± 0.14	1.04 ± 0.14	0.01 (− 0.02 to 0.05)	0.36
Urinary Ca excretion (mmol/24 h)	4.95 ± 2.40	4.07 ± 1.74	− 0.88 (− 1.39 to − 0.36)	**0.01**
Urinary P excretion (mmol/24 h)	31 ± 9	28 ± 10	− 3 (− 5 to − 0)	**0.04**
Joint and inflammation markers				
Urinary CTX-II excretion (ng/24 h)[a]	1688 (841–1873)	2050 (1534–2632)		**< 0.001**
COMP (µg/L)	206 ± 67	214 ± 67	9 (2 to 17)	**0.02**
4-Hydroxyproline (mg/L)	26.9 ± 9.0	27.3 ± 7.7	0.3 (− 1.3 to 1.9)	0.74
CRP (mg/L)[a]	1.2 [0.8–1.8]	1.0 [0.6–1.9]		0.06
Calciotrophic and bone markers				
25(OH)D (nmol/L)[b,c]	56.3 ± 21.0	59.0 ± 23.1	7.7 (2.4 to 13.0)	**0.01**
PTH (pmol/L)	4.5 ± 1.2	4.8 ± 1.5	0.3 (0.0 to 0.5)	**0.03**
CTX-I (ng/L)	341 ± 134	438 ± 151	98 (75 to 121)	**< 0.001**
Osteocalcin (µg/L)	18.7 ± 6.4	19.5 ± 5.5	0.9 (− 0.3 to 2.1)	0.13
P1NP (µg/L)[a]	38.9 [29.8–44.4]	38.6 [31.5–47.0]		0.21
FGF23 (RU/mL)[a]	76.3 [64.2–91.1]	72.9 [62.8–84.8]		0.07

Linear mixed model analyses were performed with all subjects who completed both HDI and LDI. All models were adjusted for diet and period as random/fixed effects

P-values less than 0.05 are considered significant and are highlighted in bold

PRAL potential renal acid load, *CTX-II* C-terminal cross-linked telopeptide of type II collagen, *COMP* cartilage oligomeric matrix protein, *CRP* C-reactive protein, *PTH* parathyroid hormone, *CTX-I* C-telopeptide of crosslinked collagen type I, *P1NP* procollagen-1 N-terminal peptide, *FGF23* fibroblast growth factor 23

[a]Data were log-transformed in analyses

[b]Significant interaction carryover, term added to the model

[c]Season was additionally added to the model

Furthermore, intervention studies with a concentrated protein composition derived from the milk produced by hyperimmunized cows showed improvement of joint health and stability, discomfort and pain in individuals with osteoarthritis [24, 25] and in non-osteoarthritic participants who reported having mild-to-moderate functional knee pain during/after physical activity [26]. In this latter study, no effect of this concentrated protein was found on COMP, which is in contrast with our results, while urinary CTX-II was not measured.

Upon examining dairy components protein, Ca and P, it becomes evident that the overarching influence of dairy consumption leads to significant changes in bone and joint health, rather than specific components playing a distinct role. In terms of bone turnover, circulating CTX-I concentrations decline prominently with all dairy components, indicating a role in bone health. In contrast, the minimal changes seen in osteocalcin and P1NP indicate that these markers might be less influenced by dairy and its components in the current study. Notably, urinary CTX-II and COMP excretion

Table 3 Association between different measures of dietary intake and determinants of joint, inflammation, calciotrophic, and bone markers

	Protein intake (g/kg BW/day)	P intake (g/day)	Ca intake (g/day)	PRAL (mEq/100 g diet)
Ca and P homeostasis				
Plasma Ca (mmol/L)	− 0.00	− 0.00	− 0.00	− 0.00
Plasma P (mmol/L)	− 0.01	− 0.01	− 0.01	− 0.03*
Urinary Ca (mmol/24 h)	0.56**	0.61***	0.52***	0.59**
Urinary P (mmol/24 h)	2.80***	2.78***	1.93**	2.82**
Joint and inflammation markers				
Urinary CTX-II (ng/24 h)[a]	− 0.08**	− 0.07***	− 0.07***	− 0.05*
COMP (µg/L)	− 5.94*	− 6.55**	− 5.88**	− 5.08
4-Hydroxyproline (mg/L)	0.10	− 0.15	− 0.33	− 0.40
CRP (mg/L)[b]	0.11	0.14	0.13	0.14
Calciotrophic and bone markers				
25(OH)D (nmol/L)[b]	− 1.54	− 1.60	− 1.80	− 3.13*
PTH (pmol/L)	− 0.04	− 0.05	− 0.04	− 0.05
CTX-I (ng/L)	− 61.0***	− 60.8***	− 51.6***	− 51.3***
Osteocalcin (µg/L)	− 0.65	− 0.69	− 0.48	− 0.91*
P1NP (µg/L)[a]	− 0.03	− 0.03	− 0.02	− 0.02
FGF23 (RU/mL)[a]	0.02	0.03	0.03	0.01

Linear mixed model analyses were performed with all subjects who completed both high dairy intake and low dairy intake. Coefficients depict the change in determinants of joint, inflammation, calciotrophic, and bone markers per unit of dietary intake measure. All measures of dietary intake were defined as *Z*-scores

CTX-II C-terminal cross-linked telopeptide of type II collagen, *COMP* cartilage oligomeric matrix protein, *CRP* C-reactive Protein, *PTH* parathyroid hormone, *CTX-I* C-telopeptide of crosslinked collagen type I, *P1NP* procollagen-1 N-terminal peptide, *FGF23* fibroblast growth factor 23

*< 0.05, **< 0.01, ***< 0.001

[a]Data were log-transformed

[b]Season was additionally added to the model

significantly decreased with increased protein, P, and Ca intake, suggesting that these individual dairy elements may all guard against joint degradation. Importantly, extracting the individual impact of specific dairy components from the dairy intervention appeared difficult due to collinearity issues. Therefore, we could only conduct univariate analyses for each component, without adjustment for the other dairy components. For that reason, the results should be interpreted with caution.

Although HDI predictably led to a higher PRAL, this did not correlate with adverse changes in bone markers. On the contrary, a higher PRAL was associated with decreases in circulating concentrations of CTX-I and osteocalcin and a decrease in urinary excretion of CTX-II. This is not in concordance with results of a previous cross-sectional study, where an inverse association between PRAL and bone mass density among men with < 800 mg/day dietary Ca was found, while no associations were present among men aged > 60 with ≥ 800 mg/day [27]. Furthermore, in the current study, PRAL was associated with lower plasma P levels and higher urinary P excretion. Findings from a cross-sectional study support these findings regarding the association of PRAL with urinary P excretion [28]. These findings may be explained by the fact that P is the predominant urinary pH buffer, of which excretion increases with acidosis [29] and with an increased intake of P.

In the current study, the average P intake in HDI (2000 mg/day) was higher than during LDI (1300 mg/day), both exceeding the advised minimal P intake of 550 mg/day. However, they are still well below the maximum tolerated P intake (4000 mg/day). Assessing inorganic P intake proved challenging due to variation in P additives among food groups and the lack of P content labeling of food products. The increase in P intake in this study, largely from additive-free dairy products, suggests a predominantly organic P source. Although cross-sectional studies reported strong associations between P intake and plasma P levels [30, 31] previous studies that evaluated plasma P levels after 4–8 week of a P intervention did not report changes in plasma levels [32, 33] which is consistent with our findings.

FGF23, produced primarily in osteocytes, is a key regulator of P homeostasis. It reduces serum P levels by inhibiting P reabsorption and suppress the synthesis of the active form of vitamin D. While plasma P levels remained stable

following 6 weeks of HDI, FGF23 showed an upward trend. The increase in FGF23, although not statistically significant, is most likely an adaptive response to high P intake aimed at maintaining P balance. This is also supported by a significant increase in urinary P excretion. Changes in plasma FGF23 after a P intervention appear to exist in both short-term (a few weeks) [32] and long-term interventions (up to a year) [34], except for one study that reported increased FGF23 levels after 4 weeks, but not after 8 weeks [33]. Direct comparisons with these studies are infeasible since our dairy intervention was also concomitant with an increase of other nutrient levels, like Ca.

PTH, another phosphate-regulating hormone, was also assessed in our study. Typically, an increase in P intake would prompt a rise in PTH secretion, as it acts to promote the release of P from the bones and decreases its reabsorption in the kidneys. However, we observed a decrease in PTH levels following the HDI. This seemingly counterintuitive result can likely be ascribed to the concomitant increase in Ca intake observed in the HDI group. Elevated Ca levels in the blood are known to inhibit PTH secretion. The intricacies of P and Ca regulation are further complicated by the role of vitamin D. When Ca intake rises, the reduction in PTH decreases the formation of active vitamin D in the kidneys. In our study, consistent with the observed decrease in PTH, we noted a decline in vitamin D levels following HDI. Since vitamin D primarily functions to enhance absorption from the gut, the body's innate regulatory mechanisms may be working to prevent potential hypercalcemia.

Maintaining P homeostasis is crucial since elevated plasma P and FGF23 have been linked to cardiovascular disease and associated mortality [35, 36]. Also, increasing dietary P intake (> 1400 mg/day) was found to be associated with increased all-cause mortality in a healthy US population [37]. However, an overview of 12 meta-analyses, concluded that the consumption of total dairy products, with either regular or low fat content, does not adversely affect the risk of CVD [38].

One of the strengths of this study was the assessment of dairy intervention in a cross-over design with controlled dairy intake. In this study, in addition to Ca and P intake, we also measured Ca and P levels in blood and urine. A limitation of this study is that due to the relatively short intervention period only bone and joint biomarkers at the end of both intervention periods were measured. No actual endpoints such as bone mineral density or fracture risk could be assessed. In addition, we did not measure cartilage synthesis markers, such as PIINP or aggrecan. Furthermore, it may be that the 6-week intervention period was too short to demonstrate changes in P metabolism. While the amount of dairy consumed was strictly controlled, participants had the freedom to decide how to compensate for the omitted dairy. This decreased the homogeneity of dairy intervention. However, this approach allowed for diets that are more closely resemble real-world consumption patterns, thereby increasing the external validity of our findings. Lastly, in this post hoc analysis, the original study did not exclude individuals with conditions or lifestyles, such as malabsorption, alcoholism, certain medications, veganism, that might influence bone and joint health, potentially affecting the interpretation of our results.

In conclusion, HDI was associated with reduced joint and bone resorption, as well as bone turnover, in healthy overweight adults. The dietary intake of P in HDI is high (> 2000 mg/day). This did not result in changes in P metabolism, although plasma FGF23 showed a trend towards an increase. Overall, this study thus indicated thereby a beneficial effect on bone and joint health by dairy.

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Data share Data described in the manuscript, code book, and analytic code will not be made available because of privacy and ethical concerns.

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