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Systematic Review Article

Effects of Whey Proteins and Milk-basic Proteins on Bone Health Parameters: A Systematic Review and Meta-analysis of Randomized Clinical Trials

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ABSTRACT

Background and Objectives: As a major public health concern worldwide, osteoporosis causes increased risks of bone fractures and decreases in bone mineral density. The present systematic review and meta-analysis aimed to determine the effect of whey protein and milk basic protein on bone health parameters.

Materials and Methods: In this study, a systematic review and meta-analysis of randomized clinical trials study was carried out. Online databases of PubMed, Scopus and Web of Science were searched up to 20 march 2023, using controlled terms (e.g., MESH) and text words for milk protein or whey and bone-health outcomes, including lumbar-bone mineral density, hip-bone mineral density, urinary N-terminal telopeptides of type I collagen, serum C-terminal telopeptides of type I collagen, osteocalcin and insulin-like growth factor 1 levels.

Results: Outcomes were pooled as standard mean difference (SMD) and 95% confidence interval (CI) in a Random-effect meta-analysis model. Nine randomized clinical trials met the eligibility criteria and were selected for the final analysis. Analysis indicated significant decreases in N-terminal telopeptides of type I collagen [SMD, -0.89 nmol/mmol; CI, -1.69 to -0.10 %; p = 0.028] following supplementation with milk basic protein compared to the placebo group. Whey supplementation resulted in significant increases in insulin-like growth factor 1 [SMD, 3.55 nmol/l; 95% CI, 3.12 to 3.98%; P = 0.001; I^2 = 58.1%; p = 0.092]. However, no significant mean differences were seen in lumbar-bone mineral density, hip-bone mineral density, serum C-terminal telopeptides of type I collagen and osteocalcin between the two groups.

Conclusions: Whey or milk basic protein supplementation may decrease N-terminal telopeptides of type I collagen and increase insulin-like growth factor 1, particularly when adults are supplemented for 12 w or longer; however, findings on lumbar-bone mineral density, hip-bone mineral density, serum C-terminal telopeptides of type I collagen and osteocalcin are inconclusive.

Keywords: Whey protein, Milk protein, Bone, IGF, BMD, CTx, NTx

Highlights

- Osteoporosis is a public health issue worldwide, associated with increased bone fractures and decreased bone mineral density.
- Cow milk contains calcium, magnesium, vitamin K, vitamin D, phosphorus, casein lipids and isoflavones, which can meaningfully affect bone metabolism and help in maximizing bone mass.
- Milk basic protein contains active elements that strengthen proliferation and collagen synthesis of osteoblasts.
- Whey or milk basic protein is associated with decreasing urinary N-terminal telopeptides of type I collagen and increasing insulin-like growth factor in adults.

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Introduction

Osteoporosis is a public health issue worldwide, associated with increased bone fractures and decreased bone mineral density (BMD) (1, 2). This disease is often seen for reasons such as estrogen deficiency in menopause, low calcium intake and/or lack of exercise in adults, decreasing individuals' ability to do daily tasks and increasing risks of falls and fractures (3). Fractures during a person's lifetime significantly increase functional damages and decreases the quality of life, leading to increases in the financial burden on countries' healthcare systems. Numerous factors play roles in development of osteoporosis, which can be addressed as nutritional, psychological and biological factors (4–6). In addition to the highlighted factors, exercises play significant roles since sports are affordable non-pharmacological methods of treating osteoporosis (7, 8). Nutrient-rich foods such as cow milk, which has been widely used since ancient times, contain calcium, magnesium, vitamin K, vitamin D, phosphorus, casein lipids and powdered milk contains isoflaven, which can meaningfully affect bone metabolism and help in maximizing bone mass (9–11). The basic idea builds on the fact that the major components of milk basic protein (MBP) are whey protein (12) and MBP contains active elements that strengthen proliferation and collagen synthesis of osteoblasts (13, 14). Moreover, whey protein includes functional appropriate roles in bone regeneration (13, 14). In several human studies, it has been detected that MBP increases BMD (15–19). Aoe et al. (2005) reported that MBP could be one of the nutritional components that increased peak bone mass and decreased the future risk of osteoporosis in premenopausal women (20). The most significant decrease in bone turnover markers (BTM) is addressed with urine N-terminal telopeptides and serum C of type I collagen (UNTX and S-CTx) (21). These markers of bone resorption are strongly correlated with BMD response and U-NTX may be the preferred marker in clinical trials because it is not sensitive to circadian changes or affected by the consumption of foods and supplements unlike S-CTx (22). In another study, Uenishi stated that in young women with prescription of 40 mg of MBP supplement every day for six months, serum osteocalcin concentration increased significantly and MBP inhibited their excretion of urinary N-telopeptides of type-I collagen (NTx). Increases in BMD at the first stage were due to increases in bone formation and then inhibition of bone loss with MBP supplements (18). In contrast, associations between the whey protein, MBP and osteoporosis are controversial. Studies have not reported significant effects of MBP supplements on bone formation. In a study, Zou reported no significant effects of MBP supplements on BMD and bone metabolism but quantity of NTx in whole milk and milk supplement groups significantly decreased (23). Indeed, CTx and NTx can be used as major markers to assess bone loss (24, 25).

In one study, it was detected that consuming 20 g of MBP supplement every day for 2 y led to 7–8% increases in insulin-like growth factor 1 (IGF-1) hormone in group consuming MBP supplement. The hormone (IGF-1) is a factor that promotes bone growth and plays significant roles in activation of osteoblasts (26). Growth hormone secretion decreases with aging and circulating levels of IGF-1 in more than 30% of older adults are lower than those in younger adults, which includes implications for age-linked osteoporosis (27). In this study, effects of daily MBP and whey protein consumption on adult bone health factors were clarified. In most human and animal studies, MBP and whey protein have been shown to enhance bone formation, suppress bone loss and increase bone mineral density (BMD). Therefore, the current systematic review and meta-analysis of randomized controlled trials (RCTs) in healthy adults were carried out to assess effects of whey protein and MBP on bone health parameters.

Materials and Methods

This review was registered (CRD42022354024) in the International Prospective Register of Systematic Review (PROSPERO). The present study was based on the PRISMA protocol for reporting systematic reviews and meta-analyses, six parts or paragraphs; to which, the reader is kindly referred for details (28).

Data sources and search strategies

In this study, an extensive literature search was carried out using popular online databases such as PubMed, Scopus and Web of Science, up to 20 march 2023. Search strategy involved specific keywords of ("whey protein" OR "milk protein" OR "milk basic protein") AND (bone). To ensure thoroughness, study deliberately did not include exercise-related terms in the selected keywords. No restrictions on language or publication date were included. Additionally, reference lists of relevant studies were manually assessed to avoid overlooking relevant publications. Unpublished studies were not included in the review. Two independent investigators carried out a literature search.

Study selection and inclusion/exclusion criteria

Studies meeting the following eligibility criteria were analyzed for inclusion. (1) RCTs (parallel or crossover studies), (2) adult population cohorts (≥ 18 years), and (3) trials reporting mean (SD) alterations of bone health factors (lumbar-BMD, hip-BMD, Urinary NTx, serum CTx, osteocalcin and IGF) for intervention and control groups or presented necessary information for calculating the effect size. It is noteworthy that RCTs using multiple intervention groups (duration) were considered various

outcomes and data were pooled separately in these cases. Investigations and clinical trials that used animal models *in vitro* or were carried out on children (< 18 years old) were reviewed and those observational or lacked control groups were excluded from the meta-analysis. The present study used specific eligibility criteria based on the population, intervention, comparison, outcomes and study design (PICOS) approach (Table 1).

Table 1. Population, intervention, comparison, outcomes and study design criteria for the inclusion of studies

Population	Adult participants (healthy or unhealthy) aged 18 years or older
Intervention	Additional whey protein supplement (isolate, concentrate, hydrolysate or MBP) ingestion with or without exercise
Comparison	Placebo, no intervention or carbohydrate
Outcomes	Lumbar-BMD, hip-BMD, Urinary NTx, serum CTx, osteocalcin and IGF
Study design	Human randomize control trial (parallel or crossover studies)

Data extraction

Two independent investigators were responsible for the extraction of data from each eligible RCT. Pre-designed abstraction form was used to extract the following information from the full-texts of included studies: first author's name, publication year, country, characteristics of individuals (e.g., mean age, BMI and sample size), duration of intervention, type and dosage of supplementation, control group details, exercise intervention details and results. When relevant data were missing, corresponding authors were reached via emails to request their assistance. If data on bone health factors were reported in various units, these were converted to the most commonly used units.

Risks of bias assessment

In the present meta-analysis, Cochrane quality assessment tool was used to assess the quality of studies (29). This tool consisted of seven domains that were assessed for each study, including random sequence generation, allocation concealment, reporting bias, performance bias, detection bias, attrition bias and other sources of bias. Each domain received a score of "high risk" if the study included methodological flaws affecting its findings, a score of "low risk" if no defects in that domain were reported and a score of "unclear risk" if insufficient information were recorded to assess the effects. The overall risk of bias for an RCT was categorized as follows:

1. High-quality, if more than four domains had a "low risk" score; 2. moderate-quality, if two or three domains had a "low risk" score; and 3. low-quality, if one or no domains had a "low risk" score (30). Assessment of the risks of bias was carried out independently by two reviewers.

Statistical analysis

The overall effect size was estimated using the mean change and standard deviation (SD) of the relevant outcomes. When mean changes were not reported, they were calculated by analyzing changes in bone health indicators such as lumbar-BMD, hip-BMD, Urinary NTx, serum CTx, osteocalcin and IGF during the intervention. To calculate standard mean differences (SMDs), standard errors (SEs), 95% confidence intervals (CIs) and interquartile ranges (IQRs) were converted to SDs using a method described by Hozo et al. (31). If the outcome measures were only present in figures, "GetData Graph Digitizer" software was used to estimate the values. The SD change was calculated using the following formula:

$$SD = \sqrt{[(SD\ pretreatment)^2 + (SD\ posttreatment)^2 - (2R * SD\ pretreatment * SD\ posttreatment)]}$$

To assess the overall effect size, random-effect model was used, regarding variabilities between the studies. Heterogeneity was assessed using I^2 statistic and Cochrane's Q test. The I^2 value > 50% or $p < 0.05$ for the Q-test was reported as significant. Between-study heterogeneity subgroup analyses were carried out based on predefined variables such as type of supplementation (whey against MBP), intervention duration (> 36 against ≤ 36 w OR > 12 against ≤ 12 w), health condition (healthy and unhealthy) and exercising condition (exercise and no exercise). Sensitivity analysis was carried out by excluding each study to assess its effects on the pooled results. Publication bias was investigated by assessing funnel plots for asymmetry using Egger (32) and Begg (33) tests. Statistical analysis was carried out using STATA software v.14 (Stata, USA). Statistically significant values were set at $p < 0.05$.

Results

Selection and identification of studies

Out of the initial 1139 publications, 378 duplicate articles were excluded. After reviewing the remaining 761 records based on the title and abstract, 751 unrelated articles were removed. This resulted in totally ten publications to assess in full text. Two eligible articles were published using the same dataset (19, 23, 34) and the complete one was included (34). The final analysis included nine eligible RCTs (18, 20, 23, 34-39). From them, five studies assessed changes in lumbar-BMD (18, 20, 23, 36, 37), three studies focused on hip-BMD (36, 37, 39), three studies investigated NTx (18, 20, 34), three studies analyzed CTx (35, 37, 38), three studies investigated serum osteocalcin (18, 20, 34) and two studies assessed IGF (37, 39). Flow diagram of the study selection is outlined in Figure 1.

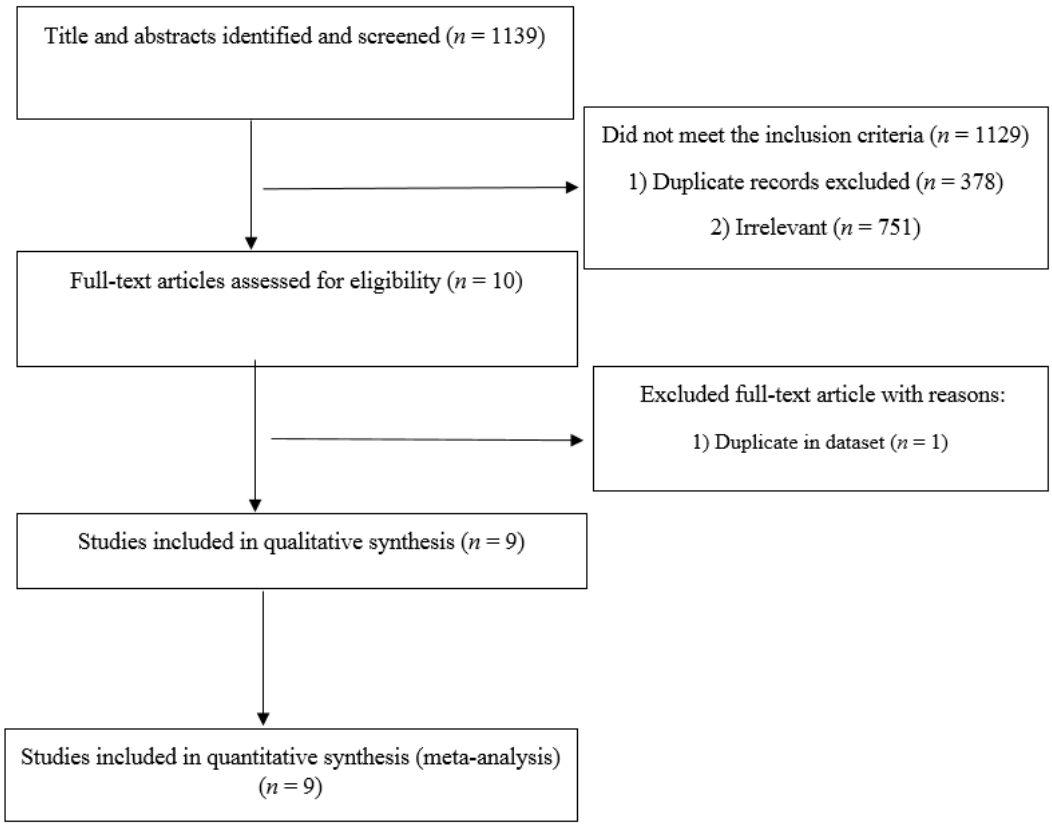


Figure 1. Flowchart of the study selection for the inclusion trials in the systematic review

Characteristics of the included studies

Characteristics of nine RCTs included in the current systematic review and meta-analysis are illustrated in Table 2. These RCTs were published between 2001 and 2022. Two studies were exclusively carried out on male subjects (36, 38), five studies on females (18, 20, 23, 34, 39) and others on the two sexes (37, 40). The total sample size included 620 individuals; out of which, 319 subjects were in the intervention group and 301 were in the control group. Age range of the participants included 18–85 y. Based on the Cochrane scores, seven studies were classified as high-quality studies (more than four items of low risks) and two were classified as moderate-quality (2–3 items of low risks). Result of the quality assessment is reported in Supplementary File 1.

Findings from the systematic review

From seven studies assessing effects of whey or MBP supplementation on BMD, four intervention arms from three studies revealed significant increasing effects (18, 20, 23), two included no significant effects (36, 37) and one included decreasing substantial effects (39). Of these, four studies were linked to MBP supplements (18, 20, 23, 34) and three were linked to whey supplements (36, 37, 39). Three studies assessed NTx changes (18, 20, 34), which all reported significant NTx decreases. Three studies assessed CTx changes (35, 37, 38); of which, one reported significant increases (37) and two described no changes (35, 38). Regarding changes in osteocalcin, two trials illustrated significant increases following MBP supplementation (18, 34) while one did not demonstrate (20) and two trials reported serum IGF, which was effective.

Findings from the meta-analysis

Overall, nine RCTs in the systematic review were included in the meta-analysis. These trials included a total sample size of 620 individuals aged 18 years and over.

Effects of whey and milk basic protein on lumbar-bone mineral density

Based on the results of six effect sizes (five studies) (18, 20, 23, 36, 37), no significant effects of whey and MBP supplementation on lumbar-BMD were described [weighted mean difference (SMD), -0.08 g/cm^2; 95CI, -0.33 to 0.16, p = 0.529] (Figure 2a). Heterogeneity between the studies was low (I^2 = 35.9%; p = 0.167). Sensitivity analysis showed that the overall effect size regarding the effects of whey and MBP supplementation on lumbar-BMD levels did not depend on a single study (CI range, -0.33, 0.16).

Table 2. Characteristics of the randomized controlled trials included in the current systematic review and meta-analysis.

Study	Participants	Country	Mean age	BMI	No. (intervention/ control)	Durations (week)	Intervention	Control	Exercise intervention	Result
Aoe et al. 2005	menopausal women (unhealthy)	Japan	50.5±3.0	INT: 21.7±2.6 CON: 21.4±3.2	27 14/13	24 WK	MBP/ 40 mg per day	50- ml placebo beverage	---	BMD-lumbar ↑ Lumbar-BMD ↑ NTx ↓ Osteocalcin ↔
Aoe et al. 2001	healthy women	Japan	28.8 ±8.7	NR	33 17/16	24 WK	MBP/ 40 mg per day	50- ml placebo beverage	---	NTx ↓ Osteocalcin ↑
Uenishi et al. 2006	healthy young women	Japan	21.3±1.2	INT: 21.0±2.3 CON: 20.7±2.3	35 17/18	24 WK	MBP/ 40 mg per day	50-ml placebo beverage	---	BMD-lumbar ↑ NTx ↓ Osteocalcin ↑
Zou et al. 2009	healthy young women	China	19.6 ± 0.6	INT: 20.7 ± 1.7 CON: 20.5 ± 2.2	53 29/24	32 WK	MBP /250 ml whole milk added with 40 mg MBP	nothing	---	BMD-lumbar ↑ BMD % ↑
Fuglsang-Nielsen et al. 2022	Adult with abdominal obesity (both)	Denmark	≥40	INT: 28±4 CON: 30±4 INT: 29±2 CON: 29±4	31 15/16 16/17	12 WK	60 g/d whey hydrolysate + 10g/d fiber Or 60 g/d whey hydrolysate + 30g/d fiber	60 g/d maltodextrin + 10g/d fiber Or 60 g/d maltodextrin + 30g/d fiber	3d per week Resistance training	CTx ↔
Kerstetter et al. 2015	healthy Men and women	USA	69.95±6.25	INT: 26.1 ± 3.4 CON: 26.1±3.4	92/79	36 OR 72 WK	45 g whey protein isolate	maltodextrin	---	BMD-hip ↔ BMD-lumbar ↔ CTx ↑ IGF ↑
kemler et al. 2020	osteosarcopenic men unhealthy	Germany	≥ 72 years	NR	21/22	72 WK	100 g protein powder (360 kcal) contained 80 g of (whey) protein	nothing	twice/week high intensity dynamic resistance exercise	BMD-hip ↑ BMD-lumbar ↑
Sefton et al. 2020	Healthy male soldiers	USA	22.10±3.51	NR	23/25	9 WK	38.6 g of protein consisting of 80% whey protein concentrate	carbohydrate in liquid-shake	physical training	CTx ↔
Zhu et al. 2011	healthy ambulant women,	Australia	70-80	INT: 26.1± 3.8 CON: 27.2 ±4.0	179 91/88	12 WK	30 g of whey protein isolate	placebo drink containing 2.1 g of protein	----	BMD-hip ↓ IGF ↑

Abbreviations. BMI: body mass index, CON: control group, INT: intervention group, WK: week, MBP: milk basic protein, BMD: bone mineral density, NTx: N-terminal telopeptide of type I collagen, NR: non-report, CTx: C-terminal telopeptide of type I collagen

Subgroup analyses by type of supplementation

Based on the results of six effect sizes, three arms from whey (36, 37) and three arms from BMD (18, 20, 23), whey or MBP supplementation included no significant effects on lumbar-BMD (p = 0.367 and p = 0.859, respectively) (Table 3).

Subgroup analyses by type of duration

Based on the results of six effect sizes, four arms from ≤ 36 w (18, 20, 23, 37) and two from > 36 (36, 37), whey and MBP supplementation included no significant effects on lumbar-BMD within duration of ≤ 36 or > 36 w (p = 0.910 and p = 0.376, respectively) (Table 3).

Effects of whey on hip-bone mineral density

Pooled effect size from three studies (36, 37, 39) containing four arms did not reveal significant changes in hip-BMD following whey protein supplementation (SMD, -0.23 g/cm^2; 95% CI, -1.17 to 0.70%; p = 0.626) (Figure 2b]. Heterogeneity between the studies was high (I^2 = 96.3%; p = 0.001). Sensitivity analysis showed that the overall effect size regarding the effects of whey supplementation on hip-BMD levels did not depend on a single study (CI range, -1.17, 0.70).

Subgroup analyses by type of duration

Based on the results of four effect sizes (36, 37, 39), two arms from ≤ 36 w (37, 39) and two from > 36 w (36, 37), whey and MBP supplementation included no significant effects on hip-BMD within duration of ≤ 36 or > 36 w (p = 0.318 and p = 0.378, respectively) (Table 3).

Table 3. The subgroup analysis of this study

	lumbar BMD					
Subcategories	Effect size, n	I^2 (%)	P-heterogeneity	SMD	(95%CI)	P-value
Type						
Whey	3	64.8	0.058	-0.17	-0.54 to 0.20	0.368
MBP	3	0.0	0.402	0.03	-0.33 to 0.40	0.859
Pooled	6	35.9	0.167	-0.08	-0.33 to 0.16	0.491
durations						
≤36wk	4	0.0	0.606	0.01	-0.22 to 0.25	0.910
>36 wk	2	80.6	0.023	-0.35	-1.13 to 0.43	0.376
Hip BMD						
Type						
whey	4	96.3	0.001	-0.23	-1.17 to 0.70	0.626
durations						
≤36 wk	2	98.1	0.001	-0.83	-2.46 to 0.80	0.318
>36 wk	2	81.1	0.021	0.36	-0.43 to 1.15	0.374
NTx						
Type						
MBP	6	84.6	0.001	-0.89	-1.69 to -0.10	0.028
durations						
≤12 wk	3	91.8	0.001	-0.78	-2.35 to -0.79	0.331
>12 wk	3	65.5	0.055	-1.02	-1.76 to -0.27	0.007
CTx						
Type						
whey	5	98.6	0.001	2.10	-0.55 to 4.75	0.121
Exercising condition						
exercise	3	0.0	0.780	-0.22	-0.59 to 0.15	0.247
No exercise	2	0.0	0.353	5.59	5.03 to 6.16	0.001
Osteocalcin						
Type						
MBP	6	93.9	0.001	0.39	-0.95 to 1.73	0.576
durations						
≤12 wk	3	95.7	0.001	-0.33	-2.60 to 1.94	0.774
>12 wk	3	93.8	0.001	1.10	0.78 to 2.99	0.251
IGF						
Type						
whey	3	58.1	0.092	3.55	3.12 to 3.98	0.001

Abbreviation: SMD: standard mean difference, CI: confidence interval, MBP: milk basic protein, wk: week,

Fig. 2a. Lumbar-BMD

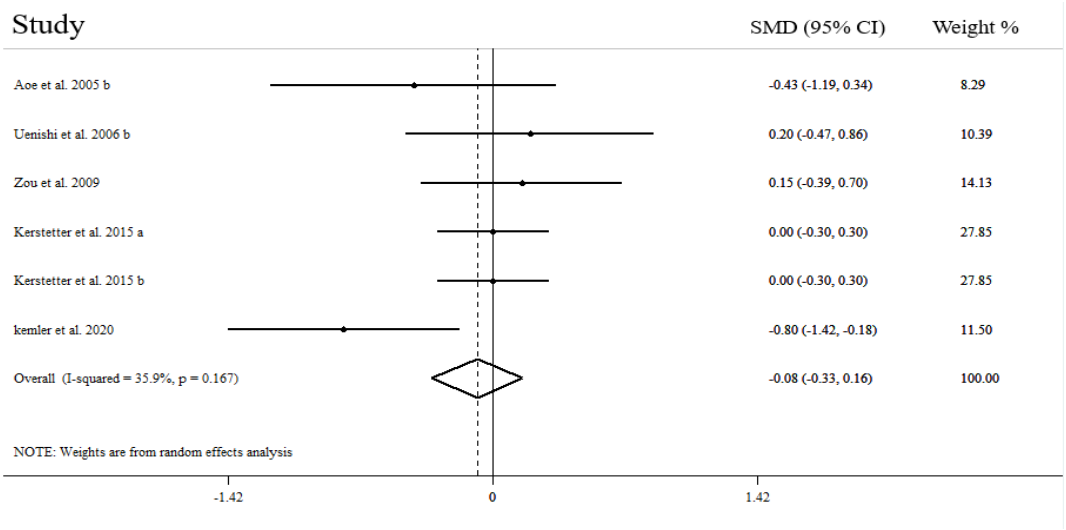


Fig. 2b. Hip-BMD

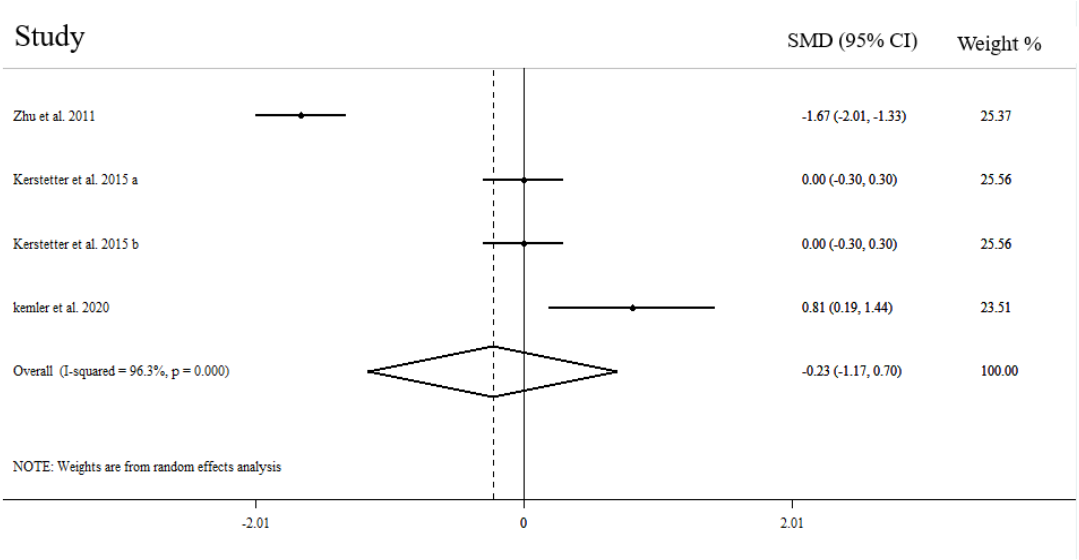


Fig. 2c. NTx

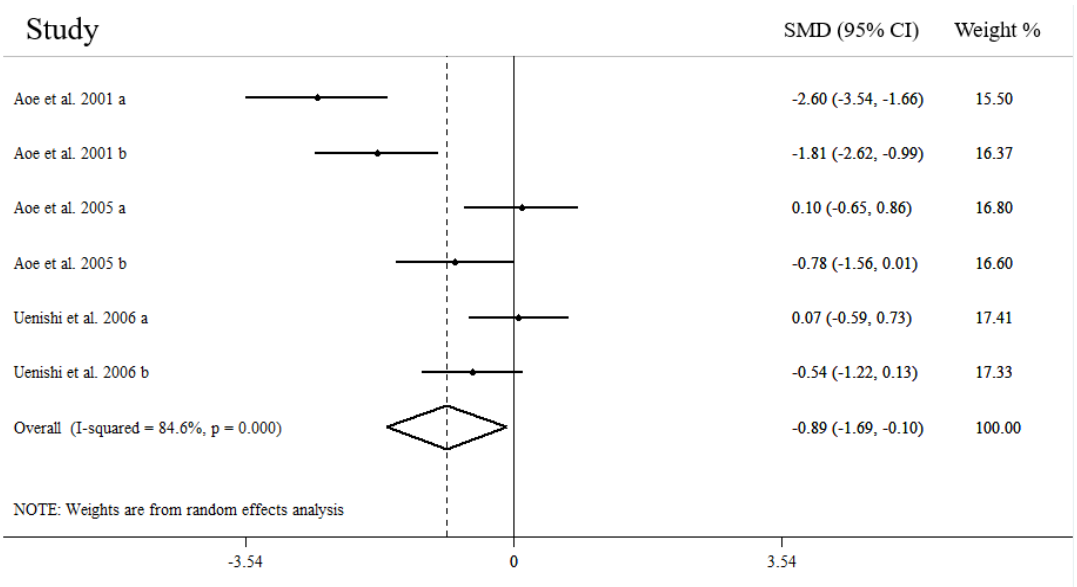


Fig. 2d. CTx

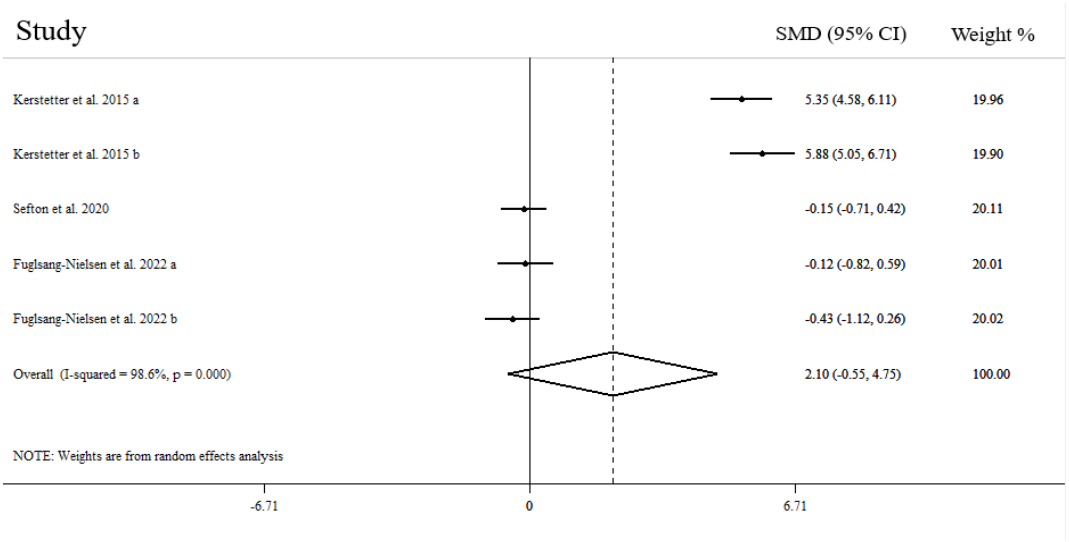


Fig. 2e. Osteocalcin

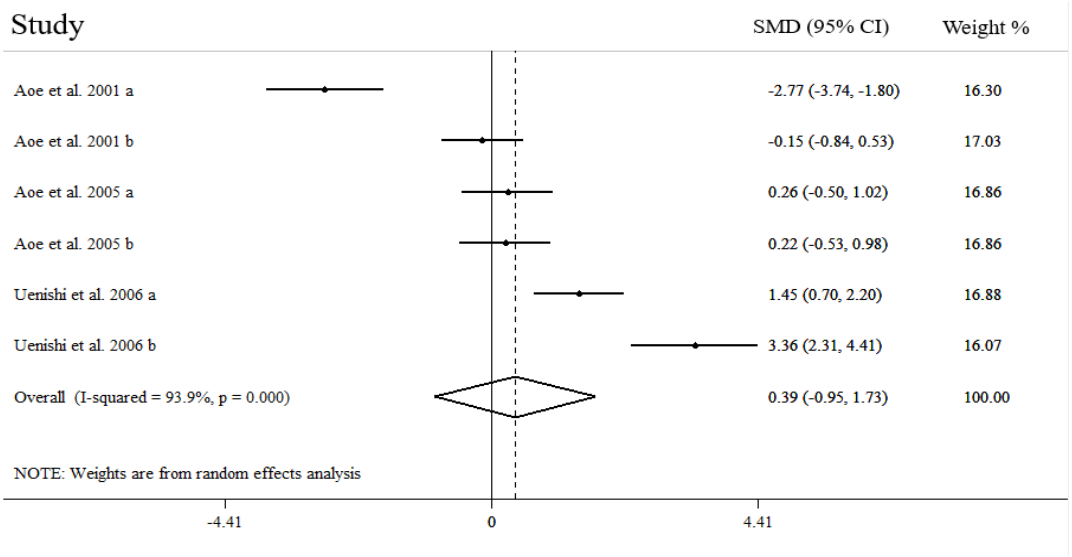


Fig. 2f. IGF

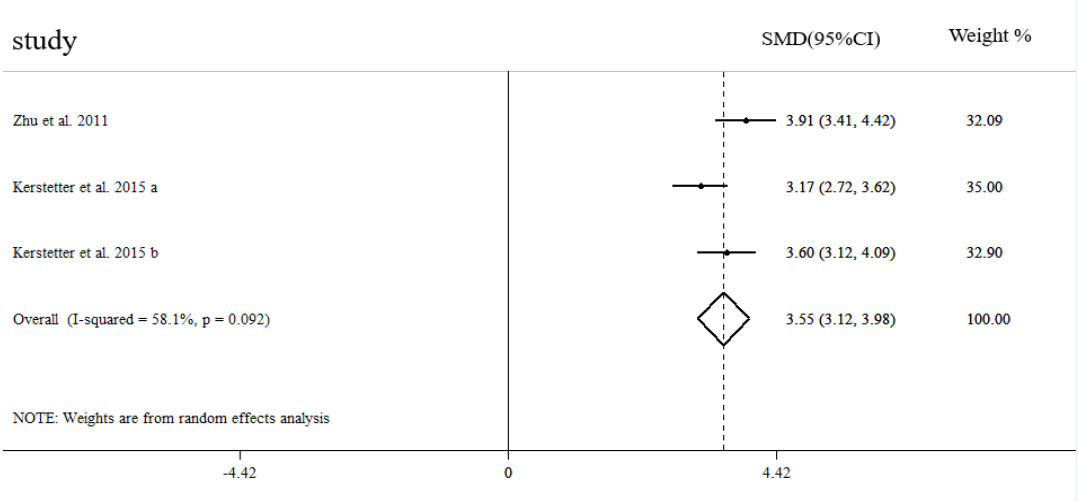


Figure 2. Forest plot for the effects of whey and MBP supplementation on (a) lumbar-BMD, (b) hip- BMD, (c) NTx, (d) CTx, (e) osteocalcin and (f) IGF

Effects of milk basic protein on urinary N-terminal telopeptides of type I collagen

In total, six effect sizes from three RCTs (18, 20, 34) were included in the meta-analysis. Combining the effect sizes, these studies showed a large heterogeneity in effect sizes (I^2 = 84.6%; p = 0.001). MBP supplementation induced significant decrease in NTx (SMD, -0.89 nmol/mmol; CI, -1.69 to -0.10%; p = 0.028) (Figure 2c]. Sensitivity analysis showed that the overall effect size regarding the effects of MBP supplementation on NTx levels depended on a single study [Aoe et al. 2001 (34)] (CI range, -1.21, 0.07).

Subgroup analyses by type of duration

Based on the results of six effect sizes, three arms from ≤ 12 w and three arms from > 12 w, MBP supplementation included no significant effects on NTX within duration of ≤ 12 (p = 331) (Table 3) and included significant decrease effects on NTx within duration of > 12 w (p = 0.007) (Table 3).

Effects of whey and whey protein on serum C-terminal telopeptides of type I collagen

Combined effect sizes of three studies (35, 37, 38) containing five arms demonstrated no significant changes in CTx following whey protein supplementation (SMD, 2.1 ng/l; 95% CI, -0.55 to 4.75%; p = 0.121) (Figure 2d]. These studies showed a large heterogeneity in effect sizes (I^2 = 98.6 %; p = 0.001). Sensitivity analysis showed that the overall effect size regarding the effects of whey supplementation on CTx levels did not depend on a single study (CI range, -0.55 to 4.75).

Subgroup analyses by exercising condition

Based on the five effect sizes, whey supplementation (three arms) included no significant effects on CTx within exercising conditions (p = 0.247) (Table 3). Pooled effects of why supplementation (two arms) on CTx within no exercising conditions included increases with significance (p = 0.001) (Table 3).

Effects of whey and milk basic protein on osteocalcin

Based on the result of three studies (18, 20, 34) containing six effect sizes, MBP supplementation failed to change osteocalcin (SMD, 0.39 ng/ml; 95% CI, -0.95 to 1.73%; p = 0.576) (Figure 2e). Heterogeneity between the studies was high (I^2 = 93.9%; p = 0.001). Sensitivity analysis showed that the overall effect size regarding the effects of MBP supplementation on osteocalcin did not depend on a single study (CI range, -0.95, 1.73).

Subgroup analyses by type of duration

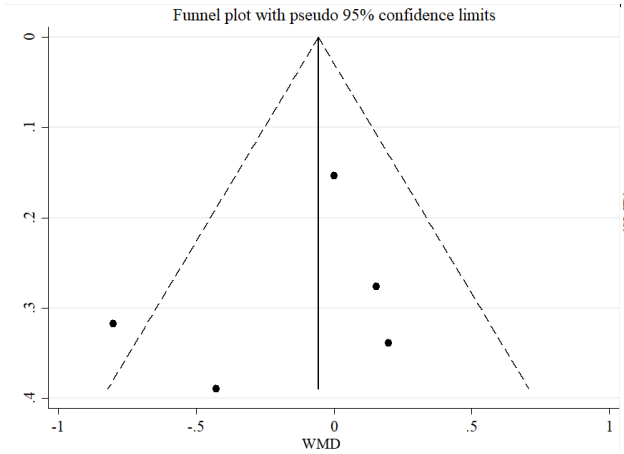
Based on the results of six effect sizes, three arms from ≤ 12 w (18, 20, 34) and three arms from > 12 w (18, 20, 34), MBP supplementation included no significant effects on osteocalcin within duration of ≤ 12 or > 12 w (p = 0.774 and p = 0.251, respectively) (Table 3).

Effects of whey supplementation on insulin-like growth factor

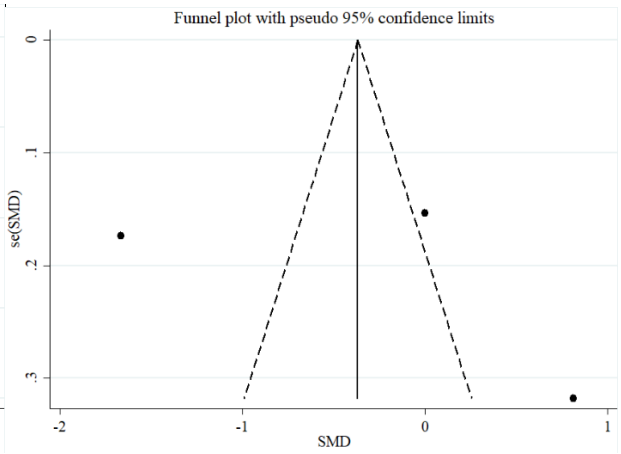
After combining three effect sizes from two studies (37, 39), pooled effects data analysis indicated that whey supplementation resulted in significant increases in IGF (SMD, 3.55 nmol/l; 95% CI, 3.12 to 3.98%; p = 0.001; I^2 = 58.1%; p = 0.092) (Figure 2f]. Sensitivity analysis showed that the overall effect size regarding whey supplementation's effects on IGF levels did not depend on a single study (CI range, 3.12, 3.98).

Publication bias

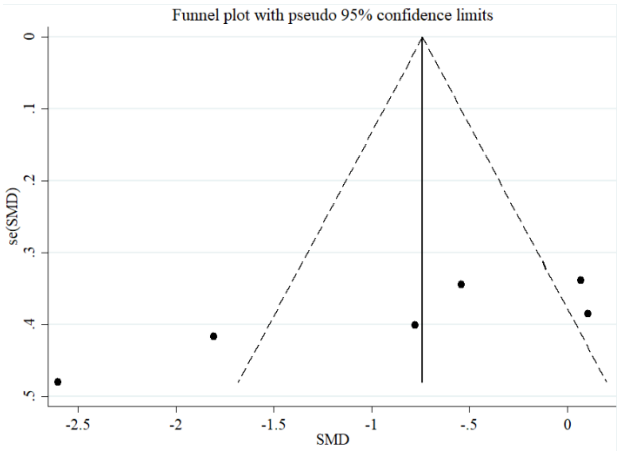
Based on Begg regression test, no evidence of publication bias were reported for studies assessing effects of whey and MBP supplementation on lumbar-BMD (p = 0.851), hip-BMD (p = 1.000), osteocalcin (p = 0.707) and IGF (p = 0.296). In addition, Eggers regression test showed no significant publication bias for lumbar-BMD (p = 0.415), hip-BMD (p = 0.763), NTx (p = 0.032), CTx (p = 0.121), osteocalcin (p = 0.856) and IGF (p = 0.090). However, a publication bias was detected for NTx (Begg, p = 0.024; Egger, p = 0.032) and CTx (Begg, p = 0.027). The current study carried out trim-and-fill method and reported that addition of one missing studies caused no effects on significances of NTX and CTx (SMD, -1.09 nmol/mmol; 95% CI, -1.89, -2.69; SMD, 1.15 ng/l; 95% CI, -1.51, 3.83, respectively) (Figure 3A–F).



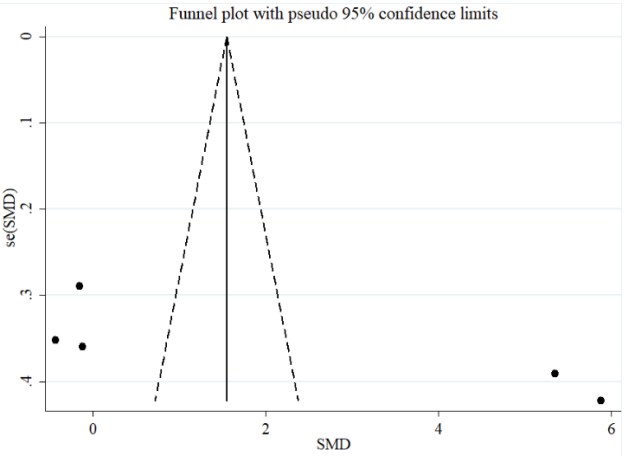
A) lumbar-BMD



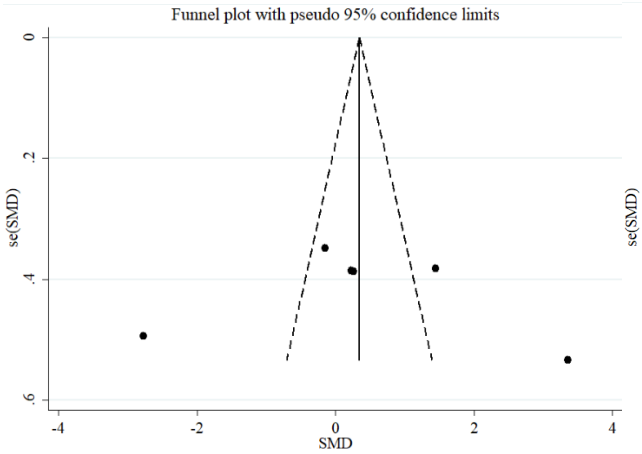
B) hip-BMD



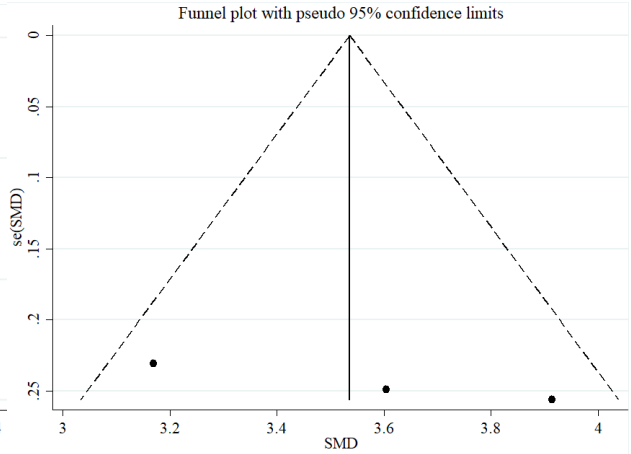
C) NTx



D) CTx



E) osteocalcin



F) IGF

Figure 3. Funnel plot for the effects of whey and MBP supplementation on (A) lumbar-BMD, (B) hip- BMD, (C) NTx, (D) CTx, (E) osteocalcin and (F) IGF

Discussion

Several factors affect maintenance of the bone mass. Protein is one of the affecting factors, which is harmful and beneficial for bone health depending on various factors, including level of proteins in the diet, protein source, calcium intake, weight loss and acid/base balance of the diet. Milk and its associated products are addressed as critical dietary components for the skeletal health since they are rich in minerals and high-quality proteins. Indeed, high quality protein in diets can include positive effects on bone health by maintaining muscle mass, increasing calcium absorption, suppressing parathyroid hormone and

stimulating insulin-like growth factor 1. Beneficial effects of dairy products on bone health have been revealed by studies, particularly in humans (41, 42). Relationships between the quantity of milk protein intakes and fracture risks varied depending on the population, dairy products and duration of consumption (43–45). Milk protein, as a complete or exclusive active component in addition to milk-derived various bioactive peptides, can carry minerals to enhance mineral bioavailability, modify bone metabolism and intercede multiple stages of bone remodeling (46–48). Therefore, a systematic review and meta-analysis was carried out to clarifying these results. To the best of the authors' knowledge, this was the first systematic review and meta-analysis that investigated effects of milk (whey) protein intake on bone health-linked mediators.

Based on the current results, MBP or whey protein intake could not change the lumbar BMD significantly. A limited number of included studies might be the reason for this result. However, another meta-analysis study by Wirunsawana et al. showed statistically significant improvements of BMD at the lumbar spine favoring whey protein and milk as the basic protein group, compared with the control group (49). Results for Hip BMP were similar and no significant changes were seen. Significantly, heterogeneity was high. The present study showed that MBP supplementation significantly decreased urinary NTx, a bone resorption marker, especially in the subgroup of more than 12 w of supplementation. This showed that duration included significant effects on the intervention studies. However, it was revealed that one of the studies significantly affected results, which included moderate risks of bias. Further high-quality studies are needed to verify effects of MBPs on NTx. Based on the results of this study, MBP consumption included no significant effects on serum concentrations of osteocalcin, a bone formation marker. High heterogeneities between the studies might be the reason for these results, although quality of the studies was high. The mechanism of how osteocalcin can affect bones is not declared; however, it has been illustrated that MBP contains active components to promote cell proliferation and collagen synthesis by the osteoblasts (50). In addition, it is verified that osteocalcin is secreted solely by the osteoblasts, including effects on bone mineralization and density. It is noteworthy that the urinary NTx excretion was linked to serum osteocalcin in group that consumed MBP, compared to the control group (20). Hence, it might indicate that MBP maintained the balance of bone remodeling via NTx and osteocalcin (20).

Regarding CTx, results showed no significant changes after milk protein (whey) consumption. However, statistically significant increases were observed in the subgroup of participants with no exercise. Exercising regularly can include specific effects on bone metabolism. Since serum CTx is an essential factor in assessing bone resorption (51, 52), a study revealed that only a 40-min downhill exercise of supra-threshold speed-enhanced momentum could increase the three osteogenic ratios (CICP/CTx, OC/CTX and BALP/CTx), which meant decreases in serum CTx levels. According to the authors, failure of anabolic outcome in 40-min laborious exercise could be a sustained increase of PTH concentration, as its high morning increase enhanced the CTx circadian rhythm (53). Similar to the present study, a possible reason for increases in serum CTx level under the effects of milk protein consumption in the subgroup of individuals with no exercise was high PTH level as the result of having no exercise. Furthermore, blood samples were collected in the early morning. It seems that further studies are necessary to assess effects of various types of exercises with protein supplementation on CTx as well.

Although providing amino acids as substrates for building matrix is crucial, protein intake has been demonstrated to positively link to the increased circulating levels of IGF-1, a bone growth-promoting factor (26, 54). The IGF-1 plays specific roles in activating osteoblast differentiation program and regulating 25-dihydroxy vitamin D3 1a-hydroxylase activity and tubular reabsorption of phosphate in kidneys (55). In verification of these contents, this meta-analysis showed significant increases in IGF-1 after consumption of whey protein, even though the heterogeneity was significant. It is further helpful if the serum procollagen type 1N propeptide (P1NP), an acceptable biomarker to assess bone formation and as a predictor of future bone fractures (51) was measured as well making the current perception clearer, especially by increasing the P1NP/CTx ratio as an indicator of bone metabolism and health (56–58).

To the best of the authors' knowledge, this study was the first systematic review and meta-analysis, which investigated effects of whey or milk protein on bone health biomarkers. In the present study, the aim included the most associated biomarkers to bone health; therefore, outcomes were limited to lumber and hip BMD, urinary NTx, serum CTx, osteocalcin and IGF-1. For the future studies, it seems necessary to assess further factors such as BMI, age, summer or winter, type of exercise and consuming other types of supplements. However, the present study did not include enough access to the information for addressing all of these factors. Moreover, the number of studies included in this meta-analysis was small. Hence, further randomized clinical trials are recommended to disclose the exact effects of consuming MBPs or whey protein on the health biomarkers of bones. Moreover, it is recommend to carry out other systematic reviews and meta-analyses to investigate effects of consuming other types of proteins on bone health parameters.

Conclusion

Several possibilities can explain discrepancies in results of studies. Various factors can affect the results about the effects of consuming MBPs or whey proteins on bone health mediators. It is recommended to carry out further specific and precise RCTs, focusing on details in this field.

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Availability of data and materials

Derived data supporting the findings of this study are available from the corresponding author on request.

Financial disclosure

The authors declare no conflict of interest.

Abbreviations

BMD: Bone mineral density
MBP: Milk basic protein
RCT: Randomized clinical trial
NTx: Urinary N-terminal telopeptides of type I collagen
CTx: Serum C-terminal telopeptides of type I collagen
SMD: Standard mean difference
CI: Confidence interval

References

1. Cauley JA. Public health impact of osteoporosis. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences. 2013;68(10):1243-51.
2. Organization WH. Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation: World Health Organization; 2003.
3. Keen R. Osteoporosis: strategies for prevention and management. Best Practice & Research Clinical Rheumatology. 2007;21(1):109-22.
4. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. New England Journal of Medicine. 1994;330(25):1769-75.
5. Santos L, Elliott-Sale KJ, Sale C. Exercise and bone health across the lifespan. Biogerontology. 2017;18(6):931-46.
6. Fitten LJ. Psychological frailty in the aging patient. Frailty: pathophysiology, phenotype and patient care. 2015;83:45-54.
7. O'Conner J, Lanyon L, MacFie H. The influence of strain rate on adaptive bone remodeling. Journal of Biomechanics. 1982;15(10):767-81.
8. Lanyon L. Using functional loading to influence bone mass and architecture: objectives, mechanisms and relationship with estrogen of the mechanically adaptive process in bone. Bone. 1996;18(1):S37-S43.
9. Bügel S. Vitamin K and bone health. Proceedings of the Nutrition Society. 2003;62(4):839-43.
10. Ishimi Y, Miyaura C, Ohmura M, Onoe Y, Sato T, Uchiyama Y, et al. Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency. Endocrinology. 1999;140(4):1893-900.
11. Scholz-Ahrens KE, Schrezenmeir J. Effects of bioactive substances in milk on mineral and trace element metabolism with special reference to casein phosphopeptides. British Journal of Nutrition. 2000;84(S1):147-53.
12. Liu J, Wiese H, Augustin W, Scholl S, Böl M. Mechanical comparison of milk and whey protein isolate fouling deposits using indentation testings. Food and Bioproducts Processing. 2020;122:145-58.
13. Takada Y, Aoe S, Kumegawa M. Whey protein stimulates the proliferation and differentiation of osteoblastic MC3T3-E1 cells. Biochemical and biophysical research communications. 1996;223(2):445-9.
14. Takada Y, Kobayashi N, Matsuyama H, Kato K, Yamamura J, Yahiro M, et al. Whey protein suppresses the osteoclast-mediated bone resorption and osteoclast cell formation. International dairy journal. 1997;7(12):821-5.
15. Aoe S, Toba Y, Yamamura J-i, Kawakami H, Yahiro M, Kumegawa M, et al. Controlled Trial of the Effects of Milk Basic Protein (MBP) Supplementation on Bone Metabolism in Healthy Adult Women. Bioscience, Biotechnology and Biochemistry. 2001;65(4):913-8.
16. Aoe S, Koyama T, Toba Y, Itabashi A, Takada Y. A controlled trial of the effect of milk basic protein (MBP) supplementation on bone metabolism in healthy menopausal women. Osteoporosis International. 2005;16(12):2123-8.
17. Toba Y, Takada Y, Matsuoka Y, Morita Y, Motouri M, Hirai T, et al. Milk basic protein promotes bone formation and suppresses bone resorption in healthy adult men. Bioscience, biotechnology and biochemistry. 2001;65(6):1353-7.
18. Uenishi K, Ishida H, Toba Y, Aoe S, Itabashi A, Takada Y. Milk basic protein increases bone mineral density and improves bone metabolism in healthy young women. Osteoporosis international. 2007;18(3):385-90.
19. Yamamura J-i, Aoe S, Toba Y, Motouri M, Kawakami H, Kumegawa M, et al. Milk basic protein (MBP) increases radial bone mineral density in healthy adult women. Bioscience, biotechnology and biochemistry. 2002;66(3):702-4.
20. Aoe S, Koyama T, Toba Y, Itabashi A, Takada Y. A controlled trial of the effect of milk basic protein (MBP) supplementation on bone metabolism in healthy menopausal women. Osteoporos Int. 2005;16(12):2123-8.
21. Delmas P, Eastell R, Garnero P, Seibel M, Stepan J, Foundation CoSAotIO. The use of biochemical markers of bone turnover in osteoporosis. Osteoporosis international. 2000;11(6):S2.
22. Henriksen DB, Alexandersen P, Bjarnason NH, Vilsbøll T, Hartmann B, Henriksen EE, et al. Role of gastrointestinal hormones in postprandial reduction of bone resorption. Journal of Bone and Mineral Research. 2003;18(12):2180-9.
23. Zou Z-Y, Lin X-M, Xu X-R, Xu R, Ma L, Li Y, et al. Evaluation of milk basic protein supplementation on bone density and bone metabolism in Chinese young women. European journal of nutrition. 2009;48(5):301-6.
24. Johansson H, Odén A, Kanis JA, McCloskey EV, Morris HA, Cooper C, et al. A meta-analysis of reference markers of bone turnover for prediction of fracture. Calcified tissue international. 2014;94(5):560-7.

25. Rosen H, Moses A, Garber J, Iloputaife I, Ross D, Lee S, et al. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. Calcified tissue international. 2000;66(2):100-3.

26. Schurch M-A, Rizzoli R, Slosman D, Vadas L, Vergnaud P, Bonjour J-P. Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture: a randomized, double-blind, placebo-controlled trial. Annals of internal medicine. 1998;128(10):801-9.

27. Perrini S, Laviola L, Carreira MC, Cignarelli A, Natalicchio A. and Francesco Giorgino. Journal of Endocrinology. 2010;205:201-10.

28. Moher D, Liberati A, Tetzlaff J, Altman DG, Group* P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of internal medicine. 2009;151(4):264-9.

29. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. Bmj. 2011;343.

30. Wu Z-J, Wang Z-Y, Gao H-E, Zhou X-F, Li F-H. Impact of high-intensity interval training on cardiorespiratory fitness, body composition, physical fitness and metabolic parameters in older adults: A meta-analysis of randomized controlled trials. Experimental gerontology. 2021;150:111345.

31. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range and the size of a sample. BMC medical research methodology. 2005;5(1):1-10.

32. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Bmj. 1997;315(7109):629-34.

33. Hayashino Y, Noguchi Y, Fukui T. Systematic evaluation and comparison of statistical tests for publication bias. Journal of epidemiology. 2005;15(6):235-43.

34. Aoe S, Toba Y, Yamamura J, Kawakami H, Yahiro M, Kumegawa M, et al. Controlled trial of the effects of milk basic protein (MBP) supplementation on bone metabolism in healthy adult women. Biosci Biotechnol Biochem. 2001;65(4):913-8.

35. Fuglsang-Nielsen R, Rakvaag E, Vestergaard P, Hermansen K, Gregersen S, Starup-Linde J. The Effects of 12-Weeks Whey Protein Supplements on Markers of Bone Turnover in Adults With Abdominal Obesity – A Post Hoc Analysis. Frontiers in Endocrinology. 2022;13.

36. Kemmler W, Kohl M, Jakob F, Engelke K, von Stengel S. Effects of High Intensity Dynamic Resistance Exercise and Whey Protein Supplements on Osteosarcopenia in Older Men with Low Bone and Muscle Mass. Final Results of the Randomized Controlled FrOST Study. Nutrients. 2020;12(8).

37. Kerstetter JE, Bihuniak JD, Brindisi J, Sullivan RR, Mangano KM, Larocque S, et al. The Effect of a Whey Protein Supplement on Bone Mass in Older Caucasian Adults. J Clin Endocrinol Metab. 2015;100(6):2214-22.

38. Sefton JM, Lyons KD, Beck DT, Haun CT, Romero MA, Mumford PW, et al. Markers of Bone Health and Impact of Whey Protein Supplementation in Army Initial Entry Training Soldiers: A Double-Blind Placebo-Controlled Study. Nutrients. 2020;12(8):2225.

39. Zhu K, Meng X, Kerr DA, Devine A, Solah V, Binns CW, et al. The effects of a two-year randomized, controlled trial of whey protein supplementation on bone structure, IGF-1 and urinary calcium excretion in older postmenopausal women. Journal of Bone and Mineral Research. 2011;26(9):2298-306.

40. Fuglsang-Nielsen R, Rakvaag E, Vestergaard P, Hermansen K, Gregersen S, Starup-Linde J. The Effects of 12-Weeks Whey Protein Supplements on Markers of Bone Turnover in Adults With Abdominal Obesity - A Post Hoc Analysis. Frontiers in endocrinology. 2022;13:832897.

41. Rozenberg S, Body J-J, Bruyere O, Bergmann P, Brandi ML, Cooper C, et al. Effects of dairy products consumption on health: benefits and beliefs—a commentary from the Belgian Bone Club and the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases. Calcified tissue international. 2016;98(1):1-17.

42. Rizzoli R, Biver E. Effects of fermented milk products on bone. Calcified tissue international. 2018;102(4):489-500.

43. Feskanich D, Meyer HE, Fung TT, Bischoff-Ferrari HA, Willett WC. Milk and other dairy foods and risk of hip fracture in men and women. Osteoporosis International. 2018;29(2):385-96.

44. Michaëlsson K, Wolk A, Langenskiöld S, Basu S, Lemming EW, Melhus H, et al. Milk intake and risk of mortality and fractures in women and men: cohort studies. Bmj. 2014;349.

45. Kalkwarf HJ, Khoury JC, Lanphear BP. Milk intake during childhood and adolescence, adult bone density and osteoporotic fractures in US women. The American journal of clinical nutrition. 2003;77(1):257-65.

46. Pandey M, Kapila S, Kapila R, Trivedi R, Karvande A. Evaluation of the osteoprotective potential of whey derived-antioxidative (YVEEL) and angiotensin-converting enzyme inhibitory (YLLF) bioactive peptides in ovariectomised rats. Food & function. 2018;9(9):4791-801.

47. Mada S, Reddi S, Kumar N, Kapila S, Kapila R. Protective effects of casein-derived peptide VLPVPQK against hydrogen peroxide–induced dysfunction and cellular oxidative damage in rat osteoblastic cells. Human & Experimental Toxicology. 2017;36(9):967-80.

48. Donida BM, Mrak E, Gravaghi C, Villa I, Cosentino S, Zacchi E, et al. Casein phosphopeptides promote calcium uptake and modulate the differentiation pathway in human primary osteoblast-like cells. Peptides. 2009;30(12):2233-41.

49. Wirunsawana K, Upala S. Impact of Whey Protein on Bone Mineral Density: a Systemic Review and Meta-analysis. Journal of Clinical Densitometry. 2018;21(4):597-8.

50. Takada Y, Aoe S, Kumegawa M. Whey protein stimulated the proliferation and differentiation of osteoblastic MC3T3-E1 cells. Biochemical and biophysical research communications. 1996;223(2):445-9.

51. Johansson H, Odén A, Kanis JA, McCloskey EV, Morris HA, Cooper C, et al. A meta-analysis of reference markers of bone turnover for prediction of fracture. Calcif Tissue Int. 2014;94(5):560-7.

52. Rosen HN, Moses AC, Garber J, Iloputaife ID, Ross DS, Lee SL, et al. Serum CTX: a new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes

with bisphosphonate therapy. Calcif Tissue Int. 2000;66(2):100-3.

53. Zheng Q, Kernozek T. Anabolic Bone Stimulus Requires a Pre-Exercise Meal and 45-Minute Walking Impulse of Suprathreshold Speed-Enhanced Momentum to Prevent or Mitigate Postmenopausal Osteoporosis within Circadian Constraints. 2021;13(11).

54. Heaney RP, McCarron DA, Dawson-Hughes B, Oparil S, Berga SL, Stern JS, et al. Dietary changes favorably affect bone remodeling in older adults. Journal of the American Dietetic Association. 1999;99(10):1228-33.

55. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors and the skeleton. Endocrine reviews. 2008;29(5):535-59.

56. Rantalainen T, Heinonen A, Linnamo V, Komi PV, Takala TE, Kainulainen H. Short-term bone biochemical response to a single bout of high-impact exercise. Journal of sports science & medicine. 2009;8(4):553-9.

57. Mosti MP, Kaehler N, Stunes AK, Hoff J, Syversen U. Maximal strength training in postmenopausal women with osteoporosis or osteopenia. Journal of strength and conditioning research. 2013;27(10):2879-86.

58. Ogata M, Ide R, Takizawa M, Tanaka M, Tetsuo T, Sato A, et al. Association between basal metabolic function and bone metabolism in postmenopausal women with type 2 diabetes. Nutrition (Burbank, Los Angeles County, Calif). 2015;31(11-12):1394-401.