Calcium supplementation for improving bone density in lactating women: a systematic review and meta-analysis of randomized controlled trials

Guoqi Cai [1], Jing Tian [1], Tania Winzenberg [1*], Feitong Wu [1*]

[1] Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

* They contributed equally to this work.

Corresponding author: Dr. Feitong Wu, Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia.

Telephone number: +613 6226 7700. E-mail: Feitong.Wu@utas.edu.au

Short running head: Calcium supplementation for BMD in lactating women

Abbreviations list:

BMC – bone mineral content

BMD – bone mineral density

CI – confidence interval

GRADE – Grading of Recommendations, Assessment, Development, and Evaluation

RCT – randomized controlled trial

WMD – weighted mean difference

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Data described in the manuscript, code book, and analytic code will not be made available because there are no additional data.

ABSTRACT

Background Clinical trials evaluating the effect of calcium supplementation on bone loss in lactating women were small and results are inconsistent.

Objective To determine the effect of calcium supplementation on bone mineral density (BMD) in lactating women.

Design Electronic search of databases was conducted from inception to January 2020. Two authors screened studies, extracted data and assessed the risk of bias of eligible studies. Percentage change in BMD was pooled using random-effects models and reported as weighted mean differences (WMD) with 95% confidence intervals (CI). Risk of bias was assessed using the Cochrane risk of bias tool.

Results Five randomized controlled trials including 567 lactating women were included. All had a high risk of bias. Mean baseline calcium intake ranged from 562 to 1333 mg/day. Compared to control groups (placebo/no intervention), calcium supplementation (600/1000 mg/day) had no significant effect on BMD at the lumbar spine (WMD=0.74% [95% CI -0.10, 1.59]; I^2=47% [95% CI 0, 81], n=527 from 5 trials) or the forearm (WMD=0.53% [95% CI -0.35, 1.42%]; I^2=55% [95% CI 0, 85], n=415 from 4 trials). BMD at other sites was assessed in single trials: calcium supplementation had a small to moderate effect on total hip BMD (WMD=3.3% [95% CI 1.5, 5.1]) but no effect on total body or femoral neck BMD.

Conclusions Overall, the meta-analysis indicates that calcium supplementation does not provide clinically important benefits for BMD in lactating women. However, there was adequate dietary intake before supplementation in some studies, and others did not measure baseline calcium intake. Advising lactating women to meet the current recommended calcium intakes (with supplement if dietary intake is low) is warranted unless new high certainty

evidence to the contrary from robust clinical trials becomes available. More research needs to be done in larger samples of women from diverse ethnic and racial groups.

Keywords: bone mineral density; calcium supplementation; lactating women; risk of bias; systematic review

Introduction

Low bone mineral density (BMD) is a major risk factor for osteoporotic fracture in the elderly (1, 2). BMD in later life is determined by both peak bone mass and the rate of subsequent bone loss (3). The higher the peak bone mass that is achieved by the early 20s, the greater the likelihood of withstanding the impacts of age-related bone loss (4). Therefore, optimizing BMD in young adulthood is critically important for preventing fractures in later life.

Numerous studies have demonstrated a remarkable bone loss in lactating women (5-8), which is likely due to increased bone resorption to meet the high calcium requirement of their infants. Moreover, there is some evidence that a longer duration of lactation could be associated with a reduction of BMD after 6 months post-weaning (9, 10), although this is not seen in all studies (11-13). Nevertheless, preventive strategies for preventing bone loss or even improving bone density in lactating women are scarce.

Calcium supplementation has potential value for BMD by reducing bone turnover, particularly in those with marginal and low dietary calcium intake (14, 15). In particular, as an average of 200 mg of calcium per day is secreted by breast-feeding secretes (16), this period could provide a window of opportunity for younger women to benefit more from calcium supplementation. However, the recommended dietary allowances for calcium intake for lactating women (17) (1000/1300 mg/day depending on age) are the same as those for non-lactating women. The main argument for this is that maternal skeletal resorption is hormonally programmed during lactation to supply the necessary calcium for breastfeeding and there is no evidence from randomized controlled trials (RCTs) and observational studies to show that increased calcium intake could prevent bone loss during lactation (18). However, RCTs examining the effect of calcium supplementation on BMD in lactating women are generally of small sample size and the results are conflicting (19-23). To our knowledge, no systematic review and meta-analysis

has been done to quantitively determine the effect of calcium supplementation on BMD in lactating women. Thus, this study aimed to address this evidence gap by determining the effect of calcium supplementation on BMD in lactating women and whether any such effect varies by baseline calcium intake, dose of calcium supplementation, co-intervention of vitamin D, duration of supplementation or breast-feeding, age or ethnicity.

Methods

Protocol registration

The protocol for the systematic review and meta-analysis was registered with PROSPERO (https://www.crd.york.ac.uk/PROSPERO, ID: CRD42015022092) (24). This study is reported as per the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) checklist (25).

Literature search

We searched EMBASE (via Ovid), MEDLINE (via PubMed), Web of Science and Cochrane Central Register of Controlled Trials (CENTRAL, via The Cochrane Library) from inception (that is, from the date the database was established) to June 2015 and updated in January 2020 for RCTs of calcium supplementations in lactating women with BMD, total body bone mineral content (BMC), fracture, quality of life or adverse events as an endpoint. See Supplementary Method for a detailed search strategy for MEDLINE. We checked bibliographies of original studies and recent review articles for additional relevant studies. We also searched ClinicalTrials.gov and the WHO trials portal (www.who.int/ictrp/en/) for clinical trials that have not been published.

77 Study selection

78 Two authors (GC and JT) independently screened titles and abstracts for all identified studies
79 and retrieved the full text of potentially relevant studies for further screening. Full-text reviews
80 were performed according to the *a priori* selection criteria which is detailed in the registered
81 protocol (24). Briefly, we included studies if they were (a) online published full-text articles or
82 conference abstracts; (b) written in any language; (c) RCTs evaluating the effect of calcium
83 supplementations (including calcium fortified food) with or without a co-intervention of
84 vitamin D for at least 3 months in lactating women who decided to breast-feed their infants for
85 at least 3 months; (d) aiming at BMD, total body BMC, fractures, quality of life or adverse
86 events as an outcome measure. If the supplementation started during pregnancy, studies were
87 included if the duration of supplementation in the postpartum period was at least double of that
88 in pregnancy. Studies eligible for the systematic review were included in the meta-analysis if
89 they provided sufficient data for pooling on any outcome measures outlined above. Given that
90 few placebo-controlled trials (n=3) were available for this review, we amended the protocol to
91 also include trials that used no intervention as control group rather than only including placebo-
92 controlled trials as originally planned.

93

94 Data extraction

95 Two authors (GC and FW) independently extracted information from each included study
96 using a data collection form. Data extracted were: (a) study characteristics (first author, year of
97 publication, study design, inclusion and exclusion criteria, sample size and duration of follow-
98 up); (b) participants' characteristics (age, ethnicity and baseline calcium intake); (c)
99 interventions (dose and duration of calcium supplementation); and (d) outcome measures
100 (techniques, sites and timing of BMD measurement). Where available, we extracted the
101 percentage of change in BMD in the calcium supplementation group and the control group at

the following sites: total hip, femoral neck, lumbar spine and forearm. As BMD at the forearm can be measured at different sites (e.g., ultradistal, proximal, distal third of radius), we used BMD at the most commonly reported site (ultradistal radius) or any other radius site if BMD at the ultradistal radius was not available. For BMD that was measured at multiple time points, we extracted data for the time points that were (a) shared by most studies included and (b) at the end of supplementation. When the outcomes were shown as graphs only, we converted graphical data to numerical data using Engauge Digitizer software (Version 10.11) (26, 27). Data on other pre-specified outcomes (i.e., total body BMC, fractures, quality of life and adverse events) were not extracted as they were not reported in any study.

Assessment of risk of bias and quality of evidence

Two authors (GC and FW) independently assessed the risk of bias using Cochrane's risk of bias assessment (28), with disagreements discussed with a third author (JT). For each outcome, we assessed quality of evidence using the GRADE (Grading of Recommendations, Assessment, Development, and Evaluation) approach, which combines risk of bias, consistency of effect, imprecision, indirectness and publication bias (29). Quality of evidence was downgraded from high (i.e., RCT) to very low based on the seriousness of each component in the GRADE. The GRADE Summary of Findings table was generated using GRADEpro Guideline Development Tool on the GRADEpro website (https://gdt.gradepro.org/app/).

Data synthesis

No studies reported fractures, quality of life, adverse events or withdrawals due to adverse events so these could not be analyzed. The most commonly shared time point at which BMD was measured was 3 (19, 21, 22) and 6 (19, 20, 22) months in three studies. Given the limited

number of studies, in the main analysis we pooled the data of BMD outcomes at the end of supplementation from all five studies (i.e. 6 months for 2 studies (20, 22) and 9 (23), 10 (21) and 18 (19) months for each of the remaining three studies), and we also pooled the data at the most commonly shared time points (i.e. 3 and 6 months). We calculated the weighted mean difference (WMD) with 95% confidence intervals (CI) between calcium supplementation and control groups of the percentage changes of BMD using DerSimonian and Laird random-effects models (30). The pooled results were presented in forest plots. All data syntheses were carried out with Review Manager software (version 5.3).

Assessment of heterogeneity

Statistical heterogeneity was assessed using the I^2 and Chi-square test. $I^2 > 50\%$ was considered substantial heterogeneity and a p-value of ≤ 0.10 in the chi-square test indicated statistical significance. The 95% CIs of the I^2 estimate were calculated based on Higgins et al. (31).

Assessment of publication bias

As only a limited number of studies were included in this systematic review and meta-analysis, we used funnel plots only to visually assess publication bias.

Subgroup analysis

The planned *a priori* subgroup analyses were not undertaken due to the small number of included studies. Those planned were by: (a) baseline calcium intake ($<$ or $\geq$ 1000 mg/day); (b) dose of calcium supplementation ($<$ or $\geq$ 1000 mg/day); (c) co-intervention of vitamin D (yes

148 or no); (d) duration of supplementation (< or ≥ 12 months); (e) duration of breast-feeding (< or
149 ≥ 6 months); (f) age (< or ≥ 30 years) and; (g) ethnicity.

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152 Sensitivity analysis

153 *A priori* sensitivity analyses were performed by omitting studies with inappropriate or unclear
154 allocation concealment and omitting studies that used no intervention as a control group. Given
155 our inability to explore heterogeneity using subgroup analyses due to the small numbers of
156 studies, we opted to address heterogeneity instead by performing a post hoc sensitivity analysis
157 excluding a single study (23) which was markedly different from the remaining studies for a
158 number of our prespecified subgroup characteristics (32) and using 6-month lactation data only
159 of an 18-month study (19). In addition, another post hoc sensitivity analysis was conducted by
160 excluding a study that was published as a conference abstract (20).

161

162 **Results**

163 Study selection

164 The flow chart of the study selection process is detailed in Figure 1. Our electronic search
165 identified 2871 potentially relevant records. Of these, 634 were excluded as duplicates and
166 2206 excluded after screening for titles and abstracts. We performed full-text screening of the
167 remaining 31 references, and five RCTs involving 567 participants (293 in the calcium
168 supplementation group and 274 in the control group) were included in this review.

169 Table 1 presents the characteristics of included studies(19-23). Four studies were performed in
170 Caucasians or predominantly Caucasians (19-22) and one in Asians (Chinese) (23). The mean

age of lactating women ranged from 27.5 to 31 years. The duration of breastfeeding ranged from 3 to 7.6 months. Of note, one study by Yu et al. started calcium supplementation when most participants had ceased lactating(23). In another, supplementation occurred both during lactation (about 6 months) and for 12-months post lactation (19). Mean calcium intake at baseline was reported in 4 studies and ranged from 562 to 1333 mg/day (20-23). The dose of calcium supplementation was 1000 mg/day in four trials (19-22) and 600 mg/day in one trial (23). Two studies provided 400 IU/day vitamin D to both the calcium supplementation and control groups (21, 22). BMD was measured by dual-energy x-ray absorptiometry (DXA) in all studies. The longest follow-up times ranged from 6 to 18 months with calcium supplementation provided during the entire follow-up. All studies compared the percentage change of BMD (%) from baseline to the end of follow-up between calcium supplementation group and control group. All studies measured BMD at the lumbar spine and four at the forearm(19-22) and single studies only reported BMD at whole body (21), total hip (23) and proximal femur (20). No studies reported total body BMC, fracture, quality of life or adverse events as outcomes. Two studies reported the mean (standard deviation [SD]) of BMD at baseline by treatment group. In the Polatti study (19), baseline lumbar spine BMD was 1.239 (0.018) g/cm^2 in the calcium supplementation group and 1.220 (0.014) g/cm^2 in the control group, and forearm BMD was 0.469 (0.009) g/cm^2 and 0.489 (0.008) g/cm^2 in the treatment and control groups, respectively. In the Yu study (23), lumbar spine BMD was 0.977 (0.099) g/cm^2 and 0.977 (0.115) g/cm^2 and total hip BMD was 0.836 (0.118) g/cm^2 and 0.849 (0.117) g/cm^2 in the calcium treatment and control groups respectively. One study had loss to follow-up of less than 5% (21), two between 5% and 20% (19, 22), one of 30% (23), and loss to follow-up was unclear in the remaining one study (20). None of the studies addressed missing data, and all of them conducted complete data analysis.

196 Assessment of risk of bias

197 Overall, assessment of risk of bias suggested a high risk of bias in all included studies
198 (Supplementary Figure 1). Adequate description of randomization was given in one study(19),
199 and the remaining four reported randomized allocation but without describing randomization
200 procedures. Allocation concealment was not clearly stated in three placebo-controlled studies
201 (20-22) and was inadequate in the remaining two studies with no intervention as controls (19,
202 23). Of the three placebo-controlled trials, two used a double-blinding design (21, 22) while
203 the other one did not state the blinding strategy (20). How missing data were handled in two
204 studies (20, 23) was not well described, indicating a potential attrition bias. No trials were
205 registered online or provided a study protocol (four completed before 2000 and one in 2011),
206 suggesting the potential for reporting bias.

207

208 Effects of calcium supplementation on BMD

209 For change in BMD from baseline to the end of calcium supplementation in each study, the
210 pooled results for lumbar spine (5 studies: 275 participants in calcium and 252 in control arms)
211 and forearm (4 studies: 217 participants in calcium and 198 in control arms) BMD are shown
212 in Figure 2 and Table 2. Compared to placebo, calcium supplementation did not significantly
213 reduce the loss of BMD at the lumbar spine (WMD 0.74% [95% CI -0.10, 1.59]; $p = 0.09$, $I^2 =$
214 47% [95% CI 0, 81]) or the forearm (WMD 0.53% [95% CI -0.35, 1.42], $p = 0.24$, $I^2 = 55\%$
215 [95% CI 0, 85]). Table 2 also provides the effect estimates from single studies for BMD of the
216 total body (21), total hip (23) and femoral neck (20). Compared to placebo, calcium
217 supplementation increased BMD of the total hip (WMD 3.30% [95% CI 1.53, 5.07]; $p < 0.001$)
218 but not the total body or femoral neck. However, the single study reporting total hip BMD (23)
219 had a substantially different design compared to the remaining four studies, as it provided

calcium supplementation mostly after the cessation of lactating (see also below sensitivity analysis).

Three- and six-month changes in BMD were reported in 3 studies for lumbar spine and 2 studies for forearm (Figure 3). Overall, calcium supplementation reduced the loss of BMD at the lumbar spine over 3 and 6 months and the forearm over 6 months, although these findings were primarily driven by one large, heavily weighted study (19).

We downgraded the quality of evidence by three levels for high risk of bias, inconsistency, imprecision, indirectness and/or publication bias for all BMD outcomes, resulting in a GRADE assessment of very low certainty of the evidence for all BMD outcomes.

Sensitivity analysis

All included studies did not perform or state allocation concealment adequately. Therefore, no sensitivity analysis was performed by omitting studies with inappropriate or unclear allocation concealment. After excluding two studies that used no intervention as control group, calcium supplementation showed no or small effect on lumbar spine BMD (WMD 0.91% [95% CI -0.06, 1.87]; p = 0.07, I^2 = 0% [95% CI 0, 85]) or forearm (WMD 1.01% [95% CI -0.003, 2.03]; p = 0.05, I^2 = 10% [95% CI 0, 86]), n=181 from 3 studies (20-22).

One study (23) was markedly different from the remaining studies in that breastfeeding was of short duration (and in fact the bulk of supplementation was given after breastfeeding ceased), calcium supplement dose was low and supplementation commenced late (3 months postpartum rather than 2 weeks or less) and participants were Chinese rather than all or predominantly white. Furthermore, this study was the only study in which lumbar spine BMD increased over the study period in both calcium and control groups. Therefore, this study was excluded in a

post hoc sensitivity analysis. In addition, change in BMD over 6 months from one study was used in this post hoc sensitivity analysis as in this study that was the point at which breastfeeding ceased (19). In this sensitivity analysis, the effect of calcium supplementation and statistical heterogeneity were both markedly reduced for lumbar spine BMD (4 studies, 455 participants, WMD 0.40% [95% CI 0.32, 0.49]; $p < 0.001$, $I^2 = 0$ [95% CI 0, 79]) and were not materially changed for forearm BMD (4 studies, 455 participants, WMD 0.54% [95% CI -0.18, 1.26]; p=0.14, $I^2 = 41\%$ [95% CI 0, 80]). Another sensitivity analysis excluding a study that was published only as a conference abstract(20) did not materially affect the results (BMD of the lumbar spine (4 studies, 448 participants, WMD 0.67% [95% CI -0.37, 1.72]; p=0.21, $I^2 = 46\%$ [95% CI 0, 82]) and forearm (3 studies, 336 participants, WMD 0.59% [95% CI -0.73, 1.90]; p=0.38, $I^2 = 61\%$ [95% CI 0, 89])).

Publication bias

Visual inspection of the funnel plots for lumbar spine BMD did not suggest publication bias, but there was possible asymmetry for forearm BMD such that publication bias could not be ruled out (Supplementary Figure 2).

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis to evaluate the effect of calcium supplementation on BMD in lactating women. At best, calcium supplementation of 600-1000 mg/day in lactating women resulted in only a small and statistically non-significant benefit for BMD of the lumbar spine (WMD 0.74% [95% CI -0.10, 1.59]) and the forearm (WMD 0.53% [95% CI -0.35, 1.42]) that was unlikely to be clinically important. Notably, all included studies had a high risk of bias and the certainty of the evidence

is assessed as very low. The effect of calcium supplementation on change in BMD over 3 and 6 months was primarily driven by the Polatti study (19) because of its larger sample size and greater precision of BMD measurement, but this study did not report baseline calcium intake. Nonetheless, the pooled results were not materially changed after excluding the Polatti study (19) and another study (23) with no intervention as control group. Evidence for effects at other sites was sparse. BMD at each of total body, total hip and femoral neck was only assessed in single studies. These reported no effects at those sites except for a 3.30% difference in change in total hip BMD in a single study (23), but it is important to note that this study (23) had different design that mainly focused on the effect of calcium supplementation during post-weaning period, making the results less comparable to those in other studies. Nevertheless, this study would suggest that calcium supplement may improve BMD recovery after lactation, and this type of design may be important to consider in future studies.

Irrespective of the poor quality of the evidence, the effect sizes for BMD at the lumbar spine, forearm, total body and femoral neck (0.33% to 1.10%) are too small to be clinically meaningful. In older people, a 10% higher BMD of the lumbar spine and femoral neck is associated with approximately 50% reduction in long-term fracture risk (33), and the effect sizes of our study are therefore unlikely to be translated into a noteworthy reduction in fracture risk. Moreover, there is no evidence that a history of lactation is associated with increased fracture risk in later life (34), so the likelihood of reducing fractures in later life through this strategy would seem low. Postpartum osteoporosis and fractures are rare and have only been described in a few case reports (35). Although bone loss during lactation may cause an increased risk of osteoporosis and fractures, Kovacs has proposed this may only be a coincidental condition caused by pre-existing low bone density for the majority of women (36) and our review suggests that it is unlikely that calcium supplementation during lactation would have sufficient benefits for BMD to prevent such fractures.

While calcium supplementation showed a moderate effect on total hip BMD, this was based on a single study with high risk of bias and in which calcium supplementation began 3 months after birth and presumably the establishment of breastfeeding, and given that the mean duration of breastfeeding tanged from 80 to 120 days, supplementation was primarily administered after breastfeeding ceased and recovery of bone mass commenced (23). This is consistent with the fact that in this study BMD increased rather than decreased in both intervention groups. The effect on the total hip in this study of 3.3% over 9 months is closer to being clinically important and this raises the question of whether calcium supplementation on cessation of rather than during breastfeeding could be beneficial. However, more evidence from high-quality RCTs is needed to address this question.

There were insufficient studies in this review to allow for a meaningful subgroup analysis by baseline calcium intake. In only two trials was the average baseline calcium intake lower than the recommended intake of 1000 mg/day for lactating women aged 18 or older (being 562 and 614 mg/day) (22, 23). The possibility of a BMD effect occurring in women with very low calcium intake cannot be ruled out and such women could be the target population in any future clinical trials. RCTs in women with low calcium intake may also provide additional evidence to support the setting of recommended dietary calcium intakes in lactating adults. Of note, a higher intake is recommended for lactating women aged 18 or younger (1300 mg/day) (17), but no trials have examined the effect of calcium supplementation in this age group. Therefore, this recommendation may also be warranted unless new evidence becomes available.

Strengths and limitations of the study

This systematic review was conducted following a prespecified, registered protocol using Cochrane methodology. We performed a comprehensive search of multiple databases and clinical trial registries without restricting language or publication status of the trials. It is therefore unlikely that we have missed any relevant trials.

The most important limitation of this review comes from the five included trials themselves. All included trials had a high risk of bias, and we have downgraded the quality of evidence to very low for all findings. The possibility of confounding cannot be ruled out because four out of the five trials included did not provide detailed information about the randomization process. However, effect sizes in this review were small and of doubtful clinical importance, and given the known tendency of trials with higher risk of bias to overestimate treatment effects (37, 38), it seems likely that the robust clinical trials required to definitively confirm this lack of effect should not be a high research priority. We included two trials using no intervention as control group due to the limited number of placebo-controlled trials based on the pre-specified protocol, but the main outcome of BMD was measured by DXA which is unlikely to be influenced by the knowledge of grouping. The exclusion of these two trials in a sensitivity analysis did not significantly change the pooled results, suggesting no impact of this issue on our conclusions. The limited number of studies precluded using subgroup analyses to explore the moderate statistical heterogeneity observed and assess potential effect modification. Instead, we addressed heterogeneity by excluding a study with markedly different characteristics of study design and population (23). The results of this analysis of the remaining four studies showed only a very small effect of calcium supplementation on lumbar spine BMD. Therefore, it may be that the main pooled result overestimates the effect of calcium supplementation in lactating women and should be interpreted with caution.

Conclusion

Overall, the meta-analysis indicates that calcium supplementation does not provide clinically important benefits for BMD in lactating women. However, the mean dietary intake was adequate before supplementation in some studies, and others did not measure baseline dietary calcium intake. Advising lactating women to meet the current recommended calcium intakes

342 (including with supplements if dietary intake is low and cannot otherwise be increased) is
343 warranted unless new high certainty evidence to the contrary from robust clinical trials
344 becomes available. More research needs to be done in larger samples of women from diverse
345 ethnic and racial groups and specifically in women with low dietary calcium intakes.

Declarations

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Tables

Table 1. Characteristics of included studies

Study	No. Ca/Control	Age, years mean (SD)	Ethnicity	Baseline Ca intake, mg/d mean (SD)	Duration of lactating	Ca dose (mg/d)	Concomitant Vitamin D	Intervention [4]		Outcome measures			Techniques of BMD measure	Sites measured
								Start	Duration	Baseline	End points	Time interval		
Cross 1995 (21)	7/8	28.2 (1.3)	White	1333 (86)	7.6 months	1000	400 IU/day	< 2 weeks postpartum	10 months	< 2 weeks postpartum	3 months lactation; 3 months postweaning	10 months	DXA	Whole-body, lumbar spine, radius (proximal 1/3, mid- and ultradistal)
Kent 1995 (20)	44/35	31 (NS)	White	1056 (284) [1]	6 months	1000	No	36 weeks pregnancy	7 months	1 week postpartum	24 weeks lactation	6 months	DXA	Lumbar spine, proximal femur (neck, trochanteric and intertrochanteric), radius and lower limb (shaft and ultradistal)
Kalkwarfa 1997 (22)	45/42	30 (3)	84% white	614 (472 to 753) [2]	6 months	1000	400 IU/day	16 ± 2 days postpartum	6 months	16 ± 2 days postpartum	3 and 6 months after enrolment	6 months	DXA	Lumbar spine, radius (shaft and ultradistal)
Polatti 1999 (19)	139/135	29.5 (3.0)	White	NS	6 months	1000	No	5-10 days postpartum	18 months	5-10 days postpartum	3, 6, 7, 12 and 18 months postpartum	18 months	DXA	Lumbar spine, radius (distance to ulna < 8mm)
Yu 2011 (23)	58/54	27.5 (4.1)	Chinese	562 (197)	3 to 4 months [3]	600	No	3 months postpartum	9 months	3 months postpartum	12 months postpartum	9 months	DXA	Lumbar spine, total hip

BMD, bone mineral density; Ca, calcium; DXA, dual-energy x-ray absorptiometry; NS, not stated; SD, standard deviation.

[1] Ca intake in the placebo group at 12 weeks lactation; baseline calcium intake was unavailable.

[2] Median (Interquartile range).

[3] Lactation occurred for a month or less of the supplementation period.

[4] Polatti 1999 and Yu 2011 did not have placebo group and used no intervention as control group.

Table 2. Summary of findings of eligible randomized controlled trials (n=5) evaluating the effect of calcium supplementation for improving bone mineral density in lactating women.

Sites of BMD measures	Absolute effect estimates			Certainty of the evidence (GRADE)	What happens
	Control group	Calcium supplementation	Difference (95% CI)		
Lumbar spine Follow up: 6 to 18 months No. of participants: 527 (5 RCTs) (19-23)	Mean 0.79% decreasing	Mean 0.05% decreasing	0.74 (95% CI -0.10, 1.59; p=0.09)	⊕⊖⊖⊖ VERY LOW (Risk of bias, inconsistency and indirectness) [1]	Calcium supplementation may have no effect for preventing the loss of lumbar spine BMD
Forearm Follow up: 6 to 18 months No. of participants: 415 (4 RCTs) (19-22)	Mean 0.27% increasing	Mean 0.80% increasing	0.53% (95% CI -0.35, 1.42; p=0.24)	⊕⊖⊖⊖ VERY LOW (Risk of bias, inconsistency and publication bias) [2]	Calcium supplementation may have no effect on the loss of forearm BMD
Total body BMD Follow up: 10 months No. of participants: 15 (1 RCT) (21)	Mean 1.0% decreasing	Mean 0.03% increasing	1.03% (95% CI -0.75, 2.81; p=0.26)	⊕⊖⊖⊖ VERY LOW (Risk of bias and imprecision) [3]	Calcium supplementation appears to not reduce the loss of whole-body BMD
Total hip BMD Follow up: 9 months No. of participants: 112 (1 RCT) (23)	Mean 3.14% increasing	Mean 6.44% increasing	3.30% (95% CI 1.53, 5.07; p<0.001)	⊕⊖⊖⊖ VERY LOW (Risk of bias, indirectness and imprecision) [4]	Calcium supplementation may have a moderate effect for reducing the loss of total hip BMD
Femoral neck BMD Follow up: 6 months No. of participants: 79 (1 RCT) (20)	Mean 6.1% decreasing	Mean 5.0% decreasing	1.10% (95% CI -0.43, 2.63; p=0.16)	⊕⊖⊖⊖ VERY LOW (Risk of bias and imprecision) [5]	Calcium supplementation may have little or no effect on the loss of femoral neck BMD

BMD: Bone mineral density; **CI:** Confidence interval; **RCT:** Randomized controlled trial.

[1] **Risk of bias**: inadequate random sequence generation, and inadequate or lack of concealment of allocation, resulting in potential selection bias; inadequate or lack of blinding of participants and personnel resulting in potential performance bias; inadequate description of missing data resulting in potential attrition bias. **Inconsistency**: Yu 2011 (23) observed a significantly larger effect of calcium supplementation compared to other trials and used no intervention as control, and Polatti 1999 (19) used no intervention as control and had a remarkably smaller variation of change in BMD than other studies. **Indirectness**: Yu 2011 (23) mainly targeted on the effect of calcium supplementation on BMD in post-lactating rather than lactating women.

[2] **Risk of bias**: inadequate random sequence generation and concealment of allocation resulting in potential selection bias; inadequate or lack of blinding of participants and personnel resulting in potential performance bias; inadequate description of missing data resulting in potential attrition bias. **Inconsistency**: Polatti 1999 (19) used no intervention as control and had a remarkably smaller variation of change in BMD than other studies. **Publication bias**: funnel plot indicates a potential publication bias.

[3] **Risk of bias**: inadequate random sequence generation and concealment of allocation resulting in potential selection bias; inadequate blinding of participants and personnel resulting in potential performance bias. **Imprecision**: only one study (21) with a very small sample size was available, and the wide confidence interval may influence clinical decision.

[4] **Risk of bias**: inadequate random sequence generation and concealment of allocation, resulting in potential selection bias; lack of blinding of participants and personnel (no intervention as control) resulting in potential performance bias; inadequate description of a high proportion of missing data (30%) may lead to potential attrition bias. **Indirectness**: Yu 2011 (23) mainly targeted on the effect of calcium supplementation on BMD in post-lactating rather than lactating women. **Imprecision**: only one study was available, and the wide confidence interval may influence clinical decision.

[5] **Risk of bias**: inadequate random sequence generation and concealment of allocation, resulting in potential selection bias; inadequate blinding of participants and personnel resulting in potential performance bias; inadequate description of missing data resulting in potential attrition bias; trial was published as a conference abstract with many details of the study unavailable. **Imprecision**: only one study was available,(20) and the wide confidence interval may influence clinical decision.

Figure Legends

Figure 1. Flow chart of the study. [1] All records from ClinicalTrials.gov and WHO trials portal were assessed and excluded.

Figure 2. Random effects meta-analysis of effect of calcium supplementation on percentage change in BMD of the lumbar spine and forearm from baseline to the end of supplementation.

Figure 3. Random effects meta-analysis of effect of calcium supplementation on percentage change in BMD of the lumbar spine and forearm from baseline to 3 or 6 months.