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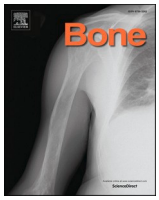

Full Length Article

Transient alendronate administration to pregnant or lactating mothers prevents bone loss in mice without adverse effects on offspring

Eri Ito [a], Yuiko Sato [b,c,d], Tami Kobayashi [b,c,d], Tomoya Soma [e], Tatsuaki Matsumoto [b], Atsushi Kimura [b], Kana Miyamoto [f], Hideo Matsumoto [a], Morio Matsumoto [b], Masaya Nakamura [b], Kazuki Sato [a], Takeshi Miyamoto [b,c,d,f,*]

[a] Institute for Integrated Sports Medicine, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan
[b] Department of Orthopedic Surgery, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan
[c] Department of Advanced Therapy for Musculoskeletal Disorders II, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan
[d] Department of Musculoskeletal Reconstruction and Regeneration Surgery, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan
[e] Division of Oral and Maxillofacial Surgery, Department of Dentistry and Oral Surgery, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan
[f] Department of Orthopedic Surgery, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan

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ABSTRACT

Changes in bone metabolism occur in mothers during pregnancy or lactation that may decrease bone mass and result in fragility fractures after partum. However, use of drugs during pregnancy or lactation to counteract these effects is often prohibited or strongly discouraged. Therefore, approaches to protect mothers from fragility fractures have not been established. Here we show that bone mineral density was significantly lower in female mice after partum than in age-matched female mice without partum. We also show that temporary administration of the bisphosphonate alendronate, either just before or just after pregnancy, to female mice was protective against bone loss due to pregnancy or lactation and had no adverse effects on offspring, such as growth retardation. Furthermore, we show that alendronate administration to female mice during lactation was effective in increasing bone mass in mothers without promoting bone abnormalities or growth retardation in offspring. Calcium levels in milk from female mice administered alendronate during lactation were equivalent to those in milk from mothers not treated with alendronate. Overall, we propose that alendronate administration to mothers could prevent bone loss and fragility fractures during pregnancy and lactation.

1. Introduction

Bone metabolic profiles dramatically change in females during pregnancy or lactation as calcium and nutrients move from the mothers to fetuses or infants, respectively [1–3]. If such calcium levels are insufficient to supply required levels of calcium to fetuses or infants, osteoclastic bone resorption and osteocytic osteolysis are activated in the mother to increase that supply [3,4]. Such bone resorption can result in reduced bone mass in women [5,6] and may underly fragility fractures experienced by mothers after partum. In particular, post-partum bone resorption activity has been demonstrated to be significantly higher in mothers who exclusively breastfed their infants than in those who fed infants with formula only or a mix of formula and breastfeeding [7]. Some mothers, however, prefer to breastfeed only; however, many drugs known to counteract bone loss are prohibited or strongly discouraged in mothers, or their safety has not been established in the context of pregnancy or lactation. Indeed, animal studies indicate that some drugs administered to mothers reach either fetuses or offspring via the placenta or through breastmilk, respectively [8], where they can exhibit teratogenic capacity or toxicity [9,10]. Thus, preventing bone loss or fragility fractures in pregnant or lactating mothers remains a challenge.

Bisphosphonates, such as alendronate, risedronate and zoledronate, are commonly used to treat patients with osteoporosis [11,12]. Bisphosphonates specifically and tightly bind to hydroxyapatite, and therefore attach to bone surfaces where they promote osteoclast apoptosis and increase bone mass in humans and other mammals [13,14]. Among bisphosphonates, alendronate is currently not

* Corresponding author at: Department of Orthopedic Surgery, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail addresses: kazuki@keio.jp (K. Sato), miyamoto@z5.keio.jp, miyamoto.takeshi@kuh.kumamoto-u.ac.jp (T. Miyamoto).

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prohibited for use in pregnant or lactating women, although it is suggested that it be administered with care [15–18]. Moreover, bisphosphonates reportedly can be transferred to offspring either through the placenta or via breastmilk [8,19]. Therefore, the safety of alendronate administration to pregnant or lactating mothers is unclear.

In the current study, we show that bone mineral density (BMD) decreased significantly after partum in untreated female mice. Temporary administration of alendronate either before or after pregnancy protected against post-partum bone loss in mouse mothers without adverse effects on offspring, such as bone malformations or other defects. Similarly, temporary alendronate administration during lactation increased bone mass in mothers without retarding growth of offspring. Calcium concentrations in milk from mothers administered alendronate were comparable to those seen in mothers administered PBS. These data show that transient alendronate administration is a safe way to protect maternal bones during pregnancy and lactation without growth retardation effects in offspring.

2. Materials and methods

2.1. Mice

All mice were maintained under specific pathogen free conditions in animal facilities accredited by the Keio University Institutional Animal Care and Use Committee. Animal protocols were approved by that Institutional Guidelines on Animal Experimentation at Keio University as described [20].

2.2. Treatment of mice with alendronate

We purchased wild-type (WT) C57/B6 female and male mice (eight-week-old) from Sankyo Labo Service (Tokyo, Japan). Female mice were first randomly divided into two groups: one to be injected with PBS (0.2 ml) and another with 20 μg alendronate (ALN) (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan). Each of those groups was then further subdivided into “pre” or “post” groups to be injected either before or after mating, respectively. Injections were subcutaneous in all cases and administered two days a week for two weeks. Mice were mated when they were 10-week-old. Mother mice or their newborns were sacrificed five days after either partum or birth, respectively.

2.3. Alendronate treatment during lactation

We purchased wild-type C57/B6 female pregnant mice from Sankyo Labo Service (Tokyo, Japan) and randomly divided them into a “PBS” group, to be injected with 0.2 ml PBS, and an “ALN” group, to be injected with 20 μg alendronate in PBS. Subcutaneous injection of mothers began the third day after partum and continued for twice a week for either two or four weeks to assess respective short- or long-term effects on offspring.

Offspring were sacrificed at either postnatal day 14 or at 10 weeks of age to analyze respective short- or long-term effects of lactation from mothers administered alendronate. Offspring were housed in the same cage with mothers until they were four weeks old, at which time they were separated by gender and housed as five per cage. Mice of both genders were used in experiments.

2.4. Milking

Milk samples were collected from mother mice 24 h after the last alendronate injection on day 14 after partum. Mother mice were separated from offspring 6 h before samples were taken. Mothers were then subcutaneously injected with 0.1 ml (1 unit) oxytocin (ASKA Animal Health Co., Ltd., Tokyo, Japan) to accelerate milk secretion. Thirty minutes later, their milk was collected using a KN-591 milking machine (Natsume Seisakusho Co., Ltd., Tokyo, Japan).

2.5. Blood sampling

Peripheral blood was collected from each mouse at sacrifice using heparinized capillary tubes. Serum was separated by centrifugation (6000 rpm, 15 min, room temperature) and stored at −80 °C until assayed.

2.6. Analysis of skeletal morphology

Femurs were removed from mice injected PBS or ALN and then fixed in 70% ethanol for analysis of bone mineral density (BMD) and bone histomorphometry. BMD of the entire femur was analyzed by Dual Energy X-ray Absorptiometry (DEXA) using a DCS-600R system (Aloka Co. Ltd., Tokyo, Japan). Changes in femur microstructure were assessed using a micro-computed tomography (micro-CT) system (CosmoScan GX; Rigaku Corporation, Tokyo, Japan). Femurs were scanned at 90 kV, 160 μA on the micro-CT scanner. Mothers and 10-week-old mice assessed for long-term effects were scanned at a voxel size 20 × 20 × 20 μm, while two-week-old mice assessed for short-term effects were scanned at a voxel size 10 × 10 × 10 μm. Three-dimensional reconstruction was performed using the TRI-BONE system (Ratoc system Engineering, Co., Ltd., Tokyo, Japan). Relevant to the femoral metaphysis, the region of interest was defined at 0.2 mm away from the growth plate. Parameters of bone morphometric analysis for the femoral metaphysis were bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), tissue mineral density of trabecular (TMD·Tb, mg/cm^3) and tissue mineral density of cortical (TMD.Ct, mg/cm^3).

Diaphyseal cortical parameters were cortical thickness (Ct.Th, μm), cortical bone area (Ct.Ar, mm^2), bone mineral content (BMC, mg) and tissue mineral density of cortical (TMD, mg/cm^3). Bone strength was analyzed by the finite element method using bone imaging of the metaphysis reconstructed using the TRI-BON system. A TRI/3D-FEM system (Ratoc System Engineering, Co., Ltd., Tokyo, Japan) was used for this analysis. A material define file was created by reflecting the BMD value. The load was defined as one distributed along the anterior to posterior axis at the longitudinal center of the model bone. Failure load of the principal stress was analyzed, and failure threshold was set to a minimum stress of 60 MPa.

2.7. Biomechanical testing

Mechanical strength was analyzed by a 3-point bending experiment using the left femur (TK-252D BONE STRENGTH TESTER; Muromachi Kikai Co., LTD., Tokyo, Japan). Femurs were collected with minimum contamination by surrounding tissue and kept in 70% ethanol at 4 °C. Femurs were tested along the antero-posterior axis with the anterior surface facing upward, and pressing force was applied vertically to the bone midshaft. The constant span length was measured at 10 mm. Each bone was tested with a loading speed of 2 mm/min until failure with a 100 N load cell.

2.8. Enzyme-linked immunosorbent assay (ELISA)

Serum TRAP5b ELISA assays were undertaken following the manufacturer's instructions (Immunodiagnostic Systems Holdings PLC., Boldon, UK) using a multiple plate analyzer (POWERSCAN HT, DS Pharma Biomedical, Osaka, Japan).

2.9. Statistical analysis

Statistical analyses were performed using an unpaired two-tailed Student's *t*-test between ALN and PBS groups (*$P < 0.05$; **$P < 0.01$; NS, not significant, throughout the paper). All data are expressed as means ± standard error.

3. Results

3.1. Decreased BMD seen after partum can be blocked by transient alendronate administration to mouse mothers either before or during pregnancy

We initially examined BMD in femoral bone of female mice after partum compared to age-matched female control mice that had not been mated. BMD was significantly decreased in female mice after partum compared to age-matched controls (Fig. 1A).

We then administered either the bisphosphonate alendronate or PBS to female mice twice a week for two weeks and then mated females with male mice, at which time drug treatment was discontinued (Fig. 1B). Five days after partum, we analyzed BMD in femoral bone of mother mice and found it was significantly greater in mice administered alendronate than in PBS-injected controls (Fig. 1C). Micro CT analysis demonstrated that BV/TV, TMD Tb., TMD Ct., Ct.Th., Ct.Ar. and BMC were significantly higher in mice administered alendronate than in controls, while Tb.Sp was significantly decreased in bones of mice administered alendronate relative to controls (Fig. 1D and E). In agreement with increased bone mass seen after alendronate administration, bone strength, as analyzed by a 3-point bending test and micro CT-based finite element analysis, was significantly higher in mothers administered alendronate than in PBS-treated controls (Figs. 1F and S1). Moreover, five days after birth, body length and weight were greater in offspring from alendronate-treated compared to control mothers (Fig. 1G), although we did not observe malformations at the macroscopic level or dwarfism among offspring of alendronate-treated mothers (data not shown). These results indicate that transient administration of alendronate to female mice prior to mating can protect the mothers from bone loss without adverse effects on offspring.

We next assessed effects of alendronate treatment of mothers during pregnancy. To do so we administered alendronate or PBS to pregnant mice immediately after mating and continued injections twice a week for two weeks (Fig. 2A). Five days after partum, BMD in femurs of mouse mothers was analyzed by DXA and found to be significantly higher in mice administered alendronate compared to PBS (Fig. 2B). Micro CT analysis also revealed significantly elevated bone mass in female mice administered alendronate relative to controls (Fig. 2C and D). Bone strength significantly increased in female mice administered

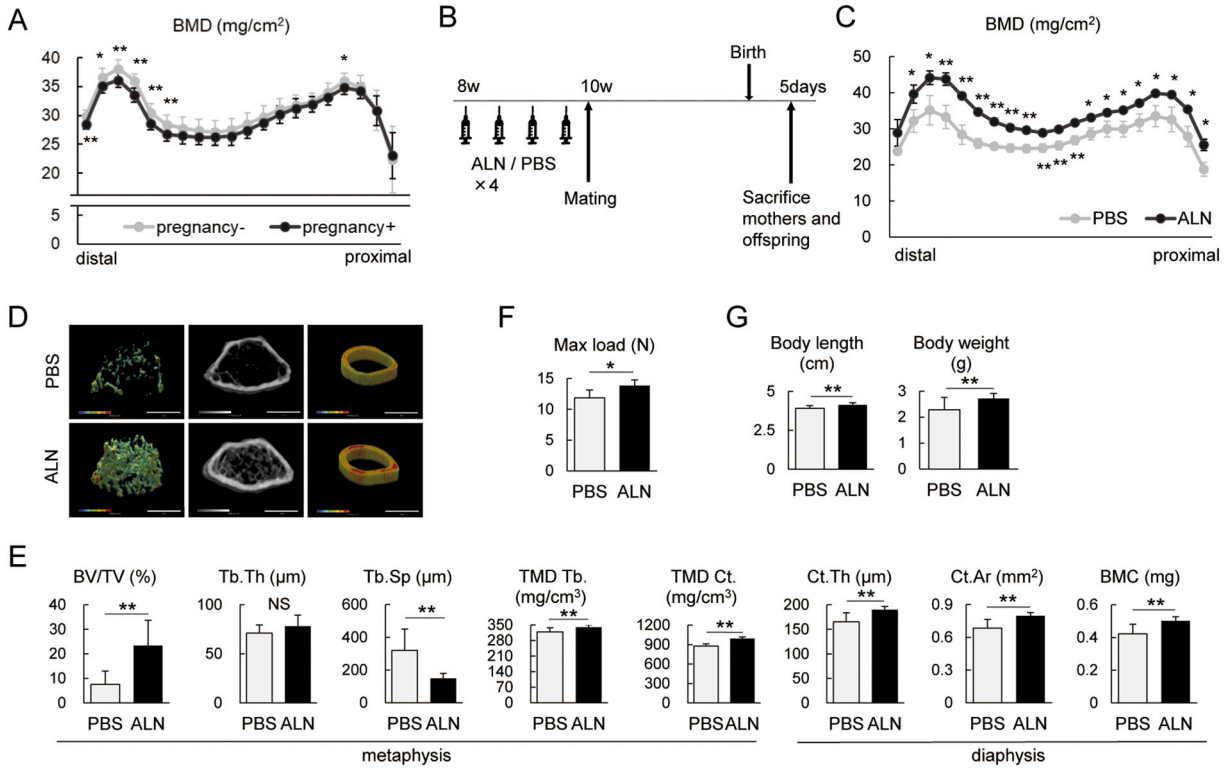

Fig. 1. Two-weeks of alendronate (ALN) treatment immediately before pregnancy suppress bone loss in mother mice, with no apparent effects on fetal growth.
(A) Bone mineral density (BMD) based on DEXA in postpartum female mice or age-matched female mice that had not undergone pregnancy. Graph shows BMD of femurs divided evenly longitudinally ($n = 15$, each).
(B) Experimental protocol. ALN (20 μg) or PBS was injected subcutaneously into eight-week-old female mice twice a week for two weeks before mating. Five days after partum, mothers and offspring were analyzed.
(C) BMD based on DEXA in postpartum female mice injected with ALN or PBS before mating. Graph shows BMD of femurs divided equally longitudinally ($n = 3$, each).
(D) Representative micro-CT images of femoral bone in female mice injected with ALN or PBS before mating. Images in trabecular (left) and total bone of metaphysis (middle) and cortical bone of diaphysis (right) are shown. Bar, 1 mm.
(E) Bone parameters in postpartum female mice injected with ALN or PBS before mating. Bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), tissue mineral density of trabecular (TMD·Tb, mg/cm^3) and tissue mineral density of cortical (TMD.Ct, mg/cm^3) of the metaphysis femur are shown. Cortical thickness (Ct. Th, μm), cortical bone area (Ct.Ar, mm^2) and bone mineral content (BMC, mg) of diaphyseal femur cortical bone are shown ($n = 10$, each).
(F) Maximum load measured at the midshaft region of a femur based on a 3-point bending test in postpartum female mice injected with ALN or PBS before mating ($n = 5$, each). N, newton.
(G) Body length and weight of five-day-old mice born to mothers injected with ALN or PBS before mating (PBS $n = 25$, ALN $n = 14$).
(A, C, E-G) Data is shown as means of indicated parameters ± S.D. (t-test; *$p < 0.05$, **$p < 0.01$, NS: not significant).

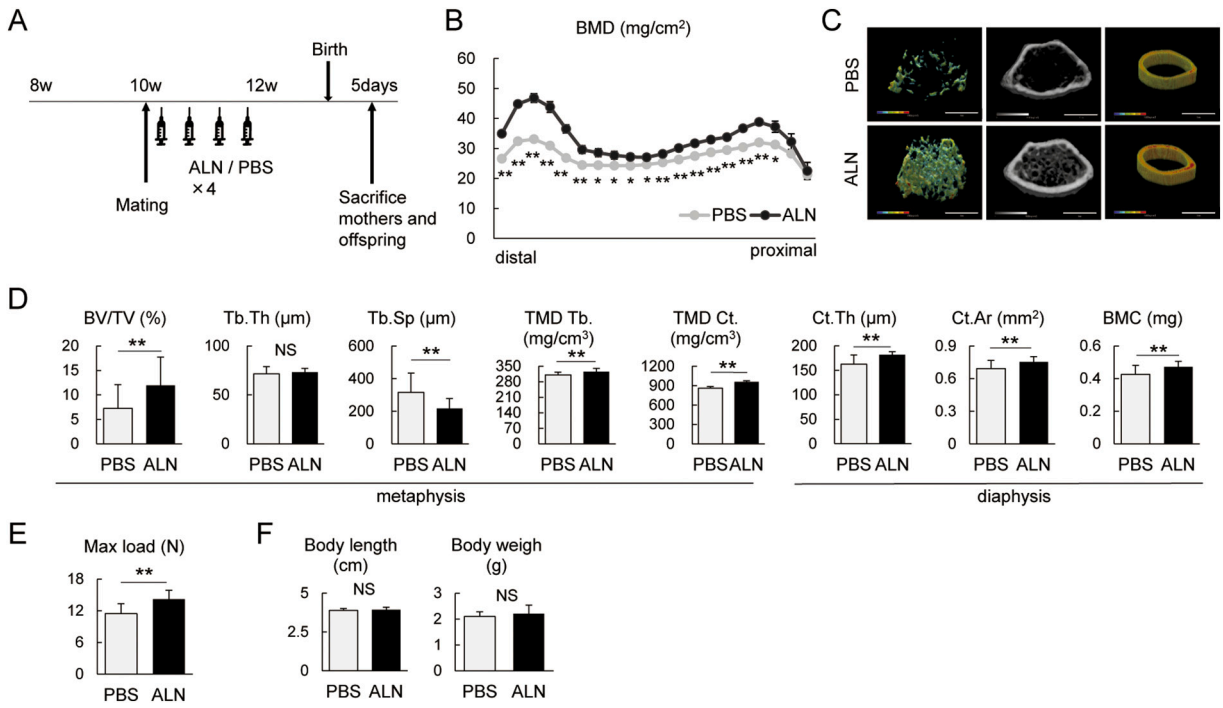

Fig. 2. Two-weeks of alendronate (ALN) treatment during pregnancy prevent bone loss in mother mice with no apparent effects on fetal growth.
(A) Experimental protocol. ALN (20 μg) or PBS was injected subcutaneously into 10-week-old female mice twice a week for two weeks immediately after mating. Five days after partum, mothers and offspring were sacrificed and analyzed.
(B) Bone mineral density (BMD) based on DEXA in postpartum female mice injected with ALN or PBS during pregnancy. Graph shows BMD of femurs divided equally longitudinally (n = 3, each).
(C) Representative micro-CT images of femoral bone in female mice injected with ALN or PBS right after mating and during pregnancy. Images in trabecular (left) and total bone of metaphysis (middle) and cortical bone of diaphysis (right) are shown. Bar, 1 mm.
(D) Bone parameters in postpartum female mice injected with ALN or PBS during pregnancy. Bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), tissue mineral density of trabecular (TMD·Tb, mg/cm^3) and tissue mineral density of cortical (TMD.Ct, mg/cm^3) of the metaphysis femur are shown. Cortical thickness (Ct. Th, μm), cortical bone area (Ct.Ar, mm^2) and bone mineral content (BMC, mg) of diaphyseal femur cortical bone are shown (n = 14, each).
(E) Maximum load measured at the midshaft region of a femur based on a 3-point bending test in postpartum female mice injected with ALN or PBS during pregnancy (n = 5, each). N, newton.
(F) Body length and body weight of five-day-old offspring born to mothers injected with ALN or PBS during pregnancy (PBS n = 27, ALN n = 25).
(B, D-F) Data represents means of indicated parameters ± S.D. (t-test; *p < 0.05, **p < 0.01, NS: not significant).

alendronate relative to PBS-treated controls (Figs. 2E and S2). By five days after partum, body length and body weight of offspring were comparable in pups delivered to mothers administered alendronate or PBS (Fig. 2F). Finally, no malformations or dwarfism were seen in offspring delivered to mothers administered alendronate during pregnancy (data not shown).

3.2. *Transient alendronate administration after partum prevents bone loss in mouse mothers without retarding offspring growth*

To assess potential effects of short-term drug administration during lactation, mother mice were administered either alendronate or PBS starting at three days after partum and continuing for two weeks during the lactation period (Fig. 3A). Mothers administered alendronate showed significantly higher BV/TV, Tb.Th, TMD Tb., TMD Ct., Ct.Th., Ct.Ar. and TMD relative to controls, while Tb.Sp was significantly lower in alendronate-treated mothers (Fig. 3B and C), indicating significantly greater bone mass in alendronate-treated relative to control mothers. Indeed, bone strength significantly increased in female mice administered alendronate relative to PBS-treated control mice (Figs. 3D and S3). Calcium levels in milk were comparable in alendronate-treated and control mothers (Fig. 3E). Moreover, by two weeks after birth, body length was equivalent in offspring delivered to mothers in both treatment groups, but body weight was greater in offspring from mothers administered alendronate relative to controls (Fig. 3F). Serum levels of calcium and of TRAP5b, a marker of bone resorption, were comparable in offspring in both groups (Fig. 3G), as were bone parameters including BV/TV, Tb.Th, Tb.Sp, TMD Tb., TMD Ct., Ct.Th., Ct.Ar. and TMD (Fig. 3H).

We then undertook comparable analysis following long-term alendronate administration to mouse mothers, again starting at three days after delivery but then continuing for four weeks until weaning (Fig. 4A). Bone mass and bone strength were significantly higher in mothers administered alendronate versus those administered control PBS (Fig. 4B, C and D). Also, growth curves analyzing either male or female offspring of mothers in both treatment groups were comparable (Fig. 4E), as were bone parameters and strength assessed at ten weeks of age (Fig. 4F, H and S4A (males), and G, I and S4B (females)).

Taken together, our findings indicate that in mice, transient alendronate administration to mothers either just before pregnancy, during pregnancy, or during lactation can prevent bone loss in mothers without adverse effects on offspring growth.

4. Discussion

In humans, post-partum fractures in breastfeeding mothers are rare, but when they occur they can cause severe pain [17,21]. Such fractures usually occur soon after partum, with most cases of fragility fractures emerging within one month of partum [7,22]. Fragility fractures are most frequent in young mothers who breastfeed their infants

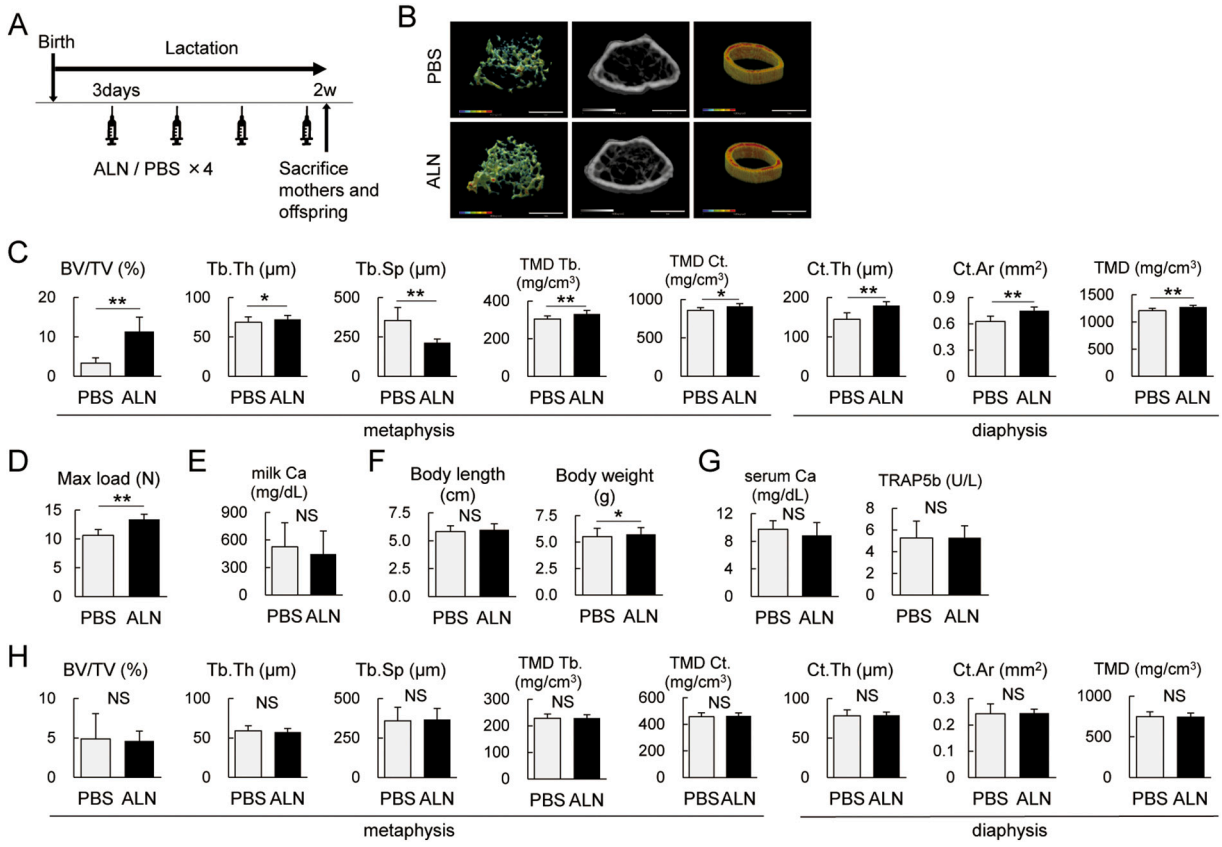

Fig. 3. Two weeks of alendronate (ALN) treatment after partum increases bone mass in mother mice without growth retardation in infants.
(A) Experimental protocol. Mother mice were injected subcutaneously ALN (20 μg) or PBS twice a week starting at three days after partum and continuing for two weeks during lactation. Mothers and offspring were sacrificed and analyzed at two weeks after partum. Milk samples were collected from mothers 24 h after the last alendronate injection at time of sacrifice.
(B) Representative micro-CT images of femoral bone in mother mice injected with ALN or PBS after partum. Images in trabecular (left) and total bone of metaphysis (middle) and cortical bone of diaphysis (right) are shown. Bar, 1 mm.
(C) Bone parameters of mother mice injected with ALN or PBS after partum. Bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), tissue mineral density of trabecular (TMD.Tb, mg/cm^3) and tissue mineral density of cortical (TMD.Ct, mg/cm^3) of the metaphysis femur are shown. Cortical thickness (Ct. Th, μm), cortical bone area (Ct.Ar, mm^2) and tissue mineral density (TMD, mg/cm^3) of diaphyseal femur cortical bone are shown (PBS $n = 24$, ALN $n = 26$).
(D) Maximum load measured at the midshaft region of a femur based on a 3-point bending test in postpartum female mice injected with ALN or PBS after partum ($n = 5$, each). N, newton.
(E) Calcium (Ca) concentration of maternal milk 24 h after ALN or PBS injection (PBS $n = 10$, ALN $n = 9$).
(F) Body length and body weight of offspring (PBS $n = 81$, ALN $n = 92$).
(G) Serum Ca and TRAP5b levels in offspring, the latter based on ELISA. (Ca: n = 10 littermates per condition. TRAP5b: PBS $n = 7$ littermates, ALN n = 5 littermates).
(H) Bone parameters of offspring. Bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), tissue mineral density of trabecular (TMD.Tb, mg/cm^3) and tissue mineral density of cortical (TMD.Ct, mg/cm^3) of the metaphysis femur are shown. Cortical thickness (Ct. Th, μm), cortical bone area (Ct.Ar, mm^2) and tissue mineral density (TMD, mg/cm^3) of diaphyseal femur cortical bone are shown (PBS $n = 57$, ALN $n = 54$).
(C-H) Data represents means of indicated parameters ± S.D. (*t*-test; $^*p < 0.05$, $^{**}p < 0.01$, NS: not significant).

exclusively, rather than in mothers who use formula or a mix of formula and breastfeeding [7]. Preventing such fractures is challenging since many drugs are prohibited during pregnancy or lactation. Here, we show that transient administration of alendronate to mothers just before pregnancy, during pregnancy or during lactation is effective to increase BMD in mouse mothers but does not result in abnormalities in their offspring.

Menopause is a well-known risk for osteoporosis development in females [23,24]. A transient menopausal state is normally seen in pregnant women or during the early period after partum, particularly during lactation. However, changes in bone metabolic profiles during pregnancy and lactation have previously not been well characterized. Recently, we demonstrated that in humans, osteoclastic but not osteoblastic activity increased in an uncoupled-manner in mothers who relied solely on breastfeeding their babies in the month after partum [7]. Generally, bone homeostasis is regulated via coupling of osteoclastic bone-resorption and osteoblastic bone-formation activities. Thus, the relatively reduced bone mass seen in breastfeeding mothers after partum strongly suggests aberrantly elevated osteoclastic than osteoblastic activity [7]. We concluded that during pregnancy or lactation osteoclasts could be considered primary targets when devising strategies to prevent bone loss or fragility fractures in women.

Here, we demonstrate that alendronate treatment blocks bone loss in mouse mothers when administered either 1) two weeks before mating, 2) two weeks after pregnancy begins, or 3) two weeks after partum. Transient alendronate administration following any of these regimes was sufficient to block bone loss in mothers without adverse effects to offspring. Alendronate has been demonstrated to have high affinity for and adherence to maternal bone [25,26].

Denosumab, a neutralizing antibody against receptor activator of nuclear factor kappa B ligand (RANKL), is known to be a strong inhibitor of osteoclastic bone-resorption [27]. However, in osteoporosis patients,

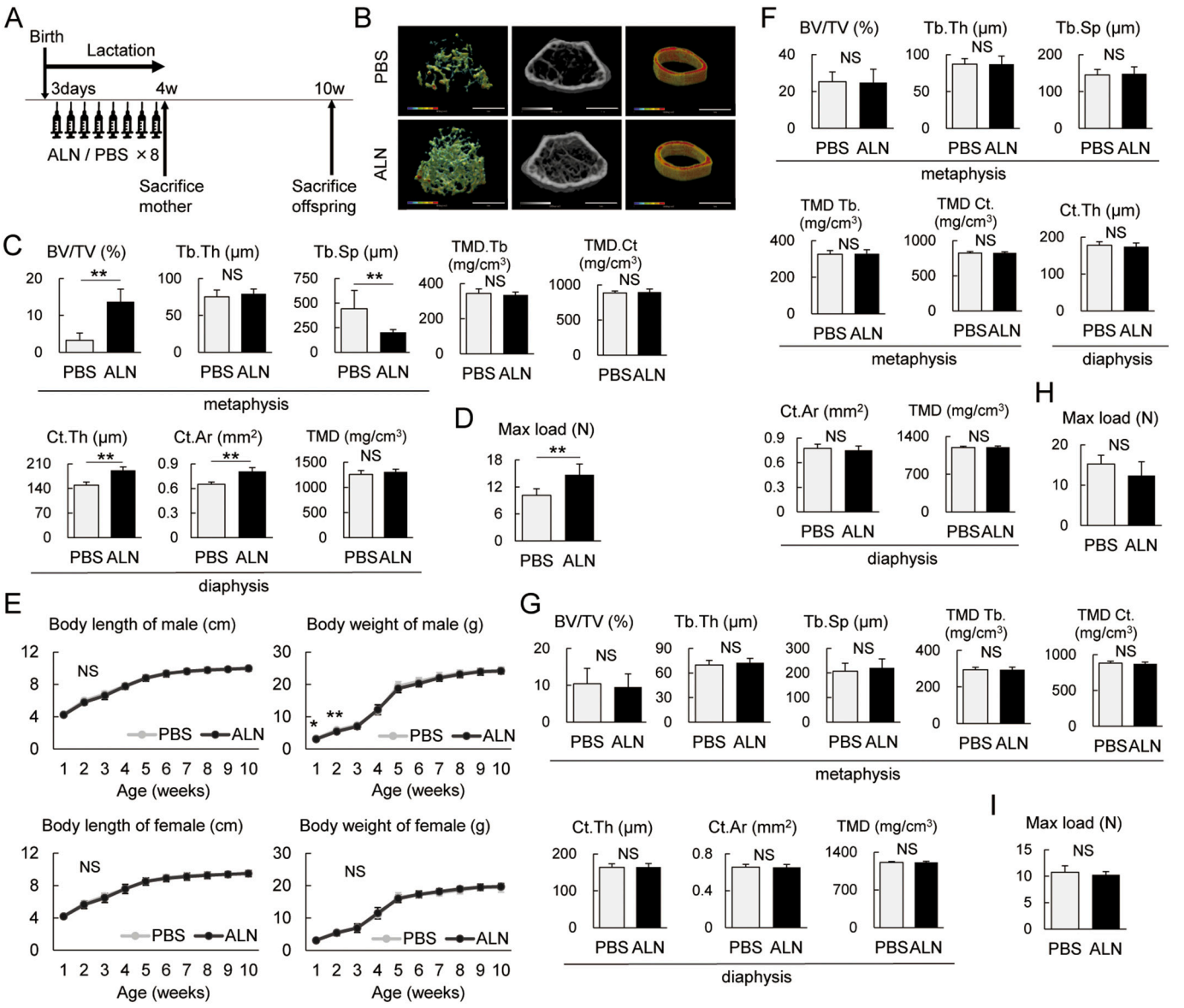

Fig. 4. Four weeks of alendronate (ALN) administration after partum increases bone mass in lactating mice without growth retardation in offspring.
(A) Experimental protocol. Mother mice were injected subcutaneously ALN (20 μg) or PBS twice a week starting at three days after partum for four weeks during lactation. Mother mice were sacrificed for analysis at four weeks after partum. Offspring were housed in the same cage with mothers and milk-fed until four-weeks-old. Offspring were fed normal diet after mothers were sacrificed. Offspring were evaluated 10 weeks after birth.
(B) Representative micro-CT images of femoral bone in mother mice injected with ALN or PBS during lactation. Images in trabecular (left) and total bone of metaphysis (left, middle) and cortical bone of diaphysis (right) are shown. Bar, 1 mm.
(C) Bone parameters of mothers injected with ALN or PBS during lactation. Bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), tissue mineral density of trabecular (TMD·Tb, mg/cm^3) and tissue mineral density of cortical (TMD.Ct, mg/cm^3) of the metaphysis femur are shown. Cortical thickness (Ct. Th, μm), cortical bone area (Ct.Ar, mm^2) and tissue mineral density (TMD, mg/cm^3) of diaphyseal femur cortical bone are shown (n = 10, each).
(D) Maximum load measured at the midshaft region of a femur based on a 3-point bending test in postpartum female mice injected with ALN or PBS during lactation (n = 5, each). N, newton.
(E) Analysis of body length (left graphs) and weight (right graphs) in male or female offspring (males: PBS n = 13, ALN n = 19; females: PBS n = 16, ALN n = 13).
(F and G) Bone parameters of male (F) or female (G) offspring. Bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), tissue mineral density of trabecular (TMD.Tb, mg/cm^3) and tissue mineral density of cortical (TMD.Ct, mg/cm^3) of the metaphysis femur are shown. Cortical thickness (Ct. Th, μm), cortical bone area (Ct.Ar, mm^2) and tissue mineral density (TMD, mg/cm^3) of diaphyseal femur cortical bone are shown (males: PBS n = 25, ALN n = 38; females: PBS n = 31, ALN n = 26).
(H and I) Maximum load measured at a midshaft region of the femur based on a 3-point bending test in male (H) or female (I) offspring (n = 5, each). N, newton.
(C-I) Data represents means of indicated parameters ± S.D. (t-test; *$p < 0.05$, **$p < 0.01$, NS: not significant).

discontinuation of denosumab treatment can promote abnormal acceleration of bone turnover, in which both bone-resorbing and bone-forming activities become elevated over levels seen before the start of treatment [28,29]. This "overshoot" beyond basal activity following denosumab discontinuation in turn decreases bone mass overall and may actually promote vertebral fractures [30]. Thus, either transient administration of denosumab or its discontinuation is generally not recommended even for osteoporotic patients [31].

Use of most drugs is prohibited or limited during pregnancy or lactation due to potential teratogenic effects, and several drugs reportedly promote adverse effects in infants [9,32]. This makes it difficult to prevent bone loss in mothers during pregnancy or lactation; moreover, among drugs used to treat osteoporosis, several reportedly promote adverse effects in rodent embryos [33]. Animal studies also show that alendronate passes through the placenta to an embryo or is transferred via milk if mothers are administered high levels of the drug [8,34]. In this study, we did not assess bone-forming activity, such as mineral apposition rate or bone formation rate, to avoid possible adverse effects to either embryos or newborn mice following injection of reagents required for those analyses and also to focus on effects of alendronate use on pregnant and lactating mothers.

Currently, we do not know why body weight and length of five-day-old offspring were greater following alendronate treatment of mothers before pregnancy. We also do not know why body length of two-week-old offspring was greater following alendronate injection of lactating mothers. Inhibition of bone remodeling in mothers by alendronate may positively support growth of embryonic or newborn mice. However, body weight and length depend greatly on both the number of embryos carried by a mother and/or the number of mouse pups nurtured by those mothers. Thus, care is needed when discussing alendronate effects on body size of offspring, and further studies are needed to address these issues.

In summary, here we show that transient administration of low levels of alendronate is sufficient to increase bone mass in mothers during pregnancy or lactation without promoting birth abnormalities or growth retardation in mouse pups. If applicable to humans, our strategy suggests a means to protect pregnant mothers from bone loss or fragility fractures after partum.

CRediT authorship contribution statement

Investigation: EI, YS and TK; conceptualization: TM (Miyamoto); data curation: TS, TM (Matsumoto), AK, and KM; funding acquisition: YS, KM, and TM; supervision; HM, MM, MN, KS, and TM; writing: TM.

Declaration of competing interest

The authors declare that they have no conflicts of interest with the contents of this article.

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Appendix A. Supplementary data

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