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Effects of Lemon Beverages on Bone Metabolism and Bone Mineral Density in Postmenopausal Women: A Double-Blind, Controlled Intervention Study with Ca-Supplemented and Unsupplemented Lemon Beverages

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Abstract

The aim of the present study was to elucidate how bone metabolism and bone mineral density are affected by the consumption of a lemon juice-containing calcium (Ca)-enriched beverage. The efficacy of this investigational product was evaluated in postmenopausal women during five months of continuous intake (intervention). This was a randomized, controlled trial. Eighty-three subjects were assigned to three groups. Using a double-blind format, the first two groups received a Ca-supplemented lemon-juice (lemon) beverage (LECA) or a Ca-unsupplemented lemon-juice (lemon) beverage (LE). The third group (control) received no intervention. Each subject in the LECA and LE groups consumed one bottle (290 mL) of their assigned investigational product every day for five consecutive months. After five months of intervention, the gain in bone mineral density at the lumbar spine was significantly larger in the LECA and LE groups than in the control group. In the femur, subjects in the LECA group gained significantly more bone mineral density than the control subjects. The largest gain in bone mineral density at the lumbar spine was observed in the LECA group. As for the concentrations of the bone resorption marker tartrate-resistant acid phosphatase 5b (TRACP-5b), subjects in the LECA group had significantly lower values than those in the control group.

Similarly, when compared with the LE and control groups, a significant decrease was detected in the LECA group in the concentrations of the bone formation markers, bone alkaline phosphatase (BAP) and osteocalcin (OC). In postmenopausal women, continuous consumption of Ca-supplemented lemon beverages improved the absorption of Ca and inhibited bone resorption. This likely blocked the function of osteoblasts and led to the suppression of bone formation, resulting in the attenuation of high-turnover bone metabolism.

Keywords

Ca-Supplemented Lemon Beverages, Bone Metabolism, Bone Mineral Density, Postmenopausal Women

1. Introduction

Osteoporosis and the subsequent risk of bone fracture are characterized by enhanced bone fragility, resulting in an increased risk of fracture, and it is usually defined as a reduction in bone mineral density (BMD) [1]. These disorders account for a significant burden of morbidity and mortality worldwide and have become a major public health problem [2]. In women with postmenopausal osteoporosis, estrogen deficiency enhances the sensitivity to parathyroid hormone (PTH), leading to an increase in bone resorption [3] [4]. This results in elevated concentrations of calcium (Ca) in the blood. High blood Ca levels then prevent the release of PTH, and, this, in turn, suppresses the production of 1,25-(OH)2 D3, which can promote Ca absorption in the intestine. Ultimately, the reduced absorption of Ca causes a decrease in BMD. Separately, estrogen deficiency leads to low levels of calcitonin. This hormone binds to its receptors on osteoclasts and inhibits the function of the cells. Thus, estrogen deficiency promotes bone resorption by regulating the direct interaction between calcitonin and its receptors. In summary, estrogen affects bone metabolism and bone mass indirectly through the activities of hormones (such as PTH and calcitonin), vitamin D, and other bone-related factors. In contrast, senile osteoporosis is caused by one or more of the following conditions [5]: reduction in dietary intake of Ca owing to aging; reduction in Ca absorption in the intestine; vitamin D deficiency (caused, for example, by a reduction in dietary intake or a decrease in biosynthesis due to a lack of ultraviolet exposure); and a reduction in vitamin D absorption caused by an impairment of its activation in the kidney. In other words, low levels of blood Ca induce mild hyperparathyroidism and increase PTH concentrations. PTH, in turn, activates osteoclast function and maintains blood Ca levels by releasing Ca from bone [6]. Thus, it is critical to maintain blood Ca levels in the normal range. A deficit in Ca homeostasis promotes bone resorption, leading to low BMD.

Citric acid is mainly responsible for the acidic flavor of lemons. Recent studies have demonstrated that this organic acid acts as a chelating agent and promotes the absorption of minerals such as Ca and iron [7] [8] [9]. Nii *et al.* [10] have also suggested that, *in vitro*, citric acid in citrus juices (including lemon juice) can solubilize Ca existing in shirasuboshi (boiled and semi-dried whitebait). We hypothesize that the chelating effect of citric acid can play an important role in preventing osteoporosis. In collaboration with Hiroshima Prefectural Akitsu Hospital and Pokka Sapporo Food & Beverage Ltd., in 2015 we investigated whether lemon drinks supplemented with Ca had any beneficial effects on bone metabolism in postmenopausal women when the drinks were continuously consumed for six months [11]. The results indicated that the concentration of the bone resorption marker tartrate-resistant acid phosphatase 5b (TRACP-5b) decreased significantly one month after study initiation. The concentration remained unchanged thereafter. Furthermore, no decrease in BMD was detected. These findings suggest that the chelating activity of citric acid facilitated Ca absorption and maintained blood Ca levels in the normal range. This likely prevented bone resorption and kept BMD unchanged. Thus, Ca-supplemented lemon beverages can potentially help inhibit osteoclast activity and maintain BMD in middle-aged and elderly women. However, many questions remained unanswered. For example, were the effects of these beverages caused by a component(s) of lemons, or were they attributable to an increased Ca absorption effect achieved by the chelating function of citric acid contained in this fruit?

In this study, the aim was to elucidate how bone metabolism and BMD are affected by the consumption of a lemon juice-containing Ca-enriched beverage. Using a double-blind experimental design, the efficacy of the beverage was examined in postmenopausal women during five-month continuous consumption (intervention).

2. Materials and Methods

2.1. Subjects

Volunteer subjects were recruited by M city public advertising. A participation condition is to be being normal and a postmenopausal woman, and the person that the ovary and the uterus which have an influence on the osteoporosis do not have a history of treatment. Exclusion criteria are the person with a history of the serious disease and the person whom it was judged to be inappropriate by the investigator. They were given detailed information about the study (such as aims and methods) at an orientation session offered prior to enrollment in the study. Written, informed consent was obtained from 97 volunteers. Of these, 83 were selected for statistical analysis. They had undergone all scheduled examinations conducted at the beginning of the study (prior to intervention with investigational products), as well as two and five months after study initiation.

This was a randomized, controlled trial. An identification number was assigned to each subject for anonymization. Subjects were divided into three groups matched for age and body mass index (BMI). Each group underwent a different intervention. In the LECA group, subjects received a Ca-supplemented lemon-juice (lemon) beverage. In the LE group, subjects received a Ca-unsupplemented lemon-juice (lemon) beverage. The third group (control) received no intervention. The placebo was not used in this study. The control group lived a usual life. The investigational products were distributed by Pokka Sapporo Food & Beverage Ltd. using a double-blind protocol. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Prefectural University of Hiroshima (Approval Number 15MH025-01).

2.2. Experimental Design and Methods

The study was conducted between December 2015 and June 2016. Each subject in the LECA and LE groups consumed one serving (290 mL) of an investigational product every day for five consecutive months. Assessments of physical measurements, BMD, and bone metabolism markers were performed at study initiation (pre-intervention) and after two and five months of intervention.

2.2.1. Physical Measurements

Age and the number of years since menopause were obtained by interview. Physical measurements consisted of determining height, weight, and BMI.

2.2.2. BMD Measurements

BMD (g/cm^2) values of the lumbar spine (L2-L4) and left femur were determined using the X-ray bone densitometer Discovery (Toyo Medic Co., Ltd., Tokyo, Japan). During the course of this study, the densitometer was calibrated 99 times with the following results: mean = 1.038 g/cm^2, SD = 0.003 g/cm^2, and CV = 0.37%. These values were within the normal ranges established by the manufacturer, Hologic, Inc. (Marlborough, MA, USA).

2.2.3. Bone Metabolism Markers

Bone Resorption Markers: The concentrations of serum TRACP-5b and urinary type I collagen cross-linked N-telopeptide (u-NTx) were measured.

Bone Formation Markers: The concentrations of bone alkaline phosphatase (BAP) and osteocalcin (OC) were measured. Blood samples collected from study subjects were centrifuged at 1500× g for 10 min to obtain serum for analyzing TRACP-5b, BAP, and OC concentrations. The analysis was carried out using commercially available kits. Similarly, u-NTx was detected in urine from subjects using a commercially available kit, without sample pretreatment. All serum and urine assays were performed by Fukuyama Medical Laboratory Inc. (Hiroshima, Japan). The kits used for these assays were: Osteolinks "TRAP-5b" (Nittobo Medical Co., Ltd., Tokyo, Japan) for serum TRACP-5b; Access Ostase (Beckman Coulter Co., Ltd., Brea, CA, USA) for BAP; BGP IRMA "LSI M" (LSI Medience Co., Ltd., Tokyo, Japan) for OC; and Osteomark (Alere Medical Co., Ltd., Tokyo, Japan) for u-NTx.

2.2.4. Investigational Products

Table 1 shows the amounts of principal ingredients in the products tested in this study. The recommended dietary reference intake for Ca for adult women is set at approximately 700 mg per day [12]. In the present study, one-half of this value (*i.e.*, 350 mg) was used as the intake amount. The amount of lemon juice in the investigational products was determined by referring to the health survey conducted by Domoto *et al.* [13]. They found that, in a lemon-growing region of Hiroshima Prefecture, individuals with high daily lemon consumption (approximately 30 mL, on average) had significantly lower blood pressure than those with low consumption. Based on this observation (the daily consumption amount was approximately 30 mL on average in the high daily lemon consumption group), the lemon juice amount was set to 30 mL. The amounts of Ca and lemon juice in the investigational products were within the ranges of values found in normally consumed foods. There have been no specific reports indicating that they can potentially pose health hazards. Thus, they were deemed safe.

2.2.5. Data Analysis

Data obtained prior to intervention are presented as means ± standard deviation. After adjusting for age and BMI, the correlations between investigational products and intervention periods were analyzed by two-way analysis of variance. The two independent variables were intervention groups (LECA, LE) or no-intervention group (control), and intervention duration [intra-individual levels at study initiation (pre-intervention), at two months of intervention, or at five months of intervention]. The dependent variable was BMD (at the lumbar spine or femur). The correlations of intervention periods with investigational products and the levels of bone metabolism markers were also analyzed using the same statistical technique and the identical independent variables. The dependent variables were the concentrations of bone metabolism markers. The normality of the distributions of BMD values and bone metabolism marker levels was assessed by histograms and the Kolmogorov-Smirnov test ($p = 0.200$). For each subject group, the net gain in BMD was determined by subtracting the pre-intervention BMD from the BMD detected after five months of intervention.

Table 1. Composition of investigational products and their dosage and administration.

	LECA Group	LE Group
Name	Ca-supplemented lemon-juice (lemon) beverage	Ca-unsupplemented lemon-juice (lemon) beverage
Intake Amount	290 mL/bottle, 1 bottle/day	
Composition	Per bottle (290 mL) Calcium 350 mg Lemon juice 30 mL Calorie 49 kcal	Per bottle (290 mL) Calcium 0 mg Lemon juice 30 mL Calorie 20 kcal
Dosage and Administration	1 bottle/day. Any time of the day	

The obtained values were then analyzed by one-way analysis of variance, followed by the Dunnett multiple comparison test. The level of significance for all tests was set at $p < 0.05$.

3. Results

3.1. Comparison of Baseline Physical Characteristics

Table 2 shows the baseline physical characteristics of the study subjects. There were no significant differences in any of these variables among the three subject groups.

3.2. Changes in BMD

There were no significant differences in the BMD of the lumbar spine among the means of the three subject groups ($p = 0.541$) or between different time points ($p = 0.902$). However, there was a significant interaction effect ($p < 0.001$) (Figure 1(a)). When the pre-intervention BMD of the lumbar spine was subtracted from the BMD after five months of intervention, positive values were obtained for the LECA and LE groups, while a negative value was obtained for the control group. The differences in these values between the LECA and control groups and between the LE and control groups were both significant (both $p < 0.001$) (Figure 1(b)).

Similarly, there were no significant differences in the BMD of the femur among the means of the three subject groups ($p = 0.709$) or between different time points ($p = 0.807$). Again, the interaction effect was significant ($p < 0.001$) (Figure 1(c)). The value obtained by subtracting the pre-intervention BMD from the BMD after five months of intervention was positive for the LECA group. For the LE and control groups, the values obtained by the same method were negative. The difference in these values between the LECA and control groups was significant ($p = 0.036$). The difference between the LE and control groups was borderline significant ($p = 0.064$) (Figure 1(d)).

Table 2. Physical characteristics of study subjects.

Clinical Variable	Intervention Group 1 (n = 28) Mean (SD) Min - Max	Intervention Group 2 (n = 28) Mean (SD) Min - Max	Control Group (n = 27) Mean (SD) Min - Max	Total (n = 83) Mean (SD) Min - Max
Age (years)	65.2 (5.2) 57.0 - 82.0	65.1 (5.7) 56.0 - 81.0	64.1 (6.7) 48.0 - 80.0	64.8 (5.8) 48.0 - 82.0
Age at Menopause (years)	48.7 (5.8) 35.0 - 60.0	49.5 (5.6) 30.0 - 55.0	51.4 (2.4) 46.0 - 55.0	49.6(4.8) 30.0 - 60.0
Height (cm)	152.8 (5.6) 143.2 - 164.1	154.4 (4.7) 147.5 - 162.5	154.0 (4.7) 141.1 - 162.8	153.7†(5.1) 141.1 - 164.1
Baseline Weight (kg)	52.3 (7.2) 40.3 - 65.7	52.9 (8.5) 41.9 - 72.9	52.4 (11.0) 30.3 - 79.5	52.5‡ (8.9) 30.3 - 79.5
Baseline BMI (kg/m^2)	22.4 (2.8) 17.8 - 29.0	22.4 (2.8) 17.8 - 29.0	22.1 (4.5) 13.3 - 34.5	22.2 (3.6) 13.3 - 34.5

†: vs 153.4 (4.8), n = 2444, by Health and Welfare Statistics Association, p = 0.192271; ‡: vs 53.9 (7.8), n = 2443 by Health and Welfare Statistics Association, p < 0.001.

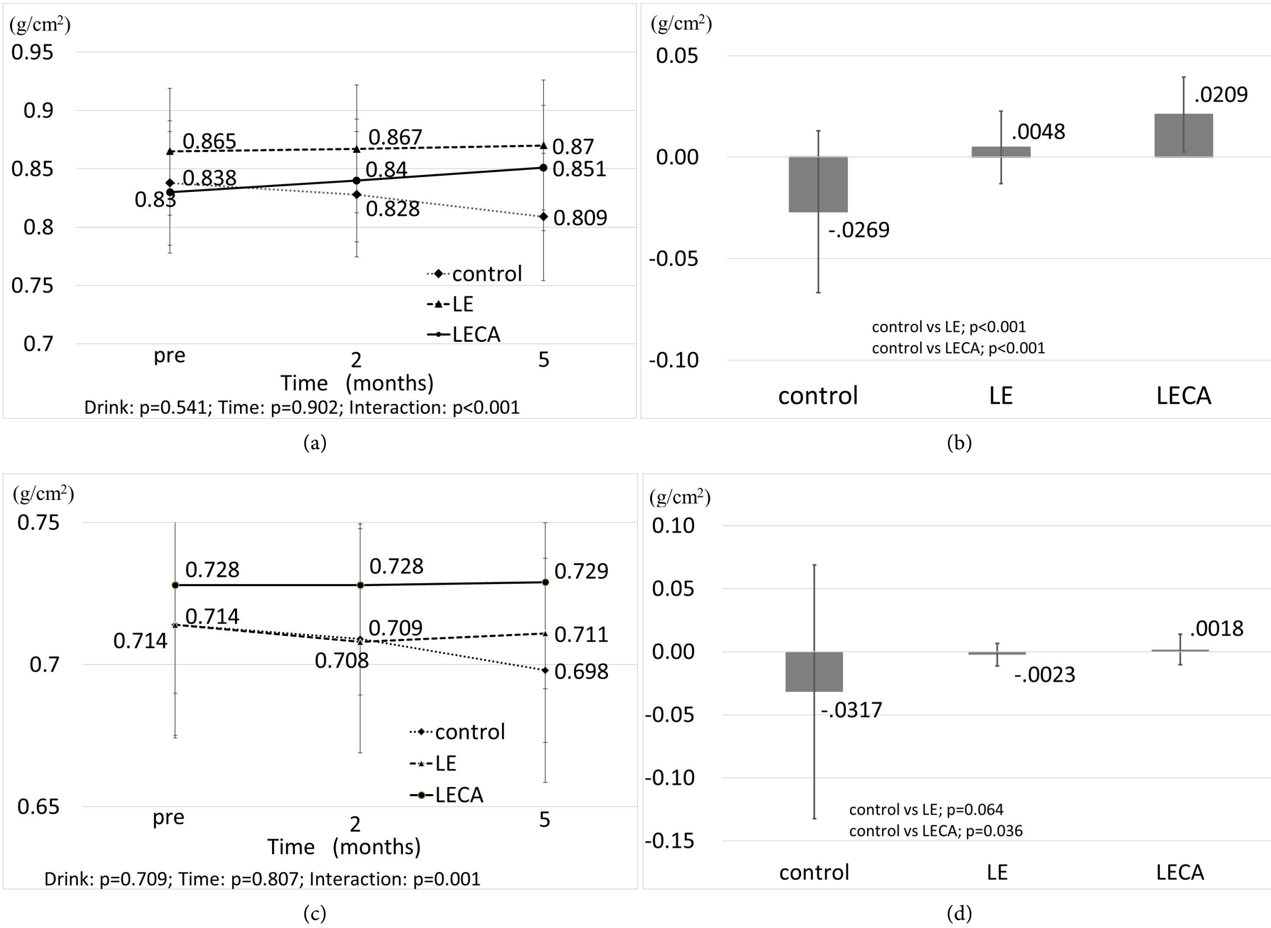

Figure 1. Relationship between investigational products and BMD. (a) BMD of the lumbar spine; (b) Changes in BMD of the lumbar spine; (c) BMD of the femur; (D) Changes in BMD of the femur. BMD: Bone Mineral Density. (a, c) Figures showed mean and SD error bar, and dotted line is control group, dashed line is LE group, solid line is LECA group. Two-way factorial analysis of variance with the kind of investigational products and intervention period as factors. In this result, they showed each of p value, investigational products are "Drink", intervention period is "Time", and interaction is "Interaction". (b, d) Figures showed the results obtained by the Dunnett multiple comparison test after analyzed by one-way analysis of variance of each subject group the margin between five months of intervention and the pre-intervention.

3.3. Changes in the Concentrations of Bone Metabolism Markers

TRACP-5b concentrations did not show any significant differences among the means of the three subject groups ($p = 0.624$) or between different time points ($p = 0.331$). However, the interaction effect was significant ($p < 0.001$) (Figure 2(a)). When the pre-intervention TRACP-5b level was subtracted from the TRACP-5b level after five months of intervention, a negative value was obtained for the LECA group, whereas positive values were obtained for the LE and control groups. The differences in these values between the LECA and LE groups and between the LECA and control groups were both significant (both $p < 0.001$) (Figure 2(b)). In the case of u-NTx, its concentrations did not differ significantly among the means of three subject groups or between different time points. There was also no significant interaction effect (Figure 2(c)). Furthermore, there were no significant differences in u-NTx levels before and after the five-month intervention (Figure 2(d)).

As for the concentrations of OC, there were no significant differences among the means of the three subject groups (p = 0.835) or between different time points (p = 0.328). The interaction effect was significant (p = 0.012) (Figure 3(a)). When the pre-intervention OC level was subtracted from the OC level after five months of intervention, a negative value was obtained for all subject groups. However, the value obtained for the LECA group was significantly smaller than that obtained for the LE group (p = 0.008) or control (p = 0.033) group (Figure 3(b)).

Finally, BAP concentrations did not show any significant differences among the means of the three subject groups (p = 0.234) or between different time points (p = 0.138), although the interaction effect was significant (p < 0.001) (Figure 3(c)). The value obtained by subtracting the pre-intervention BAP level from the BAP level after five months of intervention was negative for the LECA group. Conversely, the same calculation yielded positive values for the LE and control groups. The differences in these values between the LECA and LE groups

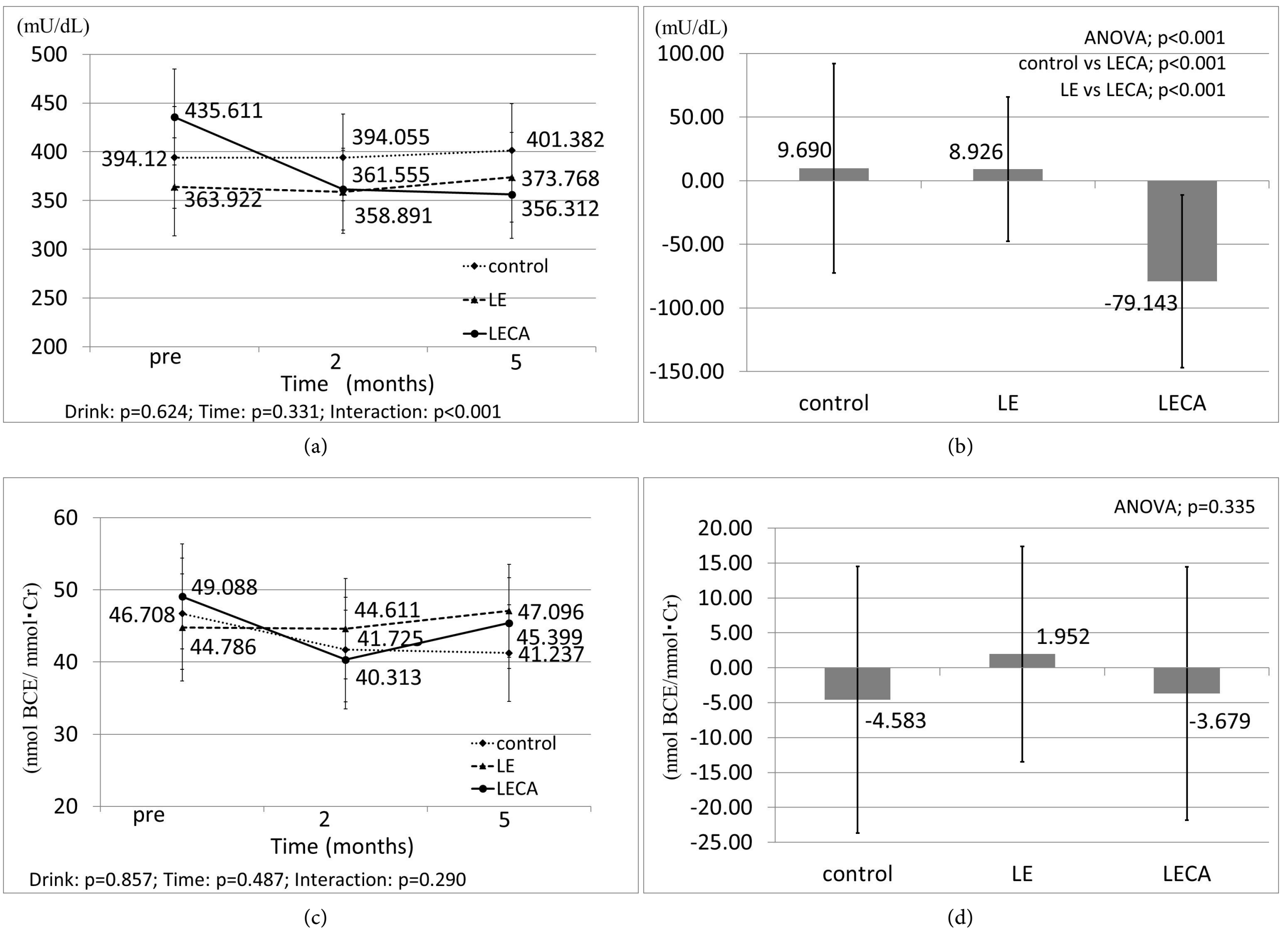

Figure 2. Relationship between investigational products and bone resorption markers. (a) Concentrations of TRACP-5b; (b) Changes in TRACP-5b concentrations; (c) Concentrations of u-NTx; (d) Changes in u-NTx concentrations. TRACP-5b: tartrate-resistant acid phosphatase type 5b. u-NTx: urinary type I collagen cross-linked N-telopeptide. The explanations in the figures of (a, c) and (b, d) are similar to figure legends of Figure 1.

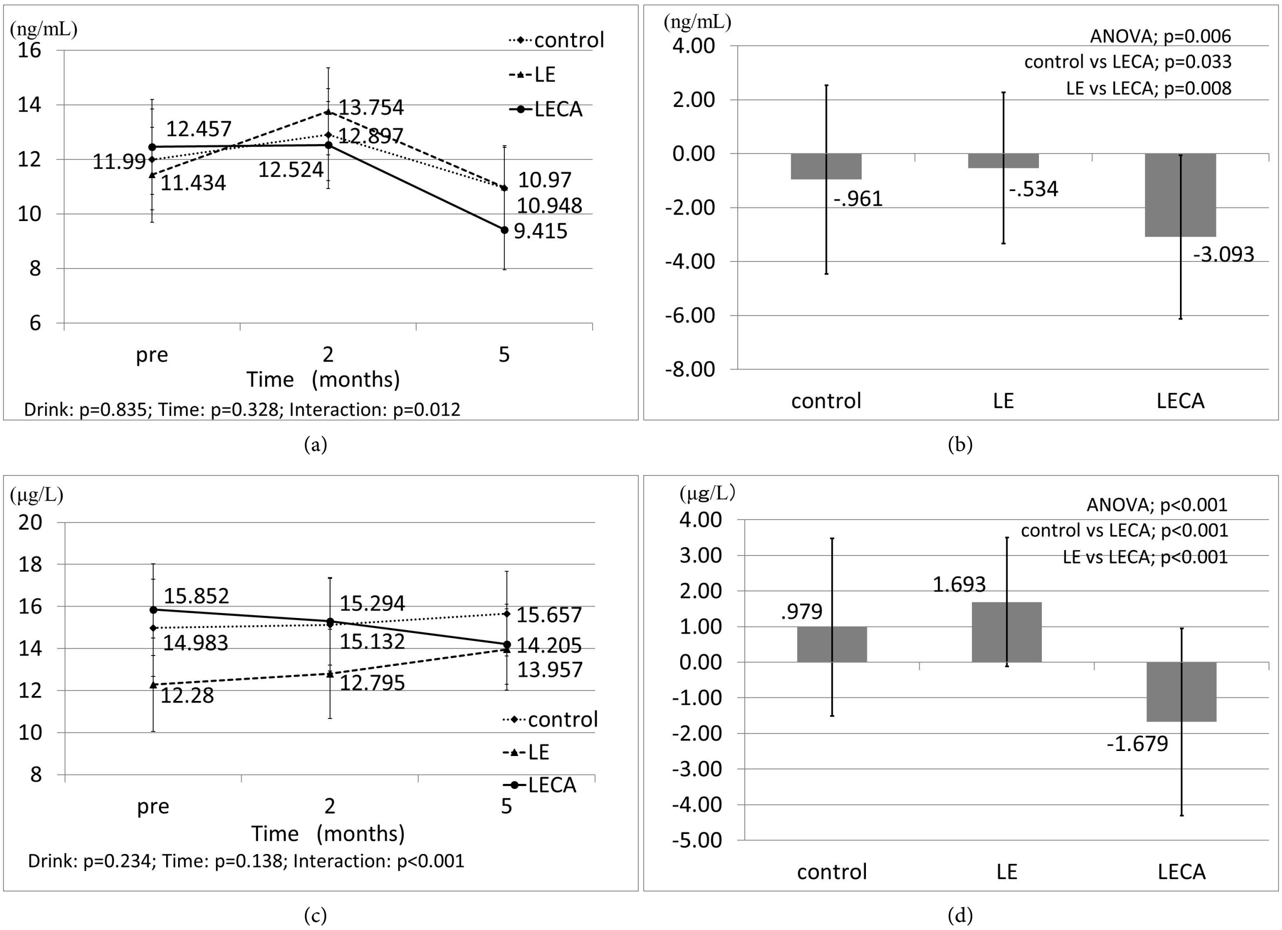

Figure 3. Relationship between investigational products and bone formation markers. (a) Concentrations of OC; (b) Changes in OC concentrations; (c) Concentrations of BAP; (d) Changes in BAP concentrations. OC: osteocalcin. BAP: bone alkaline phosphatase. The explanations in the figures of (a, c) and (b, d) are similar to figure legends of **Figure 1**.

and between the LECA and control groups were both significant (both $p < 0.001$) (**Figure 3(d)**).

In this study involving postmenopausal women, there was a significant interaction effect for the concentration of the bone resorption marker TRACP-5b. When the pre-intervention TRACP-5b level was subtracted from the TRACP-5b level after five months of intervention, a negative value was obtained for the LECA group. This value differed significantly from the positive values obtained by the same formula for the LE and control groups. Significant interaction effects were also observed for the concentrations of the bone formation markers OC and BAP. The value obtained by subtracting the pre-intervention OC level from the OC level after five months of intervention was negative for all three subject groups. However, the differences in these values between the LECA and LE groups and between the LECA and control groups were both significant. As for BAP, the value obtained by subtracting the pre-intervention level from the level after five months was negative for the LECA group and positive for the LE and control groups. The differences in these values between the LECA and LE groups and between the LECA and control groups were both significant. The results of previous studies that evaluated the effects of dietary Ca intervention are controversial. Matsumoto *et al.* [14] found significant decreases in bone resorption markers, whereas Kuwabara *et al.* [15] identified no significant changes. These discrepancies are partly due to differences in age between study participants. In the report by Kuwabara *et al.* [15], 68 institutionalized elderly subjects consumed a jelly containing Ca (200 mg) and vitamin D3 (800 IU) every day for one month. After study completion, the authors found no significant changes in the concentrations of TRACP-5b and BAP, although serum 25(OH)D levels increased significantly. Separately, Toba *et al.* [16] examined the effect of consumption of the milk basic protein (MBP), a protein assembly with basic isoelectric points, on bone metabolism markers in adult men (mean age 36.2 years). They reported that, after a 16-day intervention, the concentrations of OC and u-NTx showed a significant increase and decrease, respectively. These were both short-term studies, with intervention periods of one month or less. In the present study, each subject regularly received an investigational product for five months, and, upon study completion, subjects in the LECA group had significantly lower levels of both bone resorption and bone formation markers. We hypothesize that when Ca-supplemented lemon beverages are regularly consumed, citric acid promotes the absorption of this mineral through its chelating activity. This would help maintain blood Ca concentrations within the normal range, resulting in lower levels of bone resorption and bone formation markers. In the LECA group, normal levels of blood Ca presumably eliminated the need for supplying this mineral from bone and suppressed osteoclast-mediated bone resorption. This likely inhibited the function of osteoblasts and led to the attenuation of bone formation [11].

The increase in the BMD of the lumbar spine was calculated for each subject group by subtracting the pre-intervention value from the value obtained after five months of intervention. The results indicated that there was a significant difference in the increase between the LECA and control groups. A similar result was obtained for the BMD of the femur. Bone is constantly renewed through a process called remodeling [17]. This process elegantly regulates the balance between osteoclastic and osteoblastic activity, thereby preserving dynamic bone homeostasis. In other words, normal bone tissue is maintained through the resorption of an appropriate amount of old bone and the subsequent formation of new bone [18]. Changes in bone metabolism are induced during menopause, resulting in high bone turnover in which both bone resorption and bone formation are enhanced. However, bone resorption rates are much higher than bone formation rates, thus causing a reduction in bone mass [4] [19]. In the present study, the maintenance of blood Ca levels likely inhibited bone resorption. This must have suppressed the proliferation and differentiation of osteoblasts and caused the attenuation of high-turnover bone metabolism.

Moschonis *et al.* [20] observed a significant increase in the BMD of the lumbar spine in postmenopausal women who had received a 12-month intervention consisting of lifestyle counseling and the consumption of dairy products. Aoe *et* *al.* [21] also reported a similar observation in healthy postmenopausal women who had consumed MBP for six months. In the present study, the novel finding was that the changes in bone metabolism markers caused by the regular consumption of Ca-supplemented lemon beverages were reflected in BMD levels.

Since estrogen deficiency causes postmenopausal osteoporosis, inhibitors of bone resorption (such as estrogen and bisphosphonate preparations) have been used for the prevention and treatment of osteoporosis. In fact, Wallach *et al.* [22] found that risedronate treatment significantly increased or maintained BMD of the lumbar spine and femur in patients receiving corticosteroid therapy. Furthermore, according to a report by Yoshida *et al.* [23], serum NTx levels were greatly reduced in early postmenopausal women within one month following six-month raloxifene treatment. The authors also mentioned the inhibitory effect on bone resorption. However, while estrogen-based hormone replacement therapy can reduce the risk of fracture and colon cancer, it reportedly increases the risk of breast cancer, thrombosis, coronary artery disease, and stroke [24]. In addition, although bisphosphonate preparations can increase bone mass and prevent bone resorption and fractures, their dosing instructions are not easy to follow. An example is that, upon arising, patients need to swallow bisphosphonate tablets whole with a full glass (approximately 180 mL) of water on an empty stomach, without lying down and consuming anything other than water for at least 30 min afterwards. Thus, one of the disadvantages of bisphosphonate therapy is poor patient compliance. Indeed, several groups have previously shown suboptimal adherence to daily oral bisphosphonate therapy [25] [26] [27]. Another problem associated with the pharmacological intervention of osteoporosis is that an individual must be diagnosed with this disease (*i.e.*, BMD < 70% of the young adult mean) to receive a prescription for bisphosphonate. Considering the importance of preventive medicine in the management of osteoporosis, it would be essential to develop an alternative to conventional osteoporosis drugs that would be effective even for individuals whose BMD is 70% or higher. One can drink the Ca-supplemented lemon beverage investigated in this study regardless of the presence or absence of a disease or diagnosis. There are no instructions or restrictions as to when or how the beverage should be taken. Thus, it can be easily consumed regularly for an extended period of time.

In this study, a survey on individual diet remains to be done. Nevertheless, a new experimental result was achieved by ingesting the test beverage while having a normal life.

The physical measurements of each subject selected for the present analysis were similar to the mean values obtained from an age-matched Japanese population [28]. There were no significant difference between the average height of the subjects and Japanese women in their age group, but the subjects were below average in weight. This difference, however, is so tiny that we can generalize it. However, muscle strength varies among individuals based on a variety of elements, including their physical conditions, motivation, physical ability, nutritional status, and genetic factors [29]. Thus, it is difficult to measure strength without taking these factors into account. This illustrates a limitation of human research. Another limitation of the present study was that the participants were not hospitalized patients or institutionalized individuals. They were volunteers interested in a health study related to BMD measurement. Thus, the present subjects could have been biased towards healthy people or health-conscious individuals. Nevertheless, a novel finding suggesting that the continuous consumption of Ca-supplemented lemon beverages is effective in preventing the loss of BMD after menopause was obtained. This conclusion was based on the changes that occurred after five months of intervention in both bone metabolism markers and the BMD of the lumbar spine and femur. We expect our new discovery to become instrumental in developing preventive strategies for osteoporosis in the future. Research conducted by Nii *et al.* [30] has previously demonstrated that, in rats, the consumption of shirasuboshi together with sudachi (*Citrus sudachi*) juice leads to significant decreases in the concentrations of urinary pyridinoline and deoxypyridinoline and inhibits bone resorption. They have also reported that, in healthy male students, a diet containing shirasuboshi and sudachi juice promotes the absorption of minerals such as Ca and magnesium [31]. These observations suggest that citric acid in lemons also increased the absorption of dietary Ca in the present study. Further dietary surveys are necessary to substantiate this hypothesis.

4. Conclusion

In postmenopausal women, continuous consumption of Ca-supplemented lemon beverages improved Ca absorption and inhibited bone resorption. The suppression of bone resorption likely blocked bone formation mediated by the proliferation and differentiation of osteoblasts, resulting in the attenuation of high-turnover bone metabolism. The novel finding of the present study was that this attenuation was reflected in BMD. The present results also suggest that citric acid in lemons enhances the absorption of dietary Ca. We expect that such beverages will have an efficacy in preventing osteoporosis in the future.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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