<Research Article>

Effects of milk on the bone of a rat model of the female athlete triad

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Summary This study examined the effects of milk consumption on bone health in a rat model of the female athlete triad (FAT), a condition characterized by low energy availability, menstrual dysfunction, and osteoporosis. 6-week-old female Sprague Dawley rats were divided into four groups: control, food restricted (40% restricted), FAT (food restricted + exercise), and FAT-milk (FAT + 600 μL milk). After two weeks of high-intensity treadmill running (40 m/min, 15 min/day, 5 days/week), body weight, bone mineral density (BMD), blood biochemical markers, and calcium absorption mechanisms were analyzed. The FAT group showed significant reductions in body weight, BMD, and uterine mass, confirming the model's validity. However, milk supplementation prevented bone and uterine mass loss despite continued energy restriction. Serum osteocalcin was elevated in the FAT group, indicating high bone turnover, while *Trpv6* expression in the FAT-milk group was reduced, suggesting efficient calcium absorption due to milk intake. These findings indicate that milk consumption helps maintain bone health in energy-deficient conditions, making it a potential nutritional intervention for young female athletes at risk of osteoporosis.

Key words: Female athlete triad, Milk, Bone

1. Introduction

In recent years, there have been numerous reports of health problems in athletes because of low energy availability, and the International Olympic Committee issued a consensus statement on this subject in 2023[1]. Energy availability is calculated as energy intake minus energy consumed during exercise[2], and this is the energy used to maintain bodily function. Athletes train intensively from childhood, and low energy availability during growth retards their development[3] and can cause symptoms such as hypothalamic amenorrhea and osteoporosis in women[2]. Low energy availability, hypothalamic amenorrhea, and osteoporosis have been defined to comprise the female athlete triad (FAT), and when this is present over a long period, it causes disturbances in various physiologic functions, including the

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immune system and metabolism[1].

The most important aspect of the effective treatment of FAT is to improve energy delivery and increase energy availability. To this end, nutritional supplementation, such as through supplementary feeding, is important. An increase in energy intake or a reduction in activity can be expected to restore menstrual function and improve bone density in affected individuals[4,5]. In addition, carbohydrate supplementation of the diet can prevent reproductive dysfunction and bone loss[6]. However, greater energy intake or a reduction in activity is very difficult to achieve in athletes[7]. It has also been reported that calcium supplementation does not restore bone strength in intensively exercising individuals with low energy availability, making it difficult to prevent bone mineral loss through dietary supplementation under conditions of energy deficiency[7].

Milk has a high nutrient density and is absorbed with high efficiency. In addition, the casein phosphopeptides in milk are thought to help retain calcium and promote calcium absorption from the intestinal tract[8]. Furthermore, other factors, such as the effects of lactose and the absence of inhibitors of absorption, make milk consumption an optimal means of ensuring calcium intake and maintaining bone mass. In general, bone mineral density reaches a maximum during puberty and then declines with age. According to the published osteoporosis guidelines in Japan, insufficient bone mass when young is not only associated with a risk of fracture, but also of future osteoporosis[9]. U.S. study finds milk consumption in childhood and adolescence increases bone mass and bone density in adulthood efforts to promote a diet that includes at least one serving of milk per day for childhood and adolescent girls to reduce the risk of osteoporotic fractures are supported[10]. Thus, milk may be a beneficial nutritional supplement for women with FAT.

In this study, we supplemented the diet of a rat model of FAT with milk to determine whether this could prevent a reduction in bone mineral density.

2. Materials and methods

Animal treatments

The study was conducted with the approval of the Animal Experiment Committee of the University of Tsukuba School of Medicine (approval number 22-503), using 6-week-old female Sprague Dawley rats (SLC Japan, Tokyo, Japan). After 5 days of habituation, the rats were allocated to four groups: a control group (n=3), a food restricted group (n=3), an exercise + food restricted group (FAT, n=3), and an exercise + food restricted + milk group (FAT-milk, n=3). The experiment lasted 19 days. The following dietary restriction fees were determined from previous studies[11]. The food restricted and FAT groups were 40% food restricted of the food intake of the control group, and the FAT-milk group was designed to achieve a 40% food restricted compared to the control group by adding 600 μL of milk energy. General cow's milk was used, and the amount of milk fed was

Table1 Composition of daily diet.

Materials	control (g)	food restricted (g)	food restricted + milk(600 μL) (g)
Glucose monohydrate	62.2	37.3	24.8
Lactose	-	-	4.8
Casein	18	10.8	25.2
Cystine	0.2	0.12	0.28
Whey protein	-	-	0.7
Cottonseed oil	10	6	14
Milk fat	-	-	3.8
Ca	1.04	0.62	0.541
Energy (kcal/100 g)	412	247	247

determined by calculating the amount required per unit body mass of the rats with reference to the Japanese Food Guide Spinning Top (Ministry of Agriculture, Forestry and Fisheries of Japan, 2005), and it was allowed to soak into the food before feeding. The composition of the diet fed is shown in Table 1. Water was provided ad libitum. Exercise was performed on a treadmill (TMC-350, Melquest, Toyama, Japan) at 40 m/min for 15 min on 5 days per week. The animal housing was maintained at a temperature of 23.5 ± 2.5°C, at a humidity of 52.5% ± 12.5%, and under a 14 h/10 h light/dark cycle (lights on 5:00–19:00).

Daily data and sample collection

The body masses and food intakes of the rats were recorded daily. At the end of the experiment, isoflurane (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was administered after 3 h of fasting, and after confirmation of the disappearance of the ascending reflex, a whole-blood sample was quickly drawn from the abdominal aorta of each rat, which was then euthanized. Blood was collected into 1.5 mL tubes containing heparin and others without anticoagulant. Plasma was obtained by centrifugation of the former at 4°C and 2,500 rpm for 15 min, and serum was obtained by allowing the plain samples to stand on ice for at least 30 min, then centrifuging them at 4°C and 2,500 rpm for 10 min. After euthanasia, the femurs, uterus, jejunum, and kidneys of the rats were collected and weighed. The jejunum was collected as described previously[11]. A femur from each rat was stored at 4°C until it was used for biomechanical testing.

Measurements of bone mineral density

The bone mineral density (BMD) of each femur was measured using dual-energy X-ray absorptiometry (DXA; QDR-4500A, Hologic, MA, USA). The scans were performed in small animal mode and with high regional resolution.

Measurement of blood biochemical parameter

The plasma calcium and phosphorus concentrations were measured using a Fuji Drychem 7000V (Fujifilm, Tokyo, Japan) automatic veterinary biochemical analyzer. The corresponding slides (Fuji Drychem Slide Ca and Fuji Drychem Slide IP, Fujifilm, Tokyo, Japan) were used and measurements were performed according to the accompanying instructions. The serum osteocalcin concentration was measured using a Rat Gla-Osteocalcin High-Sensitivity EIA Kit (Takara Bio, Shiga, Japan), according to the manufacturer's instructions.

Histochemical staining and measurements

The jejunum is responsible for much of the absorption of calcium and other nutrients. Because villi greatly influence the digestion and absorption of nutrients, histochemical staining and measurement of jejunal villi were performed. The jejunum were cut open, immersed in 10% neutral buffered formalin, and fixed for 24 h. The fixative was then replaced with 70% ethanol, then portions of the tissue were paraffin-embedded and sectioned, and the sections were stained with hematoxylin and eosin (HE). Meyer's hematoxylin (1.5×method; Mutoh Chemical, Tokyo, Japan) and 1% eosin Y solution (Mutoh Chemical, Tokyo, Japan) were used for this purpose. The mucosal thickness, villus height, crypt depth, and villus width were measured for at least five villi per mouse as previously described[12]. Images were obtained using a BZ-X710 microscope (Keyence, Osaka, Japan).

cDNA synthesis and quantitative PCR

Calcium is absorbed by passive transport or by the transient receptor potential vanilloid 6 (*Trpv6*) present in small intestinal mucosal epithelial cells. Samples of rat jejunum were homogenized in RNAiso Plus (Takara Bio, Shiga, Japan) and RNA was extracted according to the instructions provided. The following procedures were performed as described previously[11]. The RNA samples were adjusted to 500 ng and reverse transcribed using PrimeScript RT Master Mix (Takara Bio, Shiga, Japan) and a thermal cycler (TP 350, Takara Bio). quantitative PCR (qPCR) was then performed using TB Green ® Premix Ex Taq ™ II (Takara Bio, Shiga, Japan). *Ywhaz* was used as the reference gene. The qPCR primer sequences used were as follows: *Ywhaz*, forward 5´-PCPGCAACGACGPACTGTCTC-3´ and reverse 5´-CCTCAGCCAAGTAGCGGTAG-3´; and the calcium absorption transporter *Trpv6*, forward 5´-ACTACCGTGATGCTAGAACGGA-3´ and reverse 5´-CTTGTCTATCTTCCACCCTCAAGAA-3´. The cycle threshold values for the target gene were normalized to those for the reference gene.

Statistical analysis

Data are presented as the mean ± SD. One-way analysis of variance and Tukey's *post-hoc* test were used to compare the groups. Statistical significance was accepted when $P < 0.05$. Statistical analyses were performed using Prism 8.4.3 software for Mac (GraphPad, CA, USA).

3. Results

Effects of milk on the body and uterine masses of the rats during exercise and dietary restriction

In previous studies, lower final body mass and weight gain have been reported in rats that were both diet-restricted and exercising[13] and we first checked that the same model of FAT could be established in the present study. The body mass, food intake, and relative uterine mass data are shown in Table 2. The food restricted, FAT, and FAT-milk groups had significantly lower final body mass than the control group, implying reproduction of the model. In addition, the relative uterine mass of the FAT group was significantly lower than those of the other three groups. However, the FAT-milk group prevented a significant decrease in relative uterine weight, suggesting rescue by milk.

Effects of milk intake during exercise and dietary restriction on bone parameters

FAT also involves abnormalities in bone. The bone mineral density of the food restricted group was significantly lower than that of the control group, and it was also significantly lower in the FAT group than in the food restricted group. However, the bone density of the FAT-milk group did not differ from that of the control group (Fig. 1A). There were no differences in the circulating calcium or phosphorus concentrations among the groups (Fig. 1B and C), but the serum osteocalcin concentrations of the FAT group were significantly higher than those of the other three groups (Fig. 1D).

Effects of the interventions on the jejunum villi

A previous study showed that FAT is associated with atrophy of the jejunum villi[13]. Therefore, we next determined whether a similar effect occurred in the present study, but found no differences in the crypt depth, mucosal thickness, or villus height in the jejunum of the various groups (Fig. 2A–C).

Effects of the interventions on calcium transporter expression in the jejunum

Because there were no differences in the circulating calcium concentration, but significant differences in the bone mineral density of the FAT and FAT-milk groups, the mRNA of the calcium absorption transporter *Trpv6* in the jejunum was next measured. This showed that *Trpv6* expression was significantly lower

Table2 Basic information in each group.

	control	food restricted	food restricted + exercise (FAT)	food restricted + exercise + milk (FAT-milk)
Initial body weight (g)	209.50 ± 5.80	197.33 ± 1.76	207.17 ± 8.93	211.17 ± 1.96
Final body weight (g)	240.33 ± 7.31**	182.50 ± 5.06	185.50 ± 9.99	181.67 ± 7.13
Food intake (g/day)	14.33 ± 0.19	8.50 ± 0.00	8.50 ± 0.00	8.00 ± 0.00
Uterus weight (g/100 g)	0.39 ± 0.04	0.27 ± 0.03	0.13 ± 0.02**	0.24 ± 0.03

All data are presented as mean ± SD (n=3).

Final body weight and Uterus weight were One-way ANOVA and Tukey's *post-hoc* test were used to compare the groups.

** Statistical difference was set at $p < 0.01$ vs other three groups.

in the FAT-milk group (0.2-fold) than in the control group. However, there were no significant differences between the other groups (Fig. 3).

4. Discussion

In the present study, we used an animal model of FAT to investigate the effects of this condition on bone and the effects of milk supplementation on these. We found that a lack of energy availability caused a decrease in uterine mass and osteoporosis, even over a short period of time, and that the addition of milk prevented this uterine weight loss and osteoporosis, in the presence of a shortage of energy availability.

Previous studies have shown low body mass and bone mineral density after 12–14 weeks[14–16] or 4 weeks[13] of spontaneous exercise and dietary restriction, respectively. In this study, we have demonstrated significantly lower body mass, relative uterine mass, and bone mineral density in rats with FAT than in normally fed rats (Table 2 and Fig. 1A). The change

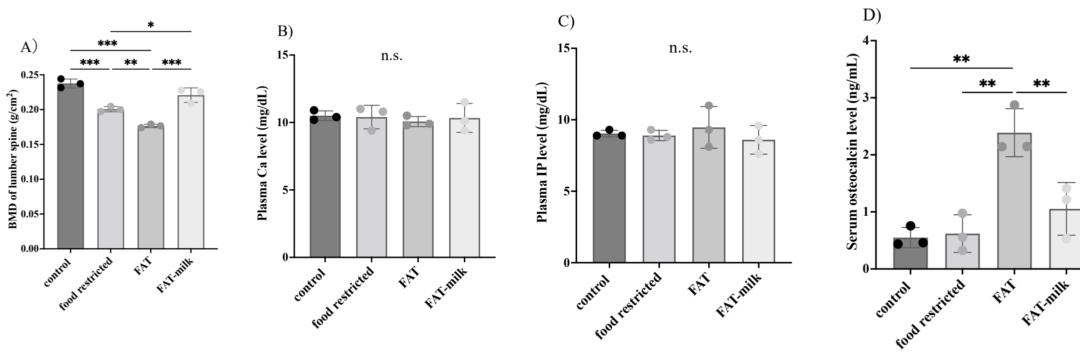

Fig. 1. Bone parameters. All data are presented as mean ± SD, and plots show values for each data (n=3). Data were One-way ANOVA and Tukey's post-hoc test were used to compare the groups. A) Bone mineral density (BMD) of lumber spine (g/cm^2). control vs FAT-milk was no significant difference. * Statistical difference was set at $p < 0.05$ food restricted vs FAT-milk. ** statistical difference was set at $p < 0.01$ food restricted vs FAT. *** Statistical difference was set at $p < 0.001$ control vs food restricted and FAT, FAT vs FAT-milk. B) Plasma calcium (Ca) concentration (mg/dL) and C) Plasma phosphorus (IP) concentration level (mg/dL) were no significant difference. D) Serum osteocalcin concentration level (ng/dL). ** Statistical difference was set at $p < 0.01$ vs other three groups.

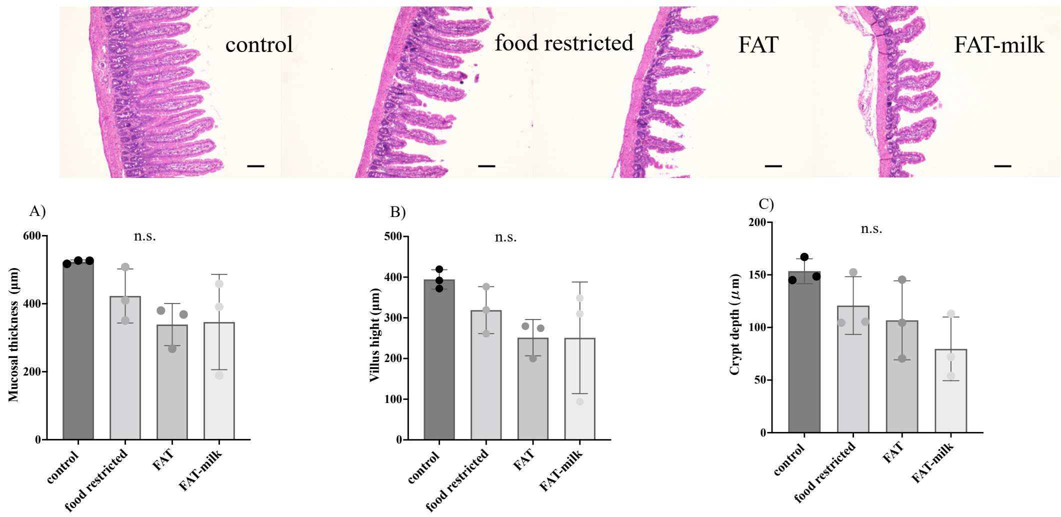

Fig. 2. Histology of the jejunum. Images of HE stained sections were obtained using an optical microscope (magnification ×100). At least 5 villi/rat were measured. Black bars indicate 100 μm. A) Mucosal thickness, B) Villus hight, C) Crypt depth. All data are presented as mean ± SD, and plots show values for each data (n=3). Data were One-way ANOVA and Tukey's post-hoc test were used to compare the groups. All data were n.s. (not significant).

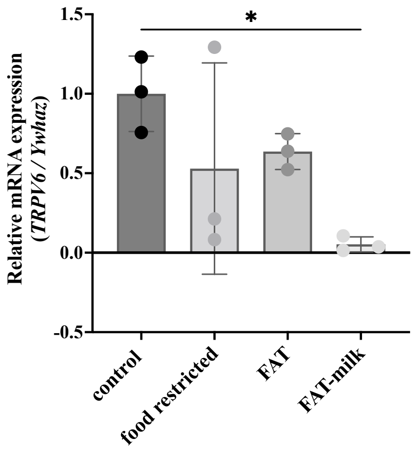

Fig. 3. Calcium transporter (*Trpv6*) gene expression in each group. The relative *Trpv6* mRNA expression data are presented as mean ± SD, and plots show values for each data (n=3). Data were One-way ANOVA and Tukey's *post-hoc* test were used to compare the groups. * Statistical difference was set at $p < 0.05$ control vs FAT - milk.

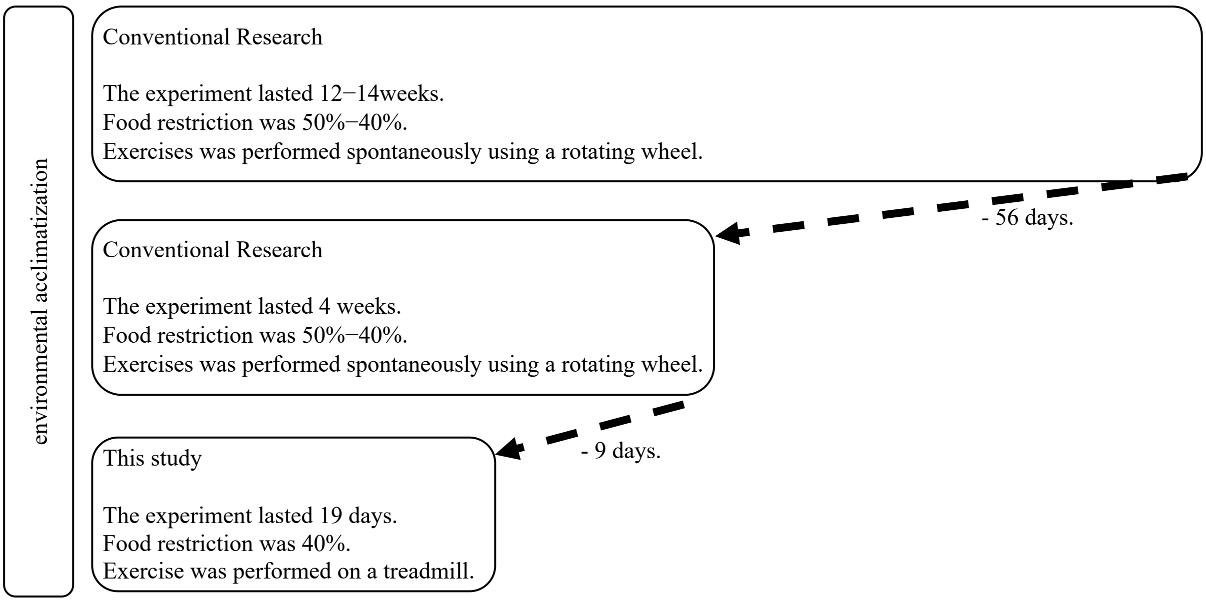

Fig. 4. Comparison of previous research and this study. In a previous study, it took 14 weeks to create a FAT model rat. However, by applying a uniform exercise load on a high-intensity treadmill, the model could be created in 19 days.

from spontaneous exercise to high-intensity treadmill running exercise suggested that the FAT model could be reproduced even after a short period of 19 days of rearing (Fig. 4). However, the FAT-milk group had significantly higher relative uterine mass and bone mineral density than the FAT group (Table 2, Fig. 1A). Therefore, we performed a molecular analysis of bone-related factors.

To maintain bone density, the levels of bone resorption and bone formation must be equal[17]. Previous studies have shown that cystatin C purified from milk basic protein inhibits cathepsin secretion by osteoclasts and suppresses bone resorption[19]. Osteocalcin is a protein involved in bone formation. In this study, the serum osteocalcin concentrations of the rats in the FAT group were high, but their bone mineral density was low, suggesting that osteoporosis associated with a high metabolic turnover was present. However, the same phenotype was not identified in the FAT-milk group.

When the concentrations of female hormones decrease, for example at menopause, the level of bone resorption exceeds that of bone formation, and high metabolic turnover-type osteoporosis develops. However, even in young people, estrogen deficiency has been reported to be associated with a large number of osteoclasts and greater bone resorption[18]. Thus, the effects of estrogen on bone is significant.

Uterine relative mass was lower in the FAT group, but not significantly reduced in the FAT-milk group. Estrogen has been reported to increase the intrauterine mucosal thickness[20], and the low relative uterine mass may have been the result of a low circulating estrogen concentration. However, milk significantly increases the circulating estrone and estradiol concentrations[21], which may have maintained the circulating estrogen concentration and the relative uterine mass of the FAT-milk group. Thus, the maintenance of circulating estrogen concentration may have prevented the reduction in bone mineral density in the FAT-milk group. The energy intake of the rats was adjusted to be the same in each of the diet-restricted groups, which suggests that an awareness of dietary composition may help prevent a reduction in bone mineral density, even when there is poor energy availability.

Calcium is absorbed from the small intestine by the simple diffusion of ions when the diet is rich in this mineral, but this process is inefficient. However, when calcium intake is insufficient or calcium requirements are high, active transport occurs through *Trpv6*, a calcium-selective cation channel that is expressed on the mucosal epithelial cell membranes in the small intestine[22]. The bone mineral density of the FAT-milk group was not low, which suggests that the calcium requirement of the FAT-milk group was not high, and indeed the *Trpv6* mRNA expression was not high. In the present study, the control group had the highest dietary calcium content, which was calculated using food composition tables[23], but there were no differences between the food restriction groups. Casein in milk is converted to casein phosphopeptide in the small intestine, and this casein phosphopeptide has been shown to have higher solubility than calcium phosphate, which enhances calcium absorption[24]. This suggests that the FAT-milk group may have absorbed their calcium by simple diffusion rather than by active transport using *Trpv6*.

Finally, the jejunum villi were not atrophic in the diet-restricted groups, whereas in a previous study of 4 weeks of food restriction, the jejunal villi of the FAT group were atrophic[13]. It is possible that jejunal villus atrophy may also have been induced in the present study if a longer period of food restriction had been used, and this possibility should be further investigated.

There were several potential limitations to the present study. First, it was not possible to assess the circulating estrogen concentrations of the rats, because it would have been necessary to know the stage of the sexual cycle of the rats, which changes over a short period of time. Second, of the possible calcium transporters, the expression of only *Trpv6* was measured, and calcium absorption is greatly influenced by active vitamin D and tight junctions in the small intestine. In addition, calcium can also be obtained from other sources, such as through reabsorption from the kidneys. Third, the amount of blood that could be collected was limited. It was not possible to measure bone formation or bone resorption markers other than osteocalcin. As for estrogen levels in the blood, they were considered based on relative uterine weight. In the future, we would like to assess these other factors and study the effect of milk on the maintenance of bone density.

5. Conclusion

To the best of our knowledge, this was the first study to model FAT over a short period of time by means of dietary restriction and high-intensity exercise for 19 days. Milk consumption resulted in the maintenance of bone mineral density and relative uterine mass, even when there was poor energy availability. These results suggest that milk consumption might help maintain the health of young athletes.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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References

1. Mountjoy M, Ackerman KE, Bailey DM, et al. 2023 International Olympic Committee's (IOC) consensus statement on Relative Energy Deficiency in Sport (REDs). Br J Sports Med, 57: 1073–1097, 2023.
2. Raj MA, Creech JA and Rogol AD. Female Athlete Triad. StatPearls Publishing, USA. (2024)
3. Gómez MPA, Weidekamm C, Aparisi F and Bazzocchi A. Sports and Metabolic Bone Disease. Semin Musculoskelet Radiol, 24: 277–289, 2020.
4. Nattiv A, Loucks AB, Manore MM, Sanborn CF, Sundgot-Borgen J, Warren MP and American College of Sports Medicine. American College of Sports Medicine position stand. The female athlete triad. Med Sci Sports Exerc, 39:1867–1882, 2007.
5. De Souza MJ, Nattiv A, Joy E, Misra M, Williams NI, Mallinson RJ, Gibbs JC, Olmsted M, Goolsby M, Matheson G and Expert Panel. 2014 Female athlete triad coalition consensus statement on treatment and return to play of the female athlete triad: 1st international conference held in San Francisco, California, May 2012 and 2nd international conference held in Indianapolis, Indiana, May 2013. Br J Sports Med, 48: 289, 2014.
6. Aikawa Y, Kakutani Y, Agata U, Hattori S, Ogata H, Kiyono K, Ezawa I and Omi N. Adequate energy intake prevents low bone mass under exercise and low intake of nutrients in young female rats. Am J Sports Sci, 7: 127–135, 2019.
7. Aikawa Y, Agata U, Kakutani Y, Kato S, Noma Y, Hattori S, Ogata H, Ezawa I and Omi N. The preventive effect of calcium supplementation on weak bones caused by the interaction of exercise and food restriction in young female rats during the period from acquiring bone mass to maintaining bone mass. Calcif Tissue Int, 98: 94–103, 2016.
8. Tenenbaum M, Deracinois B, Dugardin C, Auger J, Baniel A, Boulier A, Flahaut C, Ravallec R and Cudennec B. Digested casein phosphopeptides impact intestinal calcium transport in vitro. Food Funct, 15: 8104–8115, 2024.
9. Japan Osteoporosis Society, Japan Bone Metabolism, Society Osteoporosis Foundation. Osteoporosis prevention and treatment guidelines. (2015 Edition); 2015. (in Japanese)
10. Heidi J Kalkwarf, Jane C Khoury, Bruce P Lanphear. Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. AJCN, 77: 257-265, 2003.
11. Aoki K, Yanazawa K, Tokinoya K, Sugasawa T, Suzuki T, Yoshida Y, Nakano T, Omi N, Kawakami Y and Takekoshi K. Renalase is localized to the small intestine crypt and expressed upon the activation of NF-κB p65 in mice model of fasting-induced oxidative stress. Life Sci, 267: 118904, 2021.
12. Todorov H, Kollar B, Bayer F, et al. α-Linolenic acid-rich diet influences microbiota composition and villus morphology of the mouse small intestine. Nutrients, 12: 732, 2020.
13. Aoki K, Ebina K, Shingu H, Kioka K, Sugasawa T, Kawakami Y, Takekoshi K and Omi N. Female athlete triad affects rat intestinal morphology and sucrase-isomaltase expression. Br J Nutr, 130: 1–9, 2023.
14. Aikawa Y, Wakasugi Y, Narukawa T, Yamashita T, Sasai N, Umemura Y, Omi N and Ohtsuki M. Jump exercise and food restriction on bone parameters in young female rats. Calcif Tissue Int, 105: 557–566, 2019.
15. Aikawa Y, Agata U, Kakutani Y, Higano M, Hattori S, Ogata H, Ezawa I and Omi N. The interaction of voluntary running exercise and food restriction induces low bone strength and low bone mineral density in young female rats. Calcif Tissue Int, 97: 90–99, 2015.
16. Yanaka K, Higuchi M and Ishimi Y. Anti-osteoporotic effect of soy isoflavones intake on low bone mineral density caused by voluntary exercise and food restriction in mature female rats. J Nutr Sci Vitaminol (Tokyo), 65: 335–342, 2019.
17. Parfitt AM. The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. Metab Bone Dis Relat Res, 4: 1–6, 1982.
18. Väänänen HK and Härkönen PL. Estrogen and bone metabolism. Maturitas, 23 Suppl: S65–S69, 1996.

19. Matsuoka Y, Serizawa A, Yoshioka T, Yamamura J, Morita Y, Kawakami H, Toba Y, Takada Y and Kumegawa M. Cystatin C in milk basic protein (MBP) and its inhibitory effect on bone resorption in vitro. Biosci Biotechnol Biochem, 66: 2531–2536, 2002.
20. Naveh-Many T, Almogi G, Livni N and Silver J. Estrogen receptors and biologic response in rat parathyroid tissue and C cells. J Clin Invest, 90: 2434–2438, 1992.
21. Maruyama K, Oshima T and Ohyama K. Exposure to exogenous estrogen through intake of commercial milk produced from pregnant cows. Pediatr Int, 52: 33–38, 2010.
22. Cui M, Li Q, Johnson R and Fleet JC. Villin promoter-mediated transgenic expression of transient receptor potential cation channel, subfamily V, member 6 (TRPV6) increases intestinal calcium absorption in wild-type and vitamin D receptor knockout mice. J Bone Miner Res, 27: 2097–2107, 2012.
23. Standard Tables of Food Composition in Japan
24. Naito H, Kawakami A and Imamura T. In vivo formation of phosphopeptide with calcium-binding property in the small intestinal tract of the rat fed on casein. Agric Biol Chem, 36: 409–415, 1972.