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Dietary Essential Amino Acid Supplements Increase Bone Strength by Influencing Bone Mass and Bone Microarchitecture in Ovariectomized Adult Rats Fed an Isocaloric Low-Protein Diet

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ABSTRACT

This study was designed to investigate whether the administration of dietary essential amino acid supplements in adult rats made osteoporotic by estrogen deficiency and reduced protein intake could reverse the deleterious effects caused by these maneuvers. This animal model was selected to mimic the situation observed in elderly women in whom estrogen deficiency and/or low-protein intake (but also calcium and vitamin D deficiency) are known to contribute to the pathogenesis of osteoporosis. Six-month-old rats were ovariectomized (OVX) and fed an isocaloric 2.5% casein diet for 10 weeks or sham-operated (SHAM) and fed an isocaloric 15% casein diet. The animals fed the 2.5% casein diet were given isocaloric supplements of essential amino acids in similar relative proportion to that of casein at doses of 2.5% or 5% of total diet for an additional 16 weeks. Vertebrae, femur, and tibia bone mineral density (BMD); ultimate strength; and microtomographic histomorphometry were evaluated before and after dietary essential amino acid supplements. Essential amino acid supplements increased vertebrae, femur, and tibia bone strength in OVX rats fed a low-protein diet. The mechanical changes induced by this dietary isocaloric supplement were associated with the prevention of a further BMD decrease or even with some increases and changes in microarchitecture such as from a rod to a plate trabecular spacial configuration and increased cortical thickness. Higher insulin-like growth factor (IGF) I levels, as well as greater bone formation and reduced bone resorption as assessed by biochemical markers of bone remodeling, were found in rats receiving essential amino acid supplements. In conclusion, dietary essential amino acid supplements increased bone strength through modifications of BMD, trabecular architecture, and cortical thickness possibly by an IGF-I–mediated process. (J Bone Miner Res 2002;17:1264–1272)

Key words: elderly, nutrition, osteoporosis, insulin-like growth factor I, ovariectomy

INTRODUCTION

LOW DIETARY intake of protein, calcium, or vitamin D contributes to the pathogenesis of senile osteoporosis.[1–5] Indeed, low intake of calcium and/or protein can be associated with decreased bone mineral density (BMD) and/or an increased risk of osteoporotic fracture.[6,7] We previously established the selective effects of reduced protein intake in rats fed an isocaloric low-casein diet.[8] In this model, a decrease of BMD and bone strength was observed at all skeletal sites tested, including parts of the skeleton containing mainly cortical bone. This was associated with an early depressed bone

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formation and late increased bone resorption.[8,9] One potential link between dietary protein and bone homeostasis is insulin-like growth factor (IGF) I, and a low level of IGF-I is a risk factor for fracture.[1,10] Production and action of this growth factor are impaired under protein deficiency.[1,8,9,11–17] IGF-I administration to experimental animals increases BMD, mass, and strength.[18,19] However, the exogenous administration of IGF-I or growth hormone (GH) was unable to restore a normal growth in rats fed a low-protein diet.[14] The treatment with IGF-I/IGF-binding protein complex also failed to stimulate bone formation in adult rats fed an isocaloric low-protein diet.[9]

An apparent resistance to IGF-I at the level of bone provided the rationale to try to modulate the endogenous production of IGF-I and to attenuate the resistance to IGF-I by administering essential amino acid supplements. This was supported by the observation that protein supplements in elderly patients with hip fracture attenuated bone loss[1] while increasing circulating IGF-I. The model of adult female rats that had been made osteoporotic by ovariectomy (OVX) and by an isocaloric low-protein diet[8] was selected to test this hypothesis and to mimic the situation of elderly women with protein malnutrition and menopause. We assessed the role of protein replenishment on both IGF-I levels and bone response, BMD, architecture, and strength changes. The results indicate that essential amino acid supplements increase BMD and strength, the latter mainly through greater cortical thickness.

MATERIALS AND METHODS

Animals and treatments

All experimental designs and procedures were approved by the Animal Ethics Committee of the Geneva University Faculty of Medicine. Female Sprague–Dawley rats (RCC, Ltd., Füllinsdorf, Switzerland), housed individually at 25°C with a 12:12 h light-dark cycle, were strictly pair-fed a laboratory diet throughout the experimental period provided by Novartis Nutrition (Berne, Switzerland) containing various amounts of casein, 0.8% phosphorus, 1.1% calcium, 0.2% magnesium, 70–80% carbohydrates, and 5% fat. Isocaloric diets were produced by the addition of corn carbohydrate, thus providing the same energy intake. The animals also were given a daily dose of vitamin D dissolved in peanut oil (100 IU/kg body weight). After 2 weeks of adaptation to a 15% casein-containing diet, 6-month-old female rats underwent transabdominal OVX or a sham operation (SHAM) under anesthesia with intraperitoneal ketamine hydrochloride (100 mg/kg body weight). The effectiveness of OVX was verified at the end of the experiment by visualizing the atrophy of the uterus and absence of ovarian tissue. SHAM rats were maintained on the 15% casein-containing diet, whereas OVX rats received a 2.5% casein isocaloric diet. Ten weeks after OVX and low-protein diet, rats were randomly allocated to three groups receiving diets containing either 2.5% casein, 2.5% casein and 2.5% essential amino acids, or 2.5% casein and 5.0% essential amino acids. All the diets were isocaloric. The amino acids (leucine, lysine, isoleucine, phenylalanine, valine, DL-methionine, arginine, threonine, histidine, and tryptophane) were in a proportion similar to that found in casein (24.0, 15.0, 11.3, 9.1, 7.4, 6.8, 6.6, 6.5, and 2.4%, respectively). Before and after the treatment period, blood was sampled from the tip of the tail. Urine was collected in rats maintained in metabolic cages over 24 h for the determination of total deoxypyridinoline excretion. Before the essential amino acid supplementation period, a group of OVX rats fed a 2.5% casein diet was killed for lumbar vertebrae, tibia, and femur mechanical testing (baseline). At the end of

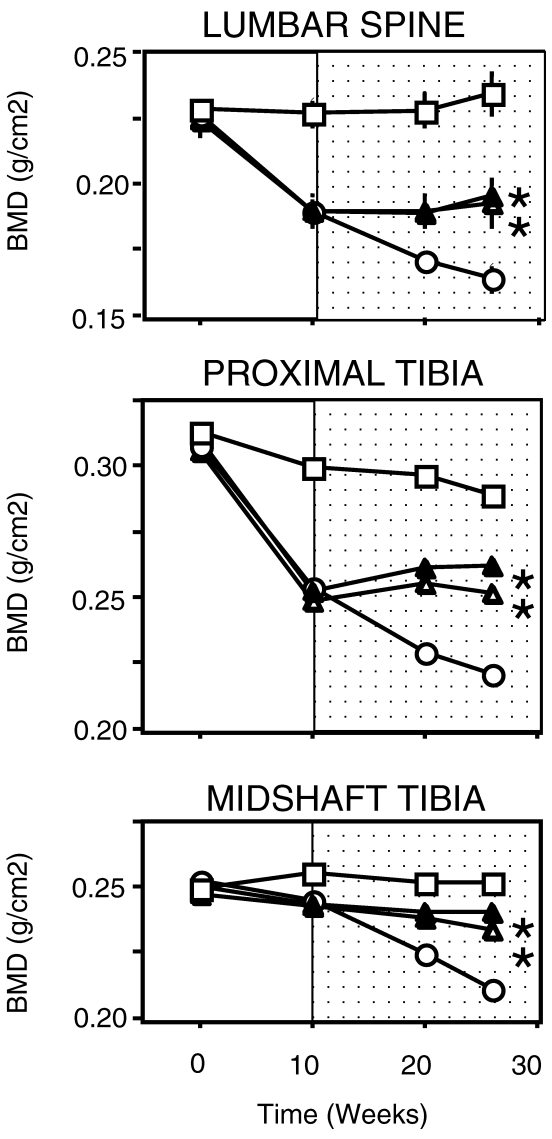

FIG. 1. Effect of isocaloric essential amino acid supplements on BMD in adult female rats made osteoporotic by OVX and a low-protein diet. BMD (g/cm^2, $n = 8–9$, and means ± SEM) was measured in vivo in OVX rats fed a low-protein diet (open circles) treated with essential amino acid supplements at a dose of 2.5% (open triangle) or 5% (closed triangle), as well as in control rats SHAM fed a 15% casein diet (open square). The treatment period is represented by a stippled area. Using ANOVA, * indicates a significant difference ($p < 0.01$) from OVX rats fed a low-protein diet.

TABLE 1. EFFECT OF ISOCALORIC EAA SUPPLEMENTS ON BMD CHANGES

	Changes from baseline (%)			
	OVX	*OVX*	*OVX*	*SHAM*
Casein	2.5	2.5	2.5	15
EAA	—	2.5	5.0	—
No. of rats	8	9	9	8
Lumbar spine	−13.6 ± 2.1	2.5 ± 5.3*	3.8 ± 3.5*	3.4 ± 3.4*
Proximal tibia	−12.9 ± 1.3	1.9 ± 0.6*	4.0 ± 1.2*	−3.6 ± 0.9*
Midshaft tibia	−5.7 ± 0.6	0.2 ± 0.3*	2.3 ± 0.8*	−0.2 ± 1.2*
Femoral neck	−12.3 ± 3.8	−5.7 ± 5.6	−1.6 ± 1.3*	3.9 ± 2.8*

Values are means ± SEM expressed in percent of pretreatment values. Treatment was started 10 weeks after the OVX and reduced protein intake. EAA supplements were administered for 16 weeks. Using ANOVA, * indicates a significant difference from OVX rats fed a low-protein diet.

EAA, essential amino acid.

the study, all groups were killed by an overdose of ketamine hydrochloride.

BMD measurements

BMD was measured by DXA using a Hologic QDR-1000 instrument adapted to small animals (Hologic, Inc., Waltham, MA, USA).[20] An ultra–high-resolution mode (line spacing, 0.254 mm, and resolution, 0.127 mm) was used with a 0.9-mm-diameter collimator. During the examination, the animals were anesthetized with ketamine hydrochloride (100 mg/kg body weight). BMD, bone mineral content (BMC), and scanned area were recorded in vivo at the level of lumbar vertebrae, tibia, and femur, as previously described.[19] Ex vivo measurements were performed at the level of the isolated vertebral body and femur in 2.5 cm of saline solution. The in vivo reproducibility was evaluated by the CV of repeated measurements with repositioning and was <1.8%. The stability of the instrument was controlled by scanning a phantom six times weekly.

Bone mechanical testing

The lumbar spine, tibia, and femur were excised immediately after death and frozen at −20°C in plastic bags. The night before mechanical testing, the bones were thawed slowly at 7°C and then maintained at room temperature. The L4 vertebrae were isolated from the lumbar spine at the level of the intervertebral discs. The vertebral pedicules were dissected out carefully to avoid any damage to the cortical shell. Because the caudal and cranial surfaces of the rat vertebral body are not parallel, 1 mm of the caudal and cranial parts of each vertebral body was embedded in methylmethacrylate cement (Technovit 4071; Heraeus Kulzer GmbH, Wehrheim, Germany) to ensure regular distribution of the compressive forces. Between the different steps of preparation, each specimen was kept immersed in physiological solution. The fibula was removed and the length of the tibia (distance from intermalleolar to intercondylar region) was measured using a caliper with electronic digital display and the middle of the shaft was determined. The tibia then was placed in the material testing machine on two supports separated by a distance of 20 mm and load was applied to the middle of the shaft, thus creating a three-point bending test. Compression testing of the proximal tibia was performed by axial compression of the tibia plateau with the shaft being fixed in methylmethacrylate cement.[8,21] Femoral neck testing was performed by maintaining the femur in a vertical position and embedding the shaft in methylmethacrylate cement up to the lesser trochanter before application of a vertical load on the femoral head.[19] The mechanical resistance to failure was tested using a servo-controlled electromechanical system (Instron 1114; Instron Corp., High Wycombe, UK) with the actuator displaced at 2 mm/minute. Both displacement and load were recorded. Ultimate strength (maximal load, newton [N]), stiffness (slope of the linear part of the curve, representing the elastic deformation, N/mm), and energy (surface under the curve, N × mm) were calculated. Reproducibility was 4.8% for vertebrae, 5.8% for proximal tibia, 3.3% for midshaft tibia, and 4.5% for femoral neck, and it was evaluated as the CV of pair sample measurements (left/right, L3–L4).

Microtomographic histomorphometry by microcomputed tomography

Parameters of mass and architecture of the vertebrae were investigated using microtomographic histomorphometry with a high-resolution microcomputed tomography (μCT) system (μCT 20; Scanco Medical AG, Bassersdorf, Switzerland) as previously described.[22,23] In summary, three-dimensional (3D) images of a vertebra were acquired with a voxel size of 20 μm in all spatial directions. No sample preparation was needed, and the vertebrae were secured in a cylindrical sample holder in air. The resulting gray-scale images were segmented using a low-pass filter to remove noise and a fixed threshold to extract the mineralized bone phase. The trabecular and cortical parts of the vertebral bodies were separated with semiautomatically drawn contours.

From the binarized images, structural indices were assessed. Relative bone volume (BV/TV), trabecular number

TABLE 2. EFFECT OF ISOCALORIC EAA SUPPLEMENTS ON BMD AND BONE MECHANICAL PROPERTIES

	OVX baseline	*OVX*	*OVX*	*OVX*	*SHAM*
Casein (%)	2.5	2.5	2.5	2.5	15
EAA (%)	—	—	2.5	5.0	—
No. of rats	6	8	9	9	8
Lumbar spine					
BMD (mg/cm^2)	103.4 ± 6.3	75.6 ± 4.5*	102.8 ± 4.8°	118.1 ± 3.3*,°	143.4 ± 4.3*,°
US (N)	95.08 ± 7.2	73.5 ± 9.2	105.4 ± 14.5	157.7 ± 15.0*,°	165.3 ± 11.9*,°
Stiffness (N/mm)	401 ± 39	282 ± 42	373 ± 48	442 ± 58	475 ± 51
Energy (N × mm)	14.9 ± 1.1	13.2 ± 2.2	18.4 ± 3.3	35.4 ± 5.5*,°	34.1 ± 4.5*,°
Proximal tibia					
BMD (mg/cm^2)	247.4 ± 3.8	220.7 ± 4*	251.3 ± 2.1°	261.7 ± 1.7*,°	288.9 ± 3.2*,°
US (N)	77.1 ± 4.4	63.1 ± 9.0	99.0 ± 6.5*,°	118.8 ± 3.0*,°	159.2 ± 8.5*,°
Stiffness (N/mm)	286 ± 56	366 ± 47	283 ± 59	364 ± 29	390 ± 37
Energy (N × mm)	24.4 ± 5.7	13.8 ± 3.5	37.7 ± 3.5*,°	37.2 ± 3.3*,°	48.5 ± 4.0*,°
Midshaft tibia					
BMD (mg/cm^2)	236.2 ± 3.4	210.8 ± 4.7*	233.1 ± 1.1°	240.3 ± 3.2°	251.4 ± 3.1*,°
US (N)	73.9 ± 4.7	55.5 ± 3.0*	81.5 ± 4.4°	77.6 ± 2.5°	87.5 ± 5.3*,°
Stiffness (N/mm)	184 ± 9	190 ± 11	216 ± 19	201 ± 11	213 ± 17
Energy (N × mm)	20.5 ± 1.6	12.1 ± 1.2*	20.37 ± 1.8°	18.73 ± 1.2°	20.2 ± 2.0°
Femoral neck					
BMD (mg/cm^2)	198.3 ± 4.0	167.1 ± 5.3*	207.9 ± 2.6°	218.2 ± 2.2*,°	253.7 ± 2.4*,°
US (N)	56.33 ± 3.1	55.3 ± 2.8	74.6 ± 3.2*,°	75.4 ± 3.7*,°	80.4 ± 5.2*,°
Stiffness (N/mm)	265 ± 31	225 ± 31	274 ± 32	275 ± 27	390 ± 23*,°
Energy (N × mm)	8.4 ± 0.8	9.0 ± 1.2	12.8 ± 2.4	13.5 ± 1.3*,°	9.1 ± 0.8

Values are means ± SEM. The EAA supplements were started 10 weeks after the OVX and reduced protein intake. They were administered for 16 weeks.

EAA, essential amino acid; US, ultimate strength (N).

* $p < 0.05$ vs. baseline and ° vs. OVX low-protein group as evaluated by ANOVA.

(Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were calculated by measuring directly the 3D distances[23,24] in the trabecular network. Connectivity density based on Euler number[25] and the structure model index (SMI) were calculated. The SMI quantifies the plate versus rod characteristics of trabecular bone,[24] where an SMI of 0 indicates a purely plate-shaped bone, an SMI of 3 a rodlike bone, and values in-between stand for a mixture of plates and rods.

Biochemical determinations

Plasma osteocalcin and IGF-I were measured by radioimmunoassay using reagents from Biomedical Technologies (Stoughton, MA, USA) for the former, and a kit from Nichols Institute (San Juan Capistrano, CA, USA) after extraction by acid-ethanol and cryoprecipitation, for the latter. Total urinary deoxypyridinoline was determined after acid hydrolysis using a kit from Quidel (Mountain View, CA, USA).

Statistical analysis

All results are expressed as mean ± SEM. Significance of difference between groups was evaluated with a one-way ANOVA followed by a Fisher's test. Possible interactions between bone density and bone dimensions in the determination of bone strength were evaluated by stepwise multiple regression analysis.

RESULTS

Effect of essential amino acid supplements on BMD

The combination of isocaloric low-protein intake and OVX induced a marked decrease of BMD at all the investigated skeletal sites (Fig. 1). At the levels of the lumbar spine, proximal tibia, and femoral neck, which are sites composed of both cancellous and cortical bone, essential amino acid supplements prevented further bone loss when introduced 10 weeks after beginning the low-protein diet (Table 1 and Fig. 1). However, BMD remained significantly lower than in SHAM controls fed a 15% casein-containing diet. Similar effects were observed when the BMD of isolated bone was measured ex vivo (Table 2). At the level of the midshaft tibia, mainly constituted of cortical bone, bone loss was delayed as compared with other sites.

Effect of essential amino acid supplements on bone strength

The BMD decrease induced by the combination of isocaloric low-protein intake and OVX was associated with

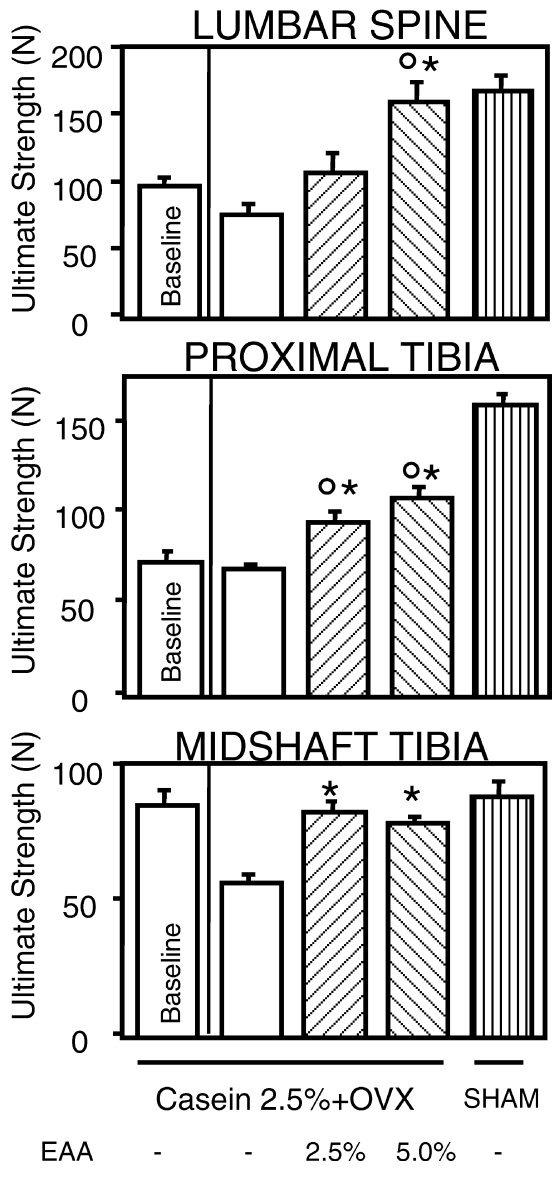

FIG. 2. Effect of isocaloric essential amino acid supplements on bone strength in adult female rats made osteoporotic by OVX and a low-protein diet. Ultimate strength (N; means ± SEM) was measured at the level of the vertebral body and proximal tibia by an axial compression test and a three-point bending test at the level of the midshaft tibia. The measurements were performed 10 weeks after OVX and of feeding a low-protein diet (baseline) and after 16 weeks of essential amino acid supplements. SHAM refers to sham-operated animals fed a 15% casein diet. Using ANOVA, * indicates a significant difference ($p < 0.01$) from baseline and ° from OVX rats fed a low-protein diet.

significant alteration of bone mechanical properties (ultimate strength, stiffness, and energy absorbed) at all the investigated sites (Table 2 and Fig. 2). At the levels of the lumbar spine, proximal tibia, and femoral neck, essential amino acid supplements dose-dependently increased bone strength (Table 2 and Fig. 2). A full recovery was observed at the level of the lumbar spine in rats receiving the supplement containing 5% essential amino acids. At the level of the midshaft tibia, mainly constituted of cortical bone, the treatment prevented the alteration of mechanical properties (Table 2 and Fig. 2). The linear regression between vertebra ultimate strength and BMD was steeper in animals receiving essential amino acid supplements (slope = 2.60, $r^2 = 0.48$, and $p = 0.004$) than in control animals (slope = 1.34, $r^2 = 0.79$, and $p = 0.0001$). A similar trend was observed when energy was analyzed for different skeletal sites studied.

Effect of essential amino acid supplements on microtomographic histomorphometry

To further investigate the relation between a marked improvement in bone strength and small changes in BMD induced by essential amino acid supplements, we analyzed bone microarchitecture by microtomographic histomorphometry, a variable that is not captured by DXA measurement.

Trabecular bone architecture changes such as decreased Tb.N, spacing connectivity, and structure (rods instead of plates) were observed in OVX rats fed a low-protein diet (Table 3 and Fig. 3). The 5% essential amino acid supplements increased trabecular BV/TV and Tb.N, and reduced trabecular spacing as compared with OVX rats fed an isocaloric low-protein diet (Table 3 and Fig. 3). This was associated with a partial recovery of the indices of connectivity and a shift of trabecular structure from rods to mixed plates and rod forms. However, trabecular BV/TV was still significantly lower in the 5% essential amino acid supplements group as compared with the SHAM group. On the other hand, a significant thickening of the cortices was observed at the level of the vertebrae in rats treated with the 2.5% and 5% essential amino acid supplements (Table 3), with values close to those of SHAM animals ($p = 0.24$ and $p = 0.42$ for 2.5% and 5% essential amino acid supplements vs. SHAM, respectively). This effect on cortical bone was observed also at all the other investigated sites. Both BV/TV and outer diameter were increased by the dietary supplements (Table 3).

Effect of essential amino acid supplements on bone remodeling

As compared with SHAM animals, OVX rats fed a low-protein diet had an increase in urinary deoxypyridinoline excretion, reflecting increased bone resorption. The serum level of the bone formation marker osteocalcin was not affected (Table 4). With essential amino acid supplements, urinary deoxypyridinoline excretion decreased and serum osteocalcin increased (Table 4) but did not reach the values of SHAM animals.

Effect of essential amino acid supplements on IGF-I

As previously shown,[8] isocaloric low-protein diet decreased plasma IGF-I (Fig. 4). In this condition, OVX was not associated, as expected, with an increment of plasma IGF-I. Essential amino acid supplements increased plasma IGF-I to levels significantly higher than in SHAM animals fed the 15% casein diet (Fig. 4). Despite the strict pair-

TABLE 3. EFFECTS OF ISOCALORIC EAA SUPPLEMENTS ON BONE ARCHITECTURE

	OVX baseline	*OVX*	*OVX*	*OVX*	*SHAM*
Casein (%)	2.5	2.5	2.5	2.5	15
EAA (%)	—	—	2.5	5.0	—
Tb.N	2.86 ± 0.45	1.13 ± 0.09	1.29 ± 0.12	2.37 ± 0.24*	3.96 ± 0.16*
Tb.Th (μm)	57.7 ± 1.0	67.4 ± 3.2	66.4 ± 1.6	67.8 ± 1.3	79.9 ± 6.4
Tb.Sp (mm)	0.468 ± 0.149	0.944 ± 0.079	0.836 ± 0.091	0.517 ± 0.111	0.256 ± 0.011
SMI	2.53 ± 0.11	2.19 ± 0.20	2.25 ± 0.10	1.98 ± 0.07°	0.87 ± 0.37°

Values are means ± SEM. Results are Tb.N, Tb.Th, Tb.Sp, and SMI. SMI is an estimate of the plate vs. the rod characteristics of trabecular bone (0 value = purely plate-shaped bone, 3 value = purely rodlike bone). The EAAs were started 10 weeks after the OVX and reduced protein intake. They were administered for 16 weeks.

EAA, essential amino acid.

* $p < 0.05$ vs. baseline and ° vs. OVX low-protein group as evaluated by ANOVA.

feeding and identical energy intake, a low-protein diet was associated with decreased body weight, which then was dose-dependently corrected by essential amino acid supplements. Body weight was 238.9 ± 5.1 g, 176.4 ± 4.8 g, 256.3 ± 8.7 g, and 266.7 ± 2.4 g in SHAM 15% casein, OVX 2.5% casein, and 2.5% and 5% essential amino acid treatment, respectively. Similar effects on muscle weight of the lower limbs were observed (4.25 ± 0.18 g, 3.06 ± 0.07 g, 4.15 ± 0.32 g, and 4.43 ± 0.20 g for the corresponding groups).

DISCUSSION

This study shows that nutritional intervention with essential amino acids is able to improve bone mechanical properties in female rats made osteoporotic by OVX and a low-protein diet. Microarchitecture assessment indicates a thickening of bone cortices. This effect is observed in association with an increase in plasma IGF-I. Analysis of biochemical markers suggests a decrease in bone resorption and a stimulation of bone formation.

To mimic the situation observed in the elderly patient with osteoporosis and a hip fracture,[1,5,8,26] adult rats were OVX and received a low-protein diet. It is well recognized that OVX results in alteration of BMD and mechanical properties, essentially at skeletal sites containing cancellous bone.[21,27–29] Skeletal sites containing mainly cortical bone are not significantly altered by estrogen deficiency, at least in rodents.[21,28,29] A reduced protein intake is associated with a marked decrease of BMD, also at mainly cortical sites, despite an identical energy intake in all the different groups. A possible explanation could be that IGF-I did not increase as usually observed after OVX.[8,21,30] Furthermore, an additional increment of cortical bone turnover could be expected in conditions of reduced protein intake. Thus, this model seems to mimic the situation of elderly women with senile osteoporosis. Previous studies indicated that no modification of BMD, strength, and turnover, and of somatotrope or gonadotrope axis could be observed in rats fed 5, 10, or 15% casein. This effect was only observed in rats fed the 2.5% casein isocaloric diet corresponding to a reduction of 50% of the minimal protein requirement in the

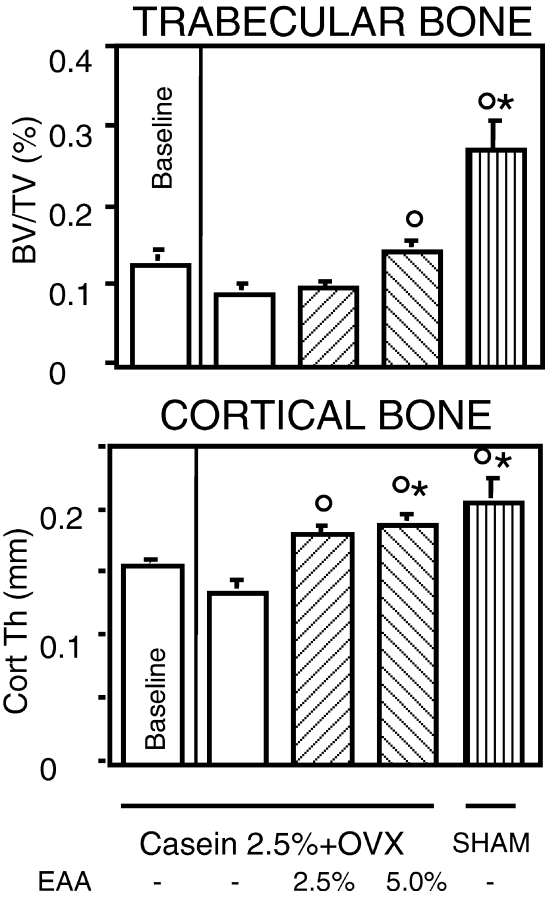

FIG. 3. Effect of isocaloric essential amino acid supplements on trabecular BV/TV and cortical bone thickness in lumbar vertebral body of adult female rats made osteoporotic by OVX and a low-protein diet. BV/TV (%; means ± SEM) and cortical thickness (Cort Th, mm) were measured by microtomographic histomorphometry (μCT) at the level of the L4 vertebra. The measurements were performed 10 weeks after OVX and of feeding a low-protein diet (baseline) and after 16 weeks of essential amino acid supplements. SHAM refers to sham-operated animals fed a 15% casein diet. All groups were fed isocaloric diets. Using ANOVA, * indicates a significant difference ($p < 0.01$) from baseline and ° from OVX rats fed a low-protein diet.

TABLE 4. EFFECTS OF ISOCALORIC EAA SUPPLEMENTS ON BIOCHEMICAL MARKERS OF BONE REMODELING

	OVX baseline	*OVX*	*OVX*	*OVX*	*SHAM*
Casein (%)	2.5	2.5	2.5	2.5	15
EAA (%)	—	—	2.5	5.0	—
No. of Rats	8	8	9	9	8
Plasma osteocalcin (nmol/liter)	1.76 ± 0.13	2.14 ± 0.13	2.26 ± 0.07	2.69 ± 0.08*,°	2.03 ± 0.07
Urinary deoxypyridinoline (nmol/24 h)	NA	3.1 ± 0.2	2.3 ± 0.4*,°	2.6 ± 0.7*	0.9 ± 0.2

Values are means ± SEM. Treatment was started 10 weeks after the OVX and reduced protein intake. Amino acid supplements were administered for 16 weeks. Determinations were performed before (baseline) and 16 weeks later. Baseline urinary deoxypyridinoline was not assessed (NA).

EAA, essential amino acid.

* $p < 0.05$ vs. OVX rats fed 2.5% casein diet and ° vs. SHAM rats as evaluated by ANOVA.

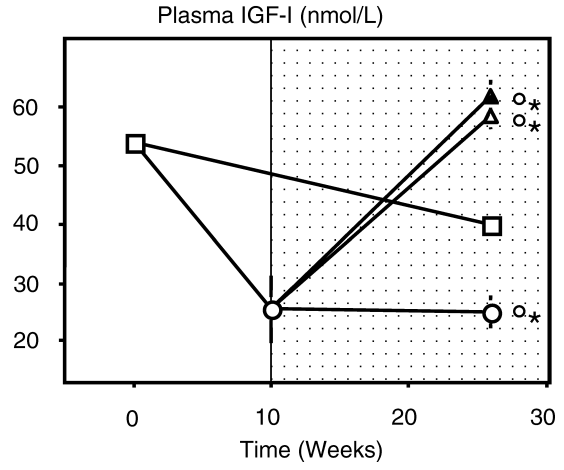

FIG. 4. Effect of isocaloric essential amino acid supplements on plasma IGF-I in adult female rats made osteoporotic by OVX and a low-protein diet. Plasma IGF-I (nmol/liter, $n = 6–9$, and means ± SEM) was measured in OVX rats fed a low-protein diet (open circles) treated with essential amino acid supplements at a dose of 2.5% (open triangle) or 5% (closed triangle), as well as in control rats SHAM fed a 15% casein diet (open square). The treatment period is represented by a stippled area. Using ANOVA, * indicates a significant difference ($p < 0.01$) from OVX rats fed a low-protein diet and ° from SHAM controls.

rat.[8] This is real so long as the energy intake is normal and the rats have stopped their growth.

The ability of nutritional intervention to restore bone strength was then investigated. The type of nutritional supplement was selected based on preliminary studies indicating that only essential amino acids (but not selected essential amino acids or nonessential amino acids) increased circulating plasma IGF-I in rats fed an isocaloric low-protein diet. The essential amino acid supplements increased bone strength at skeletal sites formed by both cortical and cancellous bone such as the vertebral body, proximal femur, and proximal tibia and also at the level of sites formed mainly by cortical bone such as the midshaft of long bones. A full restoration of mechanical properties was even observed at the level of the vertebrae. The mechanical properties were evaluated by a compression test of the intact vertebral body. This kind of sample preparation respects the cortex but also the microarchitecture of the trabeculae and their connection with the cortical shell and ensures the full integration of all the determinants of bone strength.

One of the major determinants of bone strength is BMD, which predicts up to 60% of the variance of bone strength.[19,21] The essential amino acid supplements prevented further bone loss and even increased BMD at the level of the spine, proximal tibia, and femoral neck. Nevertheless, there was a discrepancy between the moderate influence of essential amino acid supplements on BMD and the marked effect on bone strength, which resulted in a full recovery at the level of the vertebrae. It is not excluded that areal BMD measurement could preclude the detection of microarchitecture modification.

Microarchitecture determinants were investigated at the level of the vertebrae using microtomographic histomorphometry and structural assessment. Their accuracy and precision have been established previously.[22,23] This technique provides information regarding the classical parameters of trabecular bone mass as evaluated by static histomorphometry. The results indicate higher trabecular BV/TV, Tb.N, and decreased trabecular spacing in rats treated with 5% essential amino acids as compared with the time controls, as well as a prevention of further deterioration compared with baseline values. SMI distinguishes between rods and plates of bony trabeculae. Our results suggest a moderate but significant evolution of trabecular structures back from rods to a mixed plates and rods form in those animals treated with 5% essential amino acids as compared with baseline values. However, the most impressive effect concerns cortical thickness. The latter was markedly increased in both groups receiving essential amino acid supplements. An integration of trabeculae lying along the endosteal cortical surface cannot be excluded. This increase of cortical thickness, together with modification of trabecular architecture, could account for the increase of bone strength in rats fed 5% essential amino acid supplements.

OVX induces an increment of IGF-I under normal casein intake. In rats fed a low-protein diet, this does not occur.[8] Amino acid supplements increased plasma IGF-I to values even 30% higher than SHAM receiving the 15% casein diet.

Because estrogen could down-regulate the transcription of IGF-I,[31,32] this overshot of IGF-I secondary to essential amino acid supplements could possibly be explained by the lack of estrogen repression on IGF-I production. Plasma IGF-I could be responsible for the bone anabolic effect of essential amino acid supplements. Indeed, administration of IGF-I increases BMD and strength.[18,19]

To address the issue of the mechanisms involved, markers of bone remodeling such as deoxypyridinoline and osteocalcin were measured. An increase of bone resorption, as evaluated by urinary deoxypyridinoline excretion without a commensurate change of the bone formation marker osteocalcin, is found in OVX rats fed a low-protein diet. This can be interpreted as an uncoupling between resorption and formation, leading to accelerated bone loss.[8,9,17] Essential amino acid supplements may attenuate bone resorption and stimulate bone formation as indicated by changes in bone-remodeling markers, compatible with a recoupling of the two processes. Thus, a positive coupling could have led to a positive bone balance and to architectural modifications of both cortical and cancellous bone.

Despite an identical caloric intake, body weight decreased under a low-protein diet but was fully corrected by the amino acid supplements. Changes in hindlimb muscle weight followed those of body weight, with an increase under amino acid supplements.

In conclusion, nutritional intervention with essential amino acid supplements is able to increase bone strength in adult female rats made osteoporotic by OVX and a low-protein diet. The effects of such supplements in animals with a normal protein intake remain to be established. This was associated with changes in bone microarchitecture. These observations provide the preclinical basis for evaluating the effects of essential amino acid supplements in the elderly who are frail and/or osteoporotic.

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