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Effects of a Single Bout of Resistance Exercise on Calcium and Bone Metabolism in Untrained Young Males

N. Ashizawa,[1] G. Ouchi,[2] R. Fujimura,[1] Y. Yoshida,[1] K. Tokuyama,[1] M. Suzuki[1]

[1]Laboratory of Biochemistry of Exercise and Nutrition, Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan
[2]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

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Abstract. Although resistance exercise training appears to increase bone mineral density in the long term, a single bout of resistance exercise could paradoxically induce bone homeostasis disturbance, secondary to metabolic acidosis. To examine this, we obtained fasting blood and 24-hour urine samples from untrained male subjects for 5 subsequent days (control day, exercise day, and three post-exercise days), and investigated the effects of a single bout of resistance exercise on urinary calcium excretion and bone metabolism as indicated by sensitive biomarkers of bone formation and resorption. After an intense bout of resistance exercise, blood and urine became more acidic and renal net acid excretion significantly increased by 44% on the exercise day. Urinary calcium excretion significantly increased by 48% on the exercise day. Plasma procollagen type-I C-terminal concentration significantly decreased by 12% on the next day of the exercise and serum bone-specific alkaline phosphatase activity also significantly decreased by 13% and 9% on days 2 and 3, respectively, after the exercise. There was no significant change in serum osteocalcin concentration. Serum tartrate-resistant acid phosphatase activity significantly decreased by 15% on the day after the exercise and urinary deoxypyridinoline excretion decreased by 22% and 27% on days 1 and 3, respectively, after the exercise. These results suggest that the early response of bone to a bout of resistance exercise in untrained individuals was transient decreases in bone formation and resorption, whereas urinary calcium excretion increased.

Key words: Resistance exercise — Lactic acidosis — Bone formation — Bone resorption — Urinary calcium.

For the structural adaptation of bone, the intensity of the mechanical stimuli appear to be more important than the frequency of the mechanical loads [1, 2]. This is evidenced by the fact that high intensity exercises such as resistance exercise training enhance bone density and bone mass, whereas low intensity exercises such as walking and jogging may not [3, 4]. On the other hand, such high intensity exercise is associated with a transient accumulation of lactic acid [5, 6]. The degree of the acidosis induced by such a strenuous exercise, although lasting only for a few hours, was greater than that induced by ammonium chloride or protein ingestion [7, 8] which had negative effects on bone metabolism. Indeed, when neonatal mouse calvaria was cultured in medium with a reduced pH and bicarbonate concentration, an *in vitro* model of metabolic acidosis, there was a net efflux of calcium from bone [9–11], an increase in bone resorption [9–12], and a decrease in bone formation [10, 11]. Though resistance exercise training appears to increase bone mineral density in the long term, theoretically, a single bout of the resistance exercise could paradoxically induce bone metabolism disturbance, secondary to metabolic acidosis. Therefore, the purpose of this study was to investigate the effects of a single bout of resistance exercise on urinary calcium excretion and markers of bone metabolism in untrained male subjects.

Correspondence to: M. Suzuki

Materials and Methods

Subjects

A total of 14 Oriental male subjects consented to participate in this investigation. The physical characteristics of the subjects were as follows: age, 24.5 ± 0.7 years; height, 170.2 ± 1.4 cm; body weight, 66.6 ± 1.9 kg; body fat, 19.1 ± 1.2%. Most of the subjects had recreational experience with resistance training but none had participated in any regular exercise program for at least 2 years. All of the subjects were in good health and taking no medications that would alter calcium or bone homeostasis. There were four smokers among the subjects.

Experimental Protocol

The study was conducted for a total of 9 days, and the subjects consumed standardized diet containing 840 mg of calcium, as previously described [13], throughout the experimental period. The first 4 days were designed for adjustment to the standardized diet, and blood and urine samples were obtained for the subsequent 5 days (control day, exercise day, and three postexercise days). Collections of 24-hour urine samples began at 1600 hours, and overnight fasting blood samples were obtained at 0800 hours. A single bout of resistance exercise started at 1600 hours on the 6th day of the experimental period. An additional blood sample was obtained within 5 minutes after completion of the exercise to examine lactate.

Exercise Program

During the preliminary experimental period, the subjects performed each exercise with low intensity and learned accurate lift-

Table 1. Change of urinary components with a bout of resistance exercise

			Postexercise days		
	Control	Exercise	1	2	3
Calcium (mg/day)	203.9 ± 23.7	301.0 ± 29.1*	184.6 ± 23.4	187.8 ± 23.0	187.4 ± 23.3
Net acid excretion (mEq/day)	50.3 ± 4.1	72.6 ± 7.2*	58.5 ± 5.1	50.1 ± 4.8	47.7 ± 6.7
Ammonium (mEq/day)	25.5 ± 1.9	40.7 ± 4.7*	30.9 ± 3.0	26.9 ± 2.6	24.8 ± 2.8
Titratable acid (mEq/day)	24.8 ± 2.6	31.8 ± 2.8	27.6 ± 2.4	23.2 ± 2.7	22.8 ± 4.3
Creatinine (g/day)	1.7 ± 0.1	1.9 ± 0.2	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
Urine volume (ml/day)	1245 ± 204	1317 ± 106	1044 ± 508	1181 ± 142	1141 ± 123

Values are means ± SEM; n = 14
* Significantly different from control value ($P < 0.01$)

ing form in order to familiarize themselves with the resistance exercise protocol. Ten days before the experimental period, one repetition maximum (1RM) test was performed for each exercise [13]. On the exercise day, the subjects performed 3 sets of 10 repetitions of each exercise (60% of 1RM for the first set and 80% of 1RM for the second and third sets, respectively). When the subjects could not complete 10 repetitions at 80% of 1RM, the load was gradually reduced just before muscular failure so that 10 repetitions could be completed with the heaviest weight. The experimental workout order was (1) bench press, (2) back press, (3) arm curl, (4) double leg extension, (5) bent leg incline sit-up, (6) lateral pull down, and (7) leg press. Back press, arm curl, and sit-up were performed using free weights and bench press; leg extension, lateral pull down, and leg press were performed on the UESAKA weight machine.

Assay

Blood samples were obtained to measure the markers of bone metabolism and blood lactate. Serum osteocalcin was determined by specific two-site radioimmunoassay for human osteocalcin (ELSA-OSTEO, CIS Bio International, Bagnols, France). Serum bone-specific alkaline phosphatase (B-ALP) activity and tartrate-resistant acid phosphatase (TRAP) activity were measured with a colorimetric method that uses p-nitrophenyl phosphate as a substrate (Monotest ALPopt and Iso ALP, Boehringer Mannheim, Germany for B-ALP; Wako Pure Chemical Industries, Japan for TRAP test), and plasma procollagen type-I C-terminal (P1CP) was determined by radioimmunoassay (Procollagen P1CP, Orion Diagnostica, Finland). Blood lactate was measured by an enzymatic method after whole blood was deproteinized with 0.6 N perchloric acid. Serum total protein was measured with a refraction method as an index of blood water content [33]. All blood samples were stored at −60°C until assayed.

Each 24-hour urine sample was divided into two portions: one-half was preserved in acid for measurement of calcium and the other half was maintained under a thin layer of toluene for the determination of renal net acid excretion and deoxypyridinoline (DPYR). Urinary calcium was determined by inductively coupled argon plasma atomic emission spectrophotometer (ICAP-757V Nippon Jarrell-Ash, Japan), and ammonium (NH_4^+) was determined on samples of urine deproteinized with sodium phosphotungstate by colorimetric assay (Ammonia test Wako, Wako Pure Chemical Industries, Japan). Urine titratable acidity minus bicarbonate (TA-HCO_3^-) was determined by a double titration procedure [14]. Renal net acid excretion was calculated as the sum of the urine [NH_4^+] and [TA-HCO_3^-]. Urinary DPYR was measured by enzyme-immuno assay (Pyrilinks-D, Metra Biosystems Inc., USA). Urine samples were stored at −45°C until analyzed. All analyses of blood and urine samples were performed in duplicate.

Data Analysis

The procedures of the SAS Institute were used for statistical analyses. Comparisons between time points were made using repeated-measures analysis of variance and post hoc comparisons by Dunnett's test. Linear regression analyses were carried out to determine the relation between urinary calcium excretion and renal net acid excretion. The values given in the text are means ± SEM, and the $P < 0.05$ level of significance was used.

Results

After an intense bout of resistance exercise, blood and urine became more acidic. Blood lactate concentration significantly increased from 7.6 ± 1.0 mg/dl at rest to 21.6 ± 1.6 mg/dl immediately after the completion of the resistance exercise. Renal net acid excretion also significantly increased on the exercise day (Table 1).

Urinary calcium excretion significantly increased on the exercise day (Table 1). When the percent changes in urinary calcium excretion were plotted against percent changes in renal net acid excretion after the exercise, a significant positive correlation was demonstrated (r = 0.68) (Fig. 1). Urinary DPYR excretion decreased on days 1 and 3 after the exercise. The decrease was significant 3 days after the exercise (Fig. 3). There was no significant change in urine volume or urinary creatinine excretion throughout the experimental period (Table 1).

Plasma P1CP concentration started to decrease on the exercise day and decreased further on the day after the exercise. By the end of the recovery period, the value returned to the control level. Serum B-ALP activity was significantly decreased 2 and 3 days after the exercise. There was no significant change in serum osteocalcin concentration (Fig. 2). Serum TRAP activity slightly decreased on the exercise day followed by a significant decrease the next day of the exercise. By the end of the recovery period, the value returned to the resting level. There was no significant change in serum total protein concentration throughout the experimental period (data not shown).

Discussion

The aim of the present study was to investigate whether a single bout of resistance exercise could cause observable changes in urinary calcium excretion and bone metabolism as indicated by sensitive biomarkers of bone formation and resorption. Bone formation was evaluated by measuring three proteins derived from bone-forming osteoblasts. The present study showed that a bout of resistance exercise significantly decreased plasma P1CP concentration and serum B-ALP activity with no change in serum osteocalcin con-

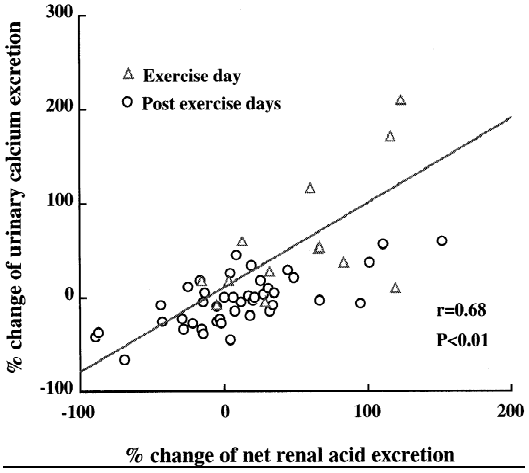

Fig. 1. Correlation between percent changes of urinary calcium excretion and renal net acid excretion after a single bout of resistance exercise. Each value on the exercise day and 3 post-exercise days were all plotted.

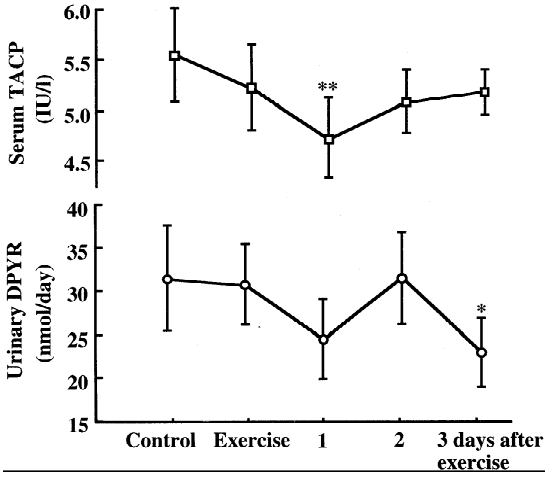

Fig. 3. Effect of a single bout of resistance exercise on serum TRAP and urinary DPYR. Values are means ± SEM. $*P < 0.05$; $**P < 0.01$; significantly different from control value.

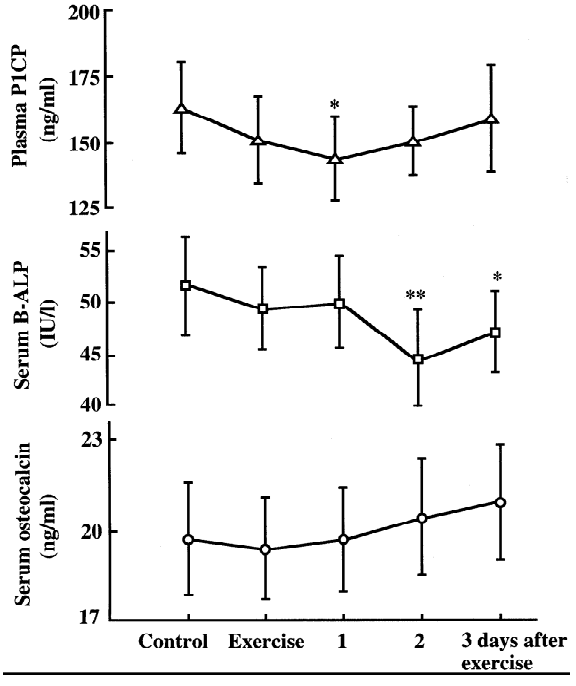

Fig. 2. Effect of a single bout of resistance exercise on plasma P1CP, serum B-ALP, and serum osteocalcin. Values are means ± SEM. $*P < 0.05$; $**P < 0.01$, significantly different from control value.

centration. The decreases in those markers of bone formation were unsuspected, because a single loading, using four-point bending and stainless steel pins inserted into caudal vertebrae, caused increase in double-labeled surface and mineral apposition rate in rat caudal vertebrae [15, 16] and in rat right tibiae [17, 18]. This apparent conflicting result may be explained by differences in the manner of imposing the load on bone. High intensity exercise such as used in this study increases the production of lactate at skeletal muscle, accompanying the generation of an equivalent number of protons that acidify the intracellular and extracellular environments. In fact, our subjects experienced a pronounced lactacidosis of 21.6 mg/dl lactate immediately after exercise and 72.6 mEq/day net renal acid excretion on the exercise day. On the other hand, previous animal studies using four-point bending or load with stainless steel pins imposed mechanical load directly on bone without muscle contraction. It is reported that metabolic acidosis suppresses osteoblastic activity, and *in vitro* studies showed that metabolic acidosis inhibited osteoblastic collagen synthesis and alkaline phosphatase activity in cultured bone [11, 19]. Lanyon [20, 21] suggested that adaptation of bone to mechanical strain was modulated by the environmental conditions surrounding bone tissue, including systemic and local hormones and nutrient status. Therefore it is possible that the osteogenic response to mechanical stimulation derived from a single bout of resistance exercise was abolished under conditions of lactic acidosis.

Urinary excretion of DPYR that derives mainly from the decomposition of bone collagen matrix and TRAP that is released from osteoclast during bone resorption were transiently decreased after the exercise. This may suggest that in spite of lactic acidosis, which potentially stimulates bone resorption, a single bout of high intensity resistance exercise transiently suppressed osteoclastic activity, and our observation is consistent with previous reports using mechanical loading which observed decreased osteoclast surface [15] and eroded surface [16] 10 days after the loading.

Another principal finding in the present study was an increase in urinary calcium excretion after resistance exercise and its strong positive correlation with net renal acid excretion, supporting the important role of acid accumulation in increasing urinary calcium excretion. It is well known that metabolic acidosis increases urinary calcium excretion in humans without a measurable increase in intestinal calcium absorption [22, 23], and the mechanism is purported to be a direct action of acidosis on renal tubular reabsorption of calcium [24]. Furthermore, increase in urinary calcium excretion was not accompanied by the increase in urinary DPYR excretion, therefore the source of the excreted calcium might not be derived via activation of osteoclast. Bushinsky et al. [25] reported that when neonatal mouse calvaria was cultured in medium with a reduced pH

and bicarbonate concentration, there was a net efflux of calcium from the bone due to a decrease in the physicochemical driving forces for mineralization in short-term (3 hours) cultures, whereas cell-mediated calcium efflux became evident in long-term cultures (>24 hours) [9]. Short-term acidosis such as that observed in this study (exercise session lasted only 45 minutes) may induce calcium release from bone because of changes in physicochemical factors. Nevertheless detailed studies are required in order to clarify the source of calcium.

In longitudinal and cross-sectional studies in humans, resistance exercise increases bone mineral density with increased bone formation [26, 27]. On the other hand, a single bout of resistance exercise decreased markers of bone formation with increased urinary calcium excretion. These suggest the possibility that there is some physiological adaptation process to increase bone mass with repeated cycles of exercise. One explanation might be that strength training increases lactate threshold and reduces blood lactate concentration at the same relative exercise intensities during submaximal exercise [28]. Furthermore, it was also reported that the capacity to transport lactate is higher in athletes than in untrained and less trained subjects [29]. These suggest that the negative effects of lactic acidosis on calcium and bone metabolism might be minimized or cleared in trained athletes, allowing an osteogenic response with loading to emerge. Another explanation for physiological adaptations to increase bone density may relate to alterations in hormonal factors. It was reported that male muscle builders have a higher circulating level of 25OHD [3, 30], $1,25(OH)_2D$ [30], IGF-1 [3], and PTH [31] than normal control subjects. Those reports concluded that it is conceivable that higher circulating levels of these hormones in muscle builders may modulate bone remodeling in favor of net bone formation.

The length of this study did not provide enough time to reflect the osteogenic response to exercise. Virttanen et al. [32] showed that short-term, high-intensity, concentric exercise decreased serum P1CP 1 hour after the exercise but increased it 2 days after the exercise, and concluded that the later increase in P1CP could reflect an adaptive process in bones after the exercise. In this regard, a more prolonged experiment should be carried out to evaluate the effect of a single bout of resistance exercise on bone metabolism.

In conclusion, the early response of bone to a bout of resistance exercise in untrained males resulted in transient decrease in bone formation and resorption, whereas urinary calcium excretion increased. It seems that a longer time span should be covered in future studies to evaluate the late effects of resistance exercise on bone biomarkers.

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