A bout of resistance exercise increases urinary calcium independently of osteoclastic activation in men

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Ashizawa, Noriko, Rei Fujimura, Kumpei Tokuyama, and Masashige Suzuki. A bout of resistance exercise increases urinary calcium independently of osteoclastic activation in men. *J. Appl. Physiol.* 83(4): 1159–1163, 1997.—Metabolic acidosis increases urinary calcium excretion in humans as a result of administration of ammonium chloride, an increase in dietary protein intake, and fasting-induced ketoacidosis. An intense bout of exercise, exceeding aerobic capacity, also causes significant decrease in blood pH as a result of increase in blood lactate concentration. In this study we investigated changes in renal calcium handling, plasma parathyroid hormone concentration, and osteoclastic bone resorption after a single bout of resistance exercise. Ten male subjects completed a bout of resistance exercise with an intensity of 60% of one repetition maximum for the first set and 80% of one repetition maximum for the second and third sets. After exercise, blood and urine pH shifted toward acidity and urinary calcium excretion increased. Hypercalciuria was observed in the presence of an increased fractional calcium excretion and an unchanged filtered load of calcium. Therefore, the observed increase in urinary calcium excretion was due primarily to decrease in renal tubular reabsorption of calcium. Likely causes of the increase in renal excretion of calcium are metabolic acidosis itself and decreased parathyroid hormone. When urinary calcium excretion increased, urinary deoxypyridinoline, a marker of osteoclastic bone resorption, decreased. These results suggest that *1*) strenuous resistance exercise increased urinary calcium excretion by decreasing renal tubular calcium reabsorption, *2*) urinary calcium excretion increased independently of osteoclast activation, and *3*) the mechanism resulting in postexercise hypercalciuria might involve non-cell-mediated physicochemical bone dissolution.

lactic acidosis; renal tubular reabsorption; bone resorption

METABOLIC ACIDOSIS increases urinary calcium excretion in humans as a result of administration of ammonium chloride (1, 22), an increase in dietary protein intake (23), and fasting-induced ketoacidosis (18) without a measurable increase in intestinal calcium absorption (1, 22, 29). Because the vast majority of body calcium is contained in bone (30), the primary candidate for the excess urinary calcium appears to be bone calcium (5, 29). Indeed, when neonatal mouse calvaria was cultured in medium with a reduced pH and HCO_3^- concentration, an in vitro model of metabolic acidosis, there was a net efflux of calcium from the bone (8, 11).

Exercise is known to influence bone mass and density, which are significantly higher in athletes than in age-matched nonexercisers. Among the exercises, it has been suggested that those that require heavy lifting with a few repetitions, which is analogous to strength training, may provide the optimal stimuli for increase in bone mineral density (BMD) (28). In this regard, cross-sectional and longitudinal studies have revealed in some cases that resistance-exercise training increases BMD (14, 20). On the other hand, such high-intensity exercise is associated with a transient accumulation of lactic acid (15), which titrates extracellular and intracelluler buffer systems, including the bone. The degree of the acidosis induced by a strenuous exercise, although lasting for only a few hours, was greater than that induced by ammonium chloride or protein ingestion (1, 22, 23), which has been shown to disturb mineral homeostasis. While resistance-exercise training appears to increase BMD in the long term, theoretically a single bout of the resistance exercise could paradoxically induce bone mineral dissolution and consequently increase urinary calcium output, secondary to metabolic acidosis. Therefore, the first purpose of this study was to examine whether hypercalciuria is induced by a single bout of resistance exercise.

In addition, there appear to be two pathways of calcium efflux from bone with metabolic acidosis: non-cell-mediated physicochemical mineral dissolution (7, 12) and osteoclastic bone resorption (2, 21). Therefore, the second purpose of this study was to examine whether the exercise-induced hypercalciuria, if it occurs, is mediated through alterations in physicochemical factors or through alterations in cell-mediated calcium efflux from bone.

MATERIALS AND METHODS

Subjects. A total of 10 Oriental male subjects signed a consent form to participate in this investigation. The physical characteristics of the subjects were the following: age, 24.3 ± 0.9 yr; height, 171.3 ± 2.2 cm; body weight, 70.1 ± 2.8 kg; and body fat, 20.7 ± 1.3%. Most of the subjects had recreational experience with resistance training, but none had participated in any regular exercise program for at least 2 yr. All of the subjects were in good health and taking no medications that would alter calcium or bone homeostasis. There were three smokers among the subjects.

Experimental protocol. The study was conducted for a total of 6 days. The subjects consumed a standardized diet containing 840 mg/day of calcium throughout the experimental period, and the first 4 days of the experimental period were designed for adjustment to the standardized diet. Deionized water was provided ad libitum throughout the experimental period. Urine samples were obtained both on *day 5* (the control day) and *day 6* (the exercise day). On the exercise day, the subjects rested for >30 min after arrival at the laboratory. After the subjects voluntarily emptied their bladders, the resting urine sample was collected for 30 min. From the onset of exercise at 1600, four 1-h urine samples were collected to 2000. To make a complete 24-h urine collection, another portion of urine (2000–1530) was also collected. Blood specimens were obtained at the midpoint of each urine collection period for the later analysis of clearances, except for first hour

after the onset of exercise, at which time blood was drawn immediately after completion of exercise. An additional blood sample was obtained at 15 min after the end of exercise (Fig. 1). On the control day, a 24-h urine collection took place during the same time period.

Exercise program. During the preliminary experimental period, the subjects performed each exercise with low intensity and learned accurate lifting form. One week before the experimental period, a one-repetition maximum (1-RM) test was performed for each exercise with a warm-up of less than five repetitions at 40–60% of the perceived maximum, followed by increasing loads until the weight could no longer be fully lifted by using correct form. The maximum weight lifted correctly was defined as the 1 RM. On the exercise day, the subjects followed 45 min of a resistance-exercise program consisting of three sets of seven exercises. 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. The intensity of the exercises was 60% of 1 RM for first set and 80% of 1 RM for second and third sets, with 1 min of rest between sets (16). Deionized water intake was allowed ad libitum throughout exercise protocol and recovery.

Assay. Blood samples were divided into four aliquots. One aliquot was transferred to a heparinized plastic tube and then covered with liquid paraffin and centrifuged immediately. The plasma was stored on ice for later analysis of ionized calcium by using an ion-selective electrode. Another aliquot of whole blood was analyzed shortly after collection for hematocrit by using the microhematocrit method and for hemoglobin by using an automated counter (Sysmex F300 Toa). Relative changes in plasma volume were calculated by using hematocrit ratios and hemoglobin concentrations (19). Another whole blood aliquot was deproteinized with 0.6 N perchloric acid to allow measurement of lactate with an enzymatic method. From the remaining blood, plasma and serum were prepared. Serum creatinine was determined by the alkaline picrate method, serum albumin by the bromocresol green method, serum calcium by the methylxylenol blue method, and serum phosphate by the Fiske-Subbarow method (Wako Pure Chemical Industries, Osaka, Japan). Plasma parathyroid hormone (PTH) was determined by two-site immunoradiometric assay (CIS Bio International, Bagnols). Albumin-corrected serum total calcium values were calculated by using a conventional formula (25). All blood samples were stored at −60°C until duplicate analysis.

Each voided urine specimen was divided into two portions. One-half was preserved in acid for measurement of calcium, and the other one-half was maintained under a thin layer of toluene for the determination of renal net acid excretion, creatinine, phosphorus, and deoxypyridinoline. Urinary calcium and phosphorus were determined by an inductively coupled argon plasma atomic emission spectrophotometer (model ICAP-757V, Nippon Jarrell-Ash, Kyoto, Japan), and NH_4^+ was determined on samples of urine deproteinized with sodium phosphotungstate by colorimetric assay (Wako Pure Chemical Industries). Urine titratable acidity minus HCO_3^- ($TA - HCO_3^-$) was determined by a double-titration procedure (24). Urinary creatinine was determined by the alkaline picrate method, and urinary deoxypyridinoline was determined by enzyme immunoassay (Pyrilinks-D, Metra Biosystems). Urine samples were stored at −45°C until analyzed, and the analyses were performed in duplicate.

Renal net acid excretion was calculated as the sum of the urine NH_4^+ and ($TA - HCO_3^-$). The creatinine clearance was assumed equal to glomerular filtration rate (GFR). Filtered load of calcium was calculated as the product of plasma ionized calcium concentration and the GFR, and fractional calcium excretion was calculated as clearance of ionized calcium divided by the GFR (3, 13).

Data analysis. The procedures of the SAS Institute were used for statistical analyses. Comparisons between time points were made by using repeated-measures analysis of variance and post hoc comparisons by Dunnett's test. Comparison of 24-h urinary calcium values on the control day and the exercise day was tested by using paired *t*-test, and linear regression analyses were carried out to determine the relationship between fractional calcium excretion and renal net acid excretion. The values given in the text are means ± SE, and the $P < 0.05$ level of significance was used.

RESULTS

After an intense bout of resistance exercise, blood and urine shifted toward acidity. Blood lactate concentration reached its peak immediately after the completion of resistance exercise and remained significantly elevated during the following 45 min (Table 1). Renal net acid excretion also significantly increased during first 2 h after the onset of exercise (Fig. 2).

Urinary calcium excretion significantly increased from 198.2 μg/min at rest to 321.6 and 350.9 μg/min during the second and third hours after the onset of exercise, respectively (Fig. 2). Urinary calcium excretion added up to 165 ± 34 mg for 24 h on the control day and 281 ± 39 mg on the exercise day ($P < 0.01$).

Plasma ionized calcium concentration decreased immediately after the completion of exercise, followed by an increase during second hour after the onset of exercise. Albumin-corrected serum total calcium significantly increased during exercise. Serum phosphate

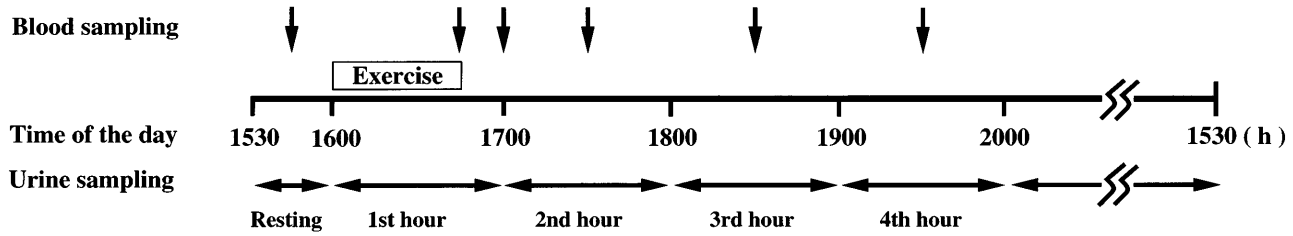

Fig. 1. Outline of study protocol during rest, exercise, and recovery periods on exercise day. Resistance exercise began at 1600 after 30 min of baseline urine collection and blood sampling. From onset of exercise, four 1-h urine samples were collected, and venous blood specimens were obtained at midpoint of each urine collection period except for 1st h from onset of exercise, at which time blood was drawn immediately after exercise. An additional blood sample was also obtained at 15 min after completion of exercise. To make a complete 24-h urine collection, another portion of urine (at 2000–1530) was also collected.

Table 1. *Blood parameters during the experimental period*

		1st Hour				
	Rest (−60 min)	0 min	15 min	2nd Hour (45 min)	3rd Hour (105 min)	4th Hour (165 min)
Ionized calcium, mg/dl	4.67 ± 0.05	4.42 ± 0.08†	4.60 ± 0.03	4.88 ± 0.04*	4.79 ± 0.05	4.83 ± 0.06
Total calcium (albumin corrected), mg/dl	9.64 ± 0.10	10.11 ± 0.12†	9.98 ± 0.11	9.95 ± 0.12	9.77 ± 0.11	9.77 ± 0.12
Albumin, g/dl	4.87 ± 0.06	5.35 ± 0.06†	5.12 ± 0.07†	5.07 ± 0.06*	4.91 ± 0.06	4.98 ± 0.05
Phosphate, mg/dl	3.55 ± 0.12	3.44 ± 0.16	2.69 ± 0.19†	2.18 ± 0.18†	1.88 ± 0.17†	2.47 ± 0.14†
Lactate, mg/dl	7.38 ± 0.92	21.40 ± 1.41†	16.24 ± 0.90†	12.36 ± 0.86†	8.56 ± 0.67	7.47 ± 0.47
PTH, pg/ml	14.17 ± 2.91	19.46 ± 4.66	16.10 ± 3.72	10.00 ± 1.93	8.72 ± 2.17*	10.87 ± 1.79
Plasma volume, %Δ		−14.17 ± 3.80†	−4.37 ± 4.50	−1.14 ± 4.73	0.87 ± 4.55	0.70 ± 3.34

Values are means ± SE of 10 subjects. Time in minutes is minutes after completion of resistance exercise. PTH, parathyroid hormone; %Δ, percent change. * Significantly different from resting values, $P < 0.05$. † Significantly different from resting values, $P \leq 0.01$.

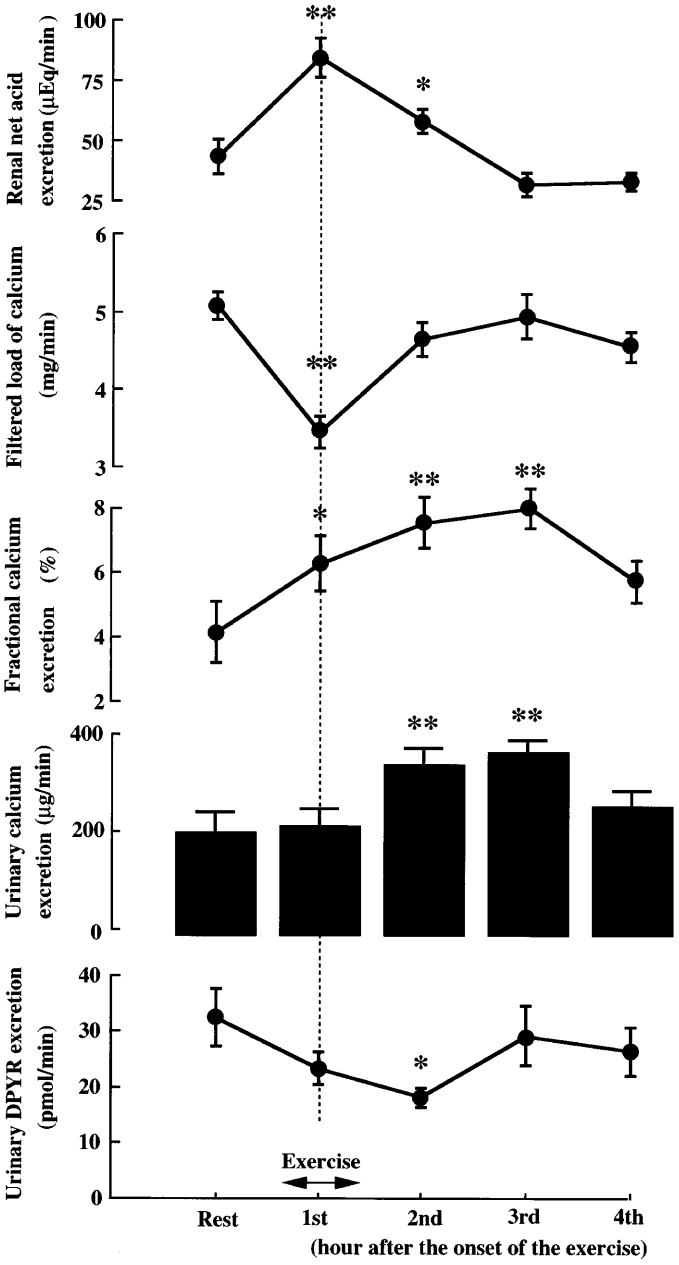

Fig. 2. Renal net acid excretion, filtered load of calcium, fractional calcium excretion, urinary calcium excretion, and urinary deoxypyridinoline (DPYR) excretion before, during, and after resistance exercise. Values are means ± SE. *Significantly different from initial value, $P < 0.05$. **Significantly different from initial value, $P < 0.01$.

concentration began to decrease 15 min after the completion of exercise and further decreased during the following 3 h. Plasma PTH concentration slightly increased immediately after the completion of exercise, followed by a significant decrease during third hour after the onset of exercise. Plasma volume significantly decreased by 14% during the first hour, and serum albumin significantly increased during first 2 h after the onset of exercise (Table 1).

Urinary deoxypyridinoline started to decrease during the first hour and further decreased during the second hour after the onset of exercise. By the end of the recovery period, the value returned toward the resting level (Fig. 2). Urinary phosphorus excretion significantly decreased during the second, third, and fourth hours after the onset of exercise. GFR estimated from creatinine clearance significantly decreased during the first hour after the onset of exercise (Table 2). Consequently, the filtered load of calcium estimated from plasma ionized calcium concentration and GFR was significantly decreased during the first hour after the onset of exercise. Fractional calcium excretion significantly increased during first 3 h after the onset of exercise (Fig. 2).

DISCUSSION

Metabolic acidosis is well known to increase urinary calcium excretion in human as a result of administration of ammonium chloride (1, 22), an increase in dietary protein intake (23), and fasting-induced ketoacidosis (18). As expected, in the present study, urinary calcium excretion significantly increased after the onset of resistance exercise with the development of a severe metabolic acidosis.

Urinary calcium excretion can be increased by an increase in the filtered load of calcium, a decrease in fractional renal reabsorption of calcium, or a combination of the two. In the present study, hypercalciuria was observed in the presence of an increased fractional calcium excretion and unchanged filtered load of calcium. Therefore, the observed increase in urinary calcium excretion caused by the exercise was due primarily to the decrease in renal tubular reabsorption of calcium.

There are several possible mechanisms by which a decrease in renal tubular calcium reabsorption may occur. One possibility is that acidosis itself directly impaired the renal tubule calcium handling. A previous

Table 2. *Urinary components and creatinine clearance during the experimental period*

	Rest	1st Hour	2nd Hour	3rd Hour	4th Hour
Urine pH	5.99 ± 0.09	5.31 ± 0.07*	5.21 ± 0.06	6.01 ± 0.30	5.97 ± 0.28
Urine volumes, ml/min	1.64 ± 0.48	0.84 ± 0.08	0.69 ± 0.06	1.16 ± 0.24	1.48 ± 0.38
Ammonium, μeq/min	19.50 ± 2.79	44.34 ± 2.83*	33.76 ± 3.33*	23.88 ± 3.47	24.17 ± 2.68
TA − HCO_3^-, μeq/min	23.97 ± 4.76	40.26 ± 5.33*	24.60 ± 2.23	7.86 ± 1.57*	8.81 ± 1.48*
Phosphorus, mg/min	1.05 ± 0.23	1.14 ± 0.24	0.35 ± 0.06*	0.11 ± 0.03*	0.17 ± 0.05*
Creatinine clearance, ml/min	108.19 ± 3.24	77.59 ± 3.64*	94.93 ± 4.05	103.36 ± 6.97	95.00 ± 4.79

Values are means ± SE of 10 subjects. TA − HCO_3^-, urine titratable acidity minus bicarbonate. * Significantly different from resting values, $P < 0.01$. No significance was found at the $P < 0.05$ level.

study in dogs showed that renal tubule fluid bicarbonate directly augmented renal calcium reabsorption (26). A significant correlation between fractional calcium excretion and renal net acid excretion ($r = 0.62$, $P < 0.01$) for the first 2 h after the onset of exercise also supports this notion. Although urinary net acid excretion returned to the control value, enhanced fractional calcium excretion was continuously observed until the third hour after the onset of exercise. This decrease in tubular calcium reabsorption that occurred during the second and third hours after the onset of exercise can be explained by a marked decrease in plasma PTH concentration. The decrease in plasma PTH was probably related to an increase in plasma ionized calcium. When exercise is completed, both serum phosphate concentration and urinary phosphorus excretion decreased, possibly reflecting a slight respiratory alkalosis caused by hyperventilation. The decrease in serum phosphate could possibly result in a sharp elevation in ionized calcium with predictable effects on PTH levels. A last point is that other than these two consecutive processes, many hormonal changes accompanying exercise, such as those of catecholamines and calcitonin, may contribute to renal tubular calcium handling and the effects of these changes remain to be studied.

What might be the source of the extra calcium being excreted in the urine after resistance exercise? It is unlikely that extracellular fluid compartments played a significant role in increased urinary calcium because when plasma volume and ionized calcium concentration decreased immediately after exercise, hypercalciuria was not observed. The most likely source of the excreted calcium is bone, because the bone is a reservoir of labile base in the form of alkaline salts of calcium (17) that can be mobilized for the defense of blood pH and plasma HCO_3^- concentrations (4, 17). In vitro studies demonstrated that the influx of protons from extracellular fluid into bone took place along with efflux of calcium from the bone into the circulation in both acute and chronic metabolic acidosis (8, 11). Furthermore, chronic metabolic acidosis was shown to induce urinary calcium excretion and consequently induced bone loss in a variety of species (3, 5). Moreover, Rubin and Bennett (27) reported that plasma calcium levels rose abruptly after intense muscular activity in vertebrates with bony skeletons but not in those with cartilaginous skeletons. We also have shown a significant increase in albumin-corrected serum total calcium without decrease in urinary calcium excretion during exercise.

Two possible mechanisms could be responsible for the bone dissolution by exercise-induced lactic acidosis. One possibility is the cell-mediated osteoclastic bone resorption (2, 21). If it was responsible for the change in urinary calcium excretion, one would expect a rise in urinary deoxypyridinoline as a marker of bone resorption. In contrast, there was a significant decrease in urinary deoxypyridinoline excretion when urinary calcium excretion significantly increased. It is, therefore, more likely that the mechanism resulting in postexercise hypercalciuria might simply involve non-cell-mediated physiochemical bone dissolution (8, 9), which is the dissolution of a fraction of the crystalline calcium hydroxyapatite compartment, independent of osteoclast activation. Indeed, when neonatal mouse calvaria was cultured in medium with a reduced pH and HCO_3^- 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-h) cultures (9, 10), whereas there was cell-mediated calcium efflux as well in chronic cultures (>24 h) (7). Short-term acidosis as such observed in this study may induce non-cell-mediated physiochemical bone dissolution.

An alternative source might be via enhanced absorption of calcium from the gut. Our study was not designed to assess the effects of exercise-induced lactic acidosis on calcium absorption from the gut; however, this possibility would appear unlikely in view of the fact that most authors failed to detect an increase of intestinal calcium absorption in complete metabolic studies in acute and chronic metabolic acidosis (1, 22, 29). Nevertheless detailed studies utilizing a variety of techniques are required to address this possibility conclusively.

In conclusion, our study demonstrated that *1*) strenuous exercise increased urinary calcium excretion by decreasing renal calcium reabsorption, *2*) urinary calcium excretion increased independent of osteoclast activation, and *3*) the mechanism resulting in postexercise hypercalciuria might simply involve non-cell-mediated physicochemical bone dissolution.

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