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Acute Effects of an Oral Calcium Load on Markers of Bone Metabolism During Endurance Cycling Exercise in Male Athletes

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Abstract. Although sport and physical activity are generally considered as positive factors for bone metabolism some endurance trainings such as running and bicycling have few or no beneficial or even deleterious effects on bone mineral density. The present study was designed to investigate the acute effect of an intensive endurance cycling exercise on biochemical bone markers. Futrthermore, the effect of the oral intake of 1g calcium load, by drinking high-calcium mineral water, just prior to and during the exercise was checked. Twelve well-trained elite male triathletes aged 23-37 years were explored. The serum concentrations of calcium, phosphate, PTH, bone alkaline phosphatase (BALP) and C-terminal cross-linking telopeptide of type 1 collagen (CTX) were measured before, during and after a 60 min 80% VO_2max cycle ergometer exercise. Since cycling exercise was accompanied by a reduction in plasma volume the total amount of biochemical bone markers was calculated. When the exercise was performed without calcium load both serum concentrations and total amount of CTX began to increase progressively 30 min after the start of the exericse and were still significantly elevated, by 45-50%, 2h after the end of the exercise. Ingestion of high-calcium mineral water completely suppressed the CTX reponse. By contrast serum concentrations and total amount of BALP fluctuated and showed no significant difference with or without calcium load. The present study demonstrates that the burst of osteoclastic activity acutely induced by an endurance cycling exercise can be suppressed by the previous intake of a calcium load afforded by drinking high-calcium mineral water.

Key words: Calcium — Bone resorption markers — CrossLaps — Sport

There is increasing evidence that physical activity is important in the development and maintenance of bone mineral density (BMD) [1]. Cross-sectional studies have shown that BMD is significantly higher in athletes than in sedentary controls, particularly in the highly strained parts of the skeleton [2]. Both retrospective and interventional studies have shown that weight-bearing and high-impact exercises are very effective in increasing BMD at sites affected by strains [3, 4]. However, the BMD of athletes participating in non–weight-bearing activities like swimming does not differ from that noninactive subjects [5]. Likewise, endurance training such as running and bicycling has few or no beneficial or even deleterious effects on bone density: In male distance runners, lumbar bone mineral content (BMC) was found to be lower than in controls [6] and was negatively correlated to the weekly distance run [7]. Similarly, endurance cyclists have a low lumbar spine bone density when compared with controls [8], and this decrease in lumbar spine density was found to be as large as 10% by some authors [9]. Furthermore, it has has been shown that cycling does not improve bone mass in youngsters [10].

Because in addition to increased physical activity optimal calcium intake is considered a major factor in maximizing peak bone mass and minimizing bone loss, studies have been undertaken to examine any interaction between physical activity and calcium intake. Results of a meta-analysis including seventeen interventional trials have demonstrated a beneficial effect of calcium intake on lumbar spine BMD when associated with physical activity, but only at calcium intakes exceeding 1000 mg/day [11]. We, therefore, decided to investigate the acute effects on bone metabolism of intensive endurance bicycling, and to determine whether the simultaneous intake of calcium could modify these effects. Biochemical bone markers can be used to evaluate the acute effects of exercise on the skeleton [12–15]; therefore, we have chosen to measure two specific and sensitive bone markers: bone alkaline phosphatase (BALP), as a marker of osteoblastic bone formation, and C-terminal cross-linking telopeptide of type I collagen (CTX), as a marker of osteoclastic bone resorption. These markers were chosen to follow the effects of endurance exercise in well-trained triathletes and to ascertain whether ingest-

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ing, just before and during the exercise, repeated quantities of high-calcium mineral water (for a total 972-mg calcium load) could acutely affect the markers of bone metabolism. The use of mineral water as a source of calcium is justified by the need to assure good hydration during intensive physical exercise.

Materials and Methods

Athletes

Twelve male caucasian well-trained endurance triathletes participated in the study. They once performed at least 6 to 12 hours per week of aerobic training (competitions not included). The mean age was 30.7 ± 4.2 years (range, 23–37 years), their mean height was 180.3 ± 4.0 cm (range, 165–198 cm), and their mean weight was 71.0 ± 3.9 kg (range, 62–80 kg). Their mean maximal oxygen uptake (VO_2max) was 61.7 ± 6.3 ml/kg b.w./min. The subjects provided written informed consent for the procedures, which were approved by the local hospital ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale).

Maximal Oxygen Uptake Measurements

Prior to the trials, the volunteers had an initial medical examination and performed a maximal exercise test on a cycle ergometer to determine aerobic threshold and maximal oxygen uptake (VO_2max), according to the American College of Sports Medicine guidelines [16]. Following the VO_2max test, a workload was selected that would elicit approximately 80% VO_2max.

Study Design

The study consisted of two exercise tests randomly distributed and separated by time intervals of at least one week. Between the two sessions the subjects were advised to continue their usual training and diet, particularly during the 3 days preceeding each session. The evening prior to the exercise test day they avoided all calcium-rich food or drink. Training and dietary records were filled in by the subjects to contribute to an accurate replication of similar dietary and exercise behaviors for all trials. Each exercise test proceeded according to the following scheme: the subjects ate a standardized continental breakfast (600 kcal, no dairy product) between 6.00 and 6.30 and came to the laboratory at 8.00. A venous catheter was placed into a forearm vein for blood sampling. Each test began at 8.30 and was divided into three periods: a one-hour rest period (from 8.30 to 9.30), a one-hour exercise period (from 9.30 to 10.30) and a two-hour recovery period (from 10.30 to 12.30). Blood was collected at 8.30, 9.00, 9.30, 10.00, 10.30, 11.00, 11.30, and 12.30.

Exercise

The subjects warmed up on a ergometer cycle for 5 minutes at 30% VO_2max before the ergometer workload was increased to a level requiring 80% VO_2max. Then, the subjects exercised at this workload for one hour. These exercises and workloads were chosen to realize a long-lasting and intensive exercise until exhaustion.

Calcium Intake and Hydration

High-calcium mineral water (486 mg/L) was ingested in 250 mL fractions every 15 minutes, beginning at 8.30 and ending at 10.15. The total volume of high-calcium water was 2 L, and the total amount of calcium was 972 mg. In the control assay, hydration of the subjects was obtained by ingesting 2 L of low-calcium mineral water (9 mg/L) administered according to the same sequence as for high-calcium mineral water.

Analyses

Serum CTX was measured using a one-step enzyme immunological test (Serum CrossLaps®, Osteometer Biotech A/S, Denmark). It recognizes two cross-linked amino acid sequences of EKAHD-ß-GGR, where the aspartic acid residue is ß-isomerized. Intraassay variation is $<7\%$ and interassay variation is $<10\%$ in the explored range of concentrations [17]. Serum bone BALP was measured by using a two-site immunoradiometric assay (Tandem®-R Ostase™, Beckman-Coulter, USA) specific for BALP with a $<15\%$ cross-reactivity with hepatic phosphatase Intraassay variation is $<5\%$ and interassay variation is $<7.5\%$ for concentrations ranging from 11.7 to 52.3 µg/L [18]. Serum parathyroid hormone (PTH) was measured by using an immunoradiometric assay for intact PTH (CIS biointernational, France); intraassay variation $<5\%$ and interassay variation $<7\%$ in the explored range of concentrations. Hematocrit was used to account for the changes in plasma volume and was measured by using microcentrifugation. Serum calcium and phosphate concentrations were measured with use of conventional methods.

Calculations

The raw hematocrit readings were multiplied by the factor (0.96×0.91) to correct for trapped plasma and to convert venous hematocrit to whole-body hematocrit. The formula given by van Beaumont et al. [19] was used to estimate the changes in plasma volume and to correct the concentrations of biochemical substances. The formula is based on measurement of hematocrit before (Ht1) and after (Ht2) the exercise and of the initial concentrations (C1) to calculate the expected concentration (CE).

$$CE = \frac{HT2(100 - Ht1)}{Ht1(100 - Ht2)} \times C1$$

The relative change in plasma content (ΔCo) or total amount was calculated from the relationship between the measured (CM) and the expected concentration (CE)

$$\Delta Co(\%) = \frac{CM - CE}{CE} \times 100$$

Statistical Analyses

The data were expressed as mean ± SD unless otherwise stated. For comparison between the different time-courses of changes in bone markers, repeated-measures two-factor ANOVA with interaction was performed by using the General Linear Models Procedure. The main effects were time (of sampling) and treatment (low-calcium water versus high-calcium water), with individuals as a random factor. When the time-by-treatment interaction was statistically significant ($P < 0.05$), Bonferroni *t*-tests of differences between means of treatment were performed at the global level of probability of 0.05. The statistical program used was StatView 5.0 from SAS Institute Inc.

Results

The time-courses of changes in hematocrit are presented in Figure 1. The mean hematocrit increased from 41.2%

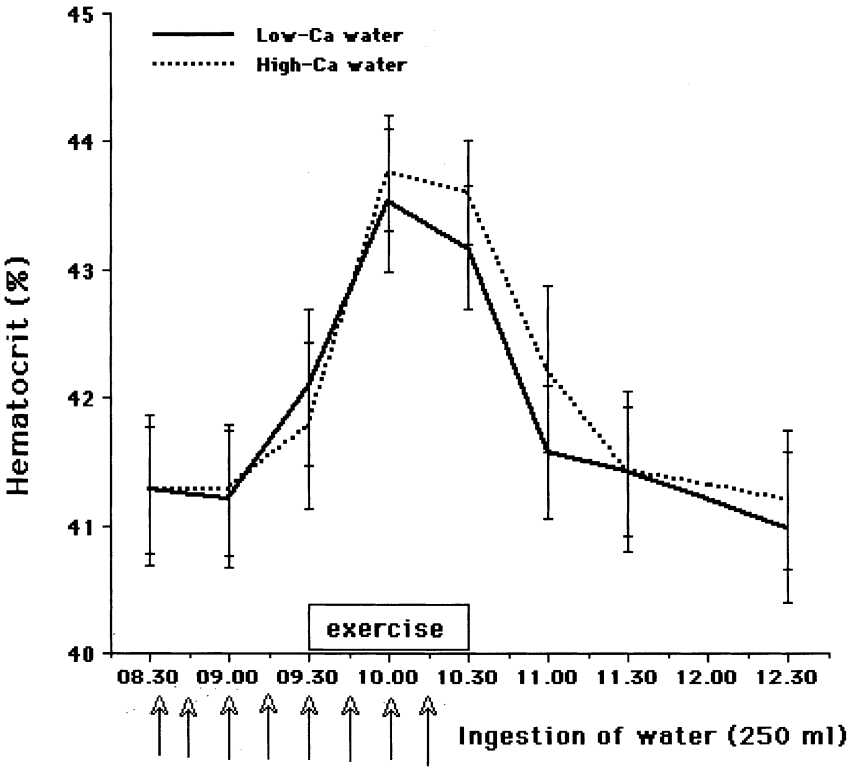

Fig. 1. Changes in hematocrit during one-hour 80% VO_2max cycling exercise. The subjects ingested either high-or low-calcium water. The values are mean ± SEM.

at rest to 43% to 44% ($P < 0.001$) 30 minutes after the start of the exercise (10.00), and it remained elevated until the end of the exercise (10.30). The calculated mean plasma volume decreased by 9% at 10.00 and by 9.9% at 10.30. There was no statistically significant difference between the two series of assays. The mean values in serum calcium, phosphate, pH, and PTH are presented in Table 1. When the subjects drank low-calcium water, the serum concentrations of calcium remained constant until the end of the exercise (10.30) and decreased slightly 30 minutes later (11.00). When they drank high-calcium water, the serum concentrations of calcium began to increase at 10.00 and reached their peak (2.51 mmol/L) at 10.30, before returning to basal values (2.35 mmol/L) at 11.00. A statistically significant rise (2.5–3 times) of serum PTH occurred during the exercise, culminating at 10.00 and at 10.30. This PTH response was partially suppressed by the calcium load. At the same time, there was a statistically significant increase in serum phosphate level ($P < 0.001$) in both series of assays. The variations of serum CTX concentrations are presented in Figure 2. When the subjects drank low-calcium water, there was a 48% increase which was completely suppressed by the intake of calcium. Similarly, when adjusted for the shifts in plasma volume, the total content of CTX (Figure 3) showed a 46% increase, which was significantly (see Table 2) suppressed by the intake of calcium. The adjusted total content of serum bone alkaline phosphatase showed no significant variations, and the intake of calcium had no significant effect.

Discussion

Both retrospective and cross-sectional studies have shown that weight-bearing exercises, such as weight-lifting, high-impact exercises, and gymnastics, improve BMD, particularly at the loaded sites [4, 20–22]. Furthermore, prospective studies have provided evidence that high BMD values characteristic of gymnasts are the result of high-impact loading rather than the consequence of a selection bias [4]. By contrast, the subjects who practice nonloading physical activities such as running, swimming, or cycling (all three are performed by triathletes) have BMD values lower than those of the subjects who practice either high-impact or weight-bearing physical activities [5, 23]. Among these nonloading activities, cycling appears to have the worst effects on BMD: In a group of *Tour de France* bikers, BMD was lower by 10% in the lumbar spine, 14% in the hip, and 17% in the Ward's triangle than in age-matched male controls [9]. In a comparison of the total and regional BMD of competitive cyclists and runners it was shown that cyclists have lower spine BMD than do controls [8]. This was confirmed in adolescent female elite athletes [24].

Because an optimal calcium intake is another factor known to maximize peak bone mass and to minimize bone loss, the possibility that combining calcium and high-impact and/or weight-bearing exercise might have additive effects on bone mass was addressed. An interaction between calcium intake and physical activity on

Table 1. Time-course of changes in mean values (mean ± SD) of serum biochemical and hormonal variables

	Time of sampling						
	08.30	09.00	09.30	10.00	10.30	11.00	11.30	12.30
Low-calcium water	Mean values							
Serum calcium (mmol/L)	2.394 ± 0.166	2.382 ± 0.179	2.403 ± 0.087	2.403 ± 0.061	2.403 ± 0.048	2.332 ± 0.149	2.337 ± 0.125	2.326 ± 0.123
Serum phosphate (mmol/L)	1.057 ± 0.202	1.049 ± 0.138	1.098 ± 0.144	1.455 ± 0.246	1.502 ± 0.250	1.253 ± 0.185	1.177 ± 0.135	1.194 ± 0.139
pH	7.400 ± 0.026	7.394 ± 0.028	7.379 ± 0.030	7.376 ± 0.041	7.397 ± 0.053	7.406 ± 0.043	7.417 ± 0.040	7.431 ± 0.030
PTH (pg/mL)	22.56 ± 6.58	24.51 ± 8.96	24.16 ± 10.43	60.67 ± 38.03	62.77 ± 34.47	31.01 ± 11.92	24.42 ± 9.24	27.92 ± 12.12
High-calcium water	Mean values							
Serum calcium (mmol/L)	2.333 ± 0.107	2.279 ± 0.087	2.347 ± 0.100	2.462 ± 0.090	2.509 ± 0.107	2.354 ± 0.102	2.353 ± 0.120	2.410 ± 0.103
Serum phosphate (mmol/L)	1.072 ± 0.155	1.075 ± 0.148	1.123 ± 0.141	1.580 ± 0.275	1.592 ± 0.275	1.278 ± 0.172	1.173 ± 0.133	1.245 ± 0.155
pH	7.397 ± 0.027	7.382 ± 0.032	7.363 ± 0.027	7.358 ± 0.049	7.386 ± 0.042	7.399 ± 0.032	7.418 ± 0.031	7.420 ± 0.019
PTH (pg/mL)	22.81 ± 7.10	21.37 ± 5.10	17.39 ± 4.47	37.47 ± 18.41	35.94 ± 16.94	18.90 ± 6.98	14.25 ± 4.33	15.71 ± 5.41

Cycle ergometry exercise was performed from 09.30 to 10.30 and 250 mL of either high-calcium or low-calcium water was ingested at every 15-minute interval from 08.30 to 10.15
PTH, Parathyroid hormone

BMD was inferred by Bonnie Specker from a meta-analysis that included seventeen trials [11]. Although there is evidence of a beneficial effect on BMD from both high-impact activity and high intakes of calcium, the demonstration of an interaction between these two factors cannot be stated unambiguously. For instance, in studies of women at different ages, a moderate level of impact physical activity and a sufficient level of calcium intake resulted in long-term improvement of the skeleton, but no significant interaction between these two factors could be demonstrated [25, 26]. A recent interventional and prospective study combining moderate-impact exercise and calcium supplementation in premenarchal girls showed a main effect of exercise at the loaded sites, femur and tibia-fibula, and a main effect of calcium at the nonloaded sites, humerus and radius-ulna, suggesting that impact exercise confers region-specific effects and calcium generalized effects on BMC [27].

To our knowledge, no studies have been published concerning a possible interaction between nonloading endurance physical activity, like bicycling, and calcium supplements. Most of the cross-sectional and longitudinal studies have used the measurement of BMD (or BMC) as an indicator of bone mass gain, but the response to exercise or to calcium supplements takes months or years to be detected with precision. The measurement of biochemical markers of bone that give insight into the cellular metabolism [28] may elucidate earlier the relationship between physical exercise and bone metabolism. As shown by the present results, the response of serum biochemical bone markers is rapid, since a statistically significant increase in serum CTX occurred one hour after the start of the training. Conversely, serum CTX can be suppressed within one hour after ingestion of calcium [29].

Some studies have used the measurement of biochemical bone markers to check the effects of endurance exercises on bone metabolism. However, their conclusions are confounded because of different duration, intensity, and types of exercise [30–33]. Furthermore, the time-points of blood or urine collection and the measured biochemical markers were different.

In contrast to outdoor exercises, indoor exercises on either treadmill or ergometer bicycle allow precise measurement of the intensity of exercise and easy collection of blood samples, so that the acute changes in biochemical parameters can be continuously monitored. For example; (1) thirty minutes of brisk treadmill walking induced a significant increase (by 40%) in urinary markers of resorption (pyridinoline and deoxypyridinoline) the day of the exercise and the day after, whereas no changes in level of either osteocalcin or alkaline phosphatase could be found 0.5, 1, 8, 24 and 32 hours after exercise [15]; (2) 17 male subjects performed a 30-minute ergometer cycling exercise, consisting of

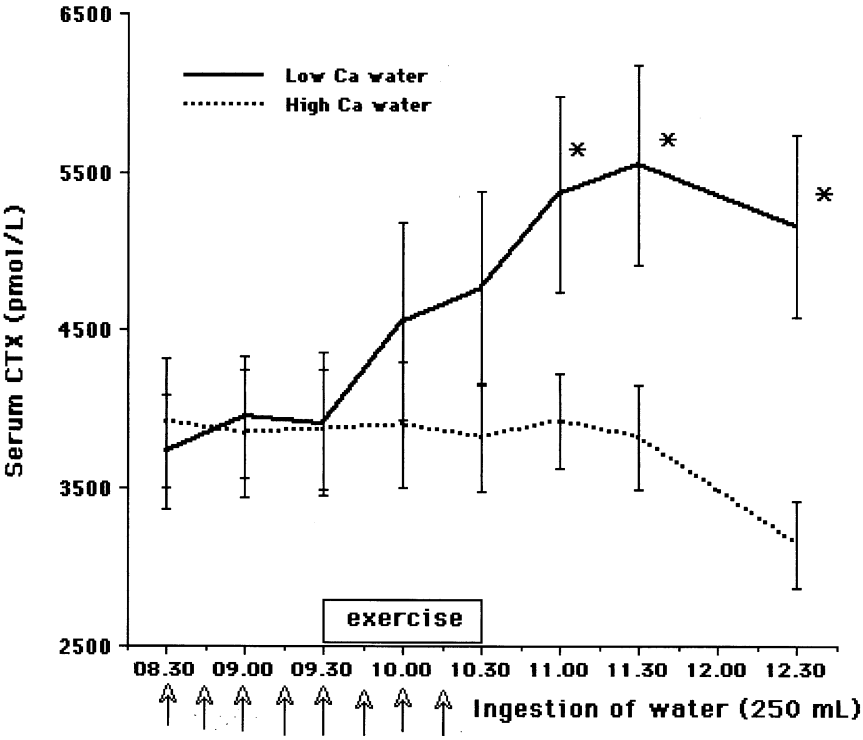

Fig. 2. Changes in concentrations of CTX during and after one-hour 80% VO_2max cycling exercise. The values are mean ± SEM. *Bonferroni *t*-tests of difference between means ($P < 0.05$) were used to compare, at each time-point, the effects of ingestion of high-calcium mineral water with those of low-calcium mineral water.

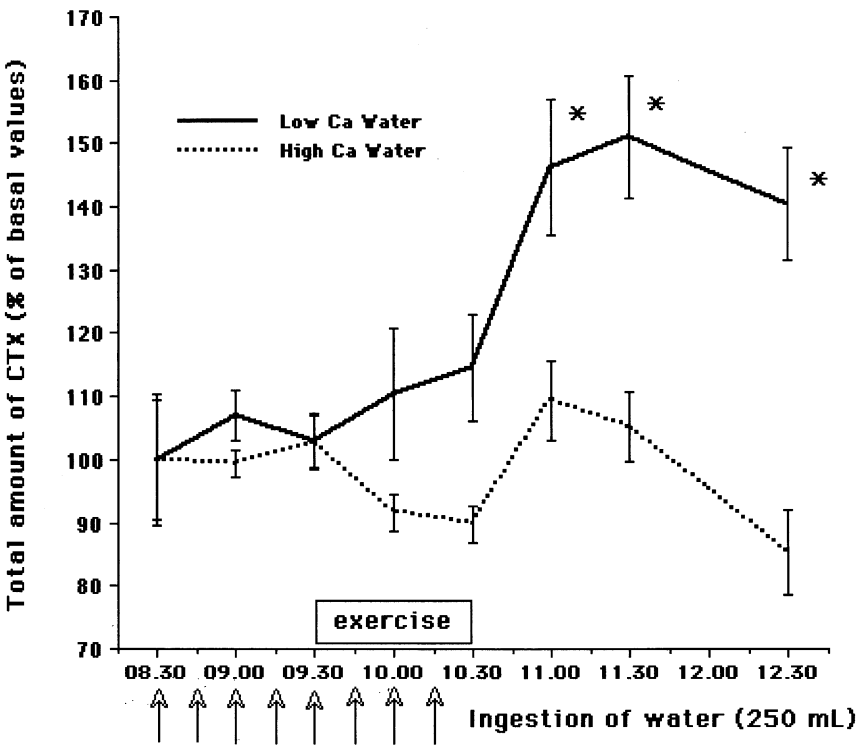

Fig. 3. Percentage changes in total amount of CTX during and after one-hour 80% VO_2max cycling exercise. The values are mean ± SEM. *Bonferroni *t*-tests of difference between means ($P < 0.05$) were used to compare at each time-point the effects of ingestion of high-calcium mineral water with those of low-calcium mineral water.

two, 5-minute warm-up stages and a 20-minute stage at 80% VO_2max during which blood was sampled at the start of the exercise and at 15-minute intervals during 2 hours after the start of the exercise. No change in osteocalcin level but a very transient increase at the end of the exercise in bone alkaline phosphatase, PICP, and PIIIP levels (all formation markers) were observed, whereas ICTP concentrations increased progressively by 10% at the end of the exercise and remained elevated thereafter [34]. In the present study, the time-course of changes in osteoclastic activity, as assessed by measurement of serum CTX, was precisely followed, before,

Table 2. Statistical analysis using repeated-measures two-factor analysis of variance with interaction for comparing the effects of ingestion of high-calcium water versus low-calcium water on concentrations of serum PTH and CTX and on the total amount of CTX during a one-hour endurance cycling exercise

Variable		Treatment *P* value	Time *P* value	Treatment* Time *P* value
Serum PTH	High-Ca vs. Low-Ca H_2O	0.0017	<‰0.0001	<‰0.0001
Serum CTX	High-Ca vs. Low-Ca H_2O	0.0195	<‰0.0001	<‰0.0001
Total amount of CTX	High-Ca vs. Low-Ca H_2O	0.0004	<‰0.0001	<‰0.0001

CTX, C-terminal cross-linking of telopeptide of type I collagen; PTH, parathyroid hormone

during, and until 2 hours after the end of exercise. The CTX response to exercise was speedy, since the serum concentrations began to rise 30 minutes after the start of exercise and reached a significantly increased (by 48%) level one hour after the end of exercise. The extent of this acute resorption effect is comparable to that of reference 15, but is much larger than that of reference 34. The difference could be due to the use of CTX, instead of ICTP, as a marker of resorption. Serum CTX, a recently developed assay, has been demonstrated to be a specific and very sensitive marker of the inhibition of osteoclastic activity after ingestion of calcium [35], and to successfully predict the bone metabolic response in patients who are receiving antiresorptive therapy [36].

Acutely elevated (from 50% to 120% of initial values) serum concentrations of PTH have been reported to occur during or immediately after intensive endurance physical exercises such as long distance running or cycling [31–33, 37–39]. In the present study, one-hour, ergometer cycling at 80% of VO_2max resulted in a high stimulation of PTH (maximal increase, 270% of initial values). This tremendous response could be related to the intensity and duration of the exercise, as compared to other reports, and also to the training of these elite athletes, since it has been shown that training enhances the PTH response to physical exercise [12, 40].

Although serum calcium is the main recognized factor regulating PTH secretion [41], the mechanism by which exercise stimulates PTH secretion is not well understood. The reported effects of physical exercise on serum calcium level are controversial. Some authors observed a drop in serum calcium [42], whereas others found either no alteration of serum calcium [41] or a rise in ionized and/or total serum calcium concentrations [40, 43, 44]. The sport-induced increase in both serum calcium and PTH levels has led some authors to conclude that the release of PTH does not involve serum calcium [38, 39]. In the present study, the assay without calcium load (ingestion of low-calcium water) showed a large increase in PTH despite the absence of any simultaneous decrease in serum calcium concentration. It should be remembered that during intensive exercise, two physiological events occur i.e., loss of calcium by sweating and increase in the hematocrit. The concentration of calcium in sweat induced by exercise in a hot, humid environment in healthy young men has been found to be 1.3 ± 0.9 mmol/L [45], and the sweat loss of calcium has been estimated between 45 mg and 247 mg, during an acute bout of moderate intensity [46] and per basket-ball training session, respectively [47, 48].

In the present experiment, calcium concentration measured in 45 samples was found to be 4.85 ± 1.93 mmol/L (mean ± SD) but the volume of sweat, allowing the estimation of total loss, could not be measured. Because the serum calcium levels remained constant throughout the period of exercise in the present study of non–calcium-loaded athletes, we can suppose that the loss of calcium was of the same order of magnitude as the relative decrease in plasma volume (≈10%). The contradictory reported effects of endurance exercise on serum calcium level might, therefore, be explained by the relative variations in plasma volume and in the loss of calcium in sweat. As evidence of the importance of the loss of calcium in sweat, we observed a decrease in serum calcium concentration 30 minutes after the end of the exercise cycle (at 11.00). A possible explanation could be that, at this time, the plasma volume, as indicated by hematocrit measurements, had already returned to basal value, whereas the loss of calcium in sweat had not yet been compensated. We may, therefore, hypothesize that during endurance exercise the PTH response is provoked by the loss of calcium in sweat. Serum phosphate concentrations rose significantly and similarly during the exercise sessions. This elevation was not proportionate to PTH variation, which excluded serum phosphate level at least in the present experiment, as a stimulator of PTH [49]. Variations in serum phosphate level might have been provoked by the release of phosphate from muscular ATP and/or creatine-phosphate.

Our previous studies demonstrated that the oral intake of either 1 g of calcium as calcium citrate tablets or even 170 mg of calcium as high-calcium mineral water can efficiently suppress urinary and serum CTX [29, 50]. In the present study, we show for the first time that the oral intake of a total dose of 972 mg of calcium, divided into eight repeated ingestions, completely suppressed the exercise-induced rise in CTX, as a likely consequence of the partial suppression of PTH. The use of high-calcium mineral water has the major advantage of providing a

good hydration, which is needed during intensive endurance exercise. Furthermore, the intake of high-calcium mineral water can be easily fractionated, as in the present experiment, into small doses of calcium which have been shown to be better absorbed [51].

As suggested by the results of the present study, the deleterious effects on some nonloaded bone sites, such as the lumbar spine, in athletes who perform endurance exercises like cycling could be explained by the acute bone resorption that follows prolonged intensive exercise. Ingestion of calcium prior to endurance exercise could, therefore, be a preventive measure. Further long-term assays are necessary to check the effects of calcium intakes before and during endurance exercise on the lumbar spine BMD of master cyclists. This trial could be particularly justified in adolescents who practice cycling [10].

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References

1. Chilibeck PD, Sale DG, Webber CE (1995) Exercise and bone mineral density. Sports Med 19:103–122
2. Heinonen A, Oje P, Kannus P, Sievänen H, Mänttäri A, et al. (1993) Bone mineral density of female athletes in different sports. Bone Miner 23:1–14
3. Etherington J, Harris PA, Nandra D, Hart DJ, Wolman RL, et al. (1996) The effect of weight-bearing exercise on bone mineral density: a study of female ex-elite athletes and the general population. J Bone Miner Res 11:1333–1338
4. Taaffe DR, Robinson TL, Snow CM, Marcus R (1997) High-impact exercise promotes bone gain in well-trained female athletes. J Bone Miner Res 12:255–260
5. Taaffe DR, Snow-Harter C, Connolly DA, Robinson TL, Brown MD, et al. (1995) Differential effects of swimming versus weight-bearing activity on bone mineral status of eumenorrheic athletes. J Bone Miner Res 10:586–593
6. Bilanin JE, Blanchard MS, Russek-Cohen E (1989) Lower vertebral bone density in male long distance runners. Med Sci Sports Exerc 21:66–70
7. Hetland ML, Haarbo J, Christiansen C (1993) Low bone mass and high bone turnover in male long distances runners. J Clin Endocrinol Metab 77:770–775
8. Stewart AD, Hannan J (2000) Total and regional bone density in male runners, cyclists, and controls. Med Sci Sports Exerc 32:1373–1377
9. Sabo D, Bernd L, Pfeil J, Reiter A (1996) Bone quality in the lumbar spine in high-performance athletes. Eur Spine J 5:258–263
10. Rico H, Revilla M, Hernandez ER, Gomez-Castresana F, Villa LF (1993) Bone mineral content and body composition in postpubertal cyclists boys. Bone 14:93–95
11. Specker BL (1996) Evidence for an interaction between calcium intake and physical activity on changes in bone mineral density. J Bone Miner Res 10:1539–1544
12. Salvesen H, Piehl-Aulin K, Ljunghall S (1994) Change in levels of the carboxyterminal propeptide of type I procollagen, the carboxyterminal cross-linked telopeptide of type I collagen and osteocalcin in response to exercise in well-trained men and women. Scand J Med Sci Sports 4:186–190
13. Brahm H, Piehl-Aulin K, Ljunghall S (1997) Bone metabolism during exercise and recovery: the influence of plasma volume and physical fittness. Calcif Tissue Int 61:192–198
14. Rong H, Berg U, Torring O, Sundberg CJ, Granberg B, et al. (1997) Effect of acute endurance and strength exercise on circulating calcium-regulating hormones and bone markers in young healthy males. Scand J Med Sci Sports 7:152–159
15. Welsh L, Rutherford OM, James I, Crowley C, Comer M, et al. (1997) The acute effects of exercise on bone turnover. Int J Sports Med 18:247–251
16. American College of Sports Medicine (1995) ACSM's Guidelines for Exercise Testing and Prescription (5th ed). Williams and Wilkins, Baltimore
17. Bonde M, Garnero P, Fledelius C, Ovist P, Delmas PD et al. (1997) Measurement of bone degradation products in serum using antibodies reactive with an isomerized form of 8 amino acid sequence of the C-telopeptide of type I collagen. J Bone Miner Res 12:1028–1034
18. Panigrahi K, Delmas PD, Singer F, Ryan W, Reiss O et al. (1994) Characteristics of a two-site immunoradiometric assay for human skeletal alkaline phosphatase in serum. Clin Chem 40:822–828
19. van Beaumont W, Strand JC, Petrofsky JS, Hipskind SG, Greenleaf JE (1973) Changes in total plasma content of electrolytes and proteins with maximal exercise. J Appl Physiol 34:102–106
20. Bassey EJ, Ramsdale SJ (1994) Increase in femoral bone density in young women following high-impact exercise. Osteoporos Int 4:472–475
21. Bassey EJ, Ramsdale SJ (1995) Weight-bearing exercise and ground reaction forces—a 12 month randomized controlled trial of effects on bone mineral density in healthy postmenopausal women. Bone 18:405–410
22. Etherington J, Harris PA, Nandra D, Hart DJ, Wolman RL, et al. (1996) The effect of weight-bearing exercise on bone mineral density: A study of female ex-elite athletes and the general population. J Bone Miner Res 11:1333–1338
23. Robinson TL, Snow-Harter C, Taaffe DR, Gillis D, Shaw J, et al. (1995) Gymnasts exhibit higher bone mass than runners despite similar prevalence of amnorrhea and oligomenorrhea. J Bone Miner Res 10:26–35
24. Duncan CS, Blimkie CJ, Cowell CT, Burke ST, Briody JN, et al. (2002) Bone mineral density in adolescent female athletes: relationship to exercise type and muscle strength. Med Sci Sports Exerc 34:286–294
25. Uusi-Rasi K, Sievänen, Vuori I, Pasanen M, Heinonen A, et al. (1998) Associations of physical activity and calcium intake with bone mass and size in healthy women at different ages. J Bone Miner Res 13:133–142
26. Uusi-Rasi K, Sievänen H, Pasanen M, Oja P, Vuori I (2002) Associations of calcium intake and physical activity with bone density and size in premenopausal and postmenopausal women: a peripheral quantitative computed tomography study. J Bone Miner Res 17:544–552
27. Iuliano-Burns S, Saxon L, Naughton G, Gibbons K, Bass SL (2003) Regional specificity of exercise and calcium during skeletal growth in girls: a randomized controlled trial. J Bone Miner Res 18:156–162
28. Garnero P, Sornay-Rendu E, Duboeuf F, Delmas PD (1999) Markers of bone turnover predict postmenopausal forearm bone loss over 4 years: the OFELY study. J Bone Miner Res 14:1614–1621
29. Guillemant J, Le HT, Accarie C, Tezenas du Montcel S, Delabroise AM, et al. (2000) Mineral water as a source of dietary calcium: acute effects on parathyroid function and bone resorption in young men. Am J Clin Nutr 71:999–1002
30. Malm HT, Ronni-Sivula HM, Viinikka LU, Ylikorkala OR (1993) Marathon running accompanied by transient

decreases in urinary calcium and serum osteocalcin levels. Calcif Tissue Int 52:209–211
31. Langberg H, Skovgaard D, Asp S, Kjaer M (2000) Time pattern of exercise-induced changes in type I collagen turnover after prolonged endurance exercise in humans. Calcif Tissue Int 67:41–44
32. Crespo R, Revilla M, Villa LF, Usabiaga J, Leibar X, et al. (1999) Transient dissociation of bone metabolism induced by high performance exercise: a study in elite marathon runners. Calcif Tissue Int 64:287–290
33. Thorsen K, Kristoffersson A, Hultdin J, Lorentzon R (1997) Effects of moderate endurance exercise on calcium, parathyroid hormone, and markers of bone metabolism in young women. Calcif Tissue Int 60:16–20
34. Wallace JD, Cuneo RC, Lundberg PA, Rosén T, Jorgensen JOL, et al. (2000) Responses of bone markers and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. J Clin Endocrinol Metab 85:124–133
35. Villa I, Saccon B, Rubinacci A (2000) Response to osteoclast activity inhibition assessed by the determination of C-telopeptide of type I collagen in serum. Clin Chem 46:560–576
36. Christgau S, Bitsch-Jensen O, Hanover Bjarnason N, Gamwell Henriksen E, Qvist P, et al. (2000) Serum CrossLaps for monitoring the response in individuals undergoing antiresorptive therapy. Bone 26:505–511
37. Grimston SK, Tanguay KE, Gundberg CM, Hanley DA (1993) The calciotropic hormone response to changes in serum calcium during exercise in female long distance runners. J Clin Endocrinol Metab 76:867–872
38. Salvesen H, Johansson AG, Foxdal P, Wide L, Piehl-Aulin K, et al. (1994) Intact serum parathyroid hormone levels increase during running exercise in well-trained men. Calcif Tissue Int 54:256–261
39. Tsai KS, Lin JC, Chen CK, Cheng WC, Yang CH (1997) Effect of exercise and exogenous glucocorticoid on serum level of intact parathyroid hormone. Int J Sports Med 18:583–587
40. Zerath E, Holy X, Douce P, Guezennec CY, Chatard JC (1997) Effect of endurance training on postexercise parathyroid hormone levels in elderly men. Med Sci Sports Exerc 29:1139–1141
41. Brown EM (1982) PTH secretion *in vivo* and *in vitro*: regulation by calcium and other secretagogues. Miner Electrolyte Metab 8:130–150
42. Ljunghall S, Joborn H, Roxin LE, Rastad J, Wide L, et al. (1986) Prolonged low-intensity exercise raises the serum parathyroid hormone levels. Clin Endocrinol (Oxf) 25:535–542
43. Kristoffersson A, Hultdin J, Holmlund I, Thorsen K, Lorentzon R (1995) Effects of short-term maximal work on plasma calcium, parathyroid hormone, osteocalcin and biochemical markers of collagen metabolism. Int J Sports Med 16:145–149
44. Ljunghall S, Joborn H, Roxin LE, Skarfors ET, Wide LE, et al. (1988) Increase in serum parathyroid hormone levels after prolonged physical exercise. Med Sci Sports Exerc 20:122–125
45. Shirrefs SM, Maughan RJ (1997) Whole body sweat collection in humans: an improved method with preliminary data on electrolyte content. J Appl Physiol 82:336–341
46. Bullen DB, O'Toole ML, Johnson KC (1999) Calcium losses resulting from an acute bout of moderate-intensity exercise. Int J Sport Nutr 9:275–284
47. Klesges RC, Ward KD, Shelton ML, Applegate WB, Cantler ED, et al. (1996) Changes in bone mineral content in male athletes. Mechanisms of action and intervention effects. JAMA 276:226–230
48. Klesges RC, Ward KD, Harmon-Clayton K (1997) Errata. JAMA 277:24
49. Reiss E, Canterbury JM, Bercovitz MA, Kaplan EL (1970) The role of phosphate in the secretion of parathyroid hormone in man. J Clin Invest 49:2146–2149
50. Guillemant J, Le HT, Maria A, Guillemant S (2000) Acute effects of oral calcium load on parathyroid function and bone resorption in young men. Am J Nephrol 20:48–52
51. Heaney RP, Weaver CM, Fitzsimmons ML (1990) Influence of calcium load on absorption fraction. J Bone Miner Res 5:1135–1138