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ORIGINAL CONTRIBUTION

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Evidence for an acute rise of intestinal calcium absorption in response to aerobic exercise

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Summary *Background* The acute effects of physical activity on intestinal calcium (Ca) uptake and on bone metabolism are not known. *Aim of the study* To investigate the consequences of an acute aerobic exercise bout on fractional Ca absorption and on biomarkers of bone turnover. *Methods* With the use of a cross over design, eighteen male athletes, aged 25.2 (SE 0.6) years, either had to perform a 60 min run (70 % of maximal speed) or had to rest for 60 min. Intestinal Ca absorption (Fc_{240}) was assessed by the use of a stable strontium test. Moreover, calciotropic hormones and serum C-Telopeptide (CTx), a biomarker of bone collagen degradation, and serum C-terminal propeptide of type I collagen (PICP), a marker of bone collagen formation, were measured prior (t_{-60}) and 3 hours after (t_{240}) exercise or rest. *Results* Fc_{240} values were significantly enhanced in response to exercise compared to rest (16.2 ± 0.7 % vs. 14.6 ± 0.8 %; $P < 0.05$). PICP values were significantly lower in response to exercise compared to rest: –9.8 % ($P < 0.05$). Exercise did not influence serum levels of intact parathyroid hormone and calcitriol. Serum CTx levels decreased markedly between t_{-60} and t_{240} during both intervention periods (both P values < 0.001), the results being in line with the circadian rhythm of serum CTx. *Conclusions* A moderate exercise bout can induce an acute rise in fractional Ca absorption. Moreover, even in endurance-trained young men a moderate exercise bout acutely decreases bone collagen formation, while the physiologic fluctuations of the bone resorption marker CTx remain unaffected.

Key words strontium – calcium absorption – endurance exercise – bone turnover

Introduction

Weight-bearing physical exercise is important to maintain or increase bone mass [1–2]. Even moderate exercise is related to an enhanced bone mineral density in peripubertal boys and also in young men compared to controls with a low level of physical activity [3, 4]. Animal studies have demonstrated that such an increase in bone mass is the result of an enhanced formation of organic bone matrix and a higher apposition rate of minerals [5] such as calcium (Ca).

A moderate level of physical exercise can already acutely influence various Ca metabolic parameters in untrained human subjects: Alterations can include a decrease in ionized serum Ca levels [6, 7] and an increase in serum parathyroid hormone (PTH) levels [6]. In well-trained long distance runners the release of PTH is immediately enhanced by high and low intensity exercise through a mechanism that does not involve serum Ca

[8]. Since PTH is an activator of renal calcitriol synthesis these changes should also lead to *functional* alterations in Ca metabolism. However, we are not aware of data about the acute effect of an exercise bout on intestinal Ca uptake. Moreover, the acute effect of moderate exercise on organic bone matrix is not completely clear. In untrained female subjects, moderate endurance exercise resulted in an acute decrease of the bone formation marker propeptide of type I procollagen (PICP) [7] and in an acute increase in the bone resorption marker carboxyterminal telopeptide of type I collagen (CTx) [9]. In trained adult men, submaximal exercise was associated with increased serum level of bone formation and resorption markers immediately after the test procedure was finished [10].

It was the aim of the present study to evaluate the acute effect of a single moderate aerobic exercise bout on intestinal Ca absorption and to expand the knowledge on the acute effect of aerobic exercise on bone collagen markers.

Materials and Methods

Subjects

The study group consisted of eighteen male athletes (triathletes, game sports and track and field sports) with a mean age of 25.2 (SE 0.6) years, a body height of 181 (SE 1.2) cm, and a body mass index of 23.0 (SE 0.3) kg/m^2. Inclusion criterion was a minimum of sports activities of eight hours per week. Mean physical activity of the study group was 15.7 h (SE 1.7 h) per week (questionnaire). All subjects were nonsmokers. Written informed consent was obtained by each participant. The study protocol was approved by the Ethics committee of the German Sports University, Cologne, Germany.

Study protocol

The study was performed at the Department of Cardiology and Sports Medicine, German Sports University, Cologne. The experimental design is given in Fig. 1.

Performance test

At study commencement (designated pre-study), all subjects participated in an outdoor performance test as described elsewhere [11]. Briefly, subjects started to run at a speed of 2.0 m/s for 5 min. To assess individual endurance capacity speed was increased by 0.5 m/s after each run. Subjects had to continue with the test until exhaustion, in order to reach a blood lactate level of at least 4 mmol/L. This threshold was used since no steady state in blood lactate concentrations can be obtained above this level [12]. Lactate was measured in 20 µl probes of capillary blood from the earlobe. Additionally, pulse rate was determined during each run.

Assessment of nutrient intake and urine sampling

All foods and beverages eaten during the last 4 days before the rest period and the exercise period had to be listed in a prospective food record. The nutrient content of the diets was quantified using the computer program Ebis (Hohenheim, Germany) which is based on the German data collection "Bundeslebensmittelschlüssel II". Reliability of the food record has previously been demonstrated [13]. On the last protocol day a 24 h urine sample was collected from 07:00 h until 07:00 h. The first morning urine of the examination day had to be added to the collection period.

Test procedure

The time interval between the pre-study and intervention period 1/2 was 2 weeks. With the exception of the 60min run, subjects had to stay at the Department of Cardiology and Sports Medicine from 08:00 h until 13:30 h. After an overnight fast (designated t_{-60}), a blood sample of 10 ml was collected from the antecubital vein into serum monovettes without stasis in sitting position. Then, body mass was measured (shorts, cotton shirt) with a precision of 50 g and body composition was determined by bioimpedance analysis at 50 kHz (BIAMED, Cologne, Germany). At 09:00 h a standardized breakfast (a 50 g roll, 5 g butter, 200 mL apple juice) was served. Together with the breakfast, participants received 2.27 mmol of strontium (Sr) chloride hexahydrate (Merck, Wiesbaden, Germany), dissolved in the 200 ml apple juice, to assess Ca absorption rates [13]. With the use of a cross over design, subjects were then randomly assigned to 2 groups. Subjects either had to perform an aerobic 60 min run or had to rest for 60 min. The individual burden during the exercise was chosen for each participant in such a manner that by means of pulse control a speed was maintained, which corresponded to 70 % of the speed at 4 mmol/l blood lactate (see performance test). During three hours after resting or exercise subjects were not allowed to eat and drink or to perform additional physical activities. Then (four hours after the Sr-containing breakfast and three hours after rest or exercise, designated t_{240}), body mass was determined again and a second blood sample of 10 ml was taken. Aliquots of samples were frozen consecutively and were stored at –20°C until analysis.

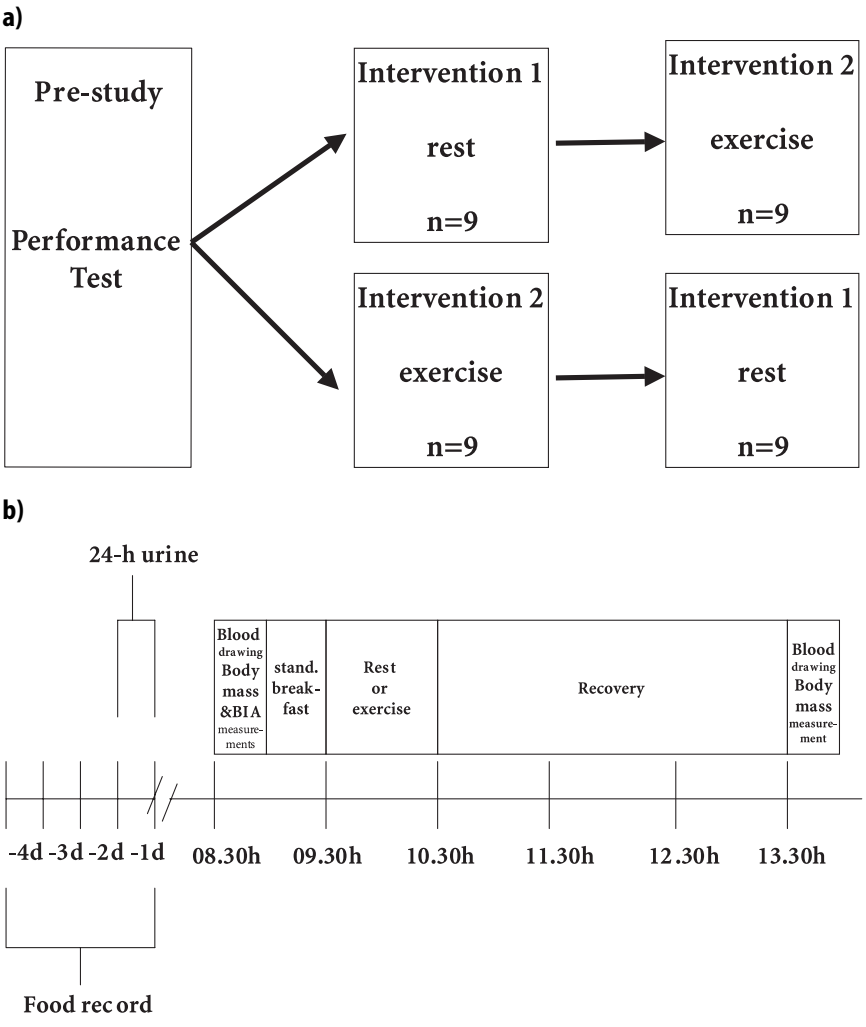

Fig. 1 Experimental design; **A**, Overall study design. **B**, Protocol for interventions 1 and 2

Analytical procedures

Biochemical parameters

All samples of each individual were measured in duplicate during the same assay sequence. Fasting serum levels of intact PTH and of CTx were determined with ELISA test kits supplied by DRG diagnostics (Marburg, Germany). The intra- and inter-assay coefficients of variation (CVs) were less than 5 % and 8 %, respectively. Serum calcitriol was measured with a test kit supplied by Immundiagnostic (Bensheim, Germany). Briefly, calcitriol samples were extracted using a double column technique and subsequently analyzed by a tritium labeled protein binding assay with calf thymus cytosol as receptor. Intra- and inter-assay CVs were 5.4 % and 9.3 % respectively. Serum Sr was measured by means of graphite furnace atomic absorption spectrophotometry (HGA–600, Perkin Elmer, Überlingen, Germany). The CV within a day was 4.8 % and that from day-to-day was 3.9 %, respectively. Serum and urine Ca was assessed using flame atomic absorption spectrometry and serum albumin concentrations were measured by a colorimetric test kit (BioMerieux, Nürtingen, Germany). CVs were all below 2.5 %. Total serum Ca was corrected with ± 0.11 mM for each 0.100 mM deviation of concomitant serum albumin from a normal mean of 0.600 mM (designated Ca_{adj}) [14]. The serum PICP levels were measured by an enzyme linked immuno assay supplied by Metra Biosystems, Osnabrück, Germany. Intra-assay and inter-assay CVs were 6.5 % and 7.2 %, respectively. Serum protein levels were measured by a test kit supplied by Boehringer, Mannheim, Germany, with a CV below 3 %. Endurance exercise has been shown to alter plasma volume [15]. Therefore, plasma volume (PV) changes after rest or exercise were used to adjust serum concentration of the biochemical markers (except for albumin-adjusted calcium and PTH, as no lactoacidosis was expected). Changes in PV were calculated by a formula given by Schmidt et al. [16]: $PV\% = [protein]_b \times [pro-$

tein]$_a^{-1} \times 100$, whereas indices $_b$ and $_a$ indicate the values before and after the test period, respectively.

Calculations

Body composition

Total body fat and total body water were calculated by bioimpedance analysis (BIA) according to the formulae of Hodgon and Fitzgerald [17] and of Kushner and Schoeller [18]. Muscle mass was determined on the basis of the BIA-measurements by a computer software, developed by the manufacturer.

Fractional Sr absorption

Calculation of fractional absorption rates (Fc) has to consider net serum strontium levels (difference between t_{240} and t_{-60}) as well as the respective distribution volume (extracellular fluid) [19]. It has been estimated that i) serum volume accounts for 5 % of body weight, ii) protein binding of calcium/strontium in serum is 33 %, and iii) interstitial fluid volume accounts for 15 % of body weight. Therefore, strontium concentrations in blood have to be multiplied by 0.15 times body weight to calculate Fc values at t_{240} [13, 20].

Statistics

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS10/Chicago, USA). Data were tested for normal distribution by the Kolmogorow Smirnov test. To test for carry-over effects, data of each subject obtained during the exercise period and the rest period were summarized. Then, results were compared separately for the group going from exercise to rest and for the group going from rest to exercise using an unpaired t-test. To test for treatment effects, the paired t-test was used. In detail, we tested for differences in body mass and body composition, nutrient intake, and urinary excretions, for differences in intestinal Sr absorption between interventions 1 and 2, for differences in the delta values (t_{240} minus t_{-60}) of the biochemical parameters between the two intervention periods, and for differences between t_{-240} and t_{-60} during each intervention. Considering the observed intra-individual variations in fractional Sr absorption, PICP and CTx levels, the statistical power ($\alpha = 0.05$; $\beta = 0.80$) was sufficient to detect differences of 7.7 %, 8.1 % and 11 %, respectively. P values below 0.05 were considered as significant. Data are presented as means ± SE.

Results

All 18 subjects performed the test procedures as planned. No carry-over effects were observed (all P values > 0.05). Energy, nutrient, and fluid intake and renal fluid loss were comparable prior to the rest period and the exercise period. Fluid intake was almost twice as high as renal fluid loss (Table 1). Body weight and body composition were comparable on the morning of the rest period and the exercise period (Table 1). Moreover, baseline Sr levels did not differ during rest and exercise. The increase in serum Sr levels after the oral bolus was, however, significantly higher in response to exercise compared to rest (Table 2). In addition, the moderate exercise resulted in a significant higher fractional Sr absorption in comparison to rest (16.2 ± 0.7 % vs. 14.6 ± 0.8 %; P < 0.05; Fig. 2).

Baseline biochemical parameters (t_{-60} values) of calcium and bone metabolism were similar at the rest period and at the exercise period (Table 2; all P values > 0.05). During the exercise period serum PICP levels decreased between t_{-60} and t_{240} (P < 0.025) while serum PICP remained constant during the rest period (Table 2). Moreover, the decrease of serum PICP in response to exercise was significant compared to rest: –9.8 % (–14.4 % in response to acute exercise compared to the rest day, –4.6 %; P < 0.05). There was an increase in serum calcitriol level between t_{-60} and t_{240} during the exercise period (P < 0.05). However, this change was not significant compared to rest (P > 0.05). Serum levels of Ca_{adj} and intact PTH remained constant between t_{-60} and t_{240} of the rest period and the exercise period. Moreover,

Table 1 Nutrition status (mean ± SE) of male athletes before two test procedures

	Rest n = 18	Exercise n = 18	Significance
Body mass and body composition			
Body mass (kg)	75.1 ± 1.9	75.3 ± 1.9	n. s.
Body fat (kg)	10.8 ± 0.7	10.9 ± 2.6	n. s.
Muscle mass (kg)	31.5 ± 0.9	31.5 ± 0.9	n. s.
Body water (kg)	47.1 ± 1.0	47.4 ± 1.0	n. s.
Energy and nutrient intake			
Energy (kJ/day)	13813 ± 3287	13560 ± 3000	n. s.
Water (ml/day)	3454 ± 819	3519 ± 927	n. s.
Protein (g/day)	117 ± 40	112 ± 26	n. s.
Fat (g/day)	126 ± 48	124 ± 38	n. s.
Carbohydrates (g/day)	399 ± 98	389 ± 99	n. s.
Dietary fiber (g/day)	30.4 ± 10.9	30.2 ± 9.6	n. s.
Alcohol (g/day)	10.2 ± 13.6	8.0 ± 9.5	n. s.
Calcium (mg/day)	1884 ± 968	2019 ± 1453	n. s.
Phosphorus (mg/day)	2457 ± 848	2274 ± 637	n. s.
24-h urine excretions			
Fluid (mL/day)	1851 ± 174	1633 ± 179	n. s.
Calcium (mmol/day)	5.1 ± 0.5	5.3 ± 0.6	n. s.
Creatinine (mmol/day)	16.3 ± 1.0	15.5 ± 1.4	n. s.

n. s. not significant

Table 2 Serum parameters (mean ± SE) of calcium metabolism and bone turnover in response to rest or exercise

	t_{-60}	t_{240}	Significance t_{-60} vs t_{240}
Protein (g/dl)			
Rest	7.10±0.18	7.68±0.19	< 0.025
Exercise	7.11±0.15	7.69±0.18	< 0.025
Albumin (µmol/L)			
Rest	691±37	758±30	< 0.05
Exercise	729±30	768±29	n. s.
Sr (µmol/L)			
Rest	0.76±0.30	30.21±1.76[a]	< 0.001
Exercise	0.69±0.31	32.82±1.36[a]	< 0.001
Ca_{adj} (mmol/L)[b]			
Rest	2.28±0.005	2.22±0.01	n. s.
Exercise	2.28±0.01	2.25±0.01	n. s.
Calcitriol (pmol/L)			
Rest	101±7	107±11	n. s.
Exercise	104±11	123±10	< 0.05
PTH (ng/mL)			
Rest	40.1±6.4	42.0±6	n. s.
Exercise	39.8±5.8	41.3±5.5	n. s.
PICP (pg/mL)			
Rest	126.1±7.1	120.7±5.9	n. s.
Exercise	122.4±9	106.5±7.0	< 0.025
CTx (pmol/L)			
Rest	7345±613	4505±557	< 0.001
Excercise	7220±419	3941±440	< 0.001

[a] 4 h after an oral bolus of 2.27 mmol Sr
[b] adjusted Ca, whereas Ca was corrected with ± 0.11 mM for each 0.100 mM deviation of concomitant serum albumin from a normal mean of 0.600 mM
n. s. not significant

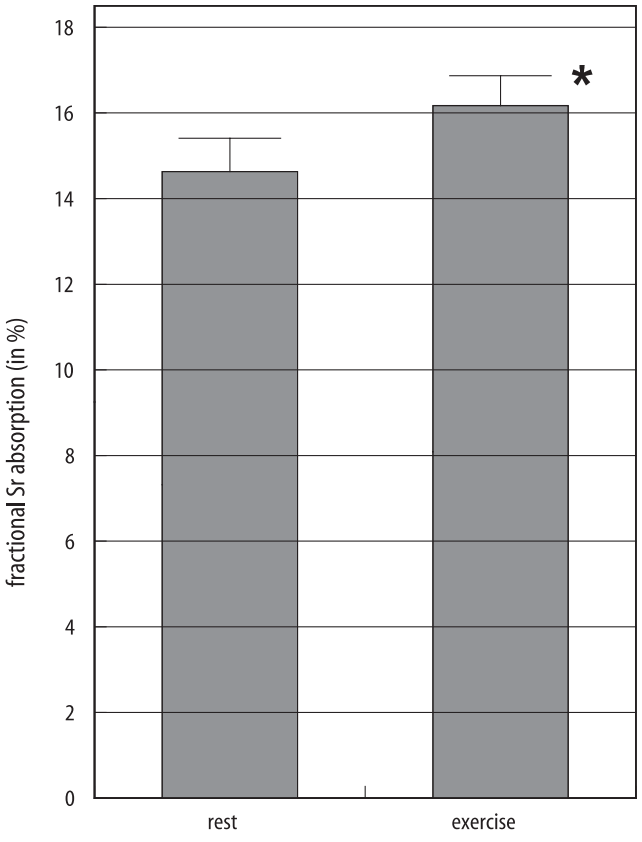

Fig. 2 Fractional Sr absorption rate of male athletes in response to acute exercise; Mean ± SE; * P < 0.05

serum CTx and protein levels did not differ in response to exercise compared to rest (both P values > 0.05). However, during both interventions there was a marked decrease in serum CTx levels between t_{-60} and t_{240} and a slight increase in serum protein between t_{-60} and t_{240}. Serum protein levels, also serum albumin levels, and serum CTX levels were, however, similar at t_{240} between rest and exercise (Table 2; P > 0.05).

Body weight did not differ at t_{240} of the exercise intervention from body weight at t_{-60} of that examination period (–0.2 ± 0.1 kg; P > 0.05). However, there was a surprising decrease in body weight after the rest period. Mean changes at t_{240} were –1.0 ± 0.1 kg in comparison to t_{-60} (P < 0.0001), with individual variations ranging from –0.2 to –1.6 kg. The body weight was significantly lower at t_{240} of the resting period in comparison to t_{240} of the exercise period (74.9 ± 1.9 kg vs 74.1 ± 1.9 kg; P < 0.01).

Discussion

As outlined in Table 1, body weight, body composition and all considered dietary factors influencing bone metabolism were similar before the two examinations. Mean Ca and phosphorus intakes were well above 1,000 mg/day covering actual recommendations [21]. Moreover, biochemical markers of Ca and bone metabolism were comparable before the two examinations (Table 2). Thus, the observed changes in serum Sr, fractional Sr absorption and serum PICP levels (Table 2 and Fig. 2) are related to the aerobic exercise.

The stable Sr test is a reliable method to investigate fractional absorption of Ca [22, 23]. Kinetics and mechanisms of Ca and Sr absorption are similar [13, 23]. The amount of Sr (2.27 mmol) is comparable to the amount of Ca normally used in Ca tracer studies [22]. A low Ca content of the standardized breakfast was chosen to reduce interference of Ca with intestinal Sr absorption. The Sr test has been used to discriminate Ca malabsorbers from normal absorbers and hyperabsorbers [13] and to study the effect of dietetic measures [24] and of physiologic changes in vitamin D status on Ca absorption [25]. Therefore, our results can be interpreted in the way that moderate endurance exercise results in an acute rise in intestinal Ca absorption.

The formula we used to calculate fractional absorption (serum Sr per liter multiplied by 0.15 kg body weight at t_{240}) is a standard method based on the as-

sumption of a constant ratio of plasma and interstitial fluid volume to body weight [13]. This equation may underestimate the Sr distribution volume of athletes. Exercise-trained men have a higher body water content, a higher plasma volume and a higher ratio of extracellular to intracellular water in comparison to sedentary controls or amateurs [26, 27]. Since the subjects served as their own controls these alterations do, however, not influence our results. Another point of concern may be an exercise-induced acute change in plasma and/or interstitial fluid volume. The similar serum protein levels after exercise and after rest (Table 2) indicate a similar plasma volume at the end of both interventions [16, 28]. Since the measurement of fractional Sr absorption was based on serum Sr and body weight measurements at t_{240}, the study procedure accounts at least in part for possible variations in fluid volume due to rest or exercise (see below). The constant body weight after the run period is in line with the moderate level of endurance exercise indicating modest substrate oxidation and/or fluid loss. The loss of body weight during the resting period was, however, an unexpected finding which cannot be explained by substrate oxidation or by fluid evaporation. The decrease in body weight during rest suggests a loss of total body fluid, e. g. interstitial fluid. It has to be mentioned that athletes can have a significant fluid loss within only a few hours during different situations of rest (e. g. during water immersion and short term immobilization), which is higher in comparison to sedentary controls [29, 30]. Thus, the unexpected results need further considerations. Data indicate that the Sr distribution volume after resting may have been overestimated by the formula we used. As a consequence, the real difference in Fc_{240} between the exercise period and the rest period may have been higher than the calculated difference.

Both intestinal Sr and intestinal Ca absorption are absorbed by an active calcitriol-mediated process and by passive diffusion [31].The active process of Ca/Sr absorption is largely limited to the proximal part of the small intestine and this process is finished within 2 hours after an oral bolus [32]. Calcitriol can induce a rapid effect on Ca absorption within 2 to 5 min, named transcaltachia [33]. Therefore, it may be that a transient rise in serum calcitriol levels, probably mediated by a transient rise in PTH levels, has influenced Sr/Ca uptake. This would be in line with the rise in serum calcitriol levels after exercise compared to baseline (Table 2). A transient rise in serum calcitriol would also explain that the difference in serum calcitriol levels in response to exercise compared to rest did not reach the level of statistical significance. Moreover, it is possible that the enhanced Ca/Sr absorption is the result of an unspecific exercise-induced effect on the gut. In young adults, a moderate aerobic exercise lasting for one hour results in an approximately 50 % prolongation of the mouth-to-cecum transit time in comparison to a similar rest period [34]. Such an effect may reduce the Ca/Sr load exposed to the absorption sites on mucosal cells and may, thus, enhance their absorbility [35]. This would also explain that the enhanced Sr/Ca absorption during the moderate exercise occurred without a change in serum Ca and intact PTH levels (Table 2).

The aerobic exercise program influenced serum PICP levels. The amount of PICP released into the blood is directly related to the number of collagen molecules formed and serum PICP correlates with histomorphometric parameters of bone formation [36]. Therefore, our data indicate a slight, but significant reduction in bone collagen formation. Others have found a decrease in serum PICP levels one and two hours after moderate exercise of approximately 7 % and 13 %, respectively [7, 10]. Data are comparable with the decrease in serum PICP levels of 9.8 %, observed in our study.

The marked reduction in serum CTx levels during both examinations is in line with the circadian rhythm of this bone resorption marker [37]. Therefore, on the basis of our data it can be ruled out that the moderate mechanical loading itself had an acute effect on serum CTx levels in this group of endurance-trained athletes.

An exercise-induced rise in intestinal Ca absorption, as observed in this study (Fig. 2), is a prerequisite for a more positive Ca balance and, thus, for a higher bone mineral accretion in comparison to sedentary controls of similar Ca intakes. Bone matrix mineralization is, however, a step that follows organic matrix synthesis [38]. The exercise-induced slight decrease in serum PICP levels and the unchanged serum CTx concentrations indicate a slight acute uncoupling of bone collagen formation and resorption processes. Consequently, the surplus of absorbed Ca cannot be deposed in additionally formed organic bone matrix. It may be that a sweat-induced Ca loss prevents an additional Ca retention [39]. However, it may also be possible that the reduced formation of organic bone matrix in response to exercise compared to rest is associated with a higher mineralization of this organic matrix.

In conclusion, our data provide evidence for an acute rise in fractional Ca absorption after a moderate aerobic exercise bout. Furthermore, data indicate that even in endurance-trained young men a moderate exercise acutely decreases bone collagen formation, while physiologic fluctuations of the bone resorption marker CTx remain unaffected.

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