SHORT TERM BONE BIOCHEMICAL RESPONSE TO A SINGLE BOUT OF HIGH-IMPACT EXERCISE

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ABSTRACT

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INTRODUCTION: Bones adapt to imposed loading environment, but the adaptive process takes years to complete. The state of skeletal turnover can be evaluated with biochemical markers of bone formation and resorption. Thus it is possible to observe the response of bone to a single bout of exercise. Currently the results concerning acute short term bone response after a single bout of loading are equivocal. Therefore, the purpose of the study was to examine the response of bone biochemical markers to a single bout of high-impact exercise.

METHODS: 18 physically active young subjects volunteered to participate. The subjects performed hopping with ankle plantarflexor muscles at 65 % of maximal ground reaction force until exhaustion. Venous blood samples were drawn before (baseline), after, 2h, on day 1 and on day 2 after the exercise. Procollagen type I amino terminal propeptide (P1NP) formation marker and carboxyterminal crosslinked telopeptide (CTx) resorption marker were analyzed from the blood samples. Marker concentrations were adjusted for changes in blood plasma volume. Non-parametric Friedman and Wilcoxon tests were used for multiple comparisons of mean values and Spearman test was used for correlation analysis.

RESULTS: CTx increased significantly two days after the exercise and P1NP one day after. There was a significant positive correlation ($r \geq 0.54$, $P \leq 0.008$) between loading variables and relative change in P1NP on day 1

DISCUSSION: Considering that only two biochemical bone turnover markers were assessed, it can be concluded that bone response to a single bout of strenuous high-impact exercise can be seen by observing the biochemical bone markers during just two days ensuing the exercise. The biochemical marker response seems to depend on exercise type even if high intensity exercises are used.

KEYWORDS: bone, biochemical, marker, exercise, short term

ABBREVIATIONS

MAPK = mitogen-activated protein kinase
BMU = basic multicellular unit
PICP = procollagen type I carboxy terminal propeptide
P1NP = procollagen type I amino terminal propeptide
OC = osteocalcin
bALP = bone-specific alkaline phosphatase
TRACP = tartrate resistant acid phosphatase
BSP = bone sialoprotein
Dpd = deoxypyridinoline
NTX = amino terminal crosslinked telopeptide
CTx = carboxy terminal crosslinked telopeptide
CSA = cross-sectional area
BMC = radial diaphysis bone mineral content
aBMD = areal bone mineral density
GRF = ground reaction force
OI = Osteogenic index
Co_A = Adjusted concentration
Co_M = Measured concentration
Hct_2 = Hematocrit after exercise
Hct_1 = Hematocrit before exercise
Co_B = Concentration before exercise
BW = Body weight

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1 INTRODUCTION

Bone serves two purposes: metabolic and mechanic. The mechanical needs define the structure of bones. (Weiner et al. 1999.) The adaptation to mechanical needs is highlighted in toughness/stiffness differences between bones. The toughness/stiffness of bone is determinated by the amount of mineralization of the bone material. There is a tradeoff between toughness and stiffness, the more mineralized material will become stiffer and less tough. This kind of toughness/stiffness adaptation seems to be caused by evolution instead of the mechanical environment the bone is subjected to. The strength of whole bone is determined by material and architectural properties. It seems that mechanical adaptation to the imposed loads during a creature's life span occurs via adapting the architectural properties of bone rather than altering the material properties. (Currey 2003.)

1.1 ORGANIZATION OF BONE TISSUE

Bone consists of bone cells (Currey 2002, 194 - 244), bone mineral, collagen (Weiner et al. 1999) and bone marrow. In addition there are blood channels in the bone (Currey 2002, 194 - 244).

1.1.1 Bone cells

Bone tissue is permeated and lined with specialized cells. *Bone-lining cell*s (figure 1)

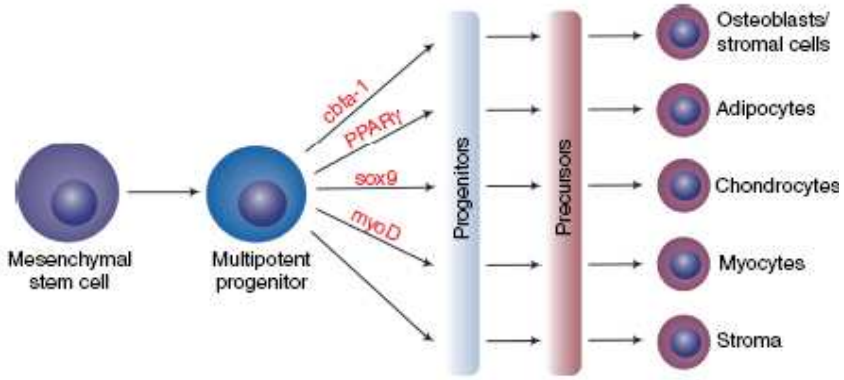

FIGURE 1. Bone-lining cells differentiate from mesenchymal stem cell in the bone marrow (Horowitz et al. 2005).

cover all surfaces of bones including the blood channels. The layer of cells outside the bone is called periosteum. Periosteum also includes the collagenous sheet covering the outer surface. The layer of cells covering the inside of bone is called the endosteum. Bone-lining cells are considered to be quiescent osteoblasts and are derived from osteoprogenitor cells. (Currey 2002, 194 - 244.)

*Osteoblast*s derive from bone-lining cells, when bone formation is initiated. The function of osteoblasts is to produce new bone by laying down new collagen matrix, osteoid, which subsequently mineralizes to form bone. (Currey 2002, 194 - 244.)

*Osteocyte*s derive from osteoblasts when osteoblast gets trapped in the osteoid it is producing thus becoming imprisoned in the hard bone tissue. Osteocytes are connected to each other via canaliculi processes. The connections between neighboring osteocyte cells are actualized through gap junctions. (Currey 2002, 194 - 244.)

*Osteoclast*s derive from precursor cells circulating in the blood stream (figure 2). The function of osteoclasts is to degrade bone. Part of the osteoclastic cell membrane forms a ruffled border under which, it can dissolve bone. (Currey 2002, 194 - 244.)

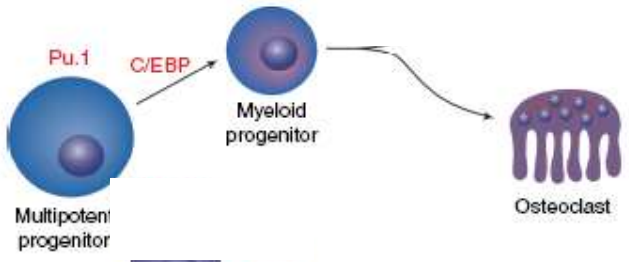

FIGURE 2. Osteoclasts arise from the hematopoetic stem cells in the bone marrow (adapted from Horowitz et al. 2005).

Osteoclastic resorption begins when osteoclast attaches to mineralized bone matrix and produces tight ring-like sealing zone. The plasma membrane opposite to bone and inside the sealing zone becomes ruffled and the resorption lacuna develops between the bone and the ruffled border membrane. Osteoclast releases acid to the resorption cavity, which will lead to degradation of the bone. Osteoclast endocytoses the degradation

products (calcium, phosphate and collagen fragments) through the ruffled border. The degradation products are thereafter released to the extracellular space. (Vaananen 2005.) When osteoclasts have done their degrading they presumably die (Currey 2002, 194 - 244).

1.1.2 Mineralized collagen matrix

The basic building block of bone is the mineralized collagen fibril. Collagen acts as a framework for plate –like carbonited apatite crystals. Together with the carbonited apatite crystals the fibril forms a crystal of non-uniform structure to all three orthogonal directions. (Weiner et al. 1999.) Mammalian bone can have two forms: woven and lamellar. Woven bone grows rapidly and its collagen is oriented randomly. Lamellar bone grows slowly (Currey 2003) and the collagen fibrils are stacked as layers with rotation between successive layers to produce rotated plywood like structure (figure 3) (Weiner et al. 1999). There are blood channels and bone cells in lamellar and woven bone. Woven and lamellar bone can be laid down to form fibrolamellar bone (Currey 2002, 194 - 244).

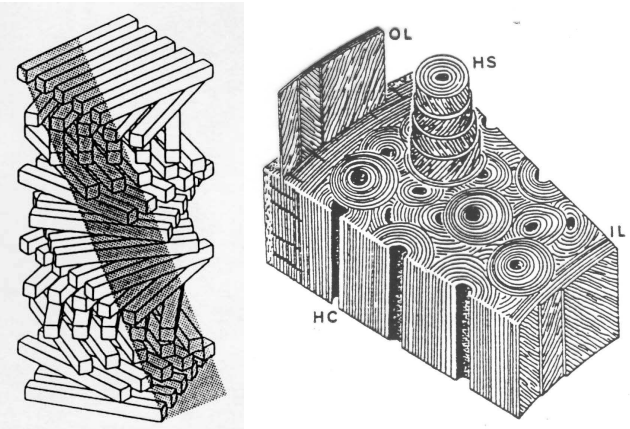

FIGURE 3. Rotated plywood structure seen in lamellar bone material. Illustration on the left from Martin et al. (1998). Illustration on the right from Giraud-Guille (1988).

Secondary remodeling of bone results in production of Haversian bone. In Haversian bone much of the bone is occupied by secondary osteons. Woven bone is superior to

Haversian bone in mechanical sense when woven bone is loaded along the grain. If however, woven bone is loaded transversely against the grain, correctly aligned Haversian bone will be superior in sense of mechanical competence. Remodeling is also used to repair microcracks caused by loading and fatigue of the bone material. (Currey 2003.)

1.1.3 Higher order organization of bone

Bone macro structure can be divided into cancellous and compact bone. Compact bone is solid with only spaces being for osteocytes, canaliculi, blood channels and resorption cavities. Cancellous bone in turn is a meshwork of bone material incorporating spaces void of bone material filled with bone marrow. The material making up the bone, cancellous and compact, is primary lamellar bone or Haversian bone in adults. (Currey 2002, 194 - 244.)

Long bones are hollow and the cavity is filled by marrow fat in adults. The marrow fat serves no essential purposes, although it may play a role in increasing the ability of bone to withstand compressive loading by preventing buckling. This kind of arrangement decreases the weight of long bones by approximately 15 % compared to correspondingly stiff solid bone from the same material (figure 3). (Currey 2003.)

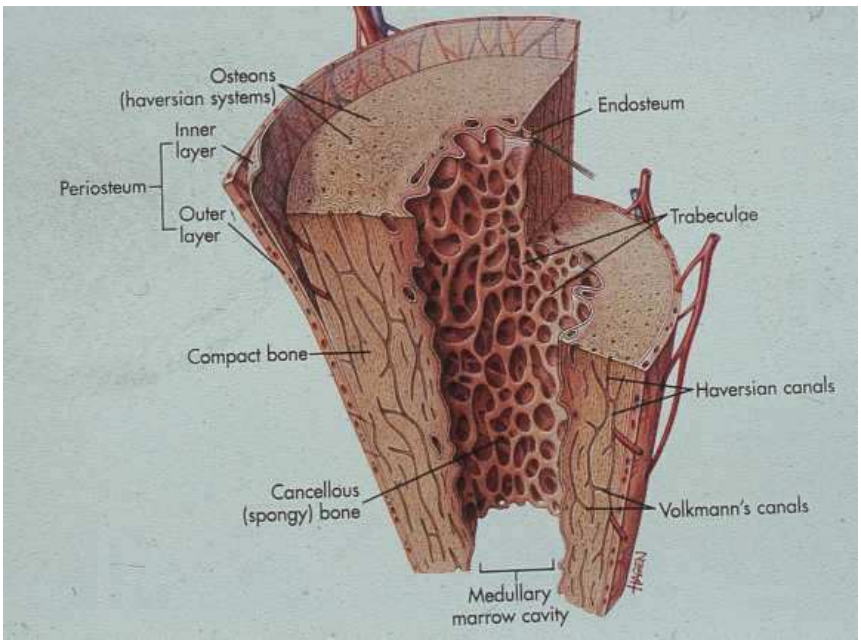

FIGURE 3. Structure of long bone (University of Bristol & University College Dublin 2001).

1.2 BONE MODELING AND REMODELING

Modeling is the process, which dominates during growth, which determines the overall shape of the bone. In modeling bone growth is retarded at some places whereas in other places the bone growth is facilitated. Bone mineral accumulation caused by modeling can be facilitated via increased mechanical usage and decreased by decreased mechanical usage. In remodeling, bone material is turned over by resorption by osteoclasts and formation by osteoblasts. If more bone is produced by the osteoblast than what is resorbed by the osteoclasts, remodeling leads to increased skeletal mass. In adults remodeling predominates as the mechanism responsible for skeletal adaptation since the bone growth. (Frost 1985).

1.2.1 Mechanotransduction

Bone remodeling cycle is initiated by mechanical signals by cellular mechanotransduction. Mechanotransduction consists of four distinct phases: 1) *mechanocoupling*, force applied to the bone is transduced into a local mechanical signal perceived by a sensor cell; 2) *biochemical coupling*, the transduction of a local mechanical signal into a biochemical signal; 3) *transmission of signal* from the sensor cell to the effector cell and 4) the *effector cell response*, the appropriate tissue-level response. (Turner & Pavalko 1998.)

During daily activities multiple mechanical factors arise in the bone tissue. Daily activities cause deformation, pressure, transient pressure waves, shear forces and dynamic electric fields. Of these possible stressors deformation (strain) and shear have been isolated as the most significant mechanical events for bone. All of the bone cells seem to be able to respond to mechanical signals. However, osteocytes seem to be advantageously situated and the microarchitecture is favourable for mechanosensing as the architecture causes amplification of the signal. The exact type of mechanosensors is yet to be revealed in bone cells, but the sensing of mechanical event leads to alteration in appropriate ion channel activities which ultimately leads to change in the activity of the cell. Mechanical signals ultimately activate mitogen-activated protein kinase

(MAPK) regardless of the cell and the response depends on the gene patterns associated with the target cell. (Rubin et al. 2006.)

1.2.2 Bone remodeling cycle

Remodeling cycle begins with recruitment of osteoclasts to the bone surface. Osteoclasts cause breakdown of the collagen matrix of bone and release of calcium and other minerals. After osteoclastic resorption the osteoblasts fill the resorption cavity with protein matrix called osteoid, which is mineralized subsequently.

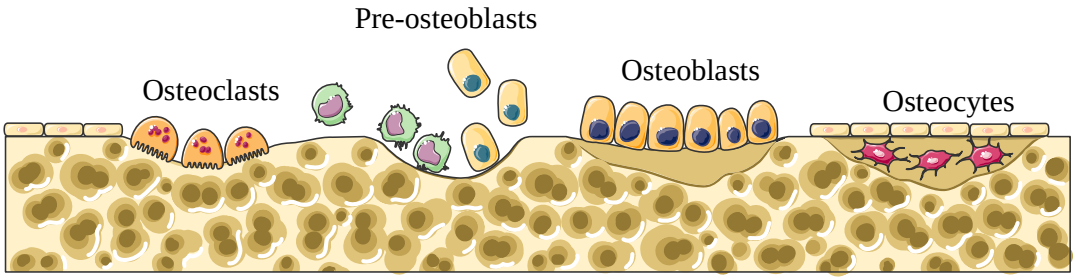

FIGURE 4. Bone remodeling cycle advancing from left to right. Adapted from Les Laboratories Servier (2005).

The cells involved in the remodeling are referred to as a basic multicellular unit (BMU). Typically resorption phase lasts 7 – 10 days and formation 2 – 3 months (figure 4). It is

TABLE 1. Chemical factors affecting bone metabolism. Reproduced from Christenson (1997).

Factor	Effect on turnover	Cells effected	Mechanism
Parathyroid hormone	Increase	Osteobasts	Increased osteoclast activation and accelerated bone loss
Thyroxine	Increase	Osteoclasts	Increased resorption
Estrogen	Decrease	Osteoblasts	Deficiency causes accelerated bone loss
Testosterone	Decrease	Osteoblasts	Deficiency causes accelerated bone loss
Vitamin D	Decrease	Osteoblasts	Deficiency causes increased activation but inhibits mineralization of osteoid matrix
Cortisol	Increase	Both	Increased resorption and inhibition of formation leading to accelerated bone loss
Calcitonin	Decrease	?	Inhibits resorption
Insulin	Decrease	Osteoblasts	Increased collagen synthesis

not unambiguously explained what causes the remodeling cycle to begin. Remodeling is regulated by local and systemic factors, which include: electrical and mechanical forces and multiple chemical factors such as hormones (table 1). About 10 % of bone material is replaced annually. (Watts 1999.)

1.3 BIOCHEMICAL MARKERS OF BONE TURNOVER

The state of the skeleton can be evaluated by many techniques. Biochemical markers respond to intervention the fastest while being minimally invasive (Watts 1999). It has been discovered, that after an acute bout of strenuous exercise there is protein leakage from the exercised muscles and connective tissue (Virtanen et al. 1993). The response to exercise can be measured from blood samples (Tähtelä 2004, Virtanen et al. 1993) and the markers can be used in estimating the bone remodeling rate (Weisman & Matkovic 2005) by comparing the ratio of resorption markers to formation markers (Christenson 1997). There is diurnal variation in bone markers and the peak levels of biochemical markers do not coincide. Thus circadian rhythm is an important contributor in intraindividual variation. (Tähtelä 2004.) Biochemical markers of bone remodeling can be measured in the serum or urine. These markers fall into 2 main categories: 1) markers of bone formation and 2) markers of bone resorption. (Weisman & Matkovic 2005.) Surplus products or fragments released into serum may be used as markers of bone formation. Products released during bone resorption can be used as markers for the catabolic events. (Ebeling & Akesson 2001.)

1.3.1 Bone formation markers

The predominant product of osteoblasts is type I collagen. Procollagen is formed in bone from α-1 and one α-2 collagen polypeptides containing hydroxylated proline and lysine residues. As procollagen is secreted from the osteoblast, the amino-terminal and carboxy-terminal regions are cleaved and type I collagen is produced. These propeptides are released into the extracellular space although a proportion of the N-terminal propeptide is also incorporated into bone. The non-helical domains of type I collagen at the amino- and carboxy-terminals are known as the N-telopeptide and the C-telopeptide

regions. Osteocalcin is also secreted by osteoblasts to the osteoid in which the mineralization takes place and bone alkaline phosphatase enzyme protein is incorporated in the membrane of osteoblasts but its release from osteoblasts remains unclear. (Ebeling & Akesson 2001.) There are 4 commonly measured markers of bone formation: 1) procollagen type I carboxy terminal propeptide [PICP], 2) procollagen type I amino terminal propeptide [P1NP], 3) osteocalcin [OC] and 4) bone-specific alkaline phosphatase [bALP]) (Weisman & Matkovic 2005). The serum concentration of P1NP reflects changes in the synthesis of new collagen. Proportion of P1NP also incorporated into bone as non-dialyzable hydroxyproline and, thus, a component of the measured fragments might represent bone resorption. Nevertheless, P1NP appears to be dynamic and sensitive marker of changes in bone formation. (Ebeling & Akesson 2001.)

1.3.2 Bone resorption markers

Bone resorption markers are mostly type I collagen degradation products. In addition tartrate resistant acid phosphatase (TRACP) and bone sialoprotein (BSP) are considered to reflect resorption. TRACP is osteoclast enzyme and BSP is osteoblast product, which is considered to be a marker of resorption because it is liberated during extracellular matrix degradation. (Tähtelä 2004.) There are 6 commonly measured markers of bone resorption: 1) hydroxyproline, 2) pyridinoline, 3) deoxypyridinoline, 4) amino terminal crosslinked telopeptide, 5) carboxy terminal crosslinked telopeptide and 6) tartrate-resistant acid phosphatase. (Weisman & Matkovic 2005.)

Bone resorption is initiated by osteoclasts. Osteoclasts attach to the bone surface and secrete acid and hydrolytic enzymes that resorb bone, releasing bone minerals and collagen fragments. Some of the collagen is completely digested by osteoclasts to its smallest units, free pyridinoline and deoxypyridinoline (Dpd) residues. The majority however, appears to be incompletely digested, resulting in the formation of pyridinium cross-links bound to fragments of the NTx α-1 and α -2 polypeptides. C-telopeptide cross-link (CTx) can be measured in serum or urine and only the β-isomer of the CTx is measured in the serum assays. (Ebeling & Akesson 2001.)

2 BONE AND EXERCISE

Mechanical loading of bone affects the quality and quantity of human bones when adequate nutrition and hormonal balance is available (Smith & Gilligan 1996). The primary forces applied to bone are caused by muscles. Muscle forces, due to leverage of the muscles, are greater than the forces caused by gravitational pull on body weight. (Burr et al. 1996.) If new forces outside normal loading range are introduced, bones will adapt to accommodate the new loads. If loading remains constant no additional bone formation occurs after bones have adapted to the new loading level. (Cullen et al. 2000.) Sievänen et al. (1996) showed in a case study a clear evidence of the dependence of bone strength to muscle forces (Sievanen et al. 1996).

The osteogenic effect of exercise increases when the interval between loadings is increased inside an exercise bout thus allowing the bone to recover from the load (Umemura et al. 2002). Rubin & Lanyon (1984) discovered with rooster ulnas that bone mass can be maintained with only a few osteogenic strain cycles (4 cycles per day taking 8 seconds in total) comparatively infrequently. Increasing the number of strain cycles / day resulted in increased bone formation. However increasing the number of strain cycles above 36 cycles / day didn't result in any additional bone mineral accrual. The strain applied was comparable to normal physiological wing flapping strains. (Rubin & Lanyon 1984.)

The translation of physical activity to cellular responses, mechanotransduction, occurs at cellular level. Fluid flow in bone tissue caused by deformation of bone is sensed by the osteocytes. The strains are amplified to responses in cellular level so that higher frequencies of strain and smaller magnitudes of strain are amplified more. Furthermore, the effect of frequency is the highest at the lowest frequencies. If strain magnitude is insufficient, no response will be seen. (Han et al. 2004.) Bone formation increases with increasing loading cycles when the intensity is held constant and the strain magnitude is not high. If intensity of the loading is increased the number of loading cycles required for response decreases. (Cullen et al. 2001.) It has been suggested that osteogenic exercises should have rapidly increasing force characteristics and sufficiently high peak

force (Heinonen 1997). An estimate of effectiveness of exercise has been developed and the index is based on exercise intensity and number of loading cycles. (Turner & Robling 2003.)

2.1 BONE STRENGTH AND EFFECTS OF AGING, BODY SIZE AND GENDER DIFFERENCES ON BONE STRENGTH

Assessment of bone strength should take shape and size of the bone into account. (Myburgh et al. 1993., Turner & Robling 2003.) Bone mass and geometrics provide an accurate measurement of the effect of activity induced changes on bone strength (Bennell et al. 2002., Brahm et al. 1998). Bone strength can be approximated from densitometric measurements. (Sievanen et al. 1998.) Although bone density measurements are important clinically assessing biochemical markers could provide more real-time information about current state of bone turnover (Christenson 1997).

In healthy people there is a linear relationship between muscle cross-sectional area (CSA) and radial diaphysis bone mineral content (BMC). During puberty women will have higher BMC/CSA ratio due to higher endocortical apposition. The difference to men is apparently caused by higher estrogen levels of women. (Schoenau et al. 2002.) Estrogen causes packing of calcium to female bones during the fertile years. After menopause bone loss is facilitated in women due to withdrawal of estrogen and the need for the calcium reservoir. Because of the extra mineral content in relation to bone strength caused by the estrogen packing phenomenon, women’s bones seem to be less responsive to loading than the bones of men. (Jarvinen et al. 2003.)

2.2 BONE MARKER RESPONSES TO AGING, EXERCISE AND INACTIVITY

There is inverse relationship between skeletal turnover rate measured with biochemical bone markers and areal bone mineral density (aBMD) in pre- and postmenopausal women (Minisola et al. 1998). Recently sustained fracture increases the biochemical bone markers at least up to two years, possibly throughout the life, after the fracture (Obrant et al. 2005). Bone resorption markers respond to initiation or removal of

therapy in approximately 3 months and formation markers respond some months later (Christenson 1997).

The acute effect of a resistance training bout is to decrease the concentrations of bone resorption and formation markers and increase the amount of urinary calcium. The increased urinary calcium is most likely not due to increasing resorption but could be induced by the effect of lowered pH. (Ashizawa et al. 1998.) Acute bout of endurance-type exercise stimulates bone resorption (Guillemant et al. 2004) and formation markers (Maimoun et al. 2006, Wallace et al. 2000). Brahm et al. (1997a) found elevated markers of bone formation during recovery from short lasting dynamic activity (Brahm et al. 1997b). In contrast Brahm et al. (1997b) didn't find any significant changes in bone biochemical markers to a bout of endurance-type exercise when the plasma fluctuations were accounted for. If the plasma fluctuations were not accounted for there were significant increases in some of the markers. (Brahm et al. 1997a.)

A bout of moderate endurance exercise reduces collagen formation (Welsh et al. 1997, Zittermann et al. 2002). Moderate intensity resistance exercise reduces bone resorption transiently and thus favorably affects bone remodeling (Whipple et al. 2004). Short-term maximal work (30 s modified Wingate test) has no effects on bone metabolism (Kristoffersson et al. 1995) nor maximal oxygen uptake test conducted by walking in increasing incline in the elderly (Maimoun et al. 2005).

3 AIMS OF THE STUDY

Currently the results concerning acute short term bone response measured from blood or urine sample after a single bout of loading are equivocal. Most of the studies on the subject have not implemented high-impact exercises [exercises used previously include resistance training (Ashizawa et al. 1998, Whipple et al. 2004), bicycling (Guillemant et al. 2004, Kristoffersson et al. 1995, Maimoun et al. 2006, Wallace et al. 2000), one-legged leg extension on bicycle ergometer (Brahm et al. 1997b), running (Brahm et al. 1997a, Zittermann et al. 2002), walking (Maimoun et al. 2005, Welsh et al. 1997)] which are most likely to provoke a response in bone (Turner 1998). Therefore, the purpose of the study was to examine the response of bone biochemical markers to single bout of high-impact exercise. Decrease in bone resorption (Ashizawa et al. 1998, Whipple et al. 2004) and formation markers (Ashizawa et al. 1998) have been observed in resistance exercise studies. In addition to high-impact exercises, resistance training is expected to have osteogenic effects (Heinonen 1997). Therefore, the working hypothesis was that bone resorption and formation markers will decrease after the high-impact exercise as is the case after resistance training.

4 METHODS

4.1 SUBJECTS

15 healthy young male students served as the subjects for the study. The subjects represented a wide range of physical activity levels ranging from sedentary to amateur athletes (exercising up to 6 times/week) with wide variety of sports. The subjects gave their written informed consent to participate. The study was conducted with the approval of the local ethical committee.

4.2 PROTOCOL

A fatiguing exercise was performed with triceps surae hopping. The subjects were instructed to jump with minimal knee bending and producing the energy for the jumps with plantar flexion of ankle. Ten to twenty continuous jumps with straight legs were performed with increasing intensity until a steady maximal level was achieved. From the jumps the maximal ground reaction force (GRF) was determined, which was then used to determine a goal level of 65% of maximal GRF for the ensuing fatiguing exercise. The subject was to reach the goal GRF level with each succeeding jump during the fatiguing exercise. Exercise was continued until the goal level of 65% of maximal GRF could not be reached for 10 successive jumps or the subject refused to continue. Subjects were provided with verbal instructions to jump higher or lower during the exercise in order to maintain the target GRF level. Verbal encouragement was provided especially during the late phases of the exercise.

Venous blood samples were drawn from medial cubital vein. Sampling was made prior to a 10 – 15 minute warm-up with bicycle ergometer with freely chosen intensity and immediately after the exhausting exercise. The subjects returned to the laboratory for sample collections at two hours after the exercise and for two succeeding days after the exercise. The follow-up blood samples were drawn at the same time of day as the before

exercise measurement. Subjects were instructed to avoid strenuous exercises during the two day follow-up period.

4.3 EXERCISE QUANTIFICATION

Hopping was conducted on a custom made force plate (University of Jyväskylä, Finland). Vertical ground reaction force was recorded for the whole duration of intensity determination and the fatiguing exercise with data acquisition analog to digital board (CED limited, Cambridge, England). The analog to digital board was controlled with Signal (Signal version 2.15) data acquisition program. The number of hops was calculated manually. Exercise intensity in terms of multiples of body weight was determined as the average of ground reaction force peaks of each hop in multiples of body weight during the exhaustive exercise. All of the ground reaction force curves of each hop were time scaled to the average length of the ground contact time and then averaged to produce a single ground reaction force curve to represent the loading. The time normalization was made by taking the fast Fourier transformation of a ground contact reaction force and then recomposing the transformed data to consist of appropriate number of data points. Another fast Fourier transformation was then taken from the representative average ground reaction force data. Osteogenic index was thereafter calculated as:

$OI = \log(1+N) \bullet \sum_{l=1}^{f_l \geq 50\,Hz} \varepsilon_l f_l$ adapted from Turner (1998).

where $\varepsilon_l = \sqrt{A_l^{\,2} + B_l^{\,2}}$, A_l = l:th cosine coefficient of the Fourier series, B_l = l:th sine coefficient of the Fourier series, f_l = l:th frequency in the Fourier series and N = the number of loading cycles. Somewhat simpler osteogenic index 2 (OI2) was thereafter calculated as:

OI2 = exercise intensity (multiples of body weight) · ln(number of jumps + 1) (Turner & Robling 2003.)

Ground reaction force analysis was conducted with MATLAB® (MATLAB® the language of technical computing, version 7.0.1.24704 (R14) service pack 1, The MathWorks, Inc.) software

4.4 BIOCHEMICAL BONE MARKERS

CTx (β-CrossLaps/serum, ECLIA assay, Roche, CV = 4.3 %) and P1NP (total P1NP, ECLIA assay, Roche, CV = 2.4 %) were analyzed from the venous blood samples with automatic immunoassay device (Elecsys 2010, Roche) with commercial electrochemiluminescence immunoassays. The blood samples were stored in -80°C until analysis. The time in storage was 6 – 18 months. Bone marker results were analyzed with measured and adjusted concentrations. The adjustment was made for changes in blood plasma volume as

$$Co_A = Co_M \bullet \frac{Hct_1 \bullet [100 - Hct_2]}{Hct_2 \bullet [100 - Hct_1]}$$ adapted from van Beaumont et al. (1973).

where Co_A = adjusted concentration, Co_M = measured concentration, Hct_2 = Hematocrit after exercise and in the follow up measurements, Hct_1 = Hematocrit before exercise.

4.5 STATISTICAL ANALYSIS

Non-parametric Friedman test was used to see whether repeated measurements of the bone markers differed. Follow-up measurements were compared to before measurement with non-parametric Wilcoxon test in accordance to the least significant difference (LSD) post hoc evaluation. Correlation between bone variables and exercise variables was checked with Spearman rank correlation coefficient. The results are reported as mean (standard deviation). The significance level was set at $P \leq 0.05$ for all statistical analyses.

5 RESULTS

Subjects and exercise quantification

Subject anthropometric values and exercise variables are presented in table 2. Maximal ground reaction force in maximum effort hopping was 6.0 (0.7) times body weight and 4.2 (0.5) times body weight during the fatiguing exercise. Osteogenic index for the exercise was 110 (30) and osteogenic index 2 was 30 (4).

TABLE 2. Subject anthropometric and fatiguing exercise loading quantification results. Reported as mean (standard deviation). GRF = ground reaction force.

Age	25 (3)
Height [cm]	177 (6)
Body mass [kg]	75 (10)
Number of hops [amount]	1250 (570)
Maximal GRF in hopping [kg]	450 (90)

Biochemical bone markers

P1NP and CTx results are reported in table 3. Difference existed between the repeated measurements of P1NP marker in measured ($P < 0.001$) and in its adjusted concentrations ($P = 0.013$), which was also the case between adjusted CTx marker measurements ($P = 0.037$).

When multiple comparisons were conducted, it was observed that, P1NP biochemical bone formation marker measured immediately after the exercise 94.3 (54.5) increased from the baseline value of 78.4 (46.7) ($P = 0.003$). Adjusting the bone markers with plasma volume shifts caused the P1NP increase seen in measured values after the exercise to disappear (figure 5). An increase for baseline adjusted P1NP value of 78.4 (46.7) to day 1 value of 85.7 (45.6) ($P = 0.049$) became evident. In addition an increase in adjusted bone resorption marker CTx was seen from value of 0.510 (0.228) to day 2 value of 0.673 (0.361) ($P = 0.020$).

TABLE 3. Biochemical bone markers before and after the fatiguing exercise. Adjusted P-values reported in comparison to before measurement. P1NP = procollagen type I amino terminal propeptide, CTx = carboxyterminal crosslinked telopeptide.

	P1NP [ng/ml]	P-value	CTx [ng/ml]	P-value
Baseline	78.4 (46.7)		0.51 (0.228)	
After	94.3 (54.5)	**0.003**	0.472 (0.182)	
2 hours	76.6 (43.9)	0.570	0.41 (0.191)	
day 1	83.4 (47.9)	0.069	0.564 (0.31)	
day 2	82 (51.7)	0.334	0.65 (0.349)	
Adjusted values				
Baseline	78.4 (46.7)		0.51 (0.228)	
After	84.9 (50.4)	0.053	0.423 (0.164)	0.191
2 hours	77.5 (44.5)	0.865	0.415 (0.194)	0.281
day 1	85.7 (45.6)	**0.049**	0.578 (0.309)	0.140
day 2	84.7 (53.5)	0.211	0.673 (0.361)	**0.020**

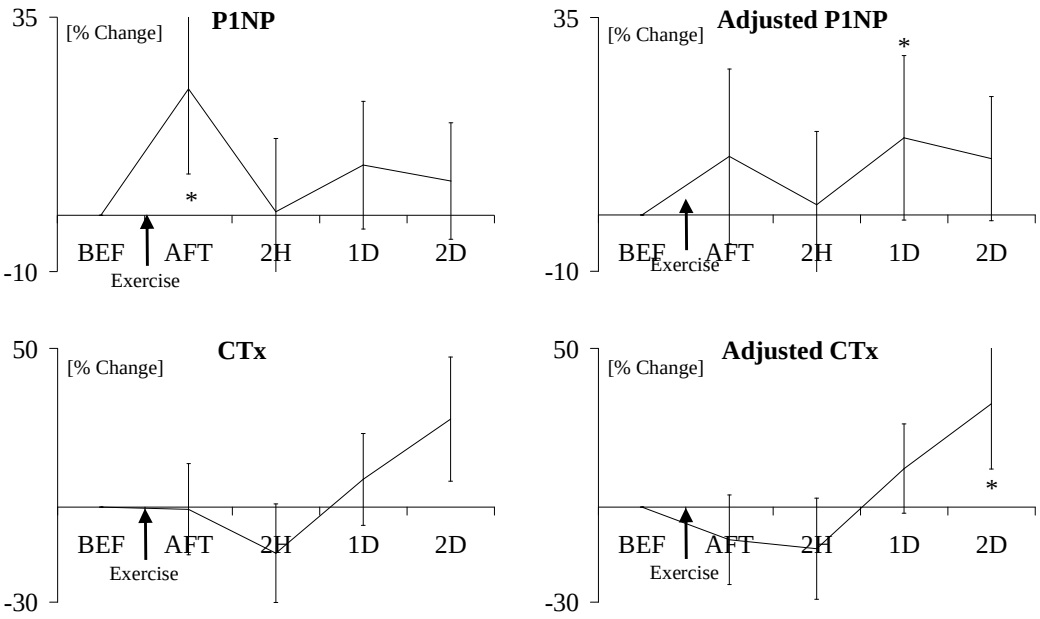

FIGURE 5. Normalized P1NP and CTx results. Measured data on left column, adjusted on the right. Normalization was conducted with before value. 95 % confidence interval reported as error bar. Asterisk = significant difference compared to before measurement at $P \leq 0.05$ level. Significance was calculated from the absolute values and is reported in table 3.

Correlations

Correlations were calculated between the percentual change in adjusted values and exercise variables. For P1NP day 1 and for CTx day 2 differed the most from before values and were used in the correlation analyses. There was significant positive correlation between exercise intensity in multiples of body weight (r = 0.49, P = 0.034), osteogenic index (r = 0.56, P = 0.019), osteogenic index 2 (r = 0.61, P = 0.010) and the percentual change in adjusted P1NP between before and one day after measurements (table 4). No other significant correlations between exercise variables and change in P1NP or CTx were seen.

TABLE 4. Correlation matrix for percentual change in adjusted P1NP between before and 1 day after and adjusted CTx between before and 2 days after biochemical bone markers and different exercise loading quantities. Spearman r- and P- values reported. Δ P1NP = percentual change in adjusted P1NP between before and one day after measurements, Δ CTx = percentual change in adjusted CTx between before and two days after measurements, GRF = ground reaction force, BW = body weight, OI = osteogenic index, OI2 = osteogenic index 2.

	Δ P1NP	Δ CTx
Number of hops	0.26	0.34
(p-value)	0.174	0.111
Maximal GRF in multiples of BW	0.38	-0.16
(p-value)	0.090	0.292
Exercise intensity in multiples of BW	**0.49**	-0.14
(p-value)	**0.034**	0.318
OI	**0.56**	-0.04
(p-value)	**0.019**	0.444
OI2	**0.61**	-0.01
(p-value)	**0.010**	0.491

6 DISCUSSION

The primary observations in the current study were as follows: Bone resorption marker CTx (Carboxyterminal crosslinked telopeptide) was elevated 2 days after the exhausting high-impact exercise. Furthermore, bone formation marker P1NP (procollagen type I amino terminal propeptide) was elevated on day 1 after the exercise. The primary findings support the results found in some of the endurance exercise and short lasting dynamic activity studies (Brahm et al. 1997a, Brahm et al. 1997b, Guillemant et al. 2004, Maimoun et al. 2005, Maimoun et al. 2006, Wallace et al. 2000). Somewhat surprisingly, the findings were in contrast with resistance training studies (Ashizawa et al. 1998, Whipple et al. 2004), in which the bone turnover has been observed to be suppressed. Even though strain rate, strain distribution and strain magnitude play major roles in determining the bone response to loading, biochemical environment affects the response of bone cells (Lanyon 1987), which may explain the apparent discrepancy between the current results and previous results form resistance training (Ashizawa et al. 1998, Whipple et al. 2004) studies.

Some recent studies have suggested that the minimal effective loading intensity to achieve gains in bone strength is around five times body weight (Jamsa et al. 2006, Vainionpaa et al. 2006) and it has been shown that osteogenic response can be excited with relatively low number of repetitions (Rubin & Lanyon 1984). It has been shown that a single bout of osteogenic loading can turn a quiescent periosteum to an active periosteum and the change takes four days to happen. The activation of the periosteum happens in a cascade of events including activation of inactive osteoblasts (Lanyon 1987). The osteogenic index of the exercise in the current study, imposed during a single bout of loading, is comparable to the osteogenic index of walking 20 minutes for 5 days a week (Turner & Robling 2003). The loading effect of walking was estimated from normal values taken from Schneider and Chao (1983). The exercise intensity used in the current study was close to the proposed minimal effective loading intensity and the number of loading cycles by far exceeded the amount needed for an osteogenic effect. Therefore, it seems plausible that the bone response observed in the current study really represents what happens at bone cellular level and was indeed caused by the

single bout of high-intensity exercise. As it has been shown, that exercise intensity plays a major role in determining the adaptive response of the bone (Turner & Robling 2003, Turner 1998), additional evidence is provided by the significant correlation seen between the percentual change in bone formation marker and exercise intensity related variables. Moreover, the fact that osteogenic index 2 fails to account for loading rate seems to make osteogenic index 2 less sensitive measure of osteogenic effect as expected (Turner & Robling 2003., Turner 1998.), therefore emphasizing the importance of loading rate in osteogenic exercises.

Even though the ratio of bone formation to resorption markers can be considered to reflect overall turnover (Christenson 1997), the results do not justify stating that bone resorption is increased after a single bout of high-impact exercise, which would be in contrast to longitudinal changes in bone with osteogenic loading (Rubin & Lanyon 1984). Bone biochemical markers were followed only two days post exercise, the increase in resorption markers was only slightly higher than that in the formation markers, and bone remodeling cycle takes 2 to 3 months to complete (Watts 1999). Thus, hypotheses concerning the amount of overall change in bone turnover, based on the current results, would be invalid. However, the main interest of the study lied in observing the short-term response of bone biochemical markers to high-impact loading and increase in resorption and formation markers was seen.

Considering that only two biochemical bone turnover markers were assessed, it can be concluded that bone response to a single bout of high-impact exercise can be seen by observing the biochemical bone markers during just two days ensuing the exercise. Moreover, the biochemical marker response seems to depend on exercise type even if high intensity exercises are used.

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