

The Effects of 10 Weeks Military Training on Heel Ultrasound and Bone Turnover

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Abstract. To measure the physiological changes in bone in response to strenuous exercise we performed a prospective study of male army recruits over 10 weeks of basic training. Measurements performed at the start and completion of training consisted of ultrasound (US) measurements of the heel: velocity of sound (VOS in m/seconds) and broadband ultrasound attenuation (BUA in dB/MHz) and bone turnover markers; osteocalcin (OC), bone-specific alkaline phosphatase (BALP), and tartrate-resistant acid phosphatase (TRAP). Forty subjects were recruited for the study and 26 completed training. Over the 10-week study period there was a significant 1.7% fall in mean VOS [mean paired difference (mpd) 27.2 m/second, SEM 9.5 (95% CI 7.5–46.8) $P = 0.009$] and a nonsignificant 3.4% increase in BUA ($P = 0.159$). There were significant falls in markers of bone formation OC [11.6%, mpd 0.11 $\mu\text{g/liter}$ (95% CI 0.07–0.14) $P < 0.001$] and BALP [13.3%, mpd 3.49 U/liter (CI 0.80–6.18) $P = 0.013$] and a nonsignificant 9.5% fall in TRAP a marker of bone resorption. The 10 recruits subsequently injured had a significantly lower VOS on entry [mean difference 24.2 m/seconds (95% CI 4.6–43.7) $P = 0.017$] and nonsignificantly raised BUA and baseline levels of all bone markers. The ultrasound changes may be accounted for by increase in trabecular separation and a fall in trabecular connectivity due to microfracture. The decrease in bone markers implies a fall in bone turnover.

Key words: Exercise — Bone — Turnover — Ultrasound — Military.

Exercise is encouraged in our society for its beneficial effects on health, particularly for the prevention of osteoporosis, obesity, and ischemic heart disease. It is well established from prospective [1, 2], cross-sectional [3], and population [4, 5] studies that bone mineral density (BMD) will increase in response to exercise. These changes in BMD reflect increases in bone mass over months or years but how they occur and the structural consequences of exercise on bone structure are unclear.

Ultrasound interacts with bone in a fundamentally different way to ionizing radiation. It correlates well with other

measures of bone density ($r = 0.73$ in calcaneal dual X-ray absorptiometry (DXA) [6]) and may predict fracture risk [7, 8] but may also reflect other qualities important in bone strength. Lower levels of velocity of sound (VOS) and broadband ultrasound attenuation (BUA) are associated with stress fracture in female army recruits [9], and higher velocity measurements are seen in professional football players compared with sedentary controls, indicating an association with weight-bearing exercise [10].

Markers of bone turnover are a useful index of metabolic changes in bone but studies of the physiological response of these markers in normal subjects during exercise have been limited. Osteocalcin (OC) and bone-specific alkaline phosphatase (BALP) are markers for bone formation [11] and osteoblast activity. Tartrate resistant acid phosphatase (TRAP) reflects the bone resorptive activity of osteoclasts. OC has been reported to fall temporarily during a 4-week exercise program [12] and plasma hydroxyproline is raised in those who are at greatest risk of military training injury [13].

Army recruits provide a unique opportunity to study a homogenous group of subjects undergoing rapidly progressive, weight-bearing, physical activity. This allows the study of the physiological changes in bone as a consequence of exercise in a young, healthy population who are not highly selected, elite athletes. We therefore performed a prospective 10-week study of a platoon of recruits in training with the aim of identifying the early physiological changes of bone structure and turnover associated with exercise.

Subjects and Methods

Subjects

The subjects for this study were a platoon drawn at random from the male recruits entering their basic military training during the summer months at the army training regiment in Pirbright, Surrey, UK. They were all new to the British regular army and had passed preliminary medical and educational assessments prior to entering training. Subjects were assessed at the start of the training period during their pre-entry medical assessments, with follow-up assessment 10 weeks later. Injury could lead to a temporary delay in training or to permanent withdrawal. Those who were injured or who withdrew from training were seen by the unit medical officer (UMO) and a clinical diagnosis was made with radiological in-

Table 1. Recruit data on entry to training

	Recruits who started training n = 40		Recruits who completed training n = 26	
	Mean/No	SD (%)	Mean/No	SD (%)
Age	18.5	1.6	18.5	1.7
Weight (kg)	67.6	6.9	68.9	7.7
Height (cm)	174.8	5.4	174.5	4.6
Body mass index	22.1	2.02	22.6	2.2
No. of subjects smoking	19	47.5	13	50
No. who drink alcohol	36	90.0	24	92.3
Weight-training (minutes/week)	111	70	110	75.0
Running preparation (miles run per week × no. of weeks preparation)	72 ^a	34 ^a	102 ^a	57 ^a
VOS on entry to training (m/second)	1628.5	35.8	1633.3	37.4
BUA (dB/Mz)	105.3	16.0	105.5	16.3
OC (µg/liter)	8.4	3.7	8.9	4.5
TRAP (U/liter)	9.2	3.0	8.4	2.7
BALP (U/liter)	28.1	15.3	26.2	16.2

^a Median values and quartile range

vestigations as necessary. Treatment and withdrawal from training was prescribed as appropriate and an injury proforma was completed and filed in the subjects' medical records. Medical records of all the subjects were examined for details of injuries not filled in on the injury proforma. A stress fracture was defined as a fracture occurring as a consequence of overuse of a limb in repetitive strenuous exercise and not as a result of direct trauma. Diagnosis was made on clinical features of crescendo onset of pain, localized bony tenderness, and exclusion of alternative soft tissue injury, although the facilities for Tc⁹⁹ radioisotope nuclear bone scan were available for the UMO as required.

Assessments

Details of ethnic origin, height, weight, and age were obtained. A self-administered questionnaire provided information on pre-entry exercise levels (including miles run per week and length of pre-entry preparation), smoking and alcohol intake, and a past medical history of sports injuries including stress fracture and anterior knee pain.

Calcaneal Bone Ultrasound Measurements

The study used the McCue Ultrasonics Ltd Cubaclinical[®] dry system osteodensitometer for measurement of the VOS and BUA across the calcaneum. This system employs coupled through-transmission, broadband single element US transducers, with a center frequency of 1.0 MHz. VOS (m/second) is the velocity of the ultrasonic wave as it passes through the heel. The BUA (dB/MHz) is a measurement of frequency-dependent attenuation of the ultrasonic wave as it passes through the heel [14]. Calibration is carried out with three quality assurance phantom models prior to scanning. The coefficient of variation (CV) in our hands, assessed by duplicate readings on 30 subjects, was 2.5% for BUA and 0.44% for VOS [15].

Bone Turnover Markers

Blood was collected at the same time of day on entry and completion of training and separated and stored at -20°C for later analysis. Serum OC was measured by competitive ELISA (NovoCalcin, Metra Biosystems, CA, USA) with intra- and inter-assay CVs of 8 and 10%, respectively, at 8 µg/liter. The sensitivity was 0.5 µg/

liter. BALP was measured by a commercial kit (Alkphase-B, Metra Biosystems) in which bone-specific alkaline phosphatase (ALP) binds to specific antibodies coated on a microtiter plate and, after washing, the activity of the enzyme was measured. The minimum detection limit was 0.7 U/liter and the intra- and interassay CV at 28 U/liter were 3.3 and 7.9%, respectively. TRAP was measured by a modification method described by Lau et al. [16]. Serum was incubated in 200 mM of citrate buffer pH 5.5 containing 80 mM sodium tartrate for 15 minutes and assayed for acid phosphatase activity using 100 mM p-NNP as a substrate on a Cobas Bio (Roche) analyzer. The sensitivity of the assay was 0.5 U/liter and the intra- and interassay CVs were 2.9 and 5.5%, respectively, at 15.0 U/liter. Pre- and post-training samples were analyzed in the same batch to reduce analytical variability.

Statistical Analysis

Data were analyzed for normality and equality of variances. Osteocalcin data were log₁₀ transformed before analysis because of non-normality. Changes in means for recruits completing the 10-week training period were assessed by paired Student's *t*-test. Recruits were subdivided into those that successfully completed training and those that sustained an injury, and the differences in means were then calculated using an unpaired Student's *t*-test. Analysis was performed using the Statistics Package for the Social Sciences (SPSS for Windows release 6.0.1).

Results

Forty male subjects were recruited into the study with a mean age of 18.5 (SD 1.6). The demographic, anthropometric, ultrasound, bone marker, and relevant questionnaire data of the 40 at entry and the 26 who successfully completed training are shown in Table 1. Subjects failed to complete training for a variety of reasons: injury, social reasons, psychological unsuitability for military service, or medical problems unrelated to training (e.g., complex migraine). All recruits were caucasian as determined by the questionnaire.

Ten of the 40 male recruits (25%) sustained an injury. Twenty-six of the 40 recruits passed basic training (65%), 5 (19.2%) of whom sustained an injury. There was a variety of soft-tissue injuries recorded including ankle inversion

Table 2. Changes in bone turnover markers

n = 25 (SD)	On entry	On completion	Mean paired difference	95% CI	Significance
OC ($\mu\text{g/liter}$)	8.87 (4.45)	6.47 (3.1)	2.14	1.16–3.11	<0.001 ^a
BALP (U/liter)	26.3 (16.5)	22.8 (13.1)	3.49	0.80–6.18	0.013
TRAP (U/liter)	8.43 (2.69)	7.63 (2.31)	0.80	-0.44–2.04	0.197

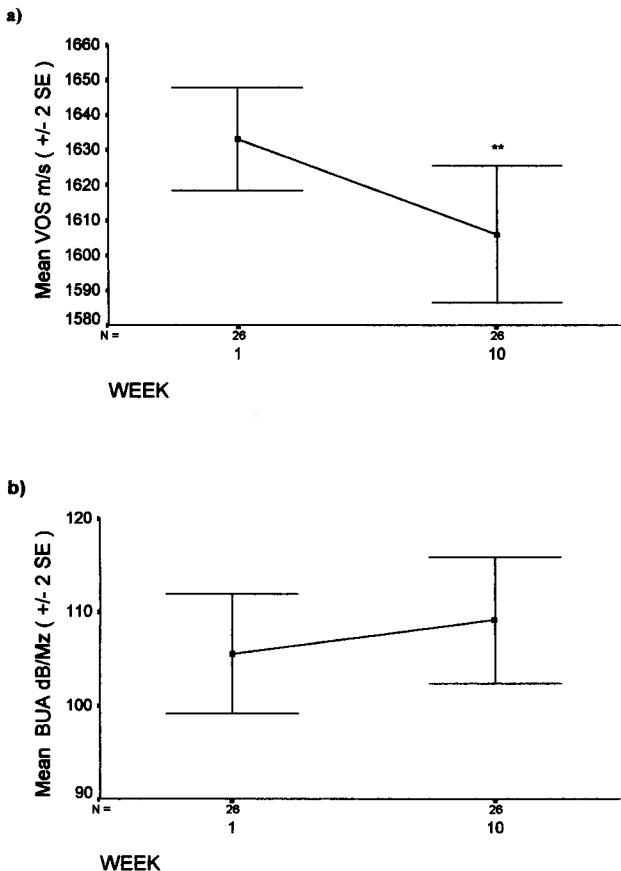
^a Based on Log_{10} OC

Fig. 1. (a) The fall in VOS measurements over the 10-week training period. Mean paired difference 27.2, SEM 9.5, 95% CI 7.5–46.8, $P = 0.009$. (b) The rise in BUA. Mean paired difference -3.6, 95% CI -8.7–1.5, $P = 0.159$.

injuries ($n = 2$), low back pain ($n = 2$), skin blisters ($n = 2$), knee ($n = 1$), and shin pain ($n = 1$). There were two clinically diagnosed cases of stress fracture, one in the tibia and another in the metatarsal, both of which led to training withdrawal, but there were no bone scans performed on these patients. There was a peak in injury incidence at the start of the fourth week of training (mean 22.5 days, SD 12.3).

Figure 1a shows the significant 1.7% fall in calcaneal VOS measurement in recruits who completed training (from $1633.3 \pm \text{SE } 7.3$ to 1606.1 ± 9.8 m/second, $P = 0.009$). Figure 1b shows a 3.4% increase in BUA, from 105.5 ± 3.2 – 109.2 ± 3.4 dB/MHz ($P = 0.159$). Those recruits who completed training uninjured ($n = 21$) had a significant 2.0% mean fall in VOS [mean paired difference (mpd) 33.7

m/second SE 11.2, 95% CI 10.4–57.0, $P = 0.007$], compared with injured recruits who also completed training in whom there was no significant change in VOS. There was a significant 5.9% increase in BUA in those uninjured (mpd, $6.2 \pm \text{SE } 2.2$ dB/MHz, $P = 0.009$), compared with a mean 4.2% fall in those who were injured (mpd 7.2 ± 8.2 , $P = 0.431$). The difference in the percentage change in VOS and BUA between the injured and uninjured groups was not statistically significant. The VOS measurement on entry was significantly lower in those recruits who were subsequently injured. The mean difference was 24.2 m/second, 95% CI 4.6–43.7, $P = 0.017$. The opposite was seen for BUA which was nonsignificantly higher in the injured (mean difference 5.4, 95% CI -23–12.1, $P = 0.511$).

Table 2 illustrates the change in serum OC, BALP, and TRAP over the exercise period. There were significant falls in OC (Log_{10} OC showed a 11.6% fall, with a mean paired difference of 0.106 $\mu\text{g/liter}$, 95% CI 0.068–0.144, $P < 0.001$), BALP (13.3%), and a nonsignificant fall in TRAP (9.5%) over the 10 weeks of training. Baseline concentrations of all bone markers were nonsignificantly higher in those recruits subsequently injured compared with those not injured (BALP 34.7 versus 25.9, TRAP 9.96 versus 8.98, OC 9.82 versus 7.91).

Discussion

This study has shown changes in the US measurements made at the calcanea of army recruits following the completion of 10 weeks of strenuous weight-bearing exercise. Overall, the VOS measurements fell significantly whereas BUA tended to increase. Recruits who did not suffer injury had a greater fall in VOS and increase in BUA than injured recruits. All the bone markers declined over the 10-week period, OC and BALP significantly. The number and type of injuries were consistent with the pattern of recruit training and other reports of military training injury incidence; a large peak in the incidence of injuries was seen at the beginning of the fourth week of training and thereafter the rate appeared to fall [17, 18].

There were limitations to the present study. The number of recruits followed up over the 10-week period was relatively small but this, in part, reflects the high dropout rate from military training. The findings of this study were not compared with the results of dual X-ray absorptiometry (DXA) because of logistic difficulties in interrupting the recruits' training to perform scans and the poor sensitivity of DXA to change in BMD over short time periods. Likewise, other bone markers of resorption were not used because of difficulties in obtaining standardized urine samples. The diagnosis of a stress fracture was made on clinical grounds, without bone scanning, in two cases, reflecting the observational nature of the study and local clinical practice.

Prolonged mechanical loading through weight-bearing

exercise will stimulate an increase in bone mass [19]. Studies of the acute response of bone to mechanical strain implicate the osteocyte as the cellular mediator of the effect, directing the bone modeling units of osteoblasts and osteoclasts [20]. There is a rapid response of the osteocyte to mechanical strain within a few minutes of a single period of loading and there is a loading-related increase in RNA synthesis soon after [21]. However, the events that occur between the acute molecular response of bone to strain and the long-term consequences of exercise on BMD are unclear.

Ultrasound offers a mode of examining the structural and material properties of bone *in vivo* and hence its early response to weight-bearing stress. The limitation of the technology is that the relationship between the us parameters measured and trabecular structure is not fully understood but there have been some associations reported. Ultrasound transmission velocity, VOS, is proportional to the elastic modulus, compressive, and yield strengths of the bone [22] and there is a good correlation between BUA and physical density ($r = 0.85$, $P < 0.0001$ [23]). *In vitro* studies of trabecular microstructure have demonstrated an inverse correlation between the us transmission velocity and the degree of trabecular separation [24]. BUA is negatively correlated with trabecular connectivity. In this present study therefore, the fall in VOS over the training period could suggest an increase in trabecular separation, and the rise in BUA would be associated with a fall in connectivity. This may represent a loss of trabeculae due to fatigue microfractures or possibly trabecular thinning and perforation due to remodeling [24]. Microfractures in trabeculae have been shown to reduce both the elastic modulus and material strength of the bone [25, 26], findings that are compatible with the changes in us parameters seen in this study. An alternative explanation might be that the us changes seen are due to alterations in the organic bone matrix, or tissue edema, in response to exercise.

The fall in markers of both bone formation and resorption suggests that there is an overall reduction in bone turnover in response to this level of strenuous exercise. A similar fall in OC in response to exercise has also been reported elsewhere [12]. This implies that the changes in US variables seen on exercise are not directly a consequence of active remodeling but could be a result of traumatic microfracture. The 10-week period may be too short or the weight-bearing exercise too intense to stimulate bone remodeling. Higher baseline concentrations of bone markers in those subsequently injured have also been described [13] and may be an indication of lower levels of pre-entry exercise.

The higher VOS levels seen in professional sportsmen [10], and in trained race horses [27], may reflect a highly selected, genetically determined bone structure associated with athletic performance or, alternatively, that the fall in VOS demonstrated in this study is an early adaptive structural response of bone with a subsequent rise in VOS seen after continued weight-bearing activity. These factors may also explain the higher starting levels of VOS in those recruits who escaped injury. Pre-entry fitness or genetic factors may generate sufficient bone reserves to allow extensive damage to occur before the bone fails or the recruit becomes injured in some other way. VOS measurements may act as a surrogate marker for resistance to training injury. Extending the study later in the time course of a period of training (and hence in the remodeling cycle) would allow us to demonstrate whether the changes in us and bone markers are transient and preempt increases in BMD.

In conclusion, this study has demonstrated some of the early US and bone marker changes that occur in acute response to strenuous weight-bearing exercise. These results may be explained by some initial damage to the trabecular structure and a reduction in the level of bone turnover. It remains to be determined, in a larger population, whether this is an early adaptive mechanism in the development of denser bones.

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References

1. Nelson ME, Fisher EC, Dilmanian FA, Dallal GE, Evans WJ (1991) A one-year walking program and increased dietary calcium in postmenopausal women: effects on bone. *Am J Clin Nutr* 53:1304–1311
2. Nichols DL, Sanborn CF, Bonnick SL, Ben-Ezra V, Gench B, Dimarco NM (1994) The effects of gymnastics training on bone mineral density. *Med Sci Sports Exerc* 26:1220–1225
3. Karlson MK, Johnell O, Obrant KJ (1993) Bone mineral density in weight lifters. *Calcif Tissue Int* 52:212–215
4. Valimaki MJ, Karkkainen M, Lamberg-Allardt, Laitinen K, Alhava E, Heikkinen J (1994) The Cardiovascular Risk in Young Finns Study Group. Exercise smoking and calcium intake during adolescence and early adulthood as determinants of peak bone mass. *Br Med J* 309:230–235
5. Zhang J, Feldblum PJ, Fortney JA (1992) Moderate physical activity and bone density among perimenopausal women. *Am J Public Health* 82:212–215
6. Waud CE, Lew R, Baran DT (1992) The relationship between ultrasound and densitometric measurements of bone mass at the calcaneus in women. *Calcif Tissue Int* 51:415–418
7. Hans D, Dargent-Molina P, Schott AM, Sebert JL, Cormier C, Kotzki PO, et al. (1996) Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. *Lancet* 348:511–514
8. Bauer DC, Gluer CC, Cauley JA, Vogt TM, Ensrud KE, Genant HK, et al. (1997) Broadband ultrasound attenuation predicts fractures strongly and independently of densitometry in older women. A prospective study. Study of Osteoporotic Fractures Research Group. *Arch Intern Med* 157:629–634
9. Kimmel DB, Lappe JM, Hise L, Laurin M, White M, Stegman MR (1996) Quantitative ultrasound predicts stress fracture during basic training of soldiers. *Osteoporosis* 96:109–113
10. Jergas M, Uffmann M, Wittenberg R, Muller P, Koster O (1992) Ultrasonic velocity measurements at weight-bearing and non-weight-bearing sites of the peripheral skeleton. The effect of physical activity in soccer players. *Rofo Fort auf dem* 157:420–424
11. Charron SA, Delmas PD, Malaval L, Chavassieux PM, Arlott M, Chapuy M, et al. (1986) Serum gla-protein in renal osteodystrophy: comparison with bone histomorphometry. *J Clin Endocrinol Metab* 63:892–897
12. Franck H, Becker F, Gurk S (1991) The effect of physical activity on bone turnover in young adults. *Exp Clin Endocrinol* 98:42–46
13. Murguia MJ, Vailas A, Mandelbaum B, Norton J, Hodgdon J, Goforth H, et al. (1988) Elevated plasma hydroxyproline. A possible risk factor associated with connective tissue injuries during overuse. *Am J Sports Med* 16:660–664

14. Moris M, Peretz A, Tjeka R, Negaban N, Wouters M, Bergmann P (1995) Quantitative ultrasound bone measurements: normal values and comparison with bone mineral density by dual X-ray absorptiometry. *Calcif Tissue Int* 57:6–10
15. Arden NK, Baker J, Hogg C, Baan K, Spector TD (1996) The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. *J Bone Miner Res* 11:530–534
16. Lau KH, Onishi T, Wergedal JE, Singer FR, Baylink DJ (1987) Characterization and assay of tartrate-resistant acid phosphatase activity in serum: potential use to assess bone resorption. *Clin Chem* 33:458–462
17. Jones BH, Bovee MW, Harris JM III, Cowan DN (1993) Intrinsic risk factors for exercise-related injuries among male and female army trainees. *Am J Sports Med* 21:705–710
18. Linenger JM, Shwayhat AF (1992) Epidemiology of podiatric injuries in US Marine recruits undergoing basic training. *J Am Podiatr Med Assoc* 82:269–271
19. Etherington J, Harris PA, Nandra D, Hart DJ, Wolman RL, Doyle DV, 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*
20. Lanyon LE (1993) Osteocytes, strain detection, bone modeling and remodeling. *Calcif Tissue Int* 53(suppl 1):S102-6; S106-7
21. Rubin CT, Lanyon LE (1987) Kappa Delta Award paper. Osteoregulatory nature of mechanical stimuli: function as a determinant for adaptive remodeling in bone. *J Orthop Res* 5: 300–310
22. Kaufman JJ, Einhorn TA (1993) Ultrasound assessment of bone. *J Bone Miner Res* 8:517–525
23. McCloskey EV, Murray SA, Charlesworth D, Miller C, Fordham J, Clifford K, et al. (1990) Assessment of broadband ultrasound attenuation in the os calcis in vitro. *Clin Sci* 78: 221–225
24. Gluer CC, Wu CY, Jergas M, Goldstein SA, Genant HK (1994) Three quantitative ultrasound parameters reflect bone structure. *Calcif Tissue Int* 55:46–52
25. Carter DR, Hayes WC (1997) Compact bone fatigue damage—1: residual strength and stiffness. *J Biomech* 10:325–337
26. Carter DR, Caler WE (1985) A cumulative damage model for bone fracture. *J Orthop Res* 3:84–90
27. Buckingham SH, McCarthy RN, Anderson GA, McCartney RN, Jeffcott LB (1992) Ultrasound speed in the metacarpal cortex—a survey of 347 thoroughbreds in training. *Equine Vet J* 24:191–195