Relation between bone turnover, oestradiol, and energy balance in women distance runners

C L Zanker, I L Swaine

Abstract
Objective—To explore relations between biochemical markers of bone turnover, indices of nutritional status, and serum oestradiol concentration in women distance runners.
Methods—Thirty three women distance runners of mean age (SD) 27.2 (1.8) years participated. Eighteen were defined as eumenorrhoeic, nine as amenorrhoeic, and six as oligomenorrhoeic. Mean (SD) running distance was 47.6 (22.4) km/week. Using bivariate correlation and regression analysis, serum levels of osteocalcin and bone alkaline phosphatase (BAP) and also urine deoxypyridinoline/creatinine (Dpyr/Cr) were correlated with mean daily energy balance, body mass index (BMI; kg/m²), and serum levels of total 3,3',5'-triiodothyronine and oestradiol within each group by menstrual status.
Results—All the amenorrhoeic women were in negative energy balance; they had a lower BMI, lower serum levels of oestriadiol, triiodothyronine, osteocalcin and BAP and a lower urine Dpyr/Cr than any of the oligomenorrhoeic or eumenorrhoeic women. These variables were also lower in oligomenorrhoeic than in eumenorrhoeic women. Positive correlations were observed between serum levels of osteocalcin or BAP and both BMI and serum oestradiol concentration in amenorrhoeic, but not in oligomenorrhoeic or eumenorrhoeic women. Urine Dpyr/Cr did not correlate with any other variable within any group. Serum oestradiol concentration correlated positively with BMI in amenorrhoeic and oligomenorrhoeic, but not eumenorrhoeic women.
Conclusions—Positive correlations between serum levels of bone formation markers, BMI and serum oestradiol concentration in our amenorrhoeic runners suggested that their reduced bone formation was linked to a low BMI and an oestrogen deficiency. Reduced bone turnover in amenorrhoeic distance runners has not previously been shown. These findings emphasise the importance of body mass and its possible link with a chronic energy deficit and hypothalamic dysfunction on bone remodelling balance in amenorrhoeic runners.

Keywords: bone turnover; women runners; amenorrhoea; oestradiol; energy deficit

Exercise is one of many factors known to influence bone mass. Weight bearing exercise, which subjects the skeleton to increased mechanical loading, has been shown to increase bone mass. However, exercise has also been linked to a reduced bone mass in some individuals. These individuals include young women who develop amenorrhoea in association with vigorous exercise training. These amenorrhoeic women have a lower bone mineral density (BMD) than their eumenorrhoeic counterparts and they continue to lose bone while their amenorrhoea persists.

The most common explanation for bone loss in young athletic women with amenorrhoea is a deficiency of sex hormones, especially oestrogens. Oestrogen deficiency has been shown to be accompanied by an increase in bone turnover, with exaggerated bone resorption. This bone remodelling imbalance has been shown after ovarian failure and can be corrected by oestrogen replacement. Consequently, it has been recommended that young athletic women with amenorrhoea are also prescribed exogenous oestrogen replacement therapy to protect their bone mass. However, this therapy appears to be less effective in reversing or even preventing, osteopenia in these athletic women. This would suggest that their bone loss is not solely attributable to a sex hormone deficiency.

Another possible explanation for this bone loss is that it is linked to a nutritional deficiency. Exercise associated amenorrhoea has been shown to stem from hypothalamic dysfunction rather than from primary ovarian failure, and it has been suggested that this hypothalamic dysfunction is induced by an energy deficit (imbalance between energy intake and expenditure). Indeed, a series of neuroendocrine and peripheral metabolic disturbances have been documented in young women with exercise associated amenorrhoea, many of which may disrupt bone remodelling. The severity of these disturbances is inversely related to body mass index (BMI) and they are reversed by weight gain. Since weight gain is also accompanied by increases in BMD in these women, it is possible that an energy deficit and the influence of this deficit on body mass and endocrine function may play a more dominant role than an oestrogen deficiency in the development of osteopenia in these women.

To date, the dynamics of bone remodelling have not been studied in young athletic women in relation to their menstrual and nutritional status. Therefore the purpose of this study was to explore relations between biochemical markers of bone turnover, indices of nutritional sta-
Amenorrhoeic women had a lower energy expenditure than eumenorrhoeic (p<0.01) and oligomenorrhoeic (p<0.01) women. The energy intake of the amenorrhoeic women was lower than that of the eumenorrhoeic and oligomenorrhoeic women (p>0.05). The energy intake of the amenorrhoeic women was lower than that of the eumenorrhoeic and oligomenorrhoeic (p>0.05). The energy intake of the amenorrhoeic women (p<0.01) and eumenorrhoeic women (p<0.001), and the oligomenorrhoeic women (p>0.05). The energy intake of the amenorrhoeic and eumenorrhoeic women (p<0.01). There was no difference between the BMI of the oligomenorrhoeic and eumenorrhoeic women (p<0.05). During the seven day assessment of energy balance, 25 of the 33 subjects were calculated to be in “energy deficit”. These 25 women included all nine amenorrhoeics, five of the six oligomenorrhoeics and 11 of the 18 eumenorrhoeics (p<0.001) and oligomenorrhoeic women (p<0.001), but there was no difference between the BMI of the oligomenorrhoeic and eumenorrhoeic women (p<0.05).

During the seven day assessment of energy balance, 25 of the 33 subjects were calculated to be in “energy deficit”. These 25 women included all nine amenorrhoeics, five of the six oligomenorrhoeics and 11 of the 18 eumenorrhoeics (p<0.001) and oligomenorrhoeic women (p<0.001), but did not differ between eumenorrhoeic and oligomenorrhoeic women (p<0.05). The energy intake of the amenorrhoeic women was lower than that of the oligomenorrhoeic (p<0.01) and eumenorrhoeic women (p<0.001), and the oligomenorrhoeic women had a lower energy intake than the eumenorrhoeic women (p<0.01). There was no difference between energy expenditure estimates for amenorrhoeics and eumenorrhoeics (p>0.05), but oligomenorrhoeic women had a lower energy expenditure than amenorrhoeic (p<0.01) and eumenorrhoeic women (p<0.01).

Table 1 gives the physical characteristics and energy balance data for the subjects. The mean (SD) duration of amenorrhoea was 2.8 (1.4) years. Amenorrhoeic women had a lower BMI than oligomenorrhoeic (p<0.001) and eumenorrhoeic women (p<0.001), but there was no evidence of this marker for creatinine (Cr) excretion. Serum oestradiol was measured using an ELISA kit (Boehringer-Mannheim), and total T	extsubscript{2}, using a chemiluminescence kit (Nichol Institute Diagnostics Ltd, Saffron Walden, Essex, UK). The intra-assay coefficients of variation for these assays were 5–8% for osteocalcin and 4–6% for BAP. Values were compared with normative control data for age-matched women which were specific to these assays. The bone resorption marker, deoxypyridinoline (D	extsubscript{pyr}) was assayed from an early morning urine sample using an enzymoimmunoassay kit (Pyrilinks D; Metra Biosystems Inc., Oxford, UK). The intra-assay coefficients of variation for these assays were 5–8% for osteocalcin and 4–6% for BAP. Values were compared with normative control data for age-matched women which were specific to these assays. The bone resorption marker, deoxypyridinoline (D	extsubscript{pyr}) was assayed from an early morning urine sample using an enzymoimmunoassay kit (Pyrilinks D; Metra Biosystems Inc.). The intra-assay coefficient of variation was 8–10%. Urine levels of this marker were corrected for creatinine (Cr) excretion. Serum oestradiol was measured using an ELISA kit (Boehringer-Mannheim), and total T	extsubscript{2}, using a chemiluminescence kit (Nichol Institute Diagnostics Ltd, Saffron Walden, Essex, UK). The intra-assay coefficients of variation for these assays were 5–8% for osteocalcin and 4–6% for BAP. Values were compared with normative control data for age-matched women which were specific to these assays. The bone resorption marker, deoxypyridinoline (D	extsubscript{pyr}) was assayed from an early morning urine sample using an enzymoimmunoassay kit (Pyrilinks D; Metra Biosystems Inc.). The intra-assay coefficient of variation was 8–10%.

Subjects and methods
Thirty three women distance runners of mean (SD) age 27.2 (1.8) years gave written informed consent and were recruited to the study, which was approved by the ethical committee of North Bedfordshire District Health Authority. All were white non-smokers, who had not taken any type of hormone medication for more than two years. Eighteen of these women were defined as eumenorrhoeic (11–13 menstrual bleeds a year for the two years before this study), nine as amenorrhoeic (absence of menses for more than two years), and six as oligomenorrhoeic (three or less menstrual bleeds per year during the preceding two years). All subjects had been running regularly for at least four years. Their volume of running training ranged from 15 to 97 km/week (mean (SD) 47.6 (22.4) km/week).

EVALUATION OF NUTRITIONAL STATUS
BMI (kg/m	extsuperscript{2}) was determined for each woman from body mass and stature. Mean daily energy balance was calculated by subtracting dietary energy intake from estimated energy expenditure measured over a seven day period. Energy intake was computed using a nutrition database program (COMP-EAT 4.0; Pond Nutrition Systems, London, UK). Subjects recorded the mass (to the nearest 2 g) of all food and beverages consumed using a pair of electronic food scales (EKS). Energy expenditure was estimated from records of activity patterns. Each subject recorded the time (to the nearest 15 minutes) that they spent engaged in each of four categories of activity. These activity categories were given a “metabolic equivalent” (MET) value which ranged from 1 to 10 (where 1 MET = energy expenditure/basal metabolic rate as described by Ainsworth et al	extsuperscript{15}). The basal metabolic rate of each subject was calculated using an equation based on age and body mass.

BIOCHEMICAL ANALYSES
Fasting blood samples were taken between 0900 and 1000 hours. These samples were taken during the early follicular phase of the menstrual cycle for eumenorrhoeic subjects. Biochemical markers of bone formation (osteocalcin and bone-specific alkaline phosphatase (BAP)) were measured in serum by using enzymoimmunoassay kits (Novocalcin and Alkphase B respectively; Metra Biosystems Ltd, Essex, UK). The intra-assay coefficients of variation for these assays were 5–8% for osteocalcin and 4–6% for BAP. Values were compared with normative control data for age-matched women which were specific to these assays. The bone resorption marker, deoxypyridinoline (D	extsubscript{pyr}) was assayed from an early morning urine sample using an enzymoimmunoassay kit (Pyrilinks D; Metra Biosystems Inc.). The intra-assay coefficient of variation was 8–10%. Urine levels of this marker were corrected for creatinine (Cr) excretion. Serum oestradiol was measured using an ELISA kit (Boehringer-Mannheim), and total T	extsubscript{2}, using a chemiluminescence kit (Nichol Institute Diagnostics Ltd, Saffron Walden, Essex, UK). The intra-assay coefficients of variation for these assays were 5–8% for osteocalcin and 4–6% for BAP. Values were compared with normative control data for age-matched women which were specific to these assays. The bone resorption marker, deoxypyridinoline (D	extsubscript{pyr}) was assayed from an early morning urine sample using an enzymoimmunoassay kit (Pyrilinks D; Metra Biosystems Inc.). The intra-assay coefficient of variation was 8–10%.

STATISTICAL ANALYSES
A Pearson's product moment correlation coefficient and regression analysis were used to explore the relations between biochemical markers of bone turnover, indices of energy availability (BMI, energy balance, T	extsubscript{2} and serum oestradiol within each group of subjects by menstrual status. Differences in group mean values for all physical characteristics and biochemical data between amenorrhoeic, eumenorrhoeic and oligomenorrhoeic women were compared using one way analysis of variance and a Tukey B test. Probability values of less than 0.05 were considered to be significant.

Table 1  Body mass index (BMI), energy intake (EI), energy expenditure (EE) and net energy balance (EB) of subjects. Values indicated as mean (SE)

<table>
<thead>
<tr>
<th></th>
<th>Amenorrhoeic (n=9)</th>
<th>Oligomenorrhoeic (n=6)</th>
<th>Eumenorrhoeic (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m	extsuperscript{2})</td>
<td>17.5 (0.2)*‡</td>
<td>19.3 (0.3)</td>
<td>19.8 (0.2)</td>
</tr>
<tr>
<td>EI (kJ/day)</td>
<td>7327 (193)*‡</td>
<td>8843 (276)*‡</td>
<td>10478 (212)</td>
</tr>
<tr>
<td>EE (kJ/day)</td>
<td>10123 (234)*†‡</td>
<td>9276 (149)*‡</td>
<td>10232 (215)</td>
</tr>
<tr>
<td>EB (kJ/day)</td>
<td>−2797 (247)*‡</td>
<td>−433 (149)</td>
<td>246 (188)</td>
</tr>
</tbody>
</table>

*Amenorrhoeic v oligomenorrhoeic, p<0.001; †amenorrhoeic v eumenorrhoeic, p<0.01; ‡amenorrhoeic v eumenorrhoeic, p<0.001; §oligomenorrhoeic v eumenorrhoeic, p<0.01.
Table 2 Serum levels of oestradiol, total 3,5,3'-triiodothyronine (T₃), osteocalcin, bone alkaline phosphatase (BAP) and urine Dpyr/Cr levels for the 33 active women. Values indicated as mean (SE)

<table>
<thead>
<tr>
<th></th>
<th>Amennorhoeic (n=9)</th>
<th>Oligomenorrhoeic (n=18)</th>
<th>Eumenorrhoeic (n=6)</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol (pmol/l)</td>
<td>46.3 (5.5)†‡</td>
<td>72.2 (4.8)†‡</td>
<td>110.7 (4.9)</td>
<td>80–180‡‡</td>
</tr>
<tr>
<td>T₃ (nmol/l)</td>
<td>1.5 (0.1)†§</td>
<td>2.1 (0.2)</td>
<td>2.6 (0.2)</td>
<td>1.3–3.2</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>7.3 (0.2)†‡</td>
<td>9.4 (0.3)¶</td>
<td>12.0 (0.2)</td>
<td>6.7–13.9</td>
</tr>
<tr>
<td>BAP (U/l)</td>
<td>4.0 (0.7)†‡</td>
<td>15.3 (0.8)</td>
<td>20.4 (0.6)</td>
<td>10.8–30.6</td>
</tr>
<tr>
<td>Dpyr/Cr (nmol/mmol Cr)</td>
<td>2.8 (0.85)‡‡</td>
<td>3.9 (0.9)**</td>
<td>4.3 (0.6)</td>
<td>2.4–5.0</td>
</tr>
</tbody>
</table>

*Amennorhoeic v oligomenorrhoeic, p<0.001; †amenorrhoeic v eumenorrhoeic, p<0.01; ‡amenorrhoeic v eumenorrhoeic, p<0.01; §oligomenorrhoeic v eumenorrhoeic, p<0.001; ¶oligomenorrhoeic v eumenorrhoeic, p<0.01; **oligomenorrhoeic v eumenorrhoeic, p<0.05. †‡Follicular phase of menstrual cycle.

**BIOCHEMICAL DATA**

Table 2 gives serum levels of oestradiol, T₃, osteocalcin, and BAP and urine Dpyr/Cr. The eumenorrhoeic women had a higher serum oestradiol concentration than the oligomenorrhoeic (p<0.001) or amenorrhoeic women (p<0.001), and the oligomenorrhoeic women had a higher serum oestradiol concentration than the amenorrhoeic women (p<0.001). Furthermore, all the amenorrhoeic and all but one of the oligomenorrhoeic women had a serum oestradiol concentration below the normal reference range for young eumenorrhoeic women during the early follicular phase of the menstrual cycle. In contrast, all of the eumenorrhoeic women exhibited a normal early follicular phase serum oestradiol concentration. Serum T₃ concentration was lower in the amenorrhoeic than the oligomenorrhoeic (p<0.01) or eumenorrhoeic women (p<0.01), but did not differ between the oligomenorrhoeic and eumenorrhoeic women (p>0.01).

Serum levels of osteocalcin and BAP were higher in the eumenorrhoeic than the oligomenorrhoeic (p<0.001; osteocalcin and BAP), and eumenorrhoeic women (p<0.001; osteocalcin and BAP), and the oligomenorrhoeic women had higher serum levels of these bone formation markers than the amenorrhoeic women (p<0.001; osteocalcin and BAP). Urine levels of Dpyr/Cr did not differ between eumenorrhoeic and oligomenorrhoeic women (p>0.05) or between amenorrhoeic and oligomenorrhoeic women (p>0.05), but it was higher in eumenorrhoeic than amenorrhoeic women (p<0.05). Figure 1 shows a scatter plot of serum osteocalcin concentration and BMI, and fig 2 shows a scatter plot of serum osteocalcin concentration and serum oestradiol concentration for eumenorrhoeic, oligomenorrhoeic, and amenorrhoeic women.

**CORRELATIONS**

Serum levels of osteocalcin and BAP correlated with BMI in amenorrhoeic (r = 0.90, p<0.001 and r = 0.88, p<0.001 respectively), but not in oligomenorrhoeic (r = 0.62, p>0.05 and r = 0.57, p>0.05) or eumenorrhoeic women (r = −0.23, p>0.05 and r = −0.27, p>0.05). These bone formation markers also correlated with serum oestradiol concentration in amenorrhoeic (r = 0.85, p<0.001 and r = 0.83, p<0.001; osteocalcin and BAP respectively), but not in oligomenorrhoeic (r = 0.58, p>0.05 and r = 0.56, p>0.05) or eumenorrhoeic women (r = −0.27, p>0.05 and r = −0.29, p>0.05). No correlations were observed between bone formation markers and either energy balance or serum T₃ concentration in any group of runners. Urine Dpyr/Cr did not correlate with BMI, energy balance or serum T₃ concentration in any subject group. Serum oestradiol concentration correlated with BMI in amenorrhoeic and oligomenorrhoeic (r = 0.92, p<0.001 and r = 0.76, p = 0.04 respectively) but not eumenorrhoeic (r = −0.27; p>0.05) runners.

**Discussion**

In this study, metabolic markers of bone turnover were shown to vary with menstrual status and nutritional indices in young women distance runners. When compared with their eumenorrhoeic counterparts, amenorrhoeic and oligomenorrhoeic runners appeared to have reduced bone turnover and, in particular, reduced bone formation. Furthermore, this reduced bone formation was associated with a low BMI and a reduced serum level of oestradiol in amenorrhoeic runners. In contrast, there was no relationship between markers of bone formation and either BMI or serum oestradiol concentration in the oligomenorrhoeic or eumenorrhoeic runners.

The interrelationship between biochemical markers of bone turnover, indices of nutritional status, and oestrogen levels has not previously been explored in young women distance runners. Nevertheless, previous research has implied that there is a bone remodelling imbalance in amenorrhoeic runners, by demonstrating that they have a lower BMD than regularly
menstruating runners,\textsuperscript{3–5} that they continue to lose bone while their amenorrhoea persists,\textsuperscript{4–7} and that their bone loss may be exacerbated in the presence of a low BMI.\textsuperscript{8–12} However, this research has not included any measurements of bone turnover, which would help to explain the interaction between oestrogen levels, energy balance, and body mass on bone remodelling in these women.

The results of the present study show that a low BMI and an oestrogen deficiency were associated with disruption of bone formation in amenorrhoeic women distance runners. Furthermore, it appeared that the magnitude of this disruption may be governed by the extent to which their BMI or serum oestriadiol concentration were reduced. However, the absence of correlations between serum levels of bone formation markers, BMI and serum oestriadiol concentration in the eumenorrhoeic runners suggested that there might be some “threshold” value of BMI, or serum oestriadiol concentration, above which bone formation is not influenced by either of these variables.

The strong correlation observed between serum oestriadiol concentration and BMI in the amenorrhoeic and oligomenorrhoeic runners was not unexpected, since it is known that the maintenance of a low BMI is associated with ovarian suppression, amenorrhoea, and an oestrogen deficiency in young women.\textsuperscript{13–15} This ovarian suppression has been shown to originate from a hypothalamic disturbance,\textsuperscript{13–15} which is accompanied by a series of metabolic changes which can influence bone remodelling.\textsuperscript{16} These metabolic changes have been shown in amenorrhoeic runners and also in young women with chronic anorexia nervosa.\textsuperscript{17} Furthermore, recent research has shown that there is reduced bone formation in anorexic women,\textsuperscript{18} which concurs with the observations of reduced bone formation in our amenorrhoeic runners. In support of the hypothesis that a chronic energy deficit and its effects on BMI may disrupt bone remodelling, studies have shown that the rate and extent of bone loss in amenorrhoeic athletes and in anorexic women is related to the magnitude of their weight loss and to the duration of the decreased BMI.\textsuperscript{4,18–21} Also, it is known that these women exhibit increases in BMD in response to refeeding and weight gain, even without resumption of their menstrual cycle.\textsuperscript{4,18–21}

Since all of the oestrogen-deficient amenorrhoeic and oligomenorrhoeic runners of the present study had a lower BMI than the eumenorrhoeic runners, it was difficult to differentiate between the possible effect of a low BMI and that of oestrogen deficiency on bone formation in these women. Many studies have shown that oestrogen-deficient women have increased bone turnover with exaggerated bone resorption.\textsuperscript{9,18,22} Thus the lower urine levels of the bone resorption marker Dpyr/Cr in our oestrogen-deficient amenorrhoeic runners contradicts previous research.\textsuperscript{22} This contradiction may be explained by differences in the aetiology of the oestrogen deficiency of our amenorrhoeic runners and that of the subjects in previous studies.\textsuperscript{9,10,22} Whereas the subjects of the previous studies were oestrogen deficient as a result of primary ovarian failure, it is known that amenorrhoea is caused by a hypothalamic disturbance.\textsuperscript{13–16} Therefore it is possible that the reduced bone resorption (as well as reduced bone formation) observed in our amenorrhoeic runners was linked to the metabolic consequences of a hypothalamic disturbance.

The observations made in this study may be of relevance to the prophylaxis and treatment of osteopenia in young athletic women with amenorrhoea. It has been suggested that these women should be prescribed oestrogen replacement to attenuate bone turnover.\textsuperscript{11,21} However, our amenorrhoeic runners already had reduced bone turnover. It therefore appeared that the stimulatory effect of an oestrogen deficiency on bone turnover in these amenorrhoeic runners was being counteracted by other factors. These findings do not contradict the use of oestrogen replacement in these runners, since there is evidence to suggest that oestrogen can induce bone formation by increasing the production of osteoblasts.\textsuperscript{10,25} Nevertheless, this study appears to emphasise the importance of body mass and its possible link with a chronic energy deficit and hypothalamic dysfunction on bone remodelling balance in amenorrhoeic runners.

The authors wish to thank the National Osteoporosis Society and De Montfort University for funding this study, Mrs Caroline Beavell and Mrs Sue Clark for their technical assistance, and Dr Edward Winter for his guidance with some of the statistical analyses.

Commentary

Osteoporosis leading to an increased risk of fracture in older women is a topic receiving much current attention. Recommendations are made frequently that potentially reversible risk factors should be dealt with. One of those risk factors is disturbance of menstrual function in young women, with lengthy periods of amenorrhea during reproductive life being regarded as important. One circumstance in which disordered menstrual function is well known to occur is in young women athletes. The present paper makes an important contribution by providing objective data on the relation between energy imbalance and markers of bone turnover in young women athletes of varying menstrual status. The authors rightly point out that it has been common belief that loss of bone density in such women is attributable to oestrogen deficiency. Their paper underlines the importance of energy deficit, carrying the significant suggestion that reversal of such energy deficit and restoration of body mass should be the preferred aims of therapy in the prevention of osteopenia in young athletes with amenorrhea. This is an area in which there has been a paucity of information, and current recommendations are that such women should be treated with oestrogens. The authors are to be commended for their useful contribution.

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