Reduced bone formation in UK Gulf War veterans: a bone histomorphometric study

J E Compston, S Vedi, A B Stephen, S Bord, A R Lyons, S J Hodges, B E Scammell

Aims: Gulf War veterans report a high prevalence of musculoskeletal symptoms. The aim of this study was to establish whether there were abnormalities in bone turnover and remodelling in a group of symptomatic subjects who had served in the Gulf War.

Methods: Iliac crest bone biopsies were obtained from 17 Gulf War veterans who were seeking litigation and compared with those of 13 age and sex matched healthy controls. Bone histomorphometry was performed using image analysis.

Results: Cancellous bone area was significantly lower in Gulf War veterans than in control subjects (p = 0.027) and this was associated with a significantly reduced mineral apposition rate (p = 0.002), mean wall width (p < 0.0001), and bone formation rate at the tissue level (p < 0.0001).

Conclusions: These results demonstrate that in this group of Gulf War veterans there was a significant reduction in bone formation at both the cellular and tissue level and this was associated with a reduction in cancellous bone area. The cause of these abnormalities is unknown but might be related to potentially harmful exposures during service in the Gulf War or to changes in lifestyle as a result of chronic ill health. The clinical relevance of the observed reduction in bone formation remains to be established.

SUBJECTS AND METHODS
Seventeen Gulf War veterans, aged 27–51 years (mean, 34.9) who were seeking litigation underwent a variety of investigations on a volunteer basis.

A n increased prevalence of symptoms and disorders has been reported by veterans of the Gulf War including chronic fatigue, headache, irritability, musculoskeletal symptoms, and depression. The existence of a specific Gulf War syndrome has been widely debated, but there is little evidence to support this concept, similar health problems being reported by veterans from other wars. Nevertheless, active military service in the Gulf War was associated with several hazardous exposures; some of these, such as exposure to the smoke of burning oil, vaccinations against biological warfare, organophosphates, and measures to protect against chemical warfare were more prominent in Gulf War veterans than in other military cohorts whereas others, such as multiple vaccinations, were used with similar frequency.

Musculoskeletal symptoms were reported with significantly greater frequency by Gulf War veterans than UK servicemen from the Bosnia conflict or those serving during the Gulf War but not deployed there. In our study, we report results from a histomorphometric study of bone biopsies that were obtained as part of a more extensive study in which Gulf War veterans seeking litigation underwent a variety of investigations on a volunteer basis.

Trans-ilial bone biopsies, 8 mm internal diameter, were obtained using a modified Bordier trephine 1 inch below and behind the anterior superior iliac spine. None of the subjects was receiving medication known to affect bone metabolism. All subjects received double tetracycline labelling before biopsy. After the biopsy procedure, biopsies were halved longitudinally, one half being snap frozen, and the other embedded in LR White medium resin (London Resin Co, London, UK). Non-decalcified sections (8 µm thick) were stained using the von Kossa and toluidine blue techniques. Histomorphometric analysis of bone remodelling and structure was performed using an in house image analysis system as described previously.

Bone area/tissue area (B.Ar/T.Ar) and osteoid perimeter/bone perimeter (O.Pm/B.Pm) were measured on von Kossa stained sections on a minimum of 25 fields from three to six sections. Tetracycline labelling was viewed by fluorescence microscopy on a minimum of six 15 µm unstained sections at ×156 magnification. The mean width of completed bone remodelling units (wall width; W.Wi) was measured on toluidine blue stained sections viewed under polarised light at ×156 magnification. The eroded perimeter was measured on toluidine blue stained sections, resorption cavities being identified under polarised light.

The mineralising perimeter (Md.Pm) was calculated as follows:

\[
Md.Pm/B.Pm \times 100 = \frac{dL.Pm + (0.5 \times sL.Pm)}{B.Pm}
\]

where dL.Pm is the double labelled perimeter and sL.Pm is the single labelled perimeter.

Abbreviations: Acf, activation frequency; B.Ar, bone area; B.Fr/B.Pm, tissue based bone formation rate; B.Pm, bone perimeter; dL.Pm, double labelled perimeter; Ip, labelling period; W.Wi, wall width; MAR, mineral apposition rate; Md.Pm, mineralising perimeter; O.Pm, osteoid perimeter; Sl.Pm, single labelled perimeter; T.Ar, tissue area; W.Wi, wall width

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The mean distance between double labels was measured directly at ×312 magnification. Measurements were made at approximately four equidistant points along each double label. A minimum of 20 labels was measured for each biopsy on a minimum of six sections.

Mineral apposition rate (MAR) was calculated as:

\[ \text{MAR (µm/day)} = \frac{L.Wi}{LP} \]

where L.Wi is the interlabel width and LP is the labelling period (12 days). The tissue based bone formation rate (BFR/B.Pm) was calculated as follows:

\[ \text{BFR/B.Pm (µm}^2/\text{µm/day)} = \text{MAR} \times (\text{M.Pm/B.Pm} \%) \]

Activation frequency was calculated as:

\[ \text{Acf (/year)} = \frac{\text{BFR/B.Pm}}{W.Wi} \]

Strut analysis, trabecular bone pattern factor, and marrow star volume were assessed as reported previously.\(^{3,13}\)

Control data were obtained from 13 healthy men who formed part of an earlier study of normal bone,\(^{3,13}\) in which iliac crest biopsies were obtained from normal subjects during general anaesthesia for a minor surgical procedure. The mean age of these controls was 36.6 years (range, 19–51). Results of histomorphometric analysis performed previously using an eye piece graticule and micrometre were used because sections were no longer available from this cohort. The measurements in biopsies from the controls and Gulf War veterans were made by the same observer (SV). Statistical analysis was performed using an unpaired Student’s t test after log transformation of the data. Results are expressed as the mean (SD).

### RESULTS

#### Demographic details and bone mineral density

The age of the veterans at the time of the study ranged between 27 and 51 years (mean, 34.9). They had spent between two and four months in the Gulf during the war and most had spent four time in a blackadder camp where there was obvious spraying of organophosphates. At least four were aware of exposure to sarin but it was not possible to obtain an accurate vaccination history. All but one complained of musculoskeletal symptoms (most commonly arthralgia) but none had a history of low trauma fracture. Seven of the men were regular cigarette smokers, six of whom smoked more than 15 daily, and four had an alcohol intake in excess of 10 units weekly (16, 40, 40, and 50 units, respectively).

The mean bone mineral density in the lumbar spine (L2–4), expressed as a Z score, was +0.55 (range, −1.6 to +2.3) and in the proximal femur (femoral neck) was +0.45 (range, −0.7 to +1.9). None of the men had osteoporosis as defined by a T score below −2.5.\(^{12}\) Body weight ranged between 64 and 120 kg (mean, 85.2).

#### Bone histology and histomorphometry

Qualitative examination of the biopsies from the Gulf War veterans revealed considerable heterogeneity, with some biopsies appearing normal and others showing reduced cancellous bone area, trabecular thinning, and reduced connectedness of the cancellous bone structure. Very few osteoclasts were seen; in addition, very few active osteoblasts were evident.

Table 1 shows the histomorphometric data. Cancellous bone area was significantly lower in the Gulf War veterans than in the controls (p = 0.027). Mineral apposition rate and mean wall width, both indices of bone formation at the cellular level, were also significantly lower in the Gulf War veterans (p = 0.002 and p < 0.0001, respectively), as were measurements that reflect bone formation at the tissue level, namely bone formation rate (p < 0.0001) and activation frequency (p = 0.0006). The eroded perimeter was significantly higher in the Gulf War veterans (p < 0.0001).

No significant differences between the groups were found in cancellous bone structure, assessed by strut analysis, marrow star volume, and trabecular bone pattern factor (data not shown).

### DISCUSSION

Our results demonstrate that in this group of Gulf War veterans there was a significant reduction in bone formation, both at the cellular and tissue level, in association with a reduction in cancellous bone area. Although the eroded perimeter was increased compared with the controls, this was generally of a small magnitude and the scarcity of osteoclasts in association with resorption cavities indicates that reduced bone formation rather than increased resorption was the most likely cause of the observed increase.

“It is of interest that the bone abnormalities observed in these Gulf War veterans were similar to those recently reported in agricultural workers with chronic organophosphate exposure as a result of sheep dipping”\(^{14}\)

The control values used in our study were obtained from healthy men in whom bone biopsies had been obtained previously.\(^{3,13}\) Because of the time that had elapsed between that study and our present one, it was not possible to obtain and quantify fresh sections so that the results of an earlier histomorphometric analysis made by the same observer (SV) were used. Although some differences may arise as a result of inter-method variations,\(^{14,17}\) these are generally small and cannot explain the observed differences between the Gulf War veterans and controls.

**Table 1** Gulf war group v controls

<table>
<thead>
<tr>
<th>Indices</th>
<th>Gulf war group</th>
<th>Control group</th>
<th>p Value (log transformed independent t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>17</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.9 (6.4)</td>
<td>36.6 (10.7)</td>
<td>&gt;0.599</td>
</tr>
<tr>
<td>Trabecular bone area (%)</td>
<td>20.5 (5.3)</td>
<td>25.3 (6.1)</td>
<td>&gt;0.027</td>
</tr>
<tr>
<td>Osteoid perimeter (%)</td>
<td>8.5 (4.5)</td>
<td>23.7 (10.3)</td>
<td></td>
</tr>
<tr>
<td>Mineralising perimeter (%)</td>
<td>4.5 (3.1)</td>
<td>11.3 (5.8)</td>
<td>&gt;0.0003</td>
</tr>
<tr>
<td>Mineral apposition rate (µ/day)</td>
<td>0.58 (0.19)</td>
<td>0.77 (0.12)</td>
<td>&gt;0.002</td>
</tr>
<tr>
<td>Bone formation rate (µm²/µm/day)</td>
<td>0.028 (0.021)</td>
<td>0.087 (0.044)</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>Activation frequency (/year)</td>
<td>0.046</td>
<td>0.54 (0.25)</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>Eroded perimeter (%)</td>
<td>5.98 (2.64)</td>
<td>1.87 (0.85)</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>Wall width (µm)</td>
<td>42.5 (4.3)</td>
<td>57.8 (7.9)</td>
<td>&gt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean (SD).
The clinical relevance of the observed reduction in bone formation remains to be established.

Indirect consequence resulting from changes in life style. Finally, the long term effects of these changes on bone fragility and fracture risk are currently unknown.

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REFERENCES