Weight loss and low body cell mass in males with lung cancer: relationship with systemic inflammation, acute-phase response, resting energy expenditure, and catabolic and anabolic hormones

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ABSTRACT

The aim of the present study was to investigate, in human lung cancer, the relationship between weight loss and the existence of a low body cell mass (BCM) on the one hand, and the putative presence of systemic inflammation, an increased acute-phase response, anorexia, hypermetabolism and changes in circulating levels of several anabolic and catabolic hormones on the other. In 20 male lung cancer patients, pre-stratified by weight loss of $\geq 10\% (n = 10)$ or of $< 10\% (n = 10)$, the following measurements were performed: BCM (by dual-energy X-ray absorptiometry/bromide dilution), circulating levels of sTNF-R55 and sTNF-R75 (soluble tumour necrosis factor receptors of molecular masses 55 and 75 kDa respectively), interleukin-6, lipopolysaccharide-binding protein, albumin, appetite (scale of 0–10), resting energy expenditure (by indirect calorimetry) and circulating levels of catabolic (cortisol) and anabolic [testosterone, insulin-like growth factor-I (IGF-I)] hormones. Compared with the patients with a weight loss of $< 10\%$, those with a weight loss of $\geq 10\%$ were characterized by higher levels of sTNF-R55 (trend towards significance; $P = 0.06$), and lower levels of albumin (27.4 compared with 34.4 mmol/l; $P = 0.02$), testosterone (13.2 compared with 21.5 nmol/l; $P = 0.01$) and IGF-I (119 compared with 184 ng/ml; $P = 0.004$). In the patient group as a whole, the percentage weight loss was significantly correlated with sTNF-R55 ($r = 0.59$, $P = 0.02$), albumin ($r = -0.63$, $P = 0.006$) and IGF-I ($r = -0.50$, $P = 0.02$) levels. Height-adjusted BCM was significantly correlated with sTNF-R55 ($r = -0.57$, $P = 0.03$), sTNF-R75 ($r = -0.50$, $P = 0.04$), lipopolysaccharide-binding protein ($r = -0.50$, $P = 0.04$), albumin ($r = 0.56$, $P = 0.02$) and resting energy expenditure/BCM ($r = -0.54$, $P = 0.03$), and there was a trend towards a correlation with IGF-I concentration ($r = 0.44$, $P = 0.06$). We conclude that, in human lung cancer, weight loss and the presence of a low BCM are associated with systemic inflammation, an increased acute-phase response and decreased levels of IGF-I. In addition, a decreased BCM is associated with hypermetabolism.

Key words: acute-phase response, body composition, cachexia, cancer, hypermetabolism, inflammation, insulin-like growth factor I, pathophysiology, performance status, testosterone.

Abbreviations: BCM, body cell mass; BCM-ix, BCM index (BCM/height$^2$); DEXA, dual-energy X-ray absorptiometry; IGF-I, insulin-like growth factor-I; IL-6, interleukin-6; LBP, lipopolysaccharide-binding protein; REE, resting energy expenditure; TNF, tumour necrosis factor; sTNF-R, soluble TNF receptor.

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INTRODUCTION

Substantial involuntary weight loss, leading eventually to cachexia, is a frequent complication of cancer that is associated with increased morbidity and mortality [1]. Several studies have shown that wasting both of fat mass and body cell mass (BCM) contribute to this weight loss [2]. As nearly all tissues that are vital for normal functioning of the body are part of the BCM [3], it is evident that wasting of this body compartment in particular can be expected to be responsible for the negative effects of weight loss on performance status and survival. Nevertheless, few cancer studies have specifically addressed this issue to date. In AIDS, however, the specific relationship between BCM wasting and a shortened survival time has been clearly established [4].

If BCM wasting is indeed an important determinant of performance status and survival in cancer, it obviously should become a key target for therapeutic intervention. In order to develop such specific treatment strategies, however, it is mandatory to gain as much insight as possible into the underlying pathophysiology. Growing evidence exists that the development of cancer cachexia is related to a large degree to a chronic, low-grade, tumour-induced activation of the host immune system [5] which, although in a mitigated form, shares several characteristics with the so-called acute-phase response that occurs after major trauma and sepsis. The latter is characterized by an increased production of cytokines [6], elevated levels of catecholamines, cortisol and glucagon [6–8], increased peripheral amino acid mobilization and hepatic amino acid uptake [6,9], enhanced hepatic gluconeogenesis and acute-phase protein production [6,10,11], increased mobilization of non-esterified fatty acids [12], and hypermetabolism [10].

Nearly all the evidence that the development of cancer cachexia is related, to a large degree, to a tumour-induced activation of the host immune system, however, has come from studies in experimental models [13–19]. Relatively few confirmatory data have come from studies in human cancer, and in particular the specific pathophysiology of cancer-related BCM wasting has barely been investigated in humans to date.

To address these issues, we have investigated, in a group of male lung cancer patients, the relationship between weight loss and the existence of a low BCM on the one hand, and the putative presence of systemic inflammation, an increased acute-phase response, anorexia, hypermetabolism and changes in circulating levels of several catabolic and anabolic hormones on the other. In addition, to obtain more insight into the specific clinical relevance of BCM wasting in cancer patients, the relationship between height-adjusted BCM and Karnofsky Performance Status was assessed.

METHODS

Patients

A total of 20 male patients newly diagnosed with cytologically or histologically proved untreated lung cancer were entered into the study. These were divided into two groups, each containing 10 patients: those with weight loss of greater than 10% of the usual body weight, and those with weight loss of less than 10% of the usual body weight. Patients were excluded if they suffered from diabetes mellitus, hyperthyroidism, manifest infection or fever. The protocol was in accordance with the Declaration of Helsinki of 1975, as revised in 1983, and was approved by the institutional review board for human research of the University Hospital Maastricht. Informed consent was obtained from all patients.

Body composition

Bone mineral mass, fat mass and lean soft tissue mass were measured in the morning, in the fasted state, by dual-energy X-ray absorptiometry (DEXA) using a total-body scanner (model DPX-L; Lunar Corp., Madison, WI, U.S.A.) that uses a constant potential X-ray source at 76 kVp and a K-edge filter (cerium) to achieve a congruent beam of stable dual-energy radiation with effective energies of 38 and 70 keV. The principles of dual-energy determination of body compartments, as well as of subject positioning and scan procedures for the scanner used, have been extensively described elsewhere [20,21]. Scans were made in the fast speed mode. Baseline calibration of the scanner was performed daily against the standard calibration block supplied by the manufacturer.

Based on the relative attenuations of the two energies through bone and soft tissue, bone mineral mass and soft tissue mass were calculated by the software provided by the manufacturer. As the ratio of beam attenuation at the lower and higher energies is inversely and linearly related to percentage fat, the latter can be derived directly from this ratio. Fat mass was calculated by multiplying the percentage fat by the assessed soft tissue mass. The precision error (1 S.D.) of the DEXA measurement for fat mass has been reported to be 1.0 kg in healthy individuals [21]. In a study in pigs, in which fat mass was assessed by DEXA as well as by chemical analysis after post-mortem homogenization, bias was small ($r = 0.99$) [22].

The volume of extracellular water was estimated by bromide dilution. The following protocol was used. On the evening before body composition assessment, at 22.00 hours, all patients drank a weighed dose of NaBr (60 mg of Br/litre of estimated total-body water) in 50 ml of tap water. Then the bottle was rinsed once with ~ 50 ml of tap water, which was also drunk. Thereafter, the patients refrained from eating and drinking. Before and 10 h after drinking the bromide water, a venous blood sample was taken. The bromide concentration in
the serum ultrafiltrate was determined by HPLC according to the anion-exchange chromatographic method [23]. Extracellular water was estimated by calculating the corrected bromide space, using the formula [23]:

\[
CBS \text{ (litres)} = \frac{\text{Br dose (mmol)}}{[\text{Br}]_{f} - [\text{Br}]_{i} (\text{mmol/l})} \times 0.90 \times 0.95
\]

where \([\text{Br}]_{i}\) is the background bromide concentration in the serum ultrafiltrate, \([\text{Br}]_{f}\) is the final bromide concentration in the serum ultrafiltrate, 0.90 is the correction factor for the distribution of bromide in the non-extracellular sites, and 0.95 is the correction factor for the Donnan equilibrium. The use of this test protocol with an overnight equilibration period of 10 h has been extensively validated in our laboratory [24].

BCM was defined as the difference between the DEXA-derived lean soft tissue mass and bromide-dilution-derived extracellular water. Using this model, the BCM will be slightly overestimated (≈ 0.5 kg), as no correction was made for non-bone extracellular solids [25]. To correlate BCM with other variables, BCM was adjusted for height by calculating the BCM index (BCM/height \(x\); BCM/height \(x\)).

**Appetite, weight and performance status**

Appetite was measured using a self-assessment numerical rating scale ranging from 0 to 10; 0 indicates absolutely no appetite and 10 indicates an extremely good appetite. Weight was measured in the morning, in the fasted state, after voiding and without clothing and shoes, to the nearest 0.1 kg by using a beam scale (SECA, Hamburg, Germany). Body weight was adjusted for height by calculating the body-mass index (weight/height \(x\)). Performance status was assessed by determining the Karnofsky Performance Status score [26]. This was performed in all patients by the same experienced physician.

**Resting energy expenditure (REE)**

REE was assessed by indirect calorimetry using a ventilated hood system (Oxycon \(\beta\); Mijnhardt, Bunnik, The Netherlands). After an overnight fast, CO\(_2\) production and \(\text{O}_2\) consumption were measured at complete rest during a period of 20 min. REE was calculated by using the abbreviated Weir formula [27]. The equipment was calibrated at the start of each experiment. The precision of the system was checked monthly by burning methanol with a theoretical respiratory quotient of 0.667 after complete combustion. Further details on the technique used in our laboratory have been described elsewhere [28]. To assess between-group differences in REE and to correlate REE with other variables, REE was adjusted for BCM (REE/BCM), as BCM is the body compartment that mainly determines the level of metabolic activity.

**Plasma and serum samples**

After an overnight fast, blood was collected by venepuncture at 08.00 hours into evacuated EDTA blood collection tubes (Sherwood Medical, Ballymoney, N. Ireland, U.K.) and evacuated integrated serum separator tubes (Sherwood Medical, St. Louis, MO, U.S.A.). The EDTA tubes were placed on ice/water and directly centrifuged for 5 min in a refrigerated (4 °C) table-top centrifuge at 3000 g. Plasma samples were stored immediately at −70 °C until analysis. After full coagulation had occurred, the serum separator tubes were also centrifuged for 5 min at 3000 g. Serum samples were stored immediately at −20 °C until analysis.

**Soluble tumour necrosis factor (TNF) receptors (sTNF-Rs) and interleukin-6 (IL-6)**

To assess systemic inflammation, plasma levels of sTNF-R55 and sTNF-R75 (sTNF-Rs of molecular mass 55 and 75 kDa respectively) [29] and of IL-6 were measured by sandwich ELISA, as described elsewhere [30,31]. In short, for measurement of sTNF-R55 and sTNF-R75, monoclonal antibodies MR1-1 and MR2-2 respectively were used for coating. Specific biotin-labelled polycyonal rabbit anti-(human sTNF-R) IgG were used as detector reagents. The standards used were recombinant human sTNF-R55 and sTNF-R75. For both assays, the limit of detection was 100 pg/ml. In the IL-6 ELISA, monoclonal antibody 5E1 was used for coating, and polyclonal rabbit anti-(human IL-6) antibodies were used as detecting reagents. The standard used was recombinant human IL-6. IL-6 could be detected with a lower detection limit of 0.02 ng/ml.

**Lipopolysaccharide-binding protein (LBP) and albumin**

To assess the hepatic acute-phase response, LBP [32,33] and albumin were measured in plasma and serum respectively. LBP was measured by sandwich ELISA, as described elsewhere [34]. In short, polyclonal rabbit anti-(recombinant human LBP) IgG was used for coating, and biotin-labelled polycyonal rabbit anti-(recombinant human LBP) IgG was used for detection. The standard used was recombinant LBP. Washing and dilution were performed in buffer that contained 40 mmol/l MgCl\(_2\) to prevent alteration by lipopolysaccharide of LBP recovery in the ELISA. The limit of detection was 200 pg/ml. Albumin was measured by the Bromocresol Purple method using a Synchron CX-7 instrument (Beckman, Mijdrecht, The Netherlands).

**Catabolic and anabolic hormones**

Cortisol, testosterone and insulin-like growth factor-1 (IGF-I) were measured in serum. Cortisol was measured by solid-phase chemiluminescent immunoassay (Immulo-lite® cortisol; Diagnostic Products, Los Angeles, CA,
Table 1  Patient characteristics stratified by weight loss
Results are means ± S.D. Abbreviations: ad, adeno; sq, squamous-cell; un, undifferentiated large-cell; sc, small-cell; COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; NSAIDs, non-steroidal anti-inflammatory drugs.

<table>
<thead>
<tr>
<th></th>
<th>Weight loss ≥ 10% (n = 10)</th>
<th>Weight loss &lt; 10% (n = 10)</th>
<th>Between-group P</th>
</tr>
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<tr>
<td>Age (years)</td>
<td>67 ± 7</td>
<td>67 ± 9</td>
<td>1.00</td>
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<tr>
<td>Tumour stage (n)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-small-cell (I–II/III/IV)</td>
<td>2/1/6</td>
<td>0/4/5</td>
<td>0.27</td>
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<tr>
<td>Small-cell (limited/ extensive)</td>
<td>1/0</td>
<td>1/0</td>
<td></td>
</tr>
<tr>
<td>Histology: ad/sq/un/sc (n)</td>
<td>2/4/3/1</td>
<td>2/4/3/1</td>
<td>1.00</td>
</tr>
<tr>
<td>Active smokers (n)</td>
<td>4</td>
<td>8</td>
<td>0.17</td>
</tr>
<tr>
<td>COPD (n)</td>
<td>5</td>
<td>2</td>
<td>0.35</td>
</tr>
<tr>
<td>FEV1 (% of predicted normal value)</td>
<td>65 ± 12</td>
<td>73 ± 17</td>
<td>0.30</td>
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<tr>
<td>Anti-inflammatory drugs:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>corticosteroids/NSAIDs (n)</td>
<td>2/1</td>
<td>1/3</td>
<td>0.49</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>35.9 ± 0.8</td>
<td>36.4 ± 0.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Pre-study weight loss (%)</td>
<td>16.6 ± 5.0</td>
<td>4.5 ± 3.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>174 ± 6</td>
<td>175 ± 5</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.6 ± 9.4</td>
<td>67.6 ± 8.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>19.7 ± 2.6</td>
<td>22.0 ± 2.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>10.2 ± 6.1</td>
<td>13.9 ± 6.6</td>
<td>0.21</td>
</tr>
<tr>
<td>Bone mineral mass (kg)</td>
<td>2.57 ± 0.21</td>
<td>2.83 ± 0.44</td>
<td>0.11</td>
</tr>
<tr>
<td>Extracellular water (litres)</td>
<td>17.8 ± 2.3</td>
<td>18.6 ± 2.2</td>
<td>0.43</td>
</tr>
<tr>
<td>BCM (kg)</td>
<td>27.5 ± 4.0</td>
<td>30.0 ± 2.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Appetite (0–10)</td>
<td>5.4 ± 3.0</td>
<td>6.6 ± 1.5</td>
<td>0.27</td>
</tr>
<tr>
<td>REE/BCM (kJ·day⁻¹·kg⁻¹)</td>
<td>243 ± 33</td>
<td>222 ± 26</td>
<td>0.16</td>
</tr>
<tr>
<td>sTNF-R55 (ng/ml)</td>
<td>1.05 ± 0.41</td>
<td>1.39 ± 1.06</td>
<td>0.06*</td>
</tr>
<tr>
<td>sTNF-R75 (ng/ml)</td>
<td>1.53 ± 0.64</td>
<td>1.59 ± 0.97</td>
<td>0.13*</td>
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<tr>
<td>IL-6</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Detectability (%)</td>
<td>56</td>
<td>78</td>
<td>0.62</td>
</tr>
<tr>
<td>Plasma concn. (ng/ml)</td>
<td>0.15 ± 0.09</td>
<td>0.15 ± 0.19</td>
<td>0.67</td>
</tr>
<tr>
<td>LBP (µg/ml)</td>
<td>26.5 ± 15.2</td>
<td>17.5 ± 7.2</td>
<td>0.14</td>
</tr>
<tr>
<td>Albumin (mmol/l)</td>
<td>27.4 ± 5.7</td>
<td>34.4 ± 5.4</td>
<td>0.02</td>
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<tr>
<td>Haemoglobin (mmol/l)</td>
<td>8.0 ± 1.2</td>
<td>8.1 ± 0.5</td>
<td>0.75</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>621 ± 235</td>
<td>571 ± 179</td>
<td>0.60</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>13.2 ± 7.5</td>
<td>21.5 ± 5.6</td>
<td>0.01</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>119 ± 31</td>
<td>184 ± 55</td>
<td>0.004</td>
</tr>
<tr>
<td>Blood urea nitrogen (mmol/l)</td>
<td>6.0 ± 2.4</td>
<td>7.6 ± 3.4</td>
<td>0.25</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>82 ± 11</td>
<td>113 ± 44</td>
<td>0.06</td>
</tr>
<tr>
<td>Karnofsky Performance Status (%)</td>
<td>72 ± 15</td>
<td>82 ± 12</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Higher in patients with weight loss ≥ 10% after adjustment for serum creatinine.

U.S.A.). The limit of detection was 5.5 nmol/l. Testosterone was measured by solid-phase ¹²⁵I radio-immunoassay (Coat-A-Count* total testosterone; Diagnostic Products). The limit of detection was 0.14 nmol/l. Total IGF-I was measured by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, U.S.A.). For this assay, the limit of detection was 6 ng/ml.

Statistical analysis
Results are given as mean ± S.D. Between-group means were compared using one-way analysis of variance (ANOVA). Differences in categoric variables were analysed by χ² test with Yates’ continuity correction or Fisher’s exact test, where appropriate. Pearson’s product–moment correlation coefficients were used to evaluate linear relationships between variables. As sTNF-R levels are linearly related to the serum creatinine concentration [35,36], between-group differences in sTNF-R levels were analysed in a co-variance model (ANCOVA) using serum creatinine as the co-variable. Also, in the analysis of linear relationships between levels of sTNF-Rs and other variables, the assessed correlation coefficients were partial correlation coefficients after adjustment for serum creatinine as co-variable. Since
BCM decreases with age [3], in the analysis of linear relationships between BCM-ix and other variables the assessed correlation coefficients were also partial correlation coefficients after adjustment for age as covariable. For all analyses the statistical package SPSS/PC+ (version 6.0 for Windows; SPSS Inc., Chicago, IL, U.S.A.) was used. Statistical significance was determined at the 5% level (two-sided).

RESULTS

In Table 1, the patients with weight loss of \(\geq 10\%\) are compared with the patients with weight loss of \(< 10\%\). There were no significant differences between the two groups in age, tumour stage, tumour histology, smoking behaviour, prevalence and severity of chronic obstructive pulmonary disease, use of anti-inflammatory medication or body temperature. Although both fat mass and BCM were lower in the group with severe weight loss, the between-group differences did not reach statistical significance. The patients with weight loss of \(\geq 10\%\) were characterized by higher sTNF-R55 levels (trend towards significance; \(P = 0.06\)) and lower levels of albumin (27.4 compared with 34.4 mmol/l; \(P = 0.02\)), testosterone (13.2 compared with 21.5 nmol/l; \(P = 0.01\)) and IGF-I (119 compared with 184 ng/ml; \(P = 0.004\)).

To clarify further the relationships between the development of weight loss and the presence of systemic inflammation (sTNF-R55, sTNF-R75 and IL-6 levels), the acute-phase response (LBP and albumin), hypermetabolism, anorexia, changes in cortisol (as catabolic hormone), and changes in testosterone and IGF-I (as anabolic hormones), correlation coefficients for these variables and percentage weight loss were assessed in the patient group as a whole. As can be seen in Figure 1 (upper panel), the development of weight loss was associated with high circulating levels of both sTNF-Rs and LBP, a high BCM-adjusted REE, high levels of cortisol, and low levels of testosterone, IGF-I and the ‘negative’ acute-phase protein albumin. However, the only correlations that reached statistical significance were those between weight loss and sTNF-R55 (\(r = 0.59, P = 0.02\)), IGF-I (\(r = -0.50, P = 0.02\)) and albumin (\(r = -0.63, P = 0.006\)).

As we explicitly aimed at identifying factors associated with wasting of BCM, the variables that were assessed with respect to weight loss were also investigated in relation to BCM-ix. As shown in Figure 1 (lower panel), the presence of a low BCM-ix was significantly correlated with high levels of sTNF-R55 \(r = 0.57, P = 0.03\) and sTNF-R75 \(r = -0.50, P = 0.04\), a high BCM-adjusted REE \(r = -0.54, P = 0.03\), and increased hepatic production of acute-phase proteins, reflected in high levels of LBP \(r = -0.50, P = 0.04\) and low levels of albumin \(r = 0.56, P = 0.02\). Although having a positive direction, the correlations between BCM-ix and appetite \(r = 0.34\), testosterone \(r = 0.36\) and IGF-I \(r = 0.44\) did not reach statistical significance. In the last case, however, significance was closely approached \((P = 0.06)\). BCM-ix was not correlated with IL-6 or cortisol concentrations.

As there is evidence from earlier studies that increased hepatic acute-phase protein production, hypermetabolism, anorexia and changes in cortisol and testosterone levels might be the result of systemic inflammation, direct correlations between plasma sTNF-R55 levels and these factors were investigated. In addition, the correlation with IGF-I was also assessed. It is shown in Figure 2 that sTNF-R55 was significantly positively correlated with REE/BCM \((r = 0.54, P = 0.03)\) and negatively correlated with albumin \((r = -0.57, P = 0.03)\). Furthermore, there were trends toward a positive cor-

**Figure 1** Diagram of Pearson correlation coefficients for the correlations between weight loss (upper panel) and BCM-ix (lower panel) and their putative determinants
relation with LBP (r = 0.48, P = 0.06) and a negative correlation with appetite (r = −0.45, P = 0.08). Although the direction of the correlation with cortisol was positive (r = 0.36), it did not reach statistical significance. The correlation coefficients for correlations with testosterone (r = −0.19) and IGF-I (r = −0.22) were low.

The Karnofsky Performance Status score was not significantly correlated with weight loss (r = 0.32, P = 0.17), body-mass index (r = 0.35, P = 0.13) or fat-mass index (r = 0.13, P = 0.59). However, the correlation with BCM-ix was clearly significant (r = 0.53, P = 0.02), and is visualized in Figure 3.

**DISCUSSION**

Substantial involuntary weight loss, leading eventually to cachexia, is a frequent complication of cancer [1]. Although this weight loss can be the result of both fat loss and wasting of the BCM [2], the present study shows that it is specifically the presence of a low BCM that is associated with a deterioration of performance status. Although the existence of this association was hypothesized in 1981 by DeWys et al. [37], it has not, to our knowledge, been explicitly confirmed since. In AIDS patients, however, BCM wasting has been specifically linked to decreased survival [4].

In animal models of cancer cachexia, many investigators have found evidence of a relationship between the development of weight loss and the presence of an increased inflammatory state, characterized by increased production of cytokines such as TNF, IL-6, interferon-γ and leukaemia inhibitory factor [14,16–19]. This increased cytokine production has been assumed to be primarily the result of a tumour-induced activation of the host immune system [38,39], although there is also evidence that these cytokines can be produced by the tumour itself [19,40,41]. It was also shown that increased production of TNF and IL-6 in particular was associated with significant wasting of muscle mass, which is the major determinant of BCM [18,42–44]. In other studies, it was observed that the exogenous administration of TNF, IL-1 and IL-6 also led to proteolysis and muscle wasting [45–49].

Several mechanisms have been proposed by which inflammation may lead to muscle and BCM wasting. Firstly, it has been postulated that inflammatory mediators have direct proteolytic effects on myocytes. For example, this was suggested by studies involving incubation of mouse diaphragms with recombinant TNF [50] and of cultured myotubes with recombinant IL-6 [51]. In addition, other investigators have demonstrated a recombinant-TNFα-induced activation of the ubiquitin-dependent proteolytic system in isolated rat soleus muscles [52]. Secondly, weight loss and BCM wasting may be related to a cytokine-induced decrease in food intake, which has been reported particularly for TNF and IL-1 [13,14,43,45,53,54]. Thirdly, cancer-related BCM wasting may be the consequence of increased utilization of amino acids due to: (1) inflammation-induced hypermetabolism [55], (2) increased amino acid uptake by the liver [56–58] associated with enhanced gluconeogenesis [59] and acute-phase protein production [18,53,60], and (3) a cytokine-triggered rise in circulating cortisol levels [11,56,61], inducing proteolysis [62]. Fourthly, BCM wasting might be related to an inflammation-related decrease in muscle protein synthesis. In this regard, TNF administration has been shown to attenuate skeletal muscle protein synthesis in rats [54]. In addition, TNF and glucocorticoids were shown, both in vivo and in vitro, to decrease the production of testosterone, an important mediator of muscle growth [63–65]. Finally, another hormone with prominent anabolic effects on muscle that has been reported to be decreased in experimental cancer cachexia [66,67] is IGF-I [68]. To our knowledge, no investigations have been carried out to date on whether this decrease is inflammation-related and/or enhances muscle wasting.

The results of the present study confirm that not only in experimental cancer, but also in human cancer, weight loss is associated with the presence of an increased systemic inflammatory state. Two other studies have reported this relationship previously. Knapp et al. [69] observed that, in stage IV breast cancer patients, the presence of severe weight loss (≥20%) was accompanied by increased blood concentrations of TNF-α, cortisol and glucagon, and Staal-van den Brekel et al. [70] reported in patients with newly diagnosed lung cancer that severe weight loss (≥10%) was associated with increased plasma levels of sTNF-Rs and of the acute-phase proteins LBP and C-reactive protein.

For the first time, the present study also specifically documents the presence of an association between systemic inflammation and wasting of BCM in human cancer, an association that, to our knowledge, has been described formerly in experimental models only [18,43–49]. In particular, the assessment of plasma sTNF-
Rs revealed this association, whereas no correlation was found between BCM wasting and plasma levels of IL-6. The latter was in accordance with results from other studies, for example in systemic lupus erythematosus, in which a significant correlation was found between disease activity and circulating levels of sTNF-R55 and sTNF-R75, but not IL-6 [71]. In addition, other investigators have reported a lack of correlation between changes in serum IL-6 (as opposed to changes in IL-6 release by peripheral blood mononuclear cells) and changes in acute-phase protein production, and postulated that local, rather than systemic, levels of IL-6 may determine its biological effects [72].

In addition to the relationship between BCM wasting and systemic inflammation, we also observed that the presence of a low BCM was associated with both increased hepatic acute-phase protein production (represented by increased levels of LBP and decreased levels of the ‘negative’ acute-phase protein albumin) and hypermetabolism. The increased hepatic acute-phase response and hypermetabolism were in their turn associated with elevated levels of sTNF-R55. This was in accordance with the results of Staal-van den Brekel et al. [70], who reported an association between elevated plasma levels of sTNF-R55 and hypermetabolism in lung cancer, and with those of Falconer et al. [73], who found a relationship between enhanced production of TNF and IL-6 by peripheral blood mononuclear cells and increased hepatic acute-phase protein production in patients with pancreatic cancer.

Although there was a trend toward a negative correlation between circulating levels of sTNF-R55 and appetite, which is in close agreement with the anorectic effects of inflammation observed in many experimental studies [13,14,43,45,53,54], we were not able to link anorexia with weight loss or BCM wasting directly. This might have been due to the relatively small sample size, but a poor correlation between subjective appetite loss and objective food intake may also have played a role.

We also found evidence that decreased IGF-I levels might be involved in the pathophysiology of cancer cachexia. However, there was no obvious relationship with systemic inflammation. In AIDS wasting and other non-cancer-related forms of malnutrition, decreased blood concentrations of IGF-I have been reported, often in combination with elevated levels of growth hormone [74,75]. These low IGF-I concentrations have largely been assumed to be the result of a decreased protein intake [76], which might also have been the case in the present study.

In accordance with earlier reports in human cancer [77], we also observed decreased testosterone levels in patients with severe weight loss. Although in a recent study in AIDS patients a significant correlation was reported between low testosterone levels and loss of BCM [75], in the present study (probably due in part to sample size) the correlation between testosterone and BCM-ix did not reach statistical significance. We were not able to confirm a relationship between low testosterone levels and increased systemic inflammation, a relationship that was suggested by studies reporting decreased testosterone production after exogenous TNF administration in vivo [63,78].

In conclusion, the present study on human lung cancer confirms that the development of weight loss is associated with the presence of an increased systemic inflammatory state. More importantly, however, a specific association was documented between systemic inflammation and low BCM values. Furthermore, it was demonstrated that decreased circulating levels of IGF-I may also be involved in the complex pathophysiology of cancer cachexia. As it has been shown that a low BCM is associated with a deterioration of performance status, and it is known from studies in other diseases that BCM wasting significantly decreases survival, our results should further encourage the search for specific therapies, in addition to treatments that aim primarily at increasing nutrient intake, that are based on a direct interference with BCM wasting and its underlying pathophysiology. Some agents deserving of further research in this regard are the non-steroidal anti-inflammatory drugs [79], n-3 fatty acids [80], IGF-I [81], branched-chain amino acids [82] and β2-adrenergic agonists [83].

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