Chapter 5

Plasma ghrelin levels are associated with anorexia but not cachexia in patients with NSCLC

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Submitted
Abstract

Background & Aims: Plasma ghrelin levels are high in patients with anorexia nervosa and low in obese subjects. We studied to what extent ghrelin levels are related with anorexia and cachexia in patients with cancer.

Methods: Fasted plasma ghrelin levels were determined as well as anorexia and cachexia in forty patients with stage III/IV NSCLC before chemotherapy. Multiple regression analyses were performed and adjusted for age and sex.

Results: Forty-three percent of patients had anorexia and 33% suffered from cachexia. Patients with anorexia had significantly higher ghrelin levels compared to patients without anorexia ($\beta$: 348.3, $p=0.031$), while ghrelin levels were not significantly different in patients with cachexia compared to patients without cachexia ($\beta$: -163.7, $p=0.354$).

Conclusions: Patients with anorexia had significantly higher ghrelin levels compared to patients without anorexia, which suggests a potential target for therapy of cancer anorexia to prevent cancer-associated weight loss and deterioration in physical functioning.
Introduction

One of the promising new therapeutic targets for cancer-associated weight loss (cachexia) is ghrelin, a 28-amino acid peptide that is the natural ligand for the growth hormone secretagogue receptor-1a (1). Ghrelin plays an important role in several physiological processes including increasing appetite by stimulating the production of orexigenic neurons such as neuropeptide Y and agouti-related protein-expressing neuron (2). Ghrelin is produced by endocrine cells of the antrum during periods of fasting (1). Ghrelin levels are high in patients with anorexia nervosa and low postprandial and in obesity (2). The evidence on changed ghrelin levels in cancer cachexia however, appears to be inconclusive. Some studies showed increased levels of ghrelin in patients with cachexia compared to patients without cachexia (3;4), other studies showed no significant differences in ghrelin levels between patients with and without cachexia (5;6). As cachexia may be accompanied by a loss of appetite (anorexia) and ghrelin is directly involved in the regulation of hunger and appetite, presence or absence of anorexia may intervene in the association between cancer cachexia and ghrelin. This was already hypothesized by Shimizu and colleagues who found a lower appetite score and higher ghrelin levels in patients with cachexia compared to patients without cachexia. Furthermore, ghrelin levels increased in patients who developed grade 2 anorexia during chemotherapy but remained stable in patients without anorexia during chemotherapy (3). To test whether anorexia rather than cachexia is associated with ghrelin, we studied associations between plasma ghrelin levels and anorexia and cachexia in patients with advanced NSCLC.

Materials and Methods

In this prospective study, patients with stage III or IV NSCLC and starting with chemotherapy were recruited at the VU University Medical Center in Amsterdam, The Netherlands.

Exclusion criteria: systemic treatment in the past month, clinically overt ascites or serious pitting edema, Diabetes Mellitus, current use of high dose of corticosteroids, presence of other active inflammatory disease (for example HIV or active colitis) and insufficient command of the Dutch language. The research protocol was approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam.
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and the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent was obtained from all participants.

**Measurements**

**Ghrelin**

Venous blood samples were collected between 7:30 and 9:00 a.m. after an overnight fast. Blood samples were collected using EDTA tubes containing 250 KIU of aprotinin (BD Diagnostics, Plymouth, UK), immediately placed on ice, and centrifuged. Plasma was stored at -80°C until assayed. Total plasma ghrelin was determined by radioimmunoassay (RIA) (Linco Research Inc., St. Charles, MO, USA). The intra-assay variation was below 4% and the inter-assay variation was below 5%. The lower limit of quantitation was 240 ng/mL. Analyses were performed at the Endocrine Laboratory of the Department of Clinical Chemistry of the VU University Medical Center.

**Anorexia**

The 12 items of the Anorexia/Cachexia subscale (A/CS) of the Functional Assessment of Anorexia/Cachexia Therapy (FAACT) questionnaire (4th version, Dutch) (7) were scored on a five-point Likert scale (0 = not at all, 1 = a little bit, 2 = somewhat, 3 = quite a bit and 4 = very much). For scoring the FAACT – A/CS, the FACIT manual was applied. A lower score indicates less appetite. A decreased appetite was defined as having a score of ≤ 37 (8).

**Cachexia**

Cachexia was defined as:

- Weight loss >5% in 6 months OR
- Weight loss >2% in 6 months in combination with BMI <20 OR
- Weight loss >2% in 6 months in combination with low SMI: L3: <55 cm²/m² for males, <39 cm²/m² for females (9), T4: <66.0 cm²/m² for males, <51.9 cm²/m² for females (cut-off value p50 for T4, comparable to L3 cut-off values in patients with SMI data on both levels, data not published)

The methods of the measurements within the diagnostic framework of cachexia are described below.

Body weight was measured (with patients wearing light indoor clothes without shoes) within 0.2 kg on a calibrated scale (Seca type 888). Self-reported body
weight six months before inclusion was assessed and a correction factor for clothes or clothes and shoes was applied when necessary. Relative weight change in six months was calculated. Body height was measured using a stadiometer; the patient was standing barefoot and height was determined to the nearest cm. BMI was calculated as the ratio of body weight (kg)/height (m)$^2$.

Skeletal muscle area (cm$^2$) was measured with SliceOmatic Software V 5.0 (Tomovision, Magog, Canada) using routine CT scans conducted for diagnostic purposes. The fourth thoracic vertebra (T4) was used for the assessment of the skeletal muscle area and in patients without evaluable T4 images, L3 images were used. The structures of muscles were quantified based on pre-established thresholds of Hounsfield Units (HU) (-29 to +150) of skeletal muscle tissue (10). Cross-sectional areas (cm$^2$) of the sum of all these muscles were computed by summing tissue pixels and multiplying by the pixel surface area for each patient. Skeletal Muscle Index (SMI) was calculated as the ratio of skeletal muscle area (cm$^2$)/height (m)$^2$.

**Statistical analysis**

Statistical analyses were performed using SPSS for Windows v.23.0 (IBM Corporation, Armonk, NY, USA). Descriptive statistics (count (%) and means ± SD) were used to describe the study sample. One-way ANOVA analysis was performed to assess differences in ghrelin levels between four groups: patients with (+) or without (-) anorexia (A) or cachexia (C). A Tukey’s multiple comparison test was performed to test which subgroups differed significantly from one another. Linear regression analyses were performed to assess differences in ghrelin levels for patients with and without anorexia and with and without cachexia. In multiple regression analyses, adjustments for age and sex were performed. A p-value of $<0.05$ was considered significant for all analyses.

**Results**

Forty patients with stage III (33%) or stage IV (68%) were recruited, of which 50% was male. Mean age was 59.6±10.3 years (table 1).
Eighteen percent of patients suffered from both anorexia and cachexia (A+C+), 26% had anorexia without cachexia (A+C-), 15% had cachexia without anorexia (A-C+) and 41% had no anorexia or cachexia (A-C-). Ghrelin levels were borderline significantly different between the four groups (p=0.052). In post-hoc analyses the A+C- patients had significantly higher ghrelin levels (1804±461) compared to A-C+ patients (1096±303, p=0.044, figure 1).

Figure 1. Fasting plasma ghrelin levels for patients with (+) or without (-) anorexia (A) and cachexia (C). Horizontal lines display mean.

**Table 1.** Patient characteristics (n=40)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (males)</td>
<td>20 (50)</td>
</tr>
<tr>
<td>Age in years†</td>
<td>59.6±10.3</td>
</tr>
<tr>
<td>Cancer stage</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>13 (33)</td>
</tr>
<tr>
<td>IV</td>
<td>27 (68)</td>
</tr>
<tr>
<td>BMI in kg/m²</td>
<td>23.9±4.0</td>
</tr>
<tr>
<td>FAACT-A/CS‡</td>
<td>38 (35-42)</td>
</tr>
<tr>
<td>Ghrelin in pg/mL†</td>
<td>1508±528</td>
</tr>
</tbody>
</table>

†Mean±sd
‡Median (IQR)
When cachexia and anorexia were analyzed separately, ghrelin levels were not significantly different between patients with or without cachexia ($\beta$: -163.7, p=0.354, adjusted for age and sex), however patients with anorexia had significantly higher ghrelin levels compared to patients without anorexia ($\beta$: 348.3, p=0.031, adjusted for age and sex, table 2).

**Table 2.** Differences in ghrelin levels for patients with anorexia and cachexia compared to patients without anorexia and cachexia

<table>
<thead>
<tr>
<th></th>
<th>B*</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia† (n=17)</td>
<td>348.3</td>
<td>33.8 to 662.8</td>
<td>0.031</td>
</tr>
<tr>
<td>Cachexia‡ (n=13)</td>
<td>-163.7</td>
<td>-517.6 to 190.2</td>
<td>0.354</td>
</tr>
</tbody>
</table>

*B: difference in ghrelin level between patients with and without condition (anorexia or cachexia), adjusted for age and sex. B>0 implies higher ghrelin levels for patients with the condition compared to patients without the condition.
†FAACT-A/CS <37 (8)
‡≥5% weight loss in the past 6 months or ≥2% weight loss in the past 6 months in combination with body mass index <20 or low skeletal muscle index (9)

**Discussion**

In NSCLC, we found that patients with anorexia had significantly higher plasma ghrelin levels compared to patients without anorexia. In contrast to previous literature, we did not find associations between ghrelin levels and cachexia. This supports the hypothesis that the earlier reported association between ghrelin levels and cachexia may be mediated through anorexia (3). We hypothesize that anorexia, and accompanying decreased food intake, may lead to increased ghrelin levels in patients with cancer, as endocrine cells of the antrum react to an empty stomach with production of ghrelin (1).

Treatment with ghrelin and ghrelin agonists has led to promising results regarding improvements in appetite, food intake, lean body mass and quality of life of patients with cancer cachexia (11;12). This suggests partial resistance to the orexigenic effects of increased ghrelin levels, as treatment with ghrelin and thereby further elevation in ghrelin levels (3- to 4-fold from baseline) may be able to restore the function of ghrelin (2). Still, no significant effect on physical functioning and survival was demonstrated (12). As anorexia may precede significant weight loss and deterioration in physical functioning, future studies should consider treatment with ghrelin in patients with cancer anorexia rather than patients with cachexia, in order to study whether significant weight loss and deterioration in physical functioning
can be prevented. One small study in 7 patients with severe anorexia showed that ghrelin infusion resulted in a marked increase in energy intake of 31% and higher meal appreciation scores, however the effect on body weight, muscle mass, physical functioning, quality of life and survival were not investigated as their sample size was too small (13).

This is the first study on plasma ghrelin levels and associations with anorexia and cachexia in patients with cancer. We need to remark that absolute reported concentrations of plasma ghrelin are dependent on the analytical method used, therefore we advise to be careful when comparing our reported absolute values with literature on ghrelin levels in humans measured with other methods.

In conclusion, patients with anorexia had significantly higher ghrelin levels compared to patients without anorexia, whereas no differences were found between patients with and without cachexia. In future studies, the effect of ghrelin (agonists) in the treatment of cancer anorexia should be evaluated to prevent severe weight loss and improve clinical outcomes such as physical functioning, quality of life and survival.
Plasma ghrelin levels and anorexia and cachexia in lung cancer

References