Mechanism of Increased Lipolysis in Cancer Cachexia

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Abstract

Loss of fat mass is a key feature of cancer cachexia and has been attributed to increased adipocyte lipolysis. The mechanism behind this alteration is unknown and was presently investigated. We studied mature s.c. fat cells and differentiated preadipocytes from 26 cancer patients with and without cachexia. Hormone-induced lipolysis and expression of lipolysis-regulating genes were determined together with body composition and in vivo lipolytic activity (fasting plasma glycerol or fatty acids related to body fat). Body fat was reduced by 40% and in vivo lipolytic activity was 2-fold increased in cachexia (P = 0.001). In mature adipocytes, the lipolytic effects of catecholamines and natriuretic peptide were 2- to 3-fold increased in cachexia (P < 0.001). This was completely counteracted by inhibiting the rate-limiting lipolysis enzyme hormone-sensitive lipase (HSL). In cachexia, the expression levels of HSL mRNA and protein were increased by 50% and 100%, respectively (P = 0.005–0.03), which strongly correlated with in vitro lipolytic stimulation (r = 0.7–0.9). The antilipolytic effect of insulin in mature fat cells and the stimulated lipolytic effect in differentiated preadipocytes were unaltered in cachexia. Patients who lost weight due to other factors than cancer cachexia had no change in adipocyte lipolysis. In conclusion, adipocyte lipolysis is increased in cancer cachexia not due to nonepigenic factors or to weight loss per se, but most probably because of enhanced expression and function of adipocyte HSL. The selective inhibition of this enzyme may prevent fat loss in cancer patients. [Cancer Res 2007;67(11):5531–7]

Introduction

Many cancer patients have a wasting syndrome termed cachexia, which is characterized by loss of skeletal muscle and adipose tissue. Although the mechanisms behind muscle wasting have been studied intensely, much less is known about the factors promoting loss of adipose tissue in cancer patients (1, 2). However, it is believed that increased fat cell lipolysis, resulting in loss of lipids from adipose tissue, is independent of malnutrition, as reviewed (1–4). Indeed, many studies have shown that increased in vivo lipolysis is a key factor behind adipose cachexia in weight-losing cancer patients (5–8).

Increased production of lipolytic factors from adipose tissue such as interleukin 6 (IL-6) and tumor necrosis factor α (TNFα) or by tumor-derived lipolytic factors such as zinc-cα2 glycoprotein (ZAG) could explain increased lipolysis in cancer cachexia (4, 9). Moreover, altered action of the major lipolysis-regulating hormones, catecholamines (stimulatory), natriuretic peptides (stimulatory), and insulin (inhibitory), may be of importance (4). These hormones regulate lipolysis by separate mechanisms, which all converge at the final rate-limiting step in lipolysis activation [i.e., hormone-sensitive lipase (HSL)] as reviewed (10). The recently described natriuretic peptide system is mediated by the cyclic guanosine 3’,5’-monophosphate (cGMP) pathway, which stimulates protein kinase G so that HSL is activated (11).

No adipocyte lipolysis mechanism has thus far been shown in human cancer cachexia. The present in vitro study was conducted to elucidate if the hormonal regulation of lipolysis in fat cells by insulin, catecholamines, and natriuretic peptides is altered in cancer cachexia. Cancer cachexia was defined as nonintentional weight loss in subjects with gastrointestinal adenocarcinoma. To control for nonspecific effects of cancer and weight loss, we included two control groups. One consisted of weight-stable cancer patients whereas a second was composed of weight-losing cancer patients with evidence of malnutrition (anorexia and/or gastrointestinal obstruction). The reasons for weight loss in the latter group might be different than in the cachexia group.

Materials and Methods

Patients. All patients scheduled for gastrointestinal cancer operation between January 2004 and December 2005 (n = 300 patients) were evaluated for the study and all patients who were (a) fit in spite of their cancer disease, (b) had not received prior anticancer treatment, and (c) were willing to participate were included (n = 36). The study was approved by the Ethics committee of the Karolinska Institutet. After explaining the study in detail to each patient, their informed consent was obtained. The patients were divided into three groups based on diagnosis before surgery by one of the responsible surgeons (T.A., J.P., B.I.), who did not take part in the adipose analyses. The diagnosis of each patient was blinded for those responsible for lipolysis studies (P.A.), gene expression studies (J.H.), protein expression studies (M.R.), and preadipocyte studies (V.v.H., J.L., A.D.) until all data were obtained. None of the patients had jaundice. Cancer cachexia (n = 7) was defined as prediagnosed gastrointestinal cancer with no evidence of gastrointestinal obstruction or anorexia with nausea and/or stomach pain, in combination with unintentional weight loss of >5% of the habitual weight during the last 3 months or >10% weight loss during the last 6 months. One control group (n = 11) consisted of subjects with prediagnosed gastrointestinal cancer who reported no important weight change during the last year. The second control group (n = 8) was composed of subjects with prediagnosed gastrointestinal cancer who had reported weight loss as defined for the cancer cachexia group above in combination with symptoms of anorexia, dysphagia, and/or evidence of obstructive cancer, which supposedly had caused malnutrition. The study was designed as “intention to treat.” Therefore, all included subjects were kept in the primary analyses in spite of postoperative reclassification. None in the cachexia group was reclassified. These patients had adenocarcinoma in the pancreas (n = 5), colon (n = 1), or gastric cardia (n = 1). Two weight-stable control patients did not have cancer but had chronic pancreatitis.

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Research Article


5531
Clinical examination. The patients came to the laboratory after an overnight fast. Height, weight, waist-to-hip ratio, and body composition with bioimpedance using Bodystat Quad Scand 4000 (Bodystat Ltd) were determined. A venous blood sample was obtained for the determination of lipids, glycerol, fatty acids, albumin, C-reactive protein, insulin-like growth factor I (IGF-I), leptin, noradrenaline, adrenaline, and insulin by the hospital's accredited routine chemistry laboratories, and atrial natriuretic peptide was measured using a RIA kit (Phoenix Peptides). The nutritional status was assessed by using a standardized questionnaire for oncology termed Subjective Global Assessment (SGA; ref. 12). Tumor stage was classified postoperatively according to the TNM Classification of Malignant Tumors by International Union Against Cancer, 6th edition (13).

Fat biopsies. After the clinical examination, an abdominal s.c. fat biopsy (0.5–1 g) was obtained by needle biopsy as described (14). The tissue pieces (≈5 mg each) were rapidly rinsed in saline and submitted to lipolysis investigations. One 300-mg portion of the collected adipose tissue was frozen in liquid nitrogen and kept at −70°C for later gene expression studies. This was done for all subjects except for three weight-losing controls in which a 300-mg portion was kept in the same way for subsequent Western blot analysis. This was done for six cachexia patients, eight weight-stable controls, and two weight-losing controls. We have previously shown that the tissue pieces removed and frozen in this way are free from damaged cells and blood (15).

Lipolysis. The remaining tissue from the needle biopsy (500–600 mg) was immediately processed further. Isolated fat cells were prepared by collagenase treatment of adipose tissue; fat cell size was determined; and lipolysis experiments were done on isolated fat cells as described (16). In brief, diluted fat cell suspensions (2%; vol/vol) were incubated in an albumin buffer (pH 7.4) supplemented with glucose, ascorbic acid, and, for the insulin experiments, adenosine deaminase and 10−3 mol/L of 8-bromo-cyclic AMP (8-Br-cAMP). The cells were incubated at 37°C for 2 h without (basal) or with increasing concentrations (10−16–10−5 mol/L, depending on the hormone used) of noradrenaline, atrial natriuretic peptide, insulin together with 10−2 mol/L 8-Br-cAMP, 8-Br-cAMP (10−2 mol/L) alone, or 10−2 mol/L 8-bromo-cGMP (8-Br-cGMP). Thereafter, glycerol release into the incubation medium (lipolysis index) was determined. In additional lipolysis experiments, the buffer containing 8-Br-cAMP plus adenosine deaminase was also supplemented with a maximum effective concentration (10−5 mol/L) of a highly selective inhibitor of hormone-sensitive lipase 4-isopropyl-3-methyl-2-[1-(3-{[5-(methyl-piperidine-1-yl)-methyl]oxy}-2-furyl)-loxazol-5-one, termed BAY (17).

For five cancer cachexia patients and eight weight-stable controls, sufficient amounts of the stroma fraction of adipose tissue were obtained for prediapocyte isolation. These cells were differentiated exactly as described (17) for 2 weeks, and lipolysis experiments without (basal) or with 10−5 mol/L of noradrenaline were done as described (16).

The lipolysis data were expressed in two ways: the first was glycerol release per gram of lipids in the studies of mature fat cells or per gram of protein in the prediapocyte studies. The second was as a function of basal (non-hormone stimulated) glycerol release. The latter was defined as insulin-induced values divided by 8-Br-cAMP–induced values in the antilipolytic experiments and noradrenaline- or atrial natriuretic peptide–induced values divided by basal values in the lipolytic experiments. Maximum effect was defined as the value obtained at maximum effective hormone concentration. Priority was given to expression of lipolysis as a function of basal rate because there is a strong relationship between this way of expressing lipolysis and HSL gene or protein expression in human s.c. fat cells (18).

Gene expression. Total RNA was extracted from 300 mg of adipose tissue using the RNeasy mini kit (Qiagen GmbH). The Agilent 2100 Bioanalyzer (Agilent Technologies) was used to confirm the integrity of the RNA. One microgram of total RNA from each sample was reverse transcribed to cDNA using the Omniscript RT kit (Qiagen) and random hexamer primers (Invitrogen). cDNA synthesis was done simultaneously for all subjects using the same mix of primers and RT kit. In a final volume of 25 μL, 5 ng of cDNA were mixed with 2× SYBR green PCR master mix (Bio-Rad Laboratories, Inc.) and primers (Invitrogen). The primer pairs were selected to yield a single amplicon based on dissociation curves and analysis by agarose gel electrophoresis. The primers used were 5′-GGTCTACGGCGG-GAAGATG-3′ (sense) and 5′-TTGAGGGGGAGAGGATG-3′ (antisense) for adipose triglyceride lipase, 5′-GAAGTCTGATCGTCTCCTG-3′ (sense) and 5′-GCGATGCTAGGCTAGCCT-3′ (antisense) for HSL, and 5′-TGACTACACCGGGAAAACC-3′ (sense) and 5′-TGCTCCCAACACTAAGAAC-3′ (antisense) for 18S. Quantitative real-time PCR was done with an iCycler IQ (Bio-Rad Laboratories). The mRNA levels were determined by a comparative Ct method. The subject with the highest Ct value was used as a reference from which all other Ct values for the target gene and reference gene, respectively, were subtracted from this Ct value. The Ct values were then normalized to rRNA for 18S using the formula 2ΔΔCt-target gene /2ΔΔCt-reference gene.

Protein expression. Approximately 300 mg of white adipose tissue were crushed and lysed in protein lysis buffer [1% Triton-X 100, Tris-HCl (pH 7.6), and 150 mmol/L NaCl, 4°C], supplemented with protease inhibitors [1 mmol/L phenylmethylsulfonyl fluoride and Complete (Boehringer Mannheim)], and homogenized. The homogenate was centrifuged and the infranatant was collected and saved. Protein content was assayed using BCA Protein Assay Reagent Kit (Pierce). One hundred micrograms of total cellular protein were loaded on polyacrylamide gels and separated by standard 12% SDS-PAGE. Gels were transferred to polyvinylidene fluoride membranes (Amersham Pharmacia). Blots were blocked for 1 h at room temperature in Tris-buffered saline with 0.1% Tween 20 and 5% nonfat dried milk. This was followed by an overnight incubation at 4°C in the presence of antibodies directed against HSL (19) or the control protein β-actin (Sigma). Secondary α-rabbit antibodies conjugated to horseradish peroxidase were from Sigma. Antigen-antibody complexes were detected by chemiluminescence using a detection kit (SuperSignal) from Pierce and specific bands were detected and quantified using a Chemidoc XRS system and the Quantity One Software from Bio-Rad, and expressed as the HSL/β-actin ratio.

Statistical analysis. The reported values are mean ± SD or median and range. The values with adipose tissue were considered to be normally distributed because retrospective analysis of previously investigated large cohorts using the same lipolysis and gene or protein expression methods showed a normal distribution of data (18, 20, 21). Results were compared using ANOVA and appropriate post hoc tests, Student’s unpaired or paired t test, or linear regression by the method of least squares. Kruskal-Wallis and Mann-Whitney tests were used to compare SGA and tumor score values. A power calculation was made before patient collection and was based on the previously known distribution of maximum noradrenaline-induced glycerol release divided by basal glycerol release (22). We expected the recruitment of weight-stable patients to be easier and anticipated the relative proportion between the three groups to be 2:3:2. Based on these estimations and mean and SD of noradrenaline/basal lipolysis, we calculated that we had to recruit 7 cachexia patients, 11 weight-stable control patients, and 7 weight-losing control patients to detect a 70% difference between cachexia and the two control groups at P < 0.05 (ANOVA) with 80% power using Sample Power (SPSS, Inc.).

Results

In vivo results. Regarding the clinical findings (Table 1), the groups were of comparable mean age, and gender distribution did not differ in a significant way. Body mass index (BMI) and amount of body fat were equal in the cachectic and weight-losing control groups but lower compared with the weight-stable group. Neither total body water nor lean body mass differed between the three groups but lower compared with the weight-stable group. Neither total body water nor lean body mass differed between the three groups but lower compared with the weight-stable group. Neither total body water nor lean body mass differed between the three groups but lower compared with the weight-stable group.

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but much smaller than in the weight-stable group. In addition, plasma levels of insulin, leptin, triglycerides, and serum albumin were similar in the two weight-losing groups but lower than the weight-stable group. IGF-I levels were reduced by half in the weight-losing control groups, which did not differ between each other, but there was no difference between the three groups in adipose triglyceride lipase mRNA (F = 0.50; P = 0.15).

**Lipolysis in vitro.** Insulin inhibited lipolysis in a similar concentration-dependent manner in the three groups (Fig. 1). Maximum inhibition of lipolysis varied from 55% to 60% in these groups (Fig. 1).

Noradrenaline and atrial natriuretic peptide stimulation of basal lipolysis rate was much more efficient (F = 0.001, ANOVA repeated measure) in cachectic patients as compared with the control groups, who did not differ between each other (Fig. 1). At the maximum effective concentration, noradrenaline stimulated lipolysis 5-, 3-, and 2.5-fold in the cachetic, weight-stable control, and weight-losing control groups, respectively (P = 0.01; Fig. 1). Corresponding values for natriuretic peptide were 7-, 4-, and 3-fold (P = 0.02; Fig. 1).

We also expressed lipolysis as absolute values of glycerol release (μmol/g of lipid/2 h). As shown in Table 2 this mode of expression gave similar values as above; however, some of them did not reach a level of statistical significance (P = 0.06–0.07). Furthermore, basal (nonhormonal) lipolysis did not differ between groups (Table 2).

The cAMP analogue 8-Br-cAMP caused a 7-fold stimulation of lipolysis in the cachetic group as compared with a 3-fold stimulation in the two control groups (Fig. 2). The selective HSL inhibitor BAY decreased the lipolytic effect of the cAMP analogue by 0.5-fold, respectively, in the two control groups (P = 0.03).

**Gene expression.** In the adipose tissue of cachetic patients, HSL mRNA expression was ≈50% higher compared with the two control groups, which did not differ between each other, but there was no difference between the three groups in adipose triglyceride lipase expression (Fig. 2). As regards maximum lipolytic effect of noradrenaline or atrial natriuretic peptide, both hormones showed a strong positive correlation with HSL gene expression (r = 0.58; P = 0.005 for noradrenaline and r = 0.50; P = 0.02 for atrial natriuretic peptide; graphs not shown). No such correlation was observed for adipose triglyceride lipase mRNA (r ≈ 0.15).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Cachexia</th>
<th>Weight-stable control</th>
<th>Weight-losing control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M/F</td>
<td>5/2</td>
<td>6/5</td>
<td>7/1</td>
<td>0.30</td>
</tr>
<tr>
<td>Age, y</td>
<td>64 ± 5</td>
<td>62 ± 7</td>
<td>65 ± 5</td>
<td>0.58</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.3 ± 2.5</td>
<td>27.8 ± 4.2</td>
<td>21.9 ± 2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist-hip, ratio</td>
<td>0.90 ± 0.11</td>
<td>0.98 ± 0.07</td>
<td>0.93 ± 0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>25 ± 7</td>
<td>34 ± 8</td>
<td>27 ± 4</td>
<td>0.02</td>
</tr>
<tr>
<td>Body fat, kg</td>
<td>16.3 ± 4.2</td>
<td>27.9 ± 8.3</td>
<td>17.8 ± 3.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>53.8 ± 17.1</td>
<td>55.5 ± 13.4</td>
<td>51.0 ± 11.0</td>
<td>0.78</td>
</tr>
<tr>
<td>Total body water, kg</td>
<td>42.8 ± 11.2</td>
<td>41.7 ± 9.1</td>
<td>38.6 ± 1.7</td>
<td>0.61</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>145 ± 36</td>
<td>144 ± 15</td>
<td>145 ± 20</td>
<td>0.995</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80 ± 12</td>
<td>83 ± 9</td>
<td>78 ± 4</td>
<td>0.46</td>
</tr>
<tr>
<td>Pulse, beats/min</td>
<td>71 ± 11</td>
<td>69 ± 11</td>
<td>69 ± 5</td>
<td>0.90</td>
</tr>
<tr>
<td>Fat cell volume, pl</td>
<td>299 ± 85</td>
<td>653 ± 176</td>
<td>395 ± 102</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight loss, % of habitual weight</td>
<td>18 ± 3</td>
<td>-</td>
<td>22 ± 9</td>
<td>0.73</td>
</tr>
<tr>
<td>P-Noradrenaline, nmol/L</td>
<td>2.2 ± 0.4</td>
<td>3.0 ± 1.0</td>
<td>2.4 ± 0.2</td>
<td>0.14</td>
</tr>
<tr>
<td>P-Adrenaline, nmol/L</td>
<td>0.22 ± 0.14</td>
<td>0.20 ± 0.13</td>
<td>0.25 ± 0.24</td>
<td>0.88</td>
</tr>
<tr>
<td>P-Atrial natriuretic peptide, ng/L</td>
<td>13 ± 5</td>
<td>12 ± 4</td>
<td>14 ± 4</td>
<td>0.69</td>
</tr>
<tr>
<td>P-Glucose, mmol/L</td>
<td>0.0 ± 1.1</td>
<td>6.6 ± 1.8</td>
<td>6.8 ± 0.8</td>
<td>0.69</td>
</tr>
<tr>
<td>P-Insulin, μL/L</td>
<td>6.8 ± 3.3</td>
<td>16.1 ± 11.3</td>
<td>6.6 ± 3.9</td>
<td>0.02</td>
</tr>
<tr>
<td>P-Triglycerides, mmol/L</td>
<td>1.0 ± 0.3</td>
<td>1.5 ± 0.5</td>
<td>1.0 ± 0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>P-Cholesterol, mmol/L</td>
<td>4.3 ± 1.1</td>
<td>5.2 ± 1.2</td>
<td>4.1 ± 0.6</td>
<td>0.10</td>
</tr>
<tr>
<td>P-Leptin, ng/mL</td>
<td>5.3 ± 2.2</td>
<td>20.1 ± 13.9</td>
<td>4.8 ± 2.5</td>
<td>0.002</td>
</tr>
<tr>
<td>S-IGF-I, μg/L</td>
<td>125 ± 30</td>
<td>138 ± 62</td>
<td>62 ± 50</td>
<td>0.01</td>
</tr>
<tr>
<td>S-Albumin, g/L</td>
<td>343.4 ± 5.0</td>
<td>390 ± 3.0</td>
<td>34.4 ± 1.1</td>
<td>0.01</td>
</tr>
<tr>
<td>C-reactive protein, μg/L</td>
<td>19 ± 23</td>
<td>13 ± 27</td>
<td>32 ± 34</td>
<td>0.38</td>
</tr>
<tr>
<td>P-Glycerol, μmol/L/kg body fat</td>
<td>6.2 ± 2.7</td>
<td>3.1 ± 0.7</td>
<td>4.8 ± 2.3</td>
<td>0.01</td>
</tr>
<tr>
<td>P-Fatty acids, μmol/L/kg fat</td>
<td>62 ± 25</td>
<td>27 ± 11</td>
<td>39 ± 3</td>
<td>0.001</td>
</tr>
<tr>
<td>SGA score, points</td>
<td>8 (5–12)</td>
<td>1 (1–3)</td>
<td>12 (8–18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tumor score, points</td>
<td>3 (1–4)</td>
<td>3 (0–4)</td>
<td>3 (3–4)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

NOTE: Values are mean ± SD or median (range). They were compared by ANOVA, χ² (for gender), or Kruskal-Wallis test (for SGA and tumor score). Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; P, fasting plasma; S, fasting serum.
Protein expression. The number of subjects investigated was too small for an analysis of the three separate groups. However, HSL protein expression was 2-fold increased in cachexia as compared with the two control groups (0.23 ± 0.57 versus 0.69 ± 0.34 arbitrary units; \( P = 0.032 \); Fig. 2). When all protein data were compiled, there was a strong positive correlation between HSL protein expression and noradrenaline-stimulated lipolysis (Fig. 2). As much as 50% of the variation in lipolysis (adjusted \( r^2 \)) could be explained by variations in HSL. Similar correlations were obtained with atrial natriuretic peptide (\( r = 0.67 ; P = 0.005 \)) or 8-Br-cAMP (\( r = 0.85 ; P < 0.001 \)). The results were not altered in a significant way when absolute values for HSL expression were used instead of HSL/actin ratio (\( r = 0.67–0.85 \); graphs not shown). \( \beta \)-Actin expression was not correlated with lipolysis (\( r = 0.03–0.07 \)). The mRNA and protein expression of HSL showed a very strong correlation (\( r = 0.81 ; P = 0.001 \)).

Preadipocytes. In preadipocytes (Fig. 2) differentiated to adipocytes, the lipolytic effect of noradrenaline was the same in cachexia and weight-stable control (≈8-fold stimulation of basal rate). Similar results were obtained when preadipocyte lipolysis was expressed as a function of cellular protein content (values not shown).

Four control subjects were wrongly classified, as they did not have cancer. When they were excluded, the results of the analysis presented above were essentially the same as for the whole material.

Discussion

The weight-losing control group had impaired food intake and/or evidence of gastrointestinal stricture, which presumably caused malnutrition. These problems were not found in the cachectic group. Tumor severity was similar in all three groups. The two weight-losing groups had comparable values for BMI, adipose mass, and plasma levels of leptin, insulin, triglycerides, and albumin, all of which were much lower than in weight-stable cancer patients. This suggests a similar catabolic state in the two former groups. However, there were two clear differences in the clinical characteristics of the two weight-losing groups. The weight-losing controls had much higher SGA scores than cachexia patients, and only the former patients displayed lower IGF-I values. Both SGA and IGF-I are markers of malnutrition as discussed (23, 24). Thus, our clinical data clearly suggest that the two weight-losing groups, at least in part, have different etiologies underlying their reported weight loss, malnutrition being dominant only in the control group.

We found no difference in lean body mass between the groups. However, none of our patients was extremely lean (lowest BMI was 18.6 kg/m²), indicating that we might have studied patients in an early cachexia phase when there is predominantly loss of adipose tissue. This notion is supported by a recent large study showing that body fat was lost more rapidly than lean tissue in progressive cancer cachexia (25).
Only the cachexia group had significantly increased \textit{in vivo} lipolysis although there was no evidence of increased sympathetic nervous activity or elevated levels of circulating natriuretic peptide. As regards the cellular mechanism promoting this increase in lipolysis in human cachexia, the antilipolytic effect of insulin was not significantly altered in cachexia. In contrast, the lipolytic effects of the two major lipolysis-regulating hormone systems in man (i.e., catecholamines and natriuretic peptides) were markedly increased in patients with cachexia as compared with the two control groups. Furthermore, stimulation of lipolysis with cAMP or cGMP analogues also gave increased effect in the cachexia group. Noradrenaline and natriuretic peptide stimulate adipocyte lipolysis through separate signal pathways (mediated by either cAMP or cGMP) that all converge at HSL, an enzyme that is also a target for cAMP and cGMP. The similar findings with the two cyclic nucleotides indicate that the cellular mechanism for increased lipolysis in cachexia is due to enhanced lipolytic signaling of the hormone systems at the post-receptor level because of enhanced function of HSL. Indeed, this seems to be the case because lipolysis could be inhibited to the same extent in all three groups using the highly selective HSL inhibitor BAY.

In a previous study, adipose tissue gene expression of HSL was increased in cancer patients as compared control patients (26). We found a 50% increase in HSL gene expression selectively in the cachexia group and a 2-fold increase in amount of HSL protein in adipocytes among this group as compared with the other two

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cachexia</th>
<th>Weight-stable control</th>
<th>Weight-losing control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0.98 ± 0.7</td>
<td>1.16 ± 0.67</td>
<td>1.49 ± 0.67</td>
<td>0.44</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>3.1 ± 1.7</td>
<td>1.5 ± 1.0</td>
<td>1.49 ± 0.67</td>
<td>0.06</td>
</tr>
<tr>
<td>Atrial natriuretic peptide</td>
<td>4.0 ± 1.5</td>
<td>2.1 ± 1.9</td>
<td>2.6 ± 1.2</td>
<td>0.07</td>
</tr>
<tr>
<td>8-Br-cAMP</td>
<td>5.1 ± 2.3</td>
<td>2.7 ± 2.7</td>
<td>3.1 ± 1.6</td>
<td>0.02</td>
</tr>
<tr>
<td>8-Br-cAMP + insulin</td>
<td>1.9 ± 1.0</td>
<td>1.3 ± 0.7</td>
<td>1.9 ± 0.9</td>
<td>0.31</td>
</tr>
</tbody>
</table>

NOTE: Values are mean ± SD of glycerol release (μmol/g of lipid/2 h). Values were compared by ANOVA.

Table 2. Absolute values for basal and maximum hormone-induced lipolysis

Figure 2. Experimental studies. Top left, lipolytic effect of a cAMP analogue (8-bcAMP) with or without HSL inhibitor BAY. Top right, mRNA expression of HSL and adipose triglyceride lipase (ATGL). Bottom left, relationship between HSL protein expression (ratio of actin) and lipolytic effect of noradrenaline. Bottom right, lipolysis in preadipocytes differentiated into adipocytes.
groups together. In the whole material, HSL gene and protein expression correlated strongly with the lipolytic effect of noradrenaline or natriuretic peptide and could explain 50% of the between subject variation in lipolytic effect of the hormone. Furthermore, mRNA and HSL protein levels were strongly interrelated and overexpression of HSL leads to a marked increase in adipocyte lipase activity (27). Thus, when present and previous data are taken together, it is likely that increased production of HSL protein, due to enhanced gene activity in fat cells, is the major cause behind enhanced lipolysis in cancer cachexia.

The novel lipase adipose triglyceride lipase may be important for determining fat cell lipolysis (28). In this study, however, there was no effect of cachexia on adipose triglyceride lipase expression, and the expression of this enzyme did not correlate with lipolysis. Furthermore, there was <10% residual adipocyte lipolytic activity in all groups when HSL-induced lipolysis was inhibited, suggesting no or a minor role of adipose triglyceride lipase for the altered regulation of lipolysis in cancer cachexia.

Our findings suggest that treatment of cancer cachexia with recently developed selective HSL inhibitors may be of clinical value to prevent loss of adipose tissue (29). Although loss of adipose tissue may be less harmful than muscle loss in cancer cachexia, the former seems to precede the latter. Maybe early antilipolysis treatment could slow down or prevent the progressive wasting among cancer patients.

It is unlikely that increased lipolysis in cachexia is secondary to weight loss because weight-losing control patients had the same adipocyte lipolytic activity as the weight-stable control patients. However, increased lipolysis in cachexia is not an epigenetic phenomenon because the difference in lipolysis between weight-losing control patients and cachexia disappeared in predisapocytes that had differentiated into fat cells. The influence of external factors had been eliminated in these cells. These factors could be produced within adipose tissue or they could be circulating factors produced by certain tumors. One such lipolytic mediator not investigated by us could be ZAG (4, 9). It is quite possible that ZAG-induced lipolysis is increased in human cancer cachexia because the protein stimulates lipolysis through the same pathway as catecholamines (30, 31). In rodents with experimentally induced cancer cachexia, a tumor-derived factor was shown to sensitize adipose tissue to lipolytic stimuli (32).

Are the present findings relevant for other lipolytic regulators such as IL-6 and TNFα? Probably not, because basal lipolysis was not influenced in cancer cachexia. In human fat cells, TNFα above all enhances the basal rate of adipocyte lipolysis (33). Furthermore, the stimulatory effect of IL-6 on human lipolysis is as acute, which does not fit in with our HSL observations (34).

Our studies were conducted on rather small study groups. It is very difficult to recruit cancer cachexia patients to this type of investigation. However, the study was powered to detect the observed differences in lipolysis.

In conclusion, we propose that yet unidentified factors among certain cancer patients increase the gene expression and thereby the protein production of HSL in fat cells. This enhances the stimulatory effect of lipolytic hormones and possibly of specific cachexia factors such as ZAG. Because the hormones are always present in the circulation, lipolysis is continuously activated because the action of the major antilipolytic hormone, insulin, is not altered. Therefore, HSL inhibitors may be useful in the treatment or prevention of cancer cachexia.

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