Evidence for an Important Role of CIDEA in Human Cancer Cachexia

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Abstract

Loss of fat mass in cancer cachexia is linked to increased adipocyte lipolysis; however, the fate of the excess fatty acids (FA) generated by lipolysis is not known. We investigated if the adipocyte-specific gene cell death–inducing DNA fragmentation factor–α–like effector A (CIDEA) could be involved. CIDEA mRNA expression was assessed in s.c. white adipose tissue from 23 cancer cachexia patients, 17 weight-stable cancer patients, and 8 noncancer patients. CIDEA was also overexpressed in adipocytes in vitro. CIDEA expression was increased in cancer cachexia (\(P < 0.05\)) and correlated with elevated levels of FAs and reported weight loss (\(P < 0.001\)). CIDEA overexpression in vitro increased FA oxidation 2- to 4-fold (\(P < 0.01\)), decreased glucose oxidation by 40% (\(P < 0.01\)), increased the expression of pyruvate dehydrogenase kinase (PDK) 1 and PDK4 (\(P < 0.01\)), and enhanced the phosphorylation (inactivation) of the pyruvate dehydrogenase complex (PDC). Inactivation of PDC facilitates FA oxidation by favoring the metabolism of FAs over glucose to acetyl-CoA. In accordance with the in vitro data, PDK1 and PDK4 expression correlated strongly with CIDEA expression in white adipose tissue (\(P < 0.001\)). We conclude that CIDEA is involved in adipose tissue loss in cancer cachexia and this may, at least in part, be due to its ability to inactivate PDC, thereby switching substrate oxidation in human fat cells from glucose to FAs.

Introduction

Cancer cachexia is associated with poor survival rate and impaired response to chemotherapy (1, 2). It is characterized by loss of both adipose tissue and skeletal muscle (1, 2). The former often precedes and is more rapid than the latter (3). Several factors may induce loss of adipose tissue in cancer cachexia. However, increased lipid mobilization due to enhanced adipocyte lipolysis seems to be a major course (1, 2, 4, 5). Lipolysis is the hydrolysis of triglycerides to glycerol and fatty acids (FA) in adipocytes, where triglycerides constitute >95% of adipose mass. Increased lipolysis in cancer cachexia has been shown in vivo (6–10) and results in decreased fat cell volume and size. In agreement with this, fat cells in cancer cachexia patients are smaller (6). The mechanism behind increased adipocyte lipolysis has recently been elucidated (6). It was shown that the expression of the adipocyte hormone-sensitive lipase (the rate-limiting enzyme of lipolysis) is increased in fat cells from subjects with cancer cachexia. This augments the lipolytic effects of the major stimulatory hormones in humans, which are catecholamines and natriuretic peptides (11).

FAs are the most energy-rich molecules in the body. It is unclear how they are metabolized after lipolysis in cancer cachexia. Increased FA oxidation has been shown in cancer patients, which was more pronounced among those losing weight (9, 12). Skeletal muscle is the major site for FA oxidation in humans, whereas in rodents, brown fat cells contribute as well. Recent rodent studies suggest that FA oxidation in white adipose tissue is an additional important regulator of FA oxidation (13). In weight-stable humans, adipocytes contribute only little to total FA combustion (14, 15). However, total energy expenditure in human fat cells (glucose and FA oxidation) is significant and constitutes up to 14% of whole body heat production (16). This percentage is markedly increased following body weight reduction (17).

The capacity of human white adipose tissue to oxidize FAs may increase markedly if the oxidative machinery is altered (14). A key factor in regulating the switch from glucose to FA oxidation is the pyruvate dehydrogenase complex (PDC; ref. 18). PDC plays a major role in glucose metabolism by catalyzing the oxidative decarboxylation of pyruvate to acetyl-CoA and NADH, which can then be used for the tricarboxylic acid cycle and various biosynthetic processes. PDC is a highly organized multienzyme complex (19) and is inactivated via phosphorylation of the E1 (PDC-E1) subunit of the complex (20). Phosphorylation of PDC-E1 is regulated by four pyruvate dehydrogenase kinases (PDKs), which are heterodimeric enzymes with tissue-specific distribution (18, 19). The expression level of PDK4 is regulated by the nuclear receptor peroxisome proliferator–activated receptor α, which is also activated by FAs (21). PDC needs to be active when the complete oxidation of glucose is required for the generation of energy, whereas its activity has to be suppressed when glucose is in short supply. Inactivation of PDC favors the preferential oxidation of long-chain FAs over glucose.

We hypothesized that in response to the increased lipolysis in white fat cells of cancer cachexia patients, adipocytes may activate compensatory FA oxidative pathways and suppress glucose oxidation to rid themselves of excess FAs. A putative candidate for such an adaptation is a novel gene termed cell death–inducing DNA fragmentation factor–α–like effector A (CIDEA). CIDEA belongs to a family of apoptosis genes with unclear function (22). However, recent studies suggest a role in lipid metabolism and body weight regulation. In rodents, CIDEA deficiency leads to increased FA oxidation in brown fat cells, acceleration of lipolysis, and resistance to diet-induced obesity (23, 24). In humans, unlike rodents, the gene is predominantly expressed in white fat cells and inhibits adipocyte lipolysis (25, 26). In transcriptomic profiling study, CIDEA was the most up-regulated gene in human white adipose tissue following dietary weight reduction (27).

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Furthermore, a polymorphism in the CIDEA gene reduces the risk for obesity (28).

In the present study, the possible role of CIDEA in white adipose tissue for FA oxidation in vitro and in vivo was investigated. Cancer patients with or without cachexia and control (noncancer) patients were compared. The direct effect of CIDEA on FA and glucose oxidation was investigated by overexpressing the gene in fat cells.

Materials and Methods

Patients. All patients scheduled for gastrointestinal cancer operation between January 2004 and December 2006 (about 450 patients) were evaluated for the study and patients who (a) were fit in spite of their cancer, (b) had not received prior anticancer treatment, and (c) were willing to participate were included (n = 48). The remaining ones were excluded due to severe illness, disincination, communication problems, and logistic reasons. The study was approved by the Regional Ethics committee. The investigation was explained in detail to each patient and written informed consent was obtained. None of the patients had jaundice. They were divided into three groups based on diagnosis after surgery. One group had cancer cachexia (n = 23), which was defined as gastrointestinal cancer with unintentional weight loss of >5% of the habitual weight during the previous 3 mo or >10% unintentional weight loss during the previous 6 mo (6). The primary location of cancer was pancreas (n = 10), esophagus (n = 10), stomach (n = 2), and colon (n = 1). One group (n = 17) consisted of patients with gastrointestinal cancer who reported no important weight change during the last year. The localization of malignancy was pancreas (n = 5), esophagus (n = 1), stomach (n = 3), colon (n = 4), gall bladder (n = 2), and liver (n = 2). The control group (n = 8) contained subjects with prediagnosed gastrointestinal cancer but who did not have a malignancy according to final histologic evaluations. The noncancer control patients had chronic pancreatitis (n = 5) or cholecystitis (n = 3). The study was designed as “intention to compare”; therefore, all included subjects were kept in the analyses despite a few missing values of some of the measurements.

Clinical examination. The patients came to the laboratory after an overnight fast. Height, weight, body composition by bioimpedance using Quad Scan 4000 (BodyStat Ltd.), and indirect calorimetry using Deltatrac (Datex-Engstrom) were determined. A venous blood sample was obtained for the measurement of lipids, glycerol, FA, albumin, and transferrin. Nutritional status was assessed by using a standardized questionnaire for oncology termed Subjective Global Assessment (SGA; ref. 29). Tumor stage was classified postoperatively as described previously (6). Indirect calorimetry could not be done on one weight-stable cancer patient due to technical reasons. To indirectly assess lipolytic activity in vivo, glycerol and FA concentrations were divided by body fat weight (6).

Fat biopsies. After the clinical examination, an abdominal s.c. fat biopsy (~0.5 g) was obtained by needle biopsy as described (30). Tissue pieces were rapidly rinsed in saline, frozen in liquid nitrogen, and kept at −70°C for later gene expression studies. Tissue pieces removed and frozen in this way are free from damaged cells and blood (31). In three cachexia patients, the quality of the RNA was not sufficient for mRNA analysis.

Gene expression. Total RNA was extracted from adipose tissue or 3T3-L1 cells using the RNeasy mini kit (Qiagen GmbH). RNA quality was analyzed using the Agilent 2100 Bioanalyzer (Agilent Technologies) before cDNA synthesis. cDNA synthesis was done on 1 μg of total RNA using the Omniscript RT kit (Qiagen) and random hexamer primers (Invitrogen). Quantitative real-time PCR was done in an iCycler IQ (Bio-Rad Laboratories, Inc.) using TaqMan probes (Assays-on-demand, Applied Biosystems). mRNA levels in patient material were determined using a comparative CΔ method, as described (6). In CIDEA overexpression experiments, cDNA from empty vector–transfected cells was used as the calibrator and acidic ribosomal protein 1 (Arp1) was used as the reference gene.

CIDEA expression vector. CIDEA expression vector was obtained by insertion of a PCR product corresponding to the human CIDEA cDNA into the pcDNA3.1(+) plasmid (Invitrogen). The forward and reverse primers (Invitrogen) used for amplification were 5′-GGGAATTCGGGCCCCATGGAGG-3′ and 5′-CTTCTGAGACGTGTACCAG-3′, respectively, with restriction sites for EcoRI and XhoI underlined. Constructs were verified by DNA sequencing (MWG Biotech). Protein expression was verified by in vitro translation using a Transcend Non-Radioactive Translation Detection System (Promega).

Transient transfections. 3T3-L1 cells were grown to confluence and differentiated for 3 d according to standard procedures. Cells were then seeded into 12-well plates (Corning, Inc.) at a density of ~10,000/cm². After 24 h in postdifferentiation medium, the 3T3-L1 adipocytes were transfected using Lipofectamine and Plus transfection reagents (Invitrogen) according to the manufacturer’s instructions. Transfection efficiency in 3T3-L1 cells was optimized using a green fluorescence protein–containing plasmid. Transfection efficiency was 30% to 40%. One microgram of CIDEA expression vector, 6 μL of Plus reagent, and 4 μL of Lipofectamine were used per well for transfection. Cells were analyzed after 48 h. After overexpression, levels of hCIDEA mRNA reached those detected in human mature adipocytes. Human adipocytes differentiated from s.c. precursor cells were transfected as described previously (32). Cell Death Detection ELISA Plus (Roche Diagnostics GmbH) was used to determine apoptosis and necrosis.

Western blot and immunoprecipitation. 3T3-L1 cells were transfected with CIDEA expression vector or pcDNA3 as described above. After 48 h, the medium was removed and cells were lysed in RIPA buffer [50 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L PMSE, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS] containing phosphatase inhibitors (1 mmol/L sodium orthovanadate and 1 mmol/L sodium fluoride) and proteinase inhibitor cocktail (Roche Diagnostics GmbH). Cell lysates were centrifuged at 14,000 rpm for 15 min at +4°C; the supernatant was removed and the protein concentration was measured using the bicinchoninic acid protein assay kit ( Pierce).

Four hundred micrograms of protein lysate were used for PDC immunoprecipitation according to the manufacturer’s instructions (PDC pull-down kit, Mitosciences). PDC was eluted with 1% SDS. Gel electrophoresis and blotting were done as described previously (33). Anti-phosphoserine antibody (Zymed Laboratories) was used at the concentration of 2 μg/mL to detect phosphorylation of the PDC-E1 subunit. Antibodies that were used for CIDEA detection were anti-CIDEA polyclonal antibodies C-16 and N-19 from Santa Cruz Biotechnology, ab5692 from Abcam, and AB16508 and AB16922 from Chemicon International.

Palmitate oxidation. 3T3-L1 cells were transfected with CIDEA expression vector or control pcDNA3 vector. After 48 h, the medium was removed and FA oxidation determined as described previously (34). A control, FA oxidation was stimulated for 3 h with 10 mmol/L 3-chloro-L-carboxy-phenylhydrazide (Sigma). CIDEA overexpression was controlled in each experiment, and palmitate oxidation was normalized to total protein amount in each experimental group.

Glucose oxidation. Oxidation of glucose to CO₂ in 3T3-L1 cells differentiated to adipocytes was assayed as previously described (35) with some modifications. 3T3-L1 cells were transfected with CIDEA expression vector or empty pcDNA3 vector as described above. After 48 h, the cell medium was removed and the cells were rinsed with PBS. Krebs-Ringer-HEPES (0.5 mL) containing 2% BSA and 0.55 mmol glucose was added. Cell suspensions from three wells (1.5 mL) were transferred to Carbosorb E flasks containing 1.5 mL of Krebs-Ringer-HEPES with 2% BSA, 0.55 mmol glucose, and 0.4 μCi/mL [U-14C]glucose (Amersham Biosciences). Flasks were closed and incubated at 37°C with shaking for 1.5 h. 14CO₂ was liberated by acidification with 10 N H₃SO₄, collected for 1 h on Carbosorb, and measured by scintillation counting.

Statistical analysis. Values are mean ± SD or range in text, tables, and figures. Results were compared using ANOVA and appropriate post hoc tests, χ² test, unpaired or paired t test, and linear or multiple regression analysis. Gene expression values and values for body fat CIDEA were log₁₀ transformed to obtain normal distribution. Kruskal-Wallis and Mann-Whitney tests were used to compare SGA and tumor score, respectively. A power calculation was made before starting to recruit subjects and was based on our previously known distribution of CIDEA mRNA expression in
white adipose tissue of healthy human subjects (27). Based on our previous study of cancer cachexia (6), it was decided to recruit control patients, weight-stable cancer patients, and cancer cachexia patients in the ratio of 1:2:3. This ratio was based on the assumption of a 10% experimental failure rate, and for the mean and SD of CIDEA mRNA expression we had to recruit at least 8 controls, 16 weight-stable, and 24 cachexia patients to detect a 2-fold difference between cachexia and the other two groups at <0.05 (ANOVA) with 80% power using Sample Power (SPSS, Inc.).

Results

Clinical findings. The clinical results are shown in Table 1. The subjects in the three groups were of similar age. Body mass index (BMI) was markedly decreased in subjects with cancer cachexia in comparison with the other two groups; the weight-stable cancer group had an intermediate value. The same was true for percentage of body fat and total amount of body fat. In contrast, lean body mass

![Figure 1](https://www.aacrjournals.org/doi/fig/9249.png)

**Table 1. Characteristics of study groups**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Cancer cachexia</th>
<th>Weight-stable cancer</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M/F</td>
<td>20/3</td>
<td>12/5</td>
<td>4/4</td>
<td>0.11</td>
</tr>
<tr>
<td>Age, y</td>
<td>65 ± 7</td>
<td>64 ± 10</td>
<td>59 ± 7</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.6 ± 4.2</td>
<td>25.7 ± 3.5</td>
<td>28.0 ± 7.6</td>
<td>0.016</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>18 ± 9</td>
<td>23 ± 7</td>
<td>37 ± 25</td>
<td>0.001</td>
</tr>
<tr>
<td>Body fat, kg</td>
<td>13.3 ± 9.9</td>
<td>21.2 ± 6.1</td>
<td>32.9 ± 23.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>57.9 ± 10.2</td>
<td>58.1 ± 13.3</td>
<td>46.7 ± 10.4</td>
<td>0.064</td>
</tr>
<tr>
<td>Weight loss, % of habitual weight</td>
<td>12.4 ± 7.6</td>
<td>3.9 ± 7.4</td>
<td>3.7 ± 7.3</td>
<td>0.002</td>
</tr>
<tr>
<td>P-glucose, mmol/L</td>
<td>6.1 ± 1.3</td>
<td>6.2 ± 1.5</td>
<td>6.6 ± 2.2</td>
<td>0.76</td>
</tr>
<tr>
<td>P-triglycerides, mmol/L</td>
<td>1.0 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>0.027</td>
</tr>
<tr>
<td>P-cholesterol, mmol/L</td>
<td>4.3 ± 1.1</td>
<td>4.8 ± 1.1</td>
<td>4.8 ± 1.2</td>
<td>0.25</td>
</tr>
<tr>
<td>S-albumin, g/L</td>
<td>34.9 ± 3.6</td>
<td>37.6 ± 3.5</td>
<td>38.9 ± 3.3</td>
<td>0.016</td>
</tr>
<tr>
<td>S-transferrin</td>
<td>2.1 ± 0.3</td>
<td>2.4 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td>0.031</td>
</tr>
<tr>
<td>P-glycerol, μmol/L/kg body fat</td>
<td>12.8 ± 12.8</td>
<td>4.4 ± 3.7</td>
<td>4.5 ± 4.2</td>
<td>0.016</td>
</tr>
<tr>
<td>P-FA, μmol/L/kg fat</td>
<td>97 ± 75</td>
<td>41 ± 26</td>
<td>45 ± 45</td>
<td>0.008</td>
</tr>
<tr>
<td>SGA score, points</td>
<td>10 (1–19)</td>
<td>3 (1–13)</td>
<td>4 (1–10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tumor stage, points</td>
<td>3 (1–4)</td>
<td>4 (0–4)</td>
<td></td>
<td>0.036</td>
</tr>
<tr>
<td>Respiratory quotient</td>
<td>0.796 ± 0.033</td>
<td>0.838 ± 0.066</td>
<td>0.831 ± 0.038</td>
<td>0.031</td>
</tr>
<tr>
<td>Resting energy expenditure, kcal/d</td>
<td>1,681 ± 273</td>
<td>1,698 ± 316</td>
<td>1,584 ± 191</td>
<td>0.65</td>
</tr>
</tbody>
</table>

NOTE: Values are mean ± SD or mean (range). They were compared by ANOVA, χ² test (for gender), Kruskal-Wallis test (for SGA), or Mann-Whitney test (for tumor stage).

Abbreviations: P, fasting plasma; S, fasting serum.
was similar in the two cancer groups. The cancer cachexia group reported a marked decrease in their habitual weight. Markers of anabolism (i.e., plasma triglycerides, serum albumin, and serum transferrin values) were significantly lower and SGA scores were significantly higher among cancer cachexia patients than in the other two groups. There was no difference between the weight-stable cancer patients and control subjects with respect to these parameters. Somewhat unexpectedly, tumor stage was more advanced in the weight-stable than in the weight-losing cancer group. Lipid mobilization was analyzed by measuring either plasma glycerol or plasma FA concentrations and corrected for total body fat. It was markedly increased in the cancer cachexia group when compared with the weight-stable cancer and control groups. The latter two groups did not differ between each other in lipid mobilization or respiratory quotient (RQ). In contrast, RQ was significantly decreased in the cancer cachexia group, which is indicative of increased FA oxidation. Total resting energy expenditure was similar in all three groups.

CIDEA is highly expressed in white adipose tissue of cancer cachexia patients. The average mRNA expression of CIDEA was much higher in cancer cachexia patients than in patients from the other two groups, which, in turn, did not differ between each other (Fig. 1). Figure 1 also shows a strong negative correlation between BMI or total body fat and CIDEA mRNA levels in the entire material put together. Furthermore, CIDEA mRNA correlated strongly and positively with the reported decrease in body weight in the whole material. However, there was no correlation between lean body mass and CIDEA expression (r = 0.18; graph not shown).

CIDEA expression levels correlate negatively with RQ. The relationship between in vivo fat oxidation and CIDEA expression was examined by comparing RQ with CIDEA mRNA in all subjects. A strong inverse relationship was observed between these two parameters (Fig. 2A). Therefore, low RQ (indicative of high fat oxidation) was associated with a high gene expression of CIDEA and could explain as much as 25% of the variation seen in RQ (i.e., adjusted r²). Furthermore, reported weight loss was inversely correlated with RQ (Fig. 2B). RQ tended to correlate with BMI (P = 0.052) and body fat (P = 0.077), but not with lean body mass (P = 0.23; graphs not shown). Because CIDEA expression was markedly influenced by BMI and total body fat, a multiple regression analysis was also done. When either BMI or body fat was used together with CIDEA as an independent regressor and RQ was the dependent variable, only CIDEA was a significant regressor for RQ (partial r = 0.54, P = 0.002 together with BMI; partial r = 0.59, P = 0.002 together with log₁₀ body fat). There was no effect of age when this parameter was included in the multiple regression analysis. The reported decrease in body weight correlated strongly and negatively with RQ (Fig. 2B).

Transient CIDEA overexpression increases FA oxidation and decreases glucose oxidation but does not cause cell death in adipocytes. To investigate the role of CIDEA in regulating substrate oxidation, a human CIDEA expression vector was transfected into differentiated 3T3-L1 cells and human adipocytes. Forty-eight hours posttransfection, FA oxidation was determined using 14C palmitate. Overexpression of CIDEA enhanced palmitate oxidation two to four times in 3T3-L1 cells (Fig. 2C) and up to two times in differentiated human adipocytes (P < 0.01; graph not shown). A low RQ indicates the use of FAs as the preferred substrate for energy production. We hypothesized that in CIDEA-transfected cells the oxidation of glucose to CO₂ could be decreased. Therefore, a glucose oxidation assay was done in 3T3-L1 cells transfected with either a CIDEA expression vector or a control vector (Fig. 2D). We observed that glucose oxidation in CIDEA-overexpressing cells was 40% lower (P < 0.01) than in
CIDEA and Cachexia

CIDEA increases expression of PDK1 and PDK4 in 3T3-L1 adipocytes. The expression levels of several genes involved in β-oxidation and FA turnover were examined. These included carnitine palmitoyl-CoA transferase A (Cpt-1A), Cpt-1B, carnitine-acylcarnitine translocase (CAST), and several mitochondrial matrix enzymes [acyl-CoA long-chain synthetase 1 (ACSL1) and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (EHHADH)]. The mRNA levels of all of these genes remained unchanged in CIDEA-transfected 3T3-L1 adipocytes (values not shown). In contrast, mRNA expression levels of PDK1 and PDK4 were increased in CIDEA-transfected 3T3-L1 cells compared with control vector–transfected cells (Fig. 3). Expression levels of PDK2, PDK3, and AMP-activated kinase (AMPK) remained unchanged (values not shown). PDK1 showed the highest expression level in 3T3-L1 cells when compared with other PDKs (values not shown).

CIDEA overexpression increases the phosphorylation of PDC. To assess whether increased expression of PDKs has any functional consequences, the phosphorylation of the PDC in CIDEA-transfected 3T3-L1 cells was examined. A pull-down assay using anti-PDH antibodies coupled to Sepharose beads was done using protein lysates from CIDEA or control vector–transfected cells. Heart mitochondrial lysate was used as a positive control. Anti-phosphoserine antibodies were used for the detection of immunoprecipitated proteins. We observed a 2-fold stronger band, representing the phosphorylated PDC-E1 component in lysates from CIDEA-transfected cells when compared with control cells (Fig. 3). The mitochondrial extracts (positive control) gave the strongest band of phosphorylated PDC-E1, and this may be explained by a higher content of mitochondrial proteins in these extracts or different amounts of PDC in specific tissues. We failed to visualize CIDEA as a single protein band from extracts of human adipose tissue despite trying four different commercial antibodies (figure not shown).

CIDEA expression correlates positively with expression levels of PDK1 and PDK4 in human white adipose tissue. PDK mRNA expression was examined in human white adipose tissue. There was a slight increase in the expression of PDK1 (P = 0.03) and PDK4 (P = 0.06) in cancer cachexia when compared with weight-stable cancer (graph not shown). In the entire examined white adipose tissue material, CIDEA mRNA expression correlated strongly and positively with mRNA expression levels of PDK1 (r = 0.7, P < 0.0001) and PDK4 (r = 0.8, P < 0.0001; Fig. 4). As much as 46% and 58% of the variation seen in PDK1 and PDK4, respectively, could be explained by the variation in CIDEA (i.e., adjusted r²). These correlations are specific for CIDEA because the mRNA expression of other genes involved in FA oxidation, uncoupling protein (UCP)-1, and AMPK did not correlate with CIDEA expression (Fig. 4). One subject had a very high relative CIDEA expression (>10 log 2); however, the correlation between mRNA for CIDEA and PDK1 or PDK4 remained significant (P < 0.01, r = 0.5–0.6) even when this value was excluded from the analysis.

Discussion

Results from this study suggest that CIDEA is involved in the altered lipid metabolism observed in cancer cachexia. We also confirm that lipolysis, FA mobilization, and lipid oxidation are increased in cancer cachexia when compared with weight-stable cancer patients and noncancer patients. The cancer cachexia patients showed clear signs of malnutrition. However, they displayed no evidence of lean body mass loss. Because loss of fat is faster than loss of skeletal muscle in cancer cachexia (3), it is likely that we investigated an early phase of the condition when the patients had only lost adipose tissue. The two noncachectic groups were comparable in all investigated parameters except for body composition, with weight-stable cancer patients having less body fat than noncancer patients.

Human CIDEA is predominantly expressed in white adipocytes and there is no or very low expression of CIDEA in human skeletal muscle (26). In this study, mRNA expression of CIDEA in white adipose tissue was markedly increased in patients with cancer cachexia. This is most likely explained by their low BMI and reduced adipose mass because these two parameters correlated strongly and negatively with CIDEA expression. In contrast, lean body mass was not related to CIDEA expression. It seems that total fat mass and adipocyte CIDEA expression are tightly coregulated. This is supported by previous findings in white adipose tissue of obese healthy subjects that a decrease in BMI is followed by an increase in CIDEA mRNA expression (25–27). In the present study, reported weight loss was strongly associated with CIDEA expression in white adipose tissue. Whether this is due to the loss of body weight per se or secondary to the catabolic state cannot be elucidated because prospective investigations before the development of cancer and associated cachexia are impossible to perform. However, studies of obese subjects before and after weight loss suggest that cachexia is more important than weight loss for CIDEA up-regulation (26).

RQ was decreased in cancer cachexia, and in the whole material we also observed a strong negative relationship between RQ and...
CIDEA mRNA expression, which was independent of BMI or fat mass. Patients with a high ability to oxidize lipids, including FAs, had high CIDEA expression and vice versa. Furthermore, there was a strong negative association between weight loss and RQ. Because CIDEA is absent or expressed at very low levels in skeletal muscle (26), these data suggest that CIDEA in white adipose tissue may participate in the oxidation of FAs in weight-losing subjects. Moreover, because FA mobilization is elevated in cancer cachexia due to increased adipocyte lipolysis (6), it could be speculated that CIDEA may regulate oxidation of the excess FAs. As mentioned earlier, FA oxidation in white adipose tissue can be increased by changes in the regulation of oxidative pathways. For example, overexpression of the peroxisome proliferator-activated receptor-γ coactivator 1α markedly increased the capacity of human white fat cells to oxidize FAs (34). We provide evidence that CIDEA can up-regulate FA oxidation in white fat cells via an alternative mechanism (i.e., inhibition of the PDH complex). Our findings in 3T3-L1 fat cells show that CIDEA does not regulate genes of the β-oxidation cascade, but rather attenuates the activity of the PDH complex. CIDEA overexpression up-regulates mRNA levels of PDKs, which results in increased phosphorylation of PDH-E1. These data are supported by clinical findings showing a strong, positive, and selective correlation between CIDEA and PDK1 and PDK4 mRNAs in human adipocytes. Taken together, our data suggest that CIDEA may function as a regulatory protein, which facilitates the preferential oxidation of FAs instead of glucose in white adipocytes. Under conditions of high FA mobilization such as cancer cachexia, CIDEA stimulates oxidation of excess FAs so that glucose recycling could be optimized in these cells. It is well known that the activity of PDC is inhibited in muscle tissue during starvation and other catabolic states such as diabetes (36); thus, if glucose supply is scarce or FA supply and oxidation is sufficient to meet cellular energy requirements, mammalian PDC activity is suppressed. In cancer cachexia, large quantities of adipose tissue are lost. This could indicate insufficient energy supply and result in protective mechanisms being switched on. Further proof about the involvement of CIDEA in the regulation of alternative substrate oxidation in human white adipose tissue would require direct measurements in vivo, which, unfortunately, are not possible to perform due to methodologic reasons.

UCP-1 is essential for FA oxidation in brown fat cells and can be activated in human white fat cells (34). As opposed to CIDEA, we found no correlation between UCP-1 and cachexia or fat oxidation. In addition, mRNA for CIDEA and UCP-1 did not correlate, which is in contrast to earlier findings in healthy subjects (26). A possible explanation for these divergent findings is that we have compared a cohort of patients with and a cohort without cancer cachexia.

Activation of AMPK in adipocytes up-regulates FA oxidation and inhibits catecholamine-induced lipolysis (37). A recent study showed CIDEA-mediated degradation of AMPK in murine brown adipocytes (38). High AMPK expression in brown adipocytes of CIDEA-null mice results in increased FA oxidation. It was proposed that high activity of AMPK is a mechanism causing resistance to diet-induced obesity in CIDEA−/− mice. In contrast, we did not observe altered levels of AMPK mRNA following CIDEA overexpression in white adipocytes (Fig. 4D). Cancer cachexia patients display increased catecholamine-induced lipolysis (6–10), suggesting that elevated activity of AMPK in these patients is unlikely. Although we cannot exclude AMPK involvement in CIDEA-mediated FA oxidation at the posttranscriptional level, our present and previous data do not support this mechanism in human adipocytes.

The findings on CIDEA and FA oxidation in white adipose tissue are different from those observed in brown adipose tissue of mice, where CIDEA inhibits FA oxidation (23). It was previously reported
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CIDEA is a multifunctional protein, although the exact intracellular mechanism of its function in human white adipocytes remains to be defined.

Because CIDEA inhibits (i.e., nonhormonal) lipolysis (25, 39), we made a retrospective analysis of basal adipocyte lipolysis (i.e., glycerol release) from our previous study (6) comparing cancer cachexia patients with control patients. The values were 3 ± 2 and 6 ± 4 μmol of glycerol/2 h/10^5 fat cells, respectively (P = 0.039, Mann-Whitney test). Thus, in addition to its effect on FA oxidation, the elevated CIDEA expression in cancer cachexia may also inhibit basal FA release in fat cells due to the antilipolytic effect. However, under hormone-stimulated conditions (natriuretic peptides, catecholamines), the effect of CIDEA seems to be masked by the activity of hormone-sensitive lipase.

Finally, the tumor stage score was higher in weight-stable than in weight-losing cancer patients. This excludes the possibility that increased FA oxidation in cancer cachexia is secondary to a more severe tumor stage. It should be pointed out that we have only investigated gastrointestinal cancer. Therefore, we do not know if FA oxidation is altered in other forms of tumor-induced cachexia.

On the basis of previous (6) and current findings, we propose the following model of disturbed white adipose tissue lipid mobilization in cancer cachexia, which could, at least in part, explain the loss of fat mass in this condition (Fig. 5). The expression of the rate-limiting enzyme hormone-sensitive lipase is elevated in fat cells, which enhances the lipolytic effect of stimulatory hormones and therefore increases the availability of FAs. Perhaps as a protective mechanism, the PDC of white fat cells is inactivated by CIDEA, favoring FA oxidation and decreasing the oxidation of glucose to acetyl-CoA.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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4. Kalra PR, Tigan S. Regulation of lipolysis: natriuretic peptides and the nucleus.6 Localization to the surface of lipid droplets does not explain how CIDEA regulates FA oxidation. We believe that CIDEA is localized to the mitochondria of brown fat cells (23). Recent findings have identified CIDEA as a lipid droplet–coating protein and mitochondrial localization was not confirmed (39). Instead, some cytosolic and nuclear localization was observed. In our laboratory, we have also detected CIDEA in the cytoplasm and the nucleus.6 Localization to the surface of lipid droplets does not explain how CIDEA regulates FA oxidation. We believe that CIDEA is localized to the mitochondria of brown fat cells (23). Recent findings have identified CIDEA as a lipid droplet–coating protein and mitochondrial localization was not confirmed (39). Instead, some cytosolic and nuclear localization was observed. In our laboratory, we have also detected CIDEA in the cytoplasm and the nucleus.6 Localization to the surface of lipid droplets does not explain how CIDEA regulates FA oxidation. We believe that CIDEA is localized to the mitochondria of brown fat cells (23). Recent findings have identified CIDEA as a lipid droplet–coating protein and mitochondrial localization was not confirmed (39). Instead, some cytosolic and nuclear localization was observed. In our laboratory, we have also detected CIDEA in the cytoplasm and the nucleus.6 Localization to the surface of lipid droplets does not explain how CIDEA regulates FA oxidation. We believe that CIDEA is localized to the mitochondria of brown fat cells (23). Recent findings have identified CIDEA as a lipid droplet–coating protein and mitochondrial localization was not confirmed (39). Instead, some cytosolic and nuclear localization was observed. In our laboratory, we have also detected CIDEA in the cytoplasm and the nucleus.6 Localization to the surface of lipid droplets does not explain how CIDEA regulates FA oxidation. We believe that

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