Elevated circulating fatty acid concentration is a hallmark of insulin resistance and is at least in part attributed to the action of adipose tissue–derived tumor necrosis factor-α (TNF-α) on lipolysis. Cell death–inducing DFFA (DNA fragmentation factor-α)-like effector A (CIDEA) belongs to a family of proapoptotic proteins that has five known members in humans and mice. The action of CIDEA is unknown, but CIDEA-null mice are resistant to obesity and diabetes. We investigated CIDEA in adipose tissue of obese and lean humans and mice. The mRNA was expressed in white human fat cells and in brown mouse adipocytes. The adipose mRNA expression of CIDEA in mice was not influenced by obesity. However, CIDEA expression was decreased twofold in obese humans and normalized after weight reduction. Low adipose CIDEA expression was associated with several features of the metabolic syndrome. Human adipocyte depletion of CIDEA by RNA interference stimulated lipolysis and increased TNF-α secretion by a posttranscriptional effect. Conversely, TNF-α treatment decreased adipocyte CIDEA expression via the mitogen-activated protein kinase c-Jun NH2-terminal kinase. We propose an important and human-specific role for CIDEA in lipolysis regulation and metabolic complications of obesity, which is at least in part mediated by cross-talk between CIDEA and TNF-α.

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Obesity is an excess of body fat that develops from a combination of genetic and environmental factors, leading to a disturbed balance between energy intake and energy expenditure (1). The prevalence is increasing in both developed and developing countries and has reached epidemic proportions (2,3). Obesity, in particular abdominal obesity, is closely associated with insulin resistance, non–insulin-dependent diabetes (type 2 diabetes), dyslipidemia, and cardiovascular disease (4).

Elevated circulating fatty acid levels in obesity resulting from increased basal adipocyte lipolysis could cause perturbations such as insulin resistance and type 2 diabetes (5). The exact molecular mechanisms causing activation of basal lipolysis are so far unknown (6), although the cytokine tumor necrosis factor-α (TNF-α) is a potential candidate. The production of adipose tissue–derived TNF-α is increased in human obesity (7), and TNF-α stimulates adipocyte lipolysis in rodent (8,9) and human fat cells (10,11).

The mammalian gene cell death–inducing DFFA (DNA fragmentation factor-α)-like effector A (CIDEA) was recently identified and characterized (12). CIDEA belongs to a family of proapoptotic proteins (12) that has five known members in the transcriptomes of humans and mice (13). CIDEA is highly homologous to FSP27 (12), an adipose-specific gene whose expression is associated with terminal differentiation of fat cells (14). The expression of FSP27 (also called clone 47) is regulated by the tumor necrosis pathway (15).

Although the murine expression pattern of CIDEA has been studied and CIDEA-null mice are resistant to diet-induced obesity and diabetes (16), little else is known regarding the function of the CIDEA gene. CIDEA transcripts of various lengths are present in different human tissues (e.g., heart, skeletal muscle, brain, placenta, and kidney) (12), but there are no reports about CIDEA in human adipose tissue. Preliminary results from microarray studies in our laboratory demonstrated that CIDEA is significantly expressed in human white adipose tissue (WAT). This is in contrast to mice, where CIDEA is expressed in brown adipose tissue (BAT) but not detectable in WAT (16). We investigated the role of this gene in human WAT and obesity and show a specific role for CIDEA in regulating lipolysis in white human fat cells. We found interactions between CIDEA and TNF-α that may be linked to the insulin-resistant phenotype of many obese subjects.

RESEARCH DESIGN AND METHODS
One cohort consisted of 40 men and 146 women, all healthy and free of medication but with a large interindividual range in BMI (18–50 kg/m2). The subjects were divided into an obese (n = 143, BMI >30 kg/m2) and a nonobese
n = 43) subgroup. Twelve of the obese healthy subjects (BMI 35–50 kg/m²) underwent gastric banding to treat their obesity. Fat tissue samples were obtained before and 2–4 years after surgery in a weight-stable condition. These subjects were part of a larger cohort described previously (17). All subjects were examined at 8 a.m. after an overnight fast. Their height, weight, and waist circumference were measured. Venous plasma samples were drawn for the purpose of insulin and glucose determination. Total RNA was extracted from subcutaneous adipose tissue (obese humans and mice) and mRNA levels of perilipin (a marker of lipid storage) were determined by real-time PCR using TaqMan probes. For mice, the cheaper SYBR Green–based technology for mRNA quantitation was used. The kits were used according to instructions from the manufacturers. MCP-1 and TNF-α measurements in smaller experiments. β2-MG was used as a reference gene for the TNF-α–treated samples, where β2-MG in our hands appears to be regulated by TNF-α. Instead, GAPDH was used for RNA from adipose tissue (obese humans and mice) in the current study. β2-MG mRNA levels were 50% lower in the obese compared with the nonobese subjects (25). The data are the means ± SE. Statistical analysis was performed using the Mann-Whitney U test, Wilcoxon’s signed-rank test, Kruskal-Wallis test, and Spearman’s correlation test. P < 0.05 was considered significant.

RESULTS

CIDEA is expressed in human WAT. We measured CIDEA mRNA in different WAT preparations from nine obese healthy women (aged 26–57 years, BMI 31–46 kg/m²) and eight nonobese healthy subjects (five female, three male, aged 24–42 years, BMI <30 kg/m²). The mRNA levels in adipose tissue correlated strongly with those in isolated adipocytes from the same subjects (P = 0.0008) (Fig. 1A). The ratio of CIDEA to β2-MG mRNA was ~6 times higher in cells than in tissue (P = 0.0003), mainly because of higher β2-MG levels in tissue (1.2 ± 0.9 and 5.4 ± 0.4 in cells and tissue, respectively). When we compared the CIDEA values without correcting for β2-microglobulin, the correlation was even stronger (P = 0.0006, values not shown).

CIDEA mRNA expression correlates with features of the metabolic syndrome. CIDEA mRNA was determined in subcutaneous WAT from 143 obese (BMI 38 ± 0.4 kg/m²) and 43 nonobese (BMI 24 ± 0.5 kg/m²) healthy subjects (Fig. 1B). The obese subjects were insulin resistant compared with the nonobese subjects (P < 0.0001, data not shown), as evidenced by increased values for the insulin sensitivity index HOMA (18). Furthermore, their basal lipolytic activity in subcutaneous WAT was increased twofold (P < 0.001, data not shown). CIDEA mRNA levels were 50% lower in the obese compared with the nonobese subjects (P = 0.0004), with an opposite change of GAPDH (P < 0.0001). We assessed the effect of weight reduction on CIDEA mRNA in 12 obese subjects (9 women, 3 men) undergoing bariatric surgery. At the follow-up 2–4 years after surgery, they were weight stable, and their BMI had decreased by an average of 15 kg/m².
At the same time, insulin sensitivity (HOMA) was improved ($P < 0.01$, data not shown), and the rate of basal adipocyte lipolysis in subcutaneous WAT was reduced by $40\%$ ($P < 0.05$, values not shown). After weight reduction the CIDEA mRNA levels were almost doubled ($P < 0.02$).

The relationship between clinical phenotype and CIDEA expression was investigated by dividing the 186 subjects described above into tertiles ($n = 62$) based on high ($1.20 \pm 0.15$), intermediate ($0.46 \pm 0.01$), or low ($0.23 \pm 0.01$) ratios of CIDEA to β2-MG mRNA (Fig. 2A–D). Waist circumference, fat cell volume, insulin resistance, and basal lipolytic activity in adipose tissue were highest in the lowest CIDEA tertile and lowest in the upper tertile. In general, intermediate values were recorded in the middle tertile ($P < 0.005$).

CIDEA is downregulated by TNF-α via JNK. TNF-α is associated with the metabolic syndrome because it stimulates lipolysis, is overexpressed in obesity, and involved in obesity-induced insulin resistance (24). We investigated whether TNF-α stimulation affects CIDEA expression in fat cells. CIDEA mRNA levels in differentiated preadipocytes were markedly downregulated by a 48-h incubation in 100 ng/ml TNF-α ($P = 0.012$, $n = 8$). This decrease was paralleled by a two- to threefold increase in basal lipolysis in the same cell preparations (11).

The MAPKs p38, p44/42, and JNK are activated by TNF-α in human fat cells (11,24,28). To further investigate the signaling pathway(s) between CIDEA and TNF-α, we therefore incubated differentiated preadipocytes with TNF-α in the absence or presence of specific MAPK inhibitors. The inhibitors of p44/42 (PD98059) and p38 (SB203580) did not affect the TNF-α–induced downregulation of CIDEA mRNA (data not shown), but the JNK inhibitor (SP600125) completely counteracted the downregulation of CIDEA in a concentration-dependent fashion (Fig. 3B). A maximum effect occurred at $6.7 \mu$mol/l.

Effect of CIDEA depletion on basal lipolysis, TNF-α secretion, and other TNF-α targets. Because TNF-α downregulates CIDEA expression and at the same time stimulates basal lipolysis, we investigated the putative effects of CIDEA by gene silencing. The quantitative relationship between lipolysis and CIDEA expression was determined in vitro by treating preadipocytes with siRNA duplexes directed against human CIDEA and measuring basal lipolysis as glycerol release into the medium. Using preadipocytes from nine healthy women (aged 47.0 ± 4.6 years, range 30–70; BMI 26.5 ± 0.8 kg/m², range 22.9–30.2), we performed 24-h incubations with different amounts of siRNA, which resulted in a 20–95% decrease in CIDEA expression. No effect on CIDEA mRNA levels was observed when cells were treated with siRNA without
known similarities to human sequences or when cells were treated with transfection reagent only. Basal lipolysis was measured in six of these experiments. The changes in CIDEA mRNA and glycerol release showed a strong inverse correlation (Fig. 3C) \((P = 0.013)\), indicating that CIDEA downregulation stimulates lipolysis.

To assess whether CIDEA depletion influenced TNF-α action, the levels of secreted TNF-α protein in cell media from eight of the RNA interference experiments were measured. There was a strong inverse correlation between the levels of secreted TNF-α protein and the ratio of CIDEA to GAPDH mRNA (Fig. 3D) \((P = 0.0051)\). The secreted levels of TNF-α also correlated with the glycerol release from the same cells \((P = 0.03)\) (graph not shown).

In the samples where CIDEA mRNA was inhibited to \(10%\) of control \((n = 2)\), TNF-α secretion was increased as much as fourfold.

To determine whether the effect of CIDEA silencing on TNF-α secretion was transcriptional or posttranscriptional, we measured the TNF-α mRNA levels in the siRNA-treated cells \((n = 8)\). There was no correlation between the ratio of TNF-α to GAPDH mRNA and the ratio of CIDEA to GAPDH mRNA (Fig. 4A) \((P = 0.29)\). To assess whether CIDEA downregulation resulted in a selective effect on lipolysis by TNF-α, additional measurements were performed in the siRNA-treated cells and incubation media. Adipocytes secrete a number of proteins in addition to TNF-α (rev. in 29), among them adiponectin, which is inhibited by TNF-α (30). We determined the amount of adiponectin secreted into the media of the cells in the same experiments as in Fig. 3D. The CIDEA mRNA levels in these experiments did not correlate with the amount of adiponectin \((P = 0.2)\), graph not shown.

Because the lipid droplet–associated phosphoprotein perilipin plays an important role in basal lipolysis (31,32), PLIN mRNA levels were assessed in the siRNA-treated cells with the most marked CIDEA downregulation (by 75–95\%, \(n = 4)\). In these samples PLIN mRNA levels were 50% lower than in control cells (Fig. 4B) \((P = 0.03)\). The perilipin protein levels were determined by Western blot in four RNA interference experiments. In contrast to mRNA data, there was no significant downregulation of the protein (values not shown).

MCP-1 is upregulated by TNF-α in human preadipocytes (33) and 3T3-L1 cells (34). We therefore measured the MCP-1 mRNA levels and secretion of MCP-1 protein in the same RNA interference experiments as in Fig. 3C. The ratio of MCP-1 to GAPDH mRNA did not correlate with the ratio of CIDEA to GAPDH mRNA (Fig. 4C) \((P = 0.53)\). Likewise, the amount of MCP-1 protein secreted into the cell media did not correlate with the ratio of CIDEA to GAPDH mRNA levels (Fig. 4D) \((P = 0.38)\).

CIDEA expression in mice differs qualitatively from that in humans. To study the species differences in

FIG. 2. Human adipose CIDEA mRNA levels in relation to features of the metabolic syndrome. A total of 186 obese and nonobese men and women were divided into low (■), intermediate (□), and high (●) tertiles based on ratios of CIDEA to β-2 microglobulin (β2-MG) mRNA expression in adipose tissue. Waist circumference (A), fat cell volume (B), insulin resistance (C) (HOMA index), and adipose tissue glycerol release (D) were compared between the tertiles. \(P < 0.005\) for all four parameters, Kruskal-Wallis.
CIDEA expression, we measured the mRNA levels of CIDEA in WAT and BAT from 18 mice of three different strains (C57BL/6J, 129Sv/Pas, and NMRI) that were divided into two groups and fed either normal or cafeteria diet. Data from the C57BL/6J mice on normal diet are shown in Fig. 5A–B and are representative of all three strains. CIDEA mRNA in WAT was not detectable with SYBR Green–based quantitative real-time PCR but highly so in BAT, confirming earlier studies (16) (Fig. 5A). The mRNA expression of β2-MG in the same samples showed the opposite pattern, with much higher levels in WAT than in BAT (Fig. 5B). Using the more sensitive TaqMan probes, we found ~400 times less CIDEA mRNA in WAT as compared with BAT (data not shown). This low level of expression could be nonspecific for fat cells and was not further analyzed. There was a significant diet effect on body weight increase in C57BL/6J mice (P < 0.05) and a borderline significant increase in NMRI mice (Fig. 5C) (P = 0.06). Surprisingly, the body weight increase in 129Sv/Pas mice on normal diet was marginally larger than for the mice on cafeteria diet (Fig. 5C), even though food consumption was equally increased in all three strains (values not shown). There was no effect of the diet or body weight increase in any strain regarding CIDEA mRNA (Fig. 5D).

DISCUSSION
The current study demonstrates that CIDEA is expressed in human white fat cells and that the adipose mRNA levels of CIDEA are markedly influenced by body fat status. This is in clear contrast to mice, where the gene is expressed in brown fat cells only and not influenced by obesity. In human adipose tissue, the expression of CIDEA is downregulated by obesity and normalized after weight reduction. The mRNA levels of CIDEA are inversely correlated to perturbations that are characteristic for the metabolic syndrome: abdominal obesity, enlarged fat cells, insulin resistance (high HOMA index), and increased basal lipolysis (glycerol release). The metabolic syndrome also has other clinical features not measured here, but it is nevertheless obvious that CIDEA mRNA in human WAT is strongly associated with the metabolic syndrome.

Although the RNA interference technique (rev. in 35) has been successfully used in 3T3-L1 preadipocytes (36, 37), its usefulness in human preadipocytes has not been
reported before. In our experiments, CIDEA mRNA knockdown varied between 20 and 95%, probably because of the interindividual differences of the primary cells used. The effect of CIDEA RNA interference on glycerol release from the preadipocytes correlated with the degree of mRNA downregulation. Our experiments suggest that basal lipolysis in human preadipocytes is under tonic inhibition by CIDEA and that relief of inhibition by mRNA depletion augments lipolysis. This effect most probably involves posttranscriptional modification of TNF-α because the release of this prolipolytic cytokine is stimulated by CIDEA downregulation, but there is no corresponding change in the TNF-α mRNA levels.

CIDEA–TNF-α interactions appear to be specific for lipolysis because the mRNA expression and protein secretion of a known target for TNF-α, MCP-1, were not influenced by RNA interference. Also, the secretion of the adipose-specific protein adiponectin, whose expression has been shown to be regulated by or associated with TNF-α levels, was not affected. At present we do not know why only glycerol release and not secretion of adiponectin or MCP-1 was influenced by increased TNF-α levels after CIDEA depletion. However, it is possible that glycerol stimulation is an early event, and effects of increased TNF-α levels on adiponectin and MCP-1 secretion occur at time points later than 24 h.

The signaling pathways of TNF-α to lipolysis are very complex and involve signaling through MAPKs, resulting in downregulation of G
\textsubscript{i0} (inhibitory G-protein α-subunit) as well as changes in perilipin phosphorylation and expression (11,24,28,41). In the current study, we have shown that TNF-α inhibits CIDEA expression and that this downregulation of CIDEA mRNA is mediated by JNK, one of the MAPKs that is involved in TNF-α-induced stimulation of lipolysis in human fat cells (24). It is noteworthy that the concentration-dependent relationship for the effect of the
JNK inhibitor SP600125 on CIDEA was almost identical to that previously shown on lipolysis using an experimental setting similar to the current study (11). The other two MAPKs that are also active in human fat cells, p44/42 and p38 (11,24,28), do not seem to be involved in regulating CIDEA expression. Our data suggest a reciprocal inhibition of CIDEA and TNF-α because CIDEA depletion by siRNA causes an increased secretion of TNF-α protein. It is tempting to speculate that the ability of TNF-α to inhibit CIDEA is an indirect way for this cytokine to further stimulate lipolysis by counteracting the inhibitory effects of CIDEA on TNF-α release. Conversely, the direct way would be through signaling to perilipin and/or other lipolytic regulators.

In the current study, we have only investigated effects of CIDEA depletion. Studies of stable overexpression would require human fat cell lines that are not available. A human fat cell line derived from BAT (42) differs in many qualitative parameters from white adipocytes and can therefore not be used. Transient overexpression is not useful because such ectopic CIDEA expression causes excessive apoptosis in other cell systems (12).

In accordance with previous studies (16), we found that CIDEA is virtually not expressed in WAT in mice. Therefore, CIDEA expression is completely different in mice compared with humans. Humans have easily detectable levels in both WAT and isolated adipocytes. An interesting observation is that the mouse strains displaying low CIDEA levels (C57BL/6J and NMRI) were those that developed diet-induced obesity. It has been suggested that CIDEA may induce obesity through processes in BAT of mice (43), but our results would rather indicate a reverse correlation.

Based on the current results, we propose the following mode of action of CIDEA in human WAT (Fig. 6): CIDEA cross-talks with TNF-α, and this has consequences for lipolysis. CIDEA decreases the availability of TNF-α by inhibiting cytokine secretion predominantly through post-transcriptional mechanisms, which in turn counteracts the ability of TNF-α to stimulate lipolysis. TNF-α downregulates the expression of CIDEA through signaling via JNK, which in turn increases the availability of TNF-α and thereby lipolytic stimulation. This cross-talk seems to be specific for humans. In obesity CIDEA expression is downregulated probably because of increased TNF-α action. This elevates the basal lipolytic activity and could be an important contributing factor to elevated circulating fatty acid levels in obesity. Other adipocyte factors responsible for increased basal lipolysis could be additional cytokines and insulin resistance in the fat cells. Because CIDEA is expressed in adipocytes of WAT, it is possible that cell-specific pharmacological agents that either up-
regulate CIDEA expression or alter the interactions between TNF-α and CIDEA (such as JNK inhibitors) could be useful therapeutic agents to decrease fatty acid levels in obese individuals and thereby ameliorate the disadvantageous effects of these lipids.

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