A Human-Specific Role of Cell Death–Inducing DFFA (DNA Fragmentation Factor-α)-Like Effector A (CIDEA) in Adipocyte Lipolysis and Obesity

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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 NH_2-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. *Diabetes* 54:1726–1734, 2005

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/m^2). The subjects were divided into an obese (n = 143, BMI >30 kg/m^2) and a nonobese

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BAT, brown adipose tissue; CIDEA, cell death–inducing DFFA (DNA fragmentation factor-α)-like effector A; HOMA, homeostasis model assessment; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; siRNA, short interfering RNA; TNF-α, tumor necrosis factor-α; WAT, white adipose tissue.

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($n = 43$) subgroup. Twelve of the obese healthy subjects (BMI 35–50 kg/m^2) 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 analysis of insulin and glucose and calculation of the homeostasis model assessment (HOMA) index ([plasma glucose (mmol/l) × plasma insulin (mU/l)]/22.5) (18), an indirect measure of in vivo insulin sensitivity. Thereafter, needle biopsies of subcutaneous adipose tissue (1–2 g) were obtained under local anesthesia. For experimental studies, subcutaneous adipose tissue was obtained from healthy subjects after elective cosmetic liposuction. The studies were approved by the ethical committee at Karolinska Institutet and explained in detail to each of the subjects, from whom informed consent was obtained.

Mice. A total of 18 male 7-week-old mice of three different strains (NMRI, C57BL/6J, and 129Sv/Pas, $n = 6$ from each strain; Scanbur B&K Universal, Sollentuna, Sweden) were fed normal pelleted chow (R70 Standard Diet; Lactamin, Vadstena, Sweden) with or without the addition of cafeteria diet ad libitum for 15 weeks. The mice had free access to water and were kept on a 12/12 h light/dark cycle. They were then killed by carbon dioxide anesthesia followed by cervical dislocation. The epididymal fat pads and interscapular BAT were dissected and snap frozen in liquid nitrogen for subsequent RNA isolation. All animal experimentation was performed in accordance with institutional guidelines, and ethical permission was obtained from the Northern Stockholm animal ethics committee.

Human adipose tissue and preadipocyte culture. Adipose tissue (~1 g) was immediately frozen in liquid nitrogen for subsequent RNA analysis. Fresh adipose tissue pieces were incubated as described for 2 h (19). Glycerol release to the medium was determined and related to the number of incubated fat cells, as previously described (20). Glycerol release is linear with incubation time for at least 4 h (21). Tissue was collagenase treated and mean fat cell size and mean fat cell weight determined as previously described (22). Preadipocytes were isolated from the stromal fraction of adipose tissue and differentiated into adipocytes as previously described (23), reaching full differentiation after 12–14 days. The cells were seeded out in 12-well plates at a density of ~30,000 cells/cm^2. For studies of TNF-α–induced *CIDEA* downregulation, preadipocytes were incubated in the presence of 100 ng/ml TNF-α in combination with different concentrations of the specific mitogen-activated protein kinase (MAPK) inhibitors SP600125 (for c-Jun NH_2-terminal kinase [JNK]), PD98059 (for p44/42, also termed extracellular signal–regulated kinase 1/2), and SB203580 (for stress/cytokine-activated kinase, or p38) (all from Sigma, St Louis, MO) as described before (11,24). The MAPK cascades activate different transcription factors that mediate TNF-α–regulated gene expression (24). TNF-α was added on day 10 (1 h after addition of the inhibitors) or on day 12 (alone), and the cells were incubated for 48 h before RNA isolation. Control cells were incubated in medium alone. Lipolysis in preadipocytes was assessed as glycerol release into the medium, as previously described (20).

RNA interference and protein analysis. Optimal transfection conditions of human preadipocytes were initially determined by titrating different amounts of fluorescent short interfering RNA (siRNA) duplexes and the transfection reagent RNAiFect (both from Qiagen, Hilden, Germany). Analysis by fluorescence microscopy 4–12 h later showed a transfection efficiency ranging from 5 to 70%, depending on the different conditions. These experiments served as a basis for optimizing *CIDEA* gene silencing. Cells at day 8 of differentiation (when the cells have developed lipid droplets but are not fully differentiated) were transfected with or without 1 or 2 μg of *CIDEA* siRNA (Qiagen, Hilden, Germany) and incubated for 24 h, a time point where a significant gene silencing effect was observed. Cells incubated without siRNA served as controls. To control for nonspecific gene silencing effects, parallel cells were transfected with siRNA without known similarities to human sequences or with *CIDEA* siRNA without RNAiFect. Conditioned cell media aliquots were then analyzed for glycerol content while cells were lysed for RNA or protein isolation. Perilipin protein levels were detected by Western blot and related to β-actin protein levels, as previously described (11). Protein release into the cell media was determined by a radioimmunoassay for adiponectin (Linco, St Charles, MO) and with Quantikine human immunoassays (R&D Systems, Abingdon, U.K.) for TNF-α and monocyte chemoattractant protein-1 (MCP-1). The kits were used according to instructions from the manufacturers.

mRNA quantitation. Total RNA was extracted from human or mouse adipose tissue, differentiated preadipocytes, or isolated adipocytes and reverse-transcribed as previously described (25). Quantitative real-time PCR was performed in an iCycler IQ (Bio-Rad Laboratories, Hercules, CA), using the less expensive SYBR Green–based technology for *CIDEA* quantitation in large cohorts (human and mouse) and mRNA levels of perilipin (*PLIN*), *MCP-1*, and the reference genes β-2 microglobulin (*β2-MG*; human and mouse) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). TaqMan probes (Applied Biosystems, Foster City, CA) were used for *CIDEA* and *TNF-α* measurements in smaller experiments. *β2-MG* was used as a reference gene except 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 interference and TNF-α experiments. *β2-MG* has been shown not to be affected by insulin and lipodystrophy (26,27) and was not affected by the other conditions used in the current study. The mRNA levels of the different genes were determined as previously described (25) and expressed as corrected threshold cycle (C_t) values or as ratios to *β2-MG* or *GAPDH*, as indicated. To clarify the data not expressed as percent of control, the ratio of *CIDEA* to *β2-MG* in obese humans (before weight reduction) and the mRNA levels in BAT from C57BL/6J mice on normal diet were set to 1, and the other values were expressed relative to these ratios or levels. The primer pairs for SYBR Green–based quantitative real-time PCR (Invitrogen, Tåstrup, Denmark) were designed to span exon-intron boundaries and generate a single amplicon. Dissociation curves and agarose gel electrophoresis were used to check for a single product, and BLAST (basic local alignment search tool) searches were performed to ensure that the primer pairs were specific for the different genes. Thus, the *CIDEA* primers did not display any homology with other genes in the *CIDE* family, such as *FSP27/CIDE3*. The forward and reverse primers were for human *CIDEA* 5′-CATGTATGAGATGTACTCCGTGTC-3′ and 5′-GAGTAG GACAGGAACCGCAG-3′, for human *MCP-1* 5′-GTGTCCCAAAGAAGCT-GTGA-3′ and 5′-GTTTGCTTGTCCAGGTGGT-3′, for human *β2-MG* 5′-TGCTG TCTCCATGTTTGATGTATCT-3′ and 5′-TCTCTGCTCCCCACCTCTAAGT-3′, for mouse *CIDEA* 5′-AAAGGGACAGAAATGGACAC-3′ and 5′-TTGAGACAGC CGAGGAAG-3′, and for mouse *β2-MG* 5′-CATGGCTCGCTCGGTGAC-3′ and 5′-CAGTTCAGTATGTTCGGCTTCC-3′. The primer sequences for human *GAPDH* and human *PLIN* have been described (25). All samples were run in duplicate or triplicate, and standard curves created by repeated dilutions of cDNA were used to check the PCR efficiency and reproducibility.

Statistical analysis. Data are the means ± SE. Statistical significance was determined by nonparametric methods because we could not ensure normal distribution of the parameters investigated. The Mann-Whitney *U* test, Wilcoxon's signed-rank test, Kruskal-Wallis test, and Spearman's correlation test were used. $P < 0.05$ 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/m^2) and eight nonobese healthy subjects (five female, three male, aged 24–42 years, BMI <30 kg/m^2). The mRNA levels in adipose tissue correlated strongly with those in isolated adipocytes from the same subjects ($P = 0.0008$) (Fig. 1*A*). 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 ± 0.09 and 5.4 ± 0.4 in cells and tissue, respectively). When we compared the CIDEA values without correcting for β-2 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/m^2) and 43 nonobese (BMI 24 ± 0.5 kg/m^2) healthy subjects (Fig. 1*B*). 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/m^2

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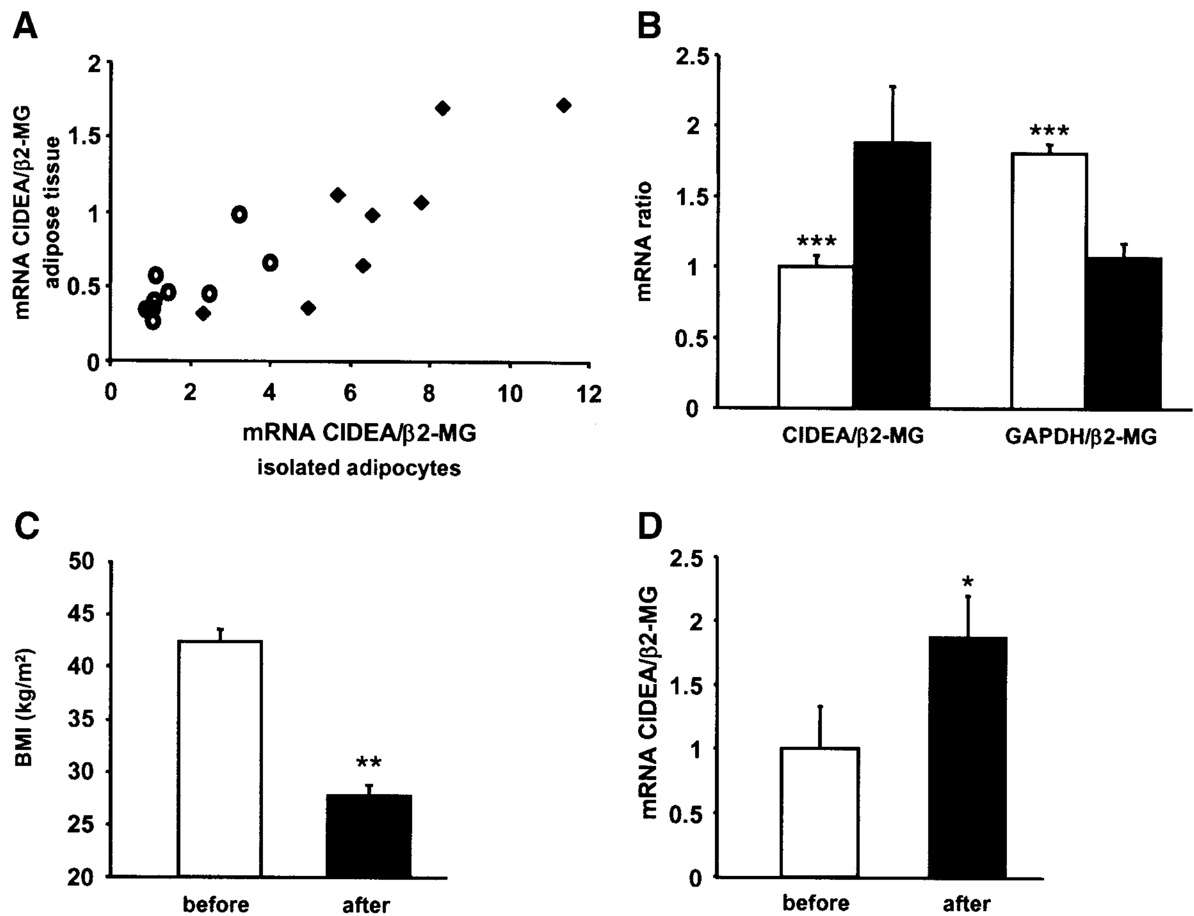

FIG. 1. ***CIDEA*** **in human WAT.** ***A*****: Ratios of** ***CIDEA*** **to β-2 microglobulin (*β2-MG*) mRNA levels in WAT and isolated adipocytes from nonobese (*n* = 8) (◆) and obese (*n* = 9) (○) men and women. *P* < 0.001, Spearman's correlation.** ***B*****: Ratios of** ***CIDEA*** **and** ***GAPDH*** **to *β2-MG* mRNA levels in WAT from obese (*n* = 143) (□) and nonobese (*n* = 43) (■) subjects.** ***C*** **and** ***D*****: BMI (*C*) and ratios of *CIDEA* to *β2-MG* adipose mRNA levels (*D*) in 12 obese subjects before (□) and after (■) weight reduction. *$P < 0.05$ and **$P < 0.01$, Wilcoxon's signed-rank test; ***$P < 0.001$, Mann-Whitney *U* test.**

(Fig. 1*C*) ($P = 0.002$). 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 (Fig. 1*D*) ($P = 0.02$).

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 ± 0.15), intermediate (0.46 ± 0.01), or low (0.23 ± 0.01) ratios of *CIDEA* to *β2-MG* mRNA (Fig. 2*A–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 ($P \leq 0.005$).

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 (Fig. 3*A*) ($P = 0.012$, $n = 8$). 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. 3*B*). A maximum effect occurred at 6.7 μmol/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/m^2, 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

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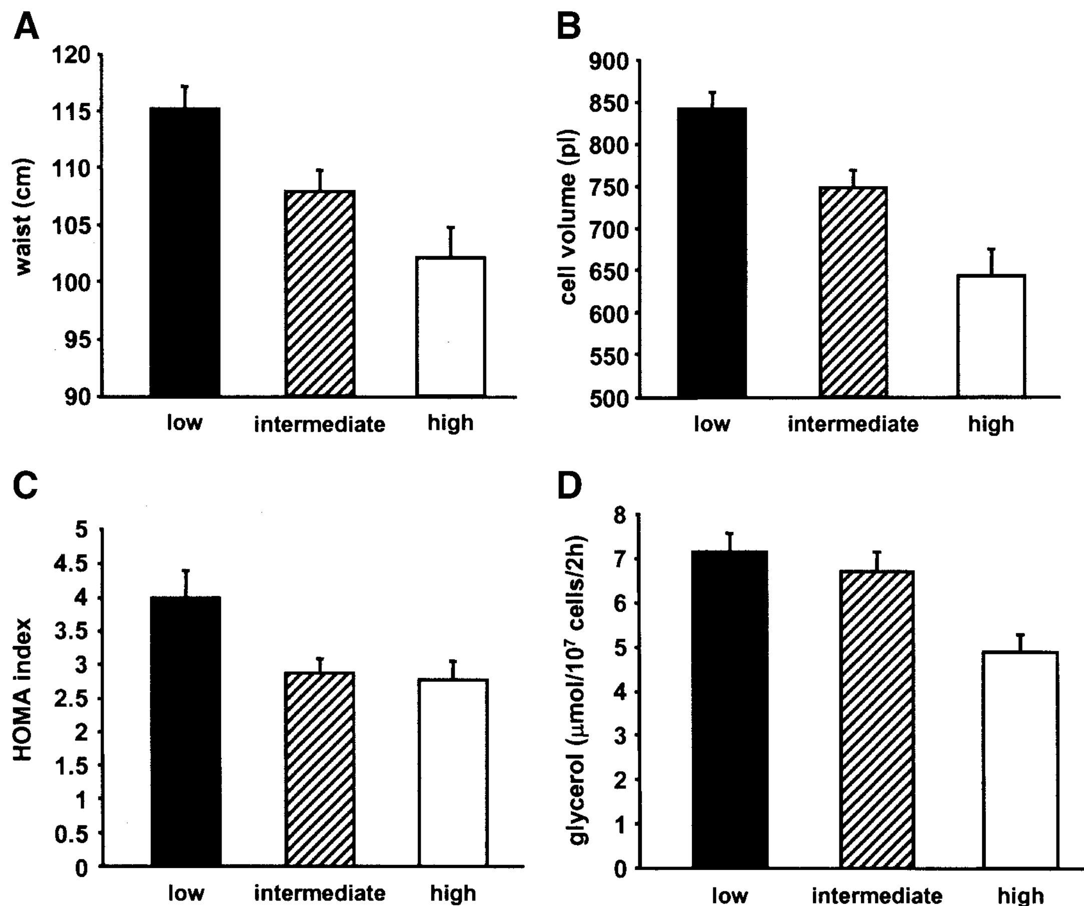

FIG. 2. Human adipose *CIDEA* mRNA levels in relation to features of the metabolic syndrome. A total of 186 obese and nonobese men and women were divided into low (■), intermediate (▨), and high (□) tertiles based on ratios of *CIDEA* to β-2 microglobulin (β*2-MG*) mRNA expression in adipose tissue. Waist circumference (*A*), fat cell volume (*B*), insulin resistance (*C*) (HOMA index), and adipose tissue glycerol release (*D*) were compared between the tertiles. $P \leq 0.005$ for all four parameters, Kruskal-Wallis.

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. 3*C*) ($P = 0.013$), 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. 3*D*) ($P = 0.0051$). 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. 4*A*) ($P = 0.29$). 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. 3*D*. 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. 4*B*) ($P = 0.03$). 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. 3*C*. The ratio of *MCP-1* to *GAPDH* mRNA did not correlate with the ratio of *CIDEA* to *GAPDH* mRNA (Fig. 4*C*) ($P = 0.53$). 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. 4*D*) ($P = 0.38$).

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

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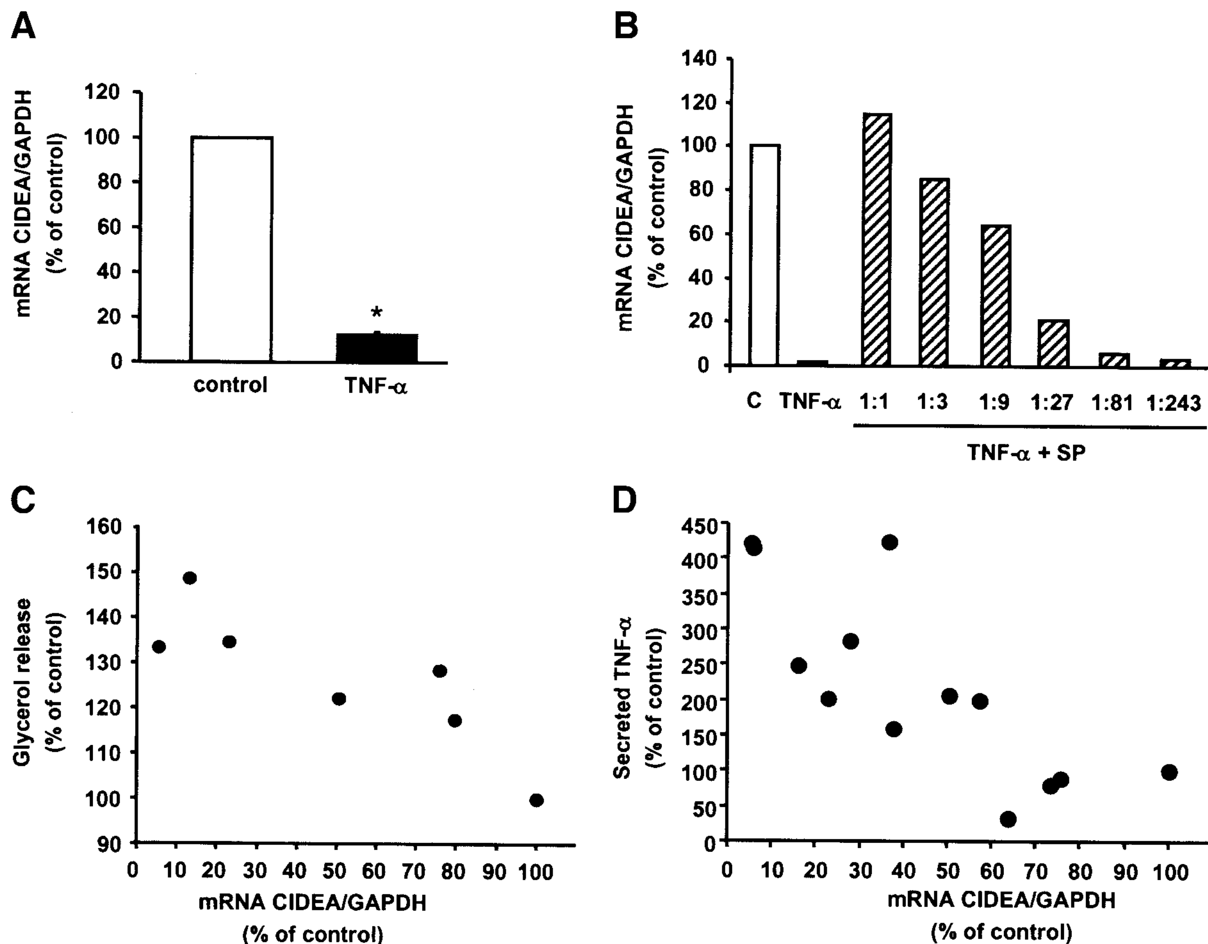

FIG. 3. Cross-talk between CIDEA and TNF-α in human preadipocytes. ***A*****: Ratios of** ***CIDEA*** **to** ***GAPDH*** **mRNA levels in differentiated preadipocytes after 48 h of incubation without (□) or with (■) TNF-α (*****n*** **= 8).** ***B*****: Ratios of** ***CIDEA*** **to** ***GAPDH*** **mRNA in differentiated preadipocytes after 48 h of incubation without (□) or with (■) TNF-α alone or with TNF-α and the indicated dilutions of the specific JNK inhibitor SP600125 (SP) (▨). The concentration 1:1 corresponds to 20 μmol/l. The figure shows one of three representative experiments.** ***C*****: Ratios of** ***CIDEA*** **to** ***GAPDH*** **mRNA levels and glycerol release in preadipocytes after 24 h of RNA interference (*****n*** **= 6).** ***P*** **< 0.05, Spearman's correlation.** ***D*****: Ratios of** ***CIDEA*** **to** ***GAPDH*** **mRNA levels and TNF-α protein secretion after 24 h of incubation with different amounts of** ***CIDEA*** **siRNA (*****n*** **= 8).** ***P*** **< 0.01, Spearman's correlation. ****P*** **< 0.05, Wilcoxon's signed-rank test.**

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. 5*A–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. 5*A*). The mRNA expression of β*2-MG* in the same samples showed the opposite pattern, with much higher levels in WAT than in BAT (Fig. 5*B*). 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. 5*C*) ($P = 0.06$). Surprisingly, the body weight increase in 129Sv/Pas mice on normal diet was marginally larger than for the mice on cafeteria diet (Fig. 5*C*), 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. 5*D*).

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

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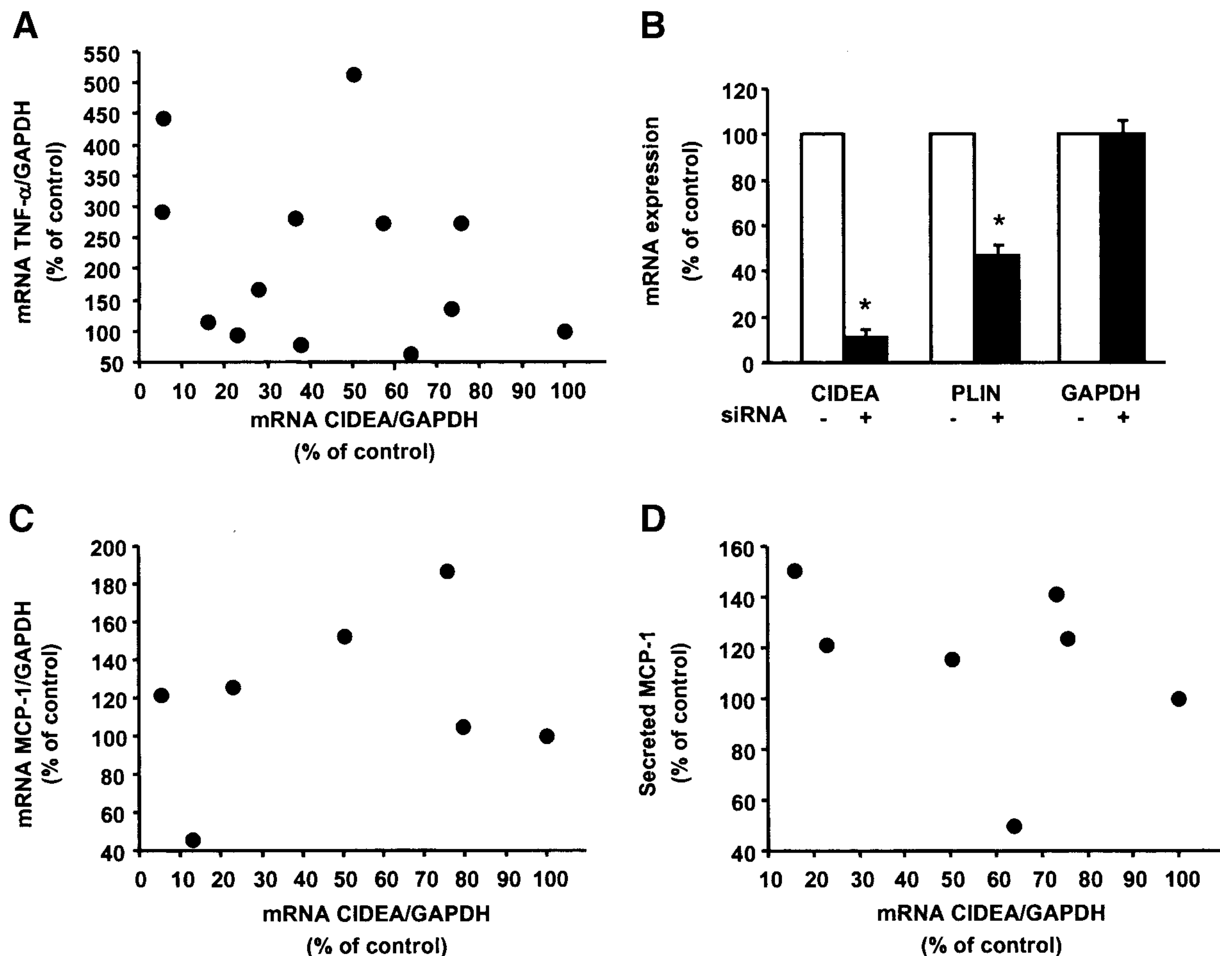

FIG. 4. Effects of *CIDEA* depletion on TNF-α mRNA levels and different TNF-α targets. *A*: Ratios of *CIDEA* and *TNF-α* to *GAPDH* mRNA levels in preadipocytes after 24 h of incubation with different amounts of CIDEA siRNA (n = 8). P = 0.29, Spearman's correlation. *B*: *CIDEA*, perilipin (*PLIN*), and *GAPDH* mRNA levels in preadipocytes after 24 h of RNA interference (n = 4). *P < 0.05, Wilcoxon's signed-rank test. *C* and *D*: Ratios of *CIDEA* and *MCP-1* to *GAPDH* mRNA levels (*C*) and MCP-1 protein secretion into cell media (*D*) after 24 h of RNA interference (n = 6). P = 0.53 and P = 0.38 for *C* and *D*, respectively; Spearman's correlation.

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 is apparently a very strong inhibitor of TNF-α secretion because adipocytes with >90% *CIDEA* depletion displayed a fourfold stimulated TNF-α release. A similar downregulation of *CIDEA* also caused a marked decrease in *PLIN* mRNA but no detectable decrease in the protein amounts of perilipin. This, however, does not exclude perilipin as an indirect target for CIDEA, putatively through TNF-α. It may, for example, necessitate a longer time to reduce the amount of protein than mRNA. Furthermore, we (25) and others (38) have previously shown discrepancies between perilipin mRNA and protein levels in human adipose tissue. In addition, CIDEA–TNF-α interactions might involve translocation and/or phosphorylation of perilipin, which were not examined here.

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 (39) or associated with (40) 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 release 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 $G_{i\alpha}$ (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

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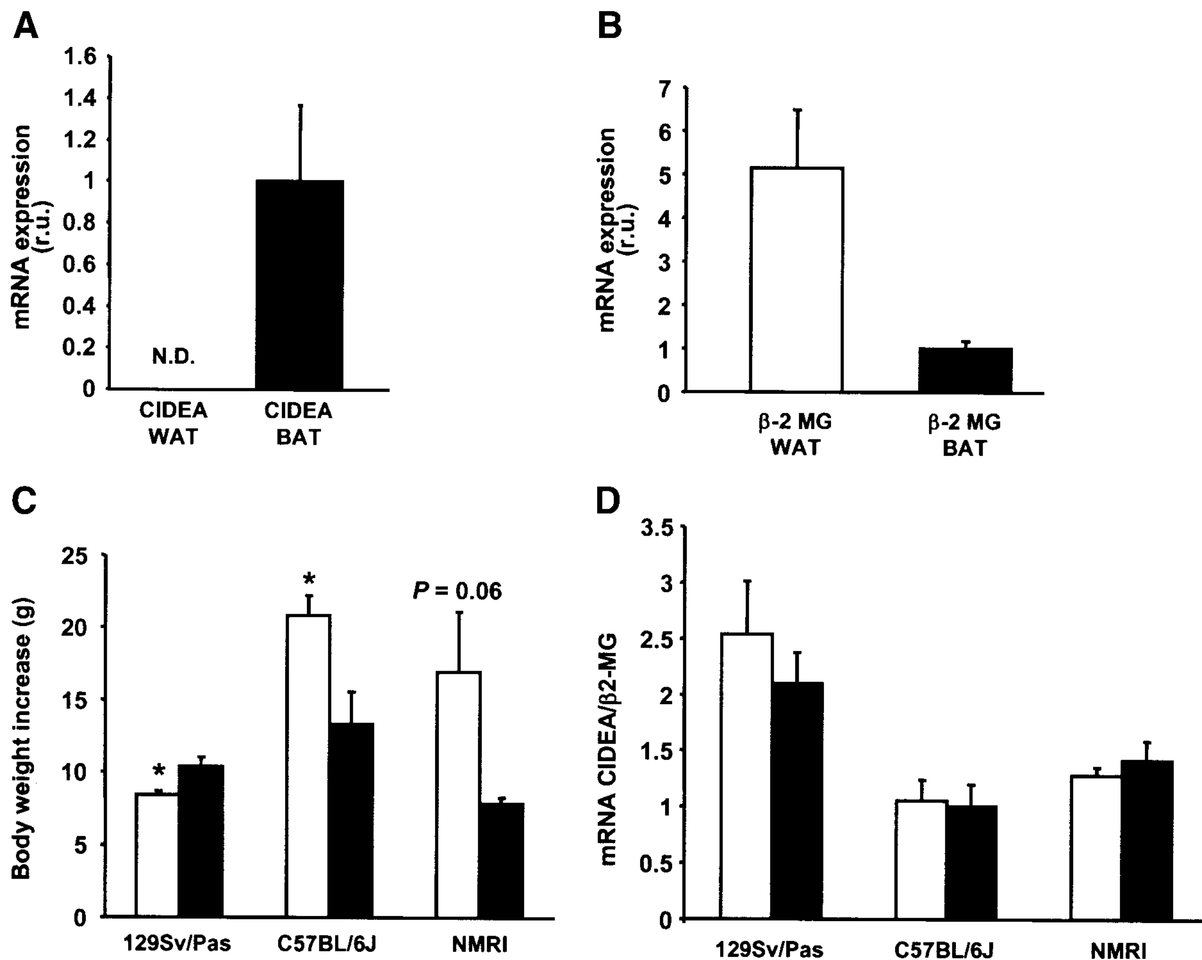

FIG. 5. ***CIDEA*** **mRNA levels in WAT and BAT of mice.** ***A*** **and** ***B*****:** ***CIDEA*** **(*****A*****) and β-2 microglobulin (*****β2-MG*****) (*****B*****) mRNA levels in WAT and BAT from C57BL/6J mice on normal diet (*****n*** **= 3).** ***C*** **and** ***D*****: Body weight increase (*****C*****) and ratios of** ***CIDEA*** **to β*****2-MG*** **mRNA levels (*****D*****) in BAT from mice of three different strains fed cafeteria diet (□) or normal diet (■). ****P*** **< 0.05, Mann-Whitney** ***U*** **test. N.D., not detectable; r.u., relative units.**

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 predominately 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-

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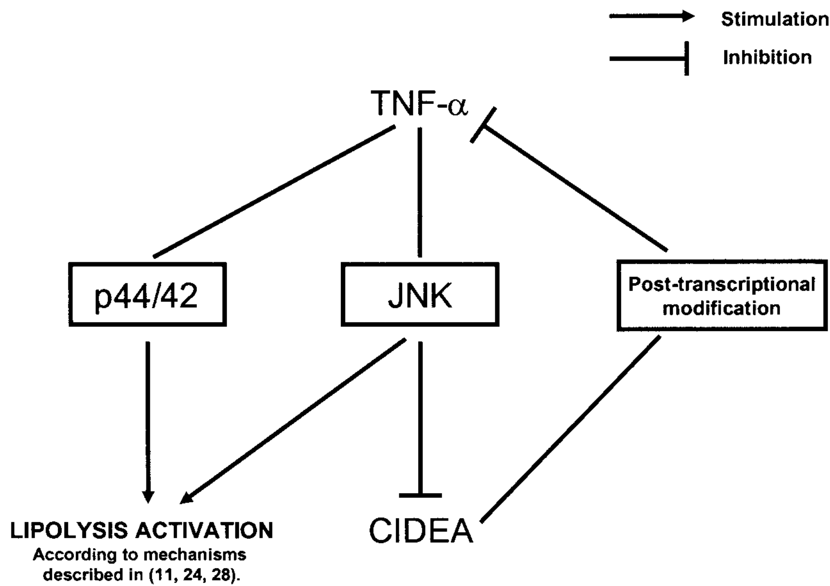

FIG. 6. Cross-talk between CIDEA and TNF-α in human adipose tissue. TNF-α stimulates basal adipocyte lipolysis via the MAPKs p44/42 and JNK and inhibits *CIDEA* expression via JNK. CIDEA inhibits TNF-α secretion via posttranscriptional mechanisms.

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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