Published in final edited form as:

Obesity (Silver Spring). 2010 February ; 18(2): 417–419. doi:10.1038/oby.2009.236.

Hepatic Expression of Cell Death–inducing DFFA-like Effector C in Obese Subjects Is Reduced by Marked Weight Loss

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Abstract

The hepatic expression of the cell death–inducing DNA fragmentation factor A–like effector family (*CIDEA*, *CIDEB*, and *CIDEC*) genes is markedly upregulated in mouse models of obesity. We evaluated the expression of *CIDE* genes in liver of obese human subjects undergoing gastric bypass surgery (GBS), at the time of surgery and again 1 year later when subjects had lost $37.6 \pm 1.4\%$ of their initial body weight. At the time of GBS, the expression of *CIDEA* ($r^2 = 0.20$, $P = 0.04$) and *CIDEC* ($r^2 = 0.32$, $P = 0.01$) was strongly correlated with BMI, whereas *CIDEB* was not ($r^2 = 0.01$, $P = 0.81$). One year after surgery, *CIDEC* expression had declined over 60% ($P = 0.02$), whereas *CIDEA* expression did not change ($P = 0.20$). These data demonstrate that, consistent with previous studies conducted in rodents, hepatic expression of *CIDEA* and *CIDEC*, but not *CIDEB*, is increased in obese humans. Moreover, the hepatic expression of *CIDEC* is downregulated by marked weight loss.

INTRODUCTION

Obesity is often associated with intrahepatic triglyceride and lipid accumulation (1). Recent studies conducted in rodent models suggest that the cell death–inducing DNA fragmentation factor A–like effector family (*CIDEA*, *CIDEB*, and *CIDEC*) are important mediators of obesity-related hepatic steatosis (2–4). Hepatic *CIDEA* and *CIDEC* expression is robustly induced in several obese mouse models (3,4), whereas *CIDEC* knockdown attenuates hepatic steatosis (4). However, we are not aware of any studies evaluating hepatic expression of *CIDE* genes in human obesity. The purpose of this study was to evaluate the relationship between adiposity and hepatic *CIDE* gene expression in obese subjects and determine whether expression of these genes is altered by weight loss.

METHODS

Study subjects

A total of 22 extremely obese men ($n = 6$) and women ($n = 16$) (BMI average = 60.8 ± 11.7 kg/m²) undergoing gastric bypass surgery (GBS) at Barnes-Jewish Hospital participated in this study. Subjects who had any liver disease, other than hepatic steatosis, consumed ≥ 20 g alcohol per day, had severe hypertriglyceridemia (≥ 200 mg/dl), or were taking medications known to

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

DISCLOSURE

The authors declared no conflict of interest.

cause hepatic dysfunction were excluded. All subjects gave written informed consent before participating in this study, which was approved by the Human Studies Committee of Washington University School of Medicine in St Louis.

Subjects were admitted to the Clinical Research Center at ~1800 hours and consumed a standard meal containing ~8 kcal/kg body weight. The following morning, after subjects fasted overnight (12 h), a catheter was inserted into a hand vein, which was heated to 55 °C by using a thermostatically controlled box, to obtain arterialized blood samples. GBS was performed the next day, and liver tissue was obtained by needle biopsy during the surgical procedure, before gastric stapling and intestinal resection were performed. One year after GBS, 10 subjects (9 women and 1 man) were readmitted to the Clinical Research Center and consumed a standard meal at ~1800 hours. The following morning, after subjects fasted for 12 h, arterialized blood samples were obtained. Liver tissue was obtained by percutaneous needle biopsy and was fixed in formalin and paraffin embedded for hematoxylin and eosin staining or immediately frozen in liquid nitrogen for RNA extraction.

RNA analyses

Total RNA was isolated from liver tissues by using RNeasy Lysis Buffer (Qiagen, Crawfordsville, IN). Real-time reverse transcription-PCR was performed using Applied Biosystems (Foster City, CA) reverse transcription kit, PowerSYBR PCR mix, and the ABI PRISM 7500 sequence detection system. The sequence of oligonucleotide primers used for quantitative reverse transcription-PCR can be found in the Supplementary Table S1 online. Arbitrary units of target gene mRNA were corrected to 36B4 RNA content to control for loading.

Histologic scoring

Scoring of steatosis was performed according to the validated method of the NIDDK NASH Cooperative Research Network (5) by a blinded pathologist. Steatosis scoring is based on percent of parenchyma occupied by fat droplets and uses a 0–3 scoring system (0 ≤ 5%; 1 = 5–33%; 2 = 34–66%; 3 ≥ 66%).

Statistical analyses

The relationships between tissue gene expression and metabolic characteristics of the study subjects were determined by using Pearson Correlation Coefficients analyses. Paired *t*-test was used to evaluate the statistical significance of the difference in values before and 1 year after GBS.

RESULTS

Relationship between *CIDE* expression and BMI

The hepatic expression of *CIDEA* and *CIDEC* (Figure 1a), but not *CIDEB* (data not shown), correlated directly with BMI. However, the hepatic expression of *CIDEA*, *CIDEB*, or *CIDEC* was not associated with plasma insulin, glucose, or triglyceride concentrations (data not shown).

Effect of GBS-induced weight loss on *CIDE* expression

At 1 year after GBS, subjects lost $37.6 \pm 1.4\%$ of their initial body weight. There was also a significant decrease in zone 3 macrovesicular steatosis in the post-GBS liver biopsies compared with the pre-GBS biopsies ($P < 0.05$) (Figure 1b,c). Although hepatic *CIDEA* expression was not changed ($P = 0.20$) 1 year after GBS (Figure 1d), hepatic *CIDEC* expression was significantly downregulated by GBS-induced weight loss ($P = 0.02$) (Figure 1d). Finally, the

expression of peroxisome proliferator-activated receptor γ was also significantly downregulated by GBS-induced weight loss ($P = 0.02$) (Figure 1d).

DISCUSSION

Data from studies conducted in mouse models of obesity-related hepatic steatosis have shown that *CIDEA* and *CIDEc* expression tracks with hepatic fat content (3,4). Our data support the notion that *CIDEc*, but not *CIDEA*, expression also tracks with intrahepatic fat content in human subjects. In addition, peroxisome proliferator-activated receptor γ , which plays a critical role in controlling hepatic *CIDE* expression in mice (4), also tracks with *CIDEc* expression in this study suggesting that this relationship is relevant in humans as well.

Interestingly, we found no association between *CIDE* expression and measures of insulin sensitivity, which differs from a previous study showing a strong relationship with adipose tissue *CIDE* expression (6). This may imply that *CIDE* gene expression is under the control of distinct regulatory circuits in these two tissues.

The molecular functions of CIDE proteins are currently unclear. As their name implies, CIDE proteins were originally identified as mediators of apoptotic cell death (7). More recently, CIDE proteins have been studied in the context of their role in regulating fatty acid metabolism. *CIDEA* and *CIDEc* coat lipid droplets and regulate lipid droplet formation and metabolism (6,8). Targeted knockdown of *CIDEc* expression protects mice from developing fatty liver, whereas *CIDEc* overexpression leads to hepatic steatosis (4). The two putative functions of CIDE proteins suggest that this family could be key sensors of cellular lipid overload and important mediators of lipotoxic cell death (9).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the research coordinators, surgeons, nurses and study participants who contributed to this work. Dr Hall is supported by the Cardiovascular Pharmacology Training grant T32-007275. This work was supported by National Institutes of Health grants DK078187, DK56341, DK37948, and RR00036 (General Clinical Research Center).

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Figure 1.

CIDE gene expression in liver of obese human subjects. (a) Scatter plots depict expression of hepatic *CIDEA* and *CIDE* in relation to BMI. (b) Representative hematoxylin and eosin–stained histologic sections from a subject before and 1 year after GBS are shown. Arrows indicate portal tracts. The round, transparent spaces between the two portal tracts are lipid droplets. (c) The graph represents scoring ($n = 9$; as described in Methods) of steatotic areas of liver before and after GBS. (d) The graph depicts expression of *CIDEA*, *CIDE*, and *PPAR* γ in liver ($n = 10$) of subjects at time of GBS and 1 year after GBS. GBS, gastric bypass surgery; *PPAR* γ , peroxisome proliferator–activated receptor- γ .