Brief Report

Hepatic Lipin 1β Expression Is Diminished in Insulin-Resistant Obese Subjects and Is Reactivated by Marked Weight Loss

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OBJECTIVE—Lipin 1 plays critical roles in controlling energy metabolism. We sought to determine the expression of lipin 1 isoforms (lipin 1α and -β) in liver and adipose tissue of obese subjects and to evaluate cellular mechanisms involved in the regulation of lipin 1 expression by physiologic stimuli.

RESEARCH DESIGN AND METHODS—The expression of lipin 1α and -β was quantified in liver and adipose tissue of extremely obese (average BMI 60.8 kg/m^2) human subjects undergoing gastric bypass surgery (GBS). Second, the expression of lipin 1 was evaluated in HepG2 cells in response to overexpression of peroxisome proliferator–activated receptor-γ coactivator (PGC)-1α under normal or hyperinsulinemic conditions.

RESULTS—The expression of lipin 1β in liver and adipose tissue was inversely related to BMI, fasting plasma insulin concentration, and the homeostasis model assessment of insulin resistance but was significantly increased by marked weight loss and insulin sensitization following GBS. Hepatic lipin 1β mRNA levels were strongly correlated with the expression of PGC-1α, and overexpression of PGC-1α in HepG2 cells increased lipin 1 expression. Conversely, hyperinsulinemic culture conditions downregulated the expression of lipin 1β, PGC-1α, and their known target genes involved in mitochondrial metabolism in HepG2 cells. Finally, overexpression of lipin 1β or PGC-1α reversed the effect of hyperinsulinemia on the expression of their target genes.

CONCLUSIONS—These studies suggest that hepatic lipin 1β and PGC-1α expression are downregulated by obesity and obesity-related metabolic perturbations in human subjects, likely due to alterations in insulin concentration or sensitivity. ***Diabetes*** **56:2395–2399, 2007**

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GBS, gastric bypass surgery; HOMA-IR, homeostasis model assessment of insulin resistance; MCAD, medium-chain acyl-CoA dehydrogenase; PAP, phosphatidic acid phosphohydrolase; PGC, peroxisome proliferator–activated receptor-γ coactivator; PPAR, peroxisome proliferator–activated receptor; SDHA, succinate dehydrogenase subunit a.

The gene encoding lipin 1 (*Lpin1*) was discovered by using a positional cloning approach to localize the causative mutation in fatty liver dystrophic (*fld*) mice. *Fld* mice completely lack lipin 1 and exhibit neonatal hepatic steatosis that spontaneously resolves shortly after weaning, life-long lipodystrophy, and insulin resistance (1–3). Based on strong sequence similarity in signature NH_2- and COOH-terminus domains, a family of lipin proteins (lipin 1, 2, and 3) has been identified in higher organisms (1). In addition, alternative splicing of the lipin 1 transcript generates two forms of lipin 1 protein, which are designated as lipin 1α and lipin 1β (4,5). The alternatively spliced exon in the lipin 1β transcript encodes an additional 33 amino acids (Fig. 1*A*) without homology in other lipin family members.

Data from yeast and vertebrate models suggest that lipin proteins have important nuclear and non-nuclear functions that regulate lipid and energy metabolism. In the cytosol, lipin proteins exhibit activity as a phosphatidic acid phosphohydrolase (PAP) (6–8), the enzyme that catalyzes the penultimate step in triglyceride synthesis. In the nucleus, yeast and vertebrate lipins are associated with chromatin and interact with transcription factors on several gene promoters (9,10). In vertebrates, the best-characterized transcription factor partners of lipin 1 are the peroxisome proliferator–activated receptor (PPAR) family (10). A direct protein-protein interaction between lipin 1 and an important PPAR coactivator protein (PGC-1α) has also been detected. Moreover, lipin 1 gene expression is robustly induced by PGC-1α in response to several physiologic stimuli in mouse liver (10). Collectively, the available data suggest that lipin 1 is a highly inducible enzyme involved in triglyceride metabolism and a transcriptional regulator that acts in a feed-forward manner to coactivate the hepatic PGC-1α–PPARα complex to increase the capacity for fatty acid catabolism (10).

Lipin 1 is also a downstream target of the insulin signaling cascade. Lipin 1 protein is phosphorylated at multiple serine and threonine residues following insulin stimulation (4,8). In addition, adipose tissue lipin 1 expression is inversely correlated with BMI and insulin resistance (11–13). These data suggest a unique interrelationship between adiposity, insulin resistance, and lipin 1 activity in adipose tissue, a key tissue involved in the metabolic pathophysiology of obesity. The liver is another organ strongly impacted by obesity and insulin resistance. However, to our knowledge, no study has evaluated the relationship

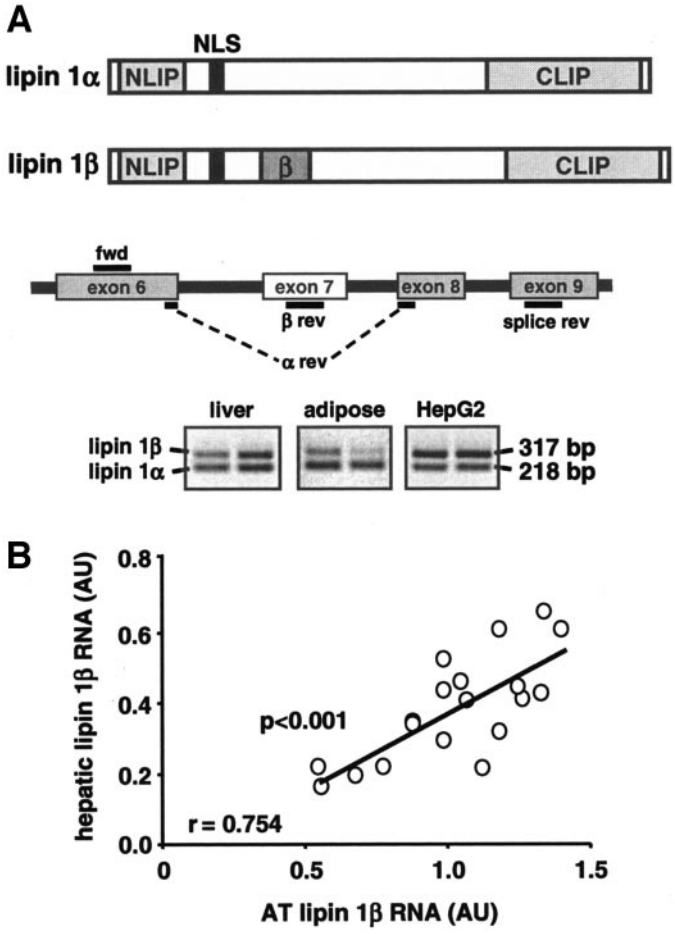

FIG. 1. Lipin 1 isoforms and their expression in human tissue. *A*: *Top*: A schematic of human lipin 1α and 1β proteins is shown. NH_2-terminal (NLIP) and COOH-terminal (CLIP) domains that are highly conserved across species and lipin family members are noted. The 33 amino acid domain that distinguishes lipin 1β from lipin 1α is also shown (β). *Middle*: The binding location for primers used in this study and a schematic representation (not to scale) of the exonic structure surrounding the alternatively spliced exon 7 are shown. *Bottom*: A representative image from agarose gel electrophoresis analysis of RT-PCR products obtained using pooled RNA from liver or adipose tissue of obese human subjects or HepG2 cells is shown. Lipin 1 "fwd" and "splice rev" primers were used for PCR analysis. Bands corresponding to transcripts lacking (lipin 1α) or containing (lipin 1β) exon 7 migrate at 218 and 317 bp, respectively. *B*: Scatter plots depict expression of hepatic lipin 1β in relation to adipose tissue (AT) lipin 1β expression in individual subjects. AU, arbitrary units.

between obesity, insulin resistance, and hepatic lipin 1 expression.

RESEARCH DESIGN AND METHODS

Twenty-seven extremely obese men ($n = 7$) and women ($n = 20$) undergoing gastric bypass surgery (GBS) at Barnes-Jewish Hospital participated in this study (supplemental Table 1 [available in an online appendix at http://dx.doi.org/10.2337/db07-0480]). Before surgery, all subjects completed a medical evaluation, including a history and physical examination and routine blood tests. Subjects were excluded if they had any history or evidence of liver disease other than nonalcoholic fatty liver disease, consumed ≥20 g alcohol/day, had severe hypertriglyceridemia (≥200 mg/dl), or were taking medications that are known to cause hepatic steatosis or liver damage. Although no subjects had a history of diabetes, diabetes was diagnosed in 13 subjects during presurgical screening. These subjects did not take diabetes medications before or after surgery. All subjects gave written informed consent before participating in this study, which was approved by the Human Studies Committee and the General Clinical Research Center Scientific Advisory Committee of Washington University School of Medicine in St. Louis, Missouri. Additional details regarding the experimental protocol, sample collection, and sample analysis can be found in the supplemental online appendix.

Statistical analyses. The relationships between tissue gene expression and metabolic characteristics of the study subjects were determined by using Pearson's correlation coefficient analysis. Nonparametric Mann-Whitney test was used to evaluate the statistical significance of the difference in values before and 1 year after GBS. Statistical comparisons for cell culture experiments were made by using ANOVA coupled to Scheffe's test. A P value ≤0.05 was considered statistically significant.

RESULTS

Analysis of lipin 1 splice variants. RT-PCR analyses demonstrated that transcripts lacking (lipin 1α; 218 bp) or containing (lipin 1β; 317 bp) exon 7 were present in both liver and adipose tissue as well as RNA from HepG2 cells (Fig. 1*A*).

Relationship between lipin 1 expression and BMI or measures of insulin resistance. Hepatic and adipose tissue lipin 1 expression was assessed in extremely obese (average BMI 60.8 ± 2.0 kg/m^2), insulin-resistant (average homeostasis model assessment of insulin resistance [HOMA-IR] 8.1 ± 0.9) subjects undergoing GBS (supplemental Table 1). The expression of lipin 1β in adipose tissue was inversely correlated with BMI ($P = 0.005$), insulin concentration ($P = 0.008$), and HOMA-IR ($P = 0.001$) (Table 1). Hepatic lipin 1β expression was also inversely related to BMI ($P = 0.04$), plasma insulin concentration ($P = 0.03$), and HOMA-IR ($P = 0.04$) (Table 1). Multivariate analyses confirmed that hepatic and adipose tissue lipin 1β expression was inversely related to plasma insulin concentration when other interdependent variables were controlled, suggesting that the decrease observed with obesity and insulin resistance was driven primarily by their relationships with plasma insulin concentration. However, no significant correlation was detected between hepatic lipin 1α expression and BMI, plasma insulin, or HOMA-IR (Table 1).

The expression of lipin 1 target genes (succinate dehydrogenase subunit a [SDHA]) and medium-chain acyl-CoA dehydrogenase (MCAD) involved in mitochondrial oxidative metabolism were also inversely related to BMI (Table 1). SDHA expression was inversely correlated with plasma insulin concentration ($P = 0.04$) and HOMA-IR ($P = 0.03$), but the expression of both SDHA and MCAD was positively correlated with hepatic lipin 1β expression (Table 1).

TABLE 1
Correlation coefficients (r) of gene expression

	SQ AT lipin 1β	Hepatic lipin 1β	Hepatic lipin 1α	Hepatic SDHA	Hepatic MCAD	Hepatic PGC-1α
BMI (kg/m^2)	−0.469*	−0.396†	0.032	−0.411†	−0.427†	0.019
Insulin (μU/ml)	−0.395*	−0.464†	0.053	−0.456†	−0.136	−0.493†
HOMA-IR	−0.713‡	−0.459†	0.048	−0.417†	−0.185	−0.616*
Hepatic lipin 1β	0.754‡	—	0.278	0.638‡	0.537*	0.450†

*$P < 0.01$; †$P < 0.05$; ‡$P < 0.001$. SQ AT, subcutaneous adipose tissue.

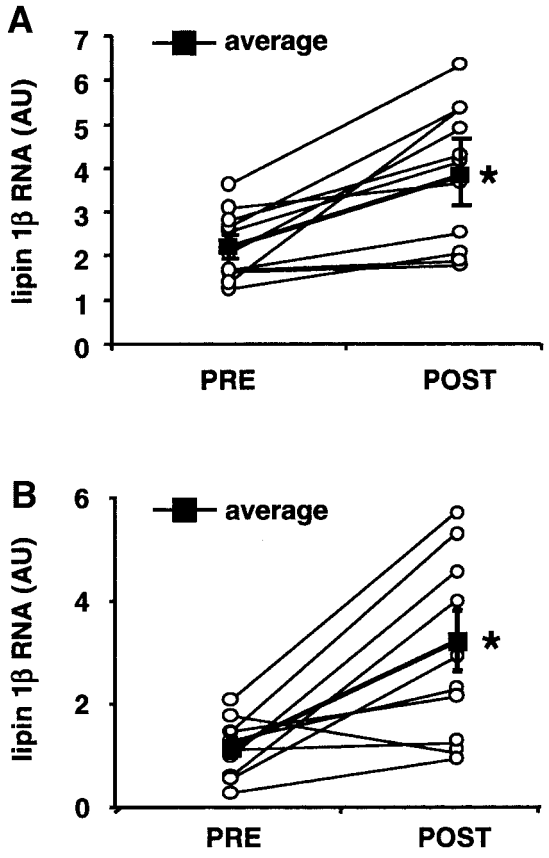

FIG. 2. The expression of lipin 1β in liver and adipose tissue is reactivated by marked weight loss. Graphs depict expression of lipin 1β liver (*A*) (n = 11) or subcutaneous adipose tissue (*B*) (n = 10) of subjects at time of GBS surgery and 1 year after GBS. The expression of lipin 1β in each individual (○) or the average of the subjects (■; ±SEM) is shown. *P < 0.05 vs. baseline average. AU, arbitrary units.

A strong relationship between hepatic and adipose tissue lipin 1β expression within an individual was detected (P = 0.001) (Fig. 1*B*). Surprisingly, hepatic lipin 1α and lipin 1β expression within an individual subject was not significantly correlated (Table 1).

Effect of GBS-induced weight loss on lipin 1 expression. Lipin 1β expression was increased ~73% in liver (P = 0.01) and 2.6-fold in adipose tissue (P = 0.01) (Fig. 2) 1 year after GBS, when subjects had lost 34.5 ± 4.1% of their initial body weight, exhibited a 66.8 ± 4.8% reduction in mean plasma insulin concentration, and displayed a 70.7 ± 5.8% reduction in mean HOMA-IR values (supplemental Table 2). In contrast, hepatic and adipose tissue lipin 1α expression was not significantly altered by GBS-induced weight loss (data not shown). Importantly, a strong correlation between hepatic and adipose tissue lipin 1β expression within an individual was also detected in subjects after weight loss (n = 10; r = 0.6129; P = 0.01).

PGC-1α gene expression. PGC-1α is a transcriptional coactivator protein that controls expression of lipin 1 in mouse liver (10). Hepatic PGC-1α expression was inversely correlated with plasma insulin concentration (P = 0.03) and HOMA-IR (P = 0.06) but not with BMI (Table 1). Lipin 1β and PGC-1α expression in liver were closely correlated in individual subjects (P = 0.03), whereas the expression of hepatic lipin 1α did not correlate with PGC-1α expression (supplemental Fig. 1).

Regulation of lipin 1 expression by PGC-1α and insulin. To explore our finding of an interrelationship between lipin 1, PGC-1α, and insulin concentration, experiments were conducted in HepG2 cells. Adenoviral-driven overexpression of PGC-1α increased lipin 1β expression 16-fold (supplemental Fig. 1). PGC-1α also significantly increased the expression of lipin 1α, but the magnitude of the increase (sevenfold) was less than that of lipin 1β (P < 0.05). Expression of lipin 1β and PGC-1α in HepG2 cells was significantly diminished by 48 h of exposure to hyperinsulinemic culture conditions (Fig. 3*A*). However, PGC-1α overexpression driven by an adenoviral vector prevented the insulin-mediated downregulation of lipin 1β expression (Fig. 3*B*). Insulin treatment also led to diminished expression of SDHA and MCAD (Fig. 3*C*). These effects of insulin were reversed by adenoviral-mediated overexpression of lipin 1β (Fig. 3*D*) or PGC-1α (data not shown).

DISCUSSION

Lipin 1 proteins play important roles in the regulation of cellular lipid and energy metabolism. In the present study, we found that hepatic and adipose tissue expression of lipin 1β, but not lipin 1α, was inversely related to BMI, plasma insulin concentration, and insulin resistance in extremely obese subjects. In addition, GBS-induced weight loss and a concomitant decrease in plasma insulin concentration were associated with a marked increase in lipin 1β expression. Moreover, hepatic lipin 1β expression correlated directly with PGC-1α mRNA levels. Overexpression of PGC-1α in HepG2 cells markedly induced lipin 1β expression and prevented the downregulation of lipin 1 gene expression caused by hyperinsulinemic culture conditions. These results demonstrate marked differences in the regulation of hepatic and adipose tissue lipin 1β and lipin 1α expression and suggest that lipin 1β dysregulation may play a role in the metabolic pathophysiology associated with obesity.

Emerging evidence suggests that lipin 1β is a downstream target of the insulin signaling pathway and that insulin is an important regulator of hepatic lipin 1β expression and activity. Lipin 1β gene expression in liver and adipose tissue is inversely related to plasma insulin concentration (11–13), and hyperinsulinemic culture conditions downregulate lipin 1β gene expression in HepG2 cells (current study). Moreover, the reduction in insulin concentration following weight loss or thiazolidinedione treatment is associated with an increase in lipin 1β expression (12,13), and streptozotocin-induced insulin deficiency leads to a marked increase in hepatic lipin 1 expression in mice (10). However, the results of these studies fail to define whether it is insulin action or insulin resistance that leads to impaired lipin 1 expression. Some arms of the insulin signaling cascade remain active in insulin-resistant liver (14). In addition, the hyperinsulinemic conditions used in this study have been shown to cause insulin resistance in cultured cells (15). Therefore, determining whether it is insulin action or insulin resistance that deactivates lipin 1β gene expression will require further study.

On the other hand, evidence also exists that lipin 1 activity is an important determinant of insulin sensitivity. For example, a single nucleotide polymorphism in the LPIN1 gene correlates with plasma insulin concentration in a population of dyslipidemic subjects (11). Quantitative

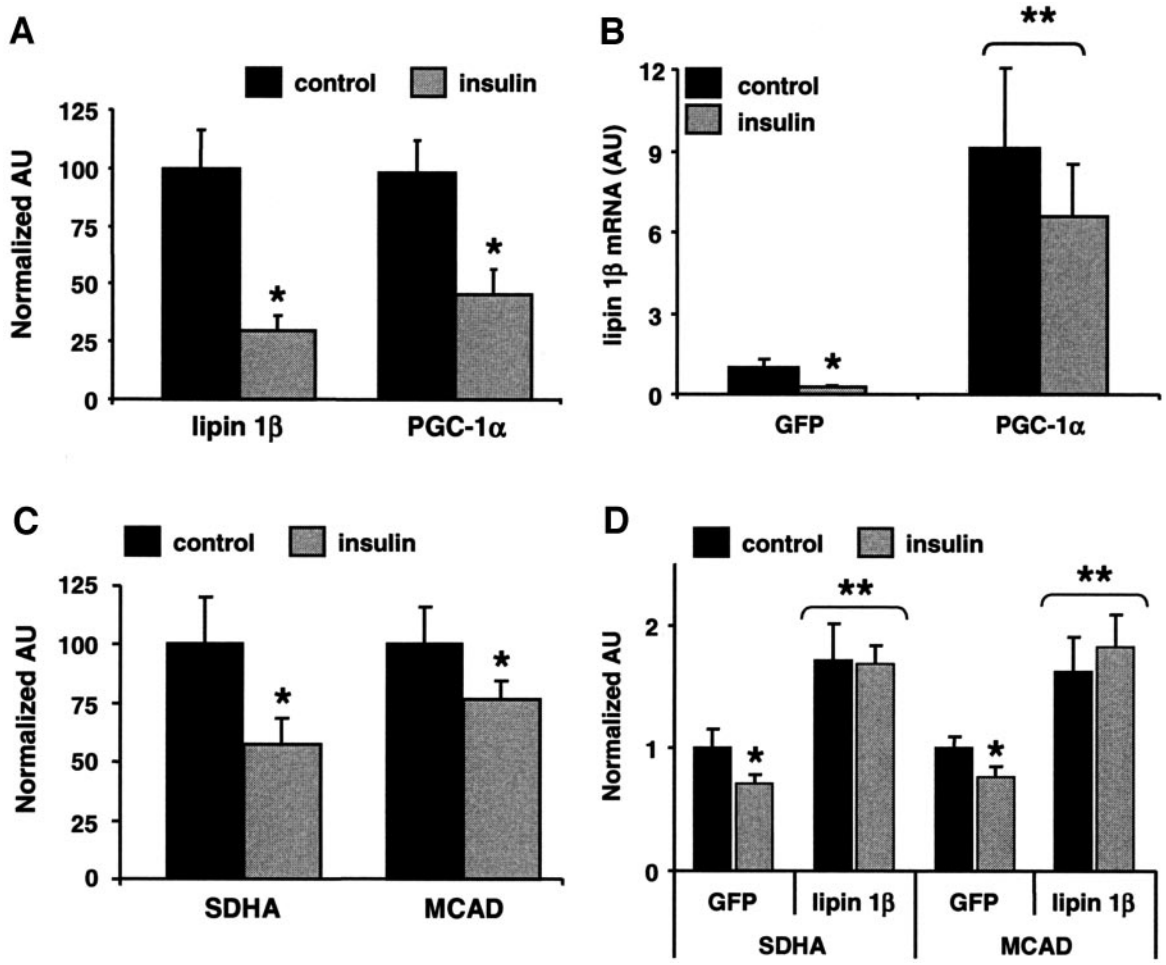

FIG. 3. The expression of lipin 1β and PGC-1α is downregulated by hyperinsulinemia in cultured HepG2 cells. *A*: The graphs depict the expression of lipin 1β and PGC-1α in mRNA isolated from HepG2 cells cultured under control or hyperinsulinemic conditions. Values are normalized (= 100) to control values. *B*: The graphs depict the expression of lipin 1β in mRNA isolated from HepG2 cells cultured under control or hyperinsulinemic conditions and infected with adenovirus-overexpressing PGC-1α and/or green fluorescent protein (GFP). Values are normalized (= 1.0) to control values. *C*: The graphs depict the expression of SDHA and MCAD in mRNA isolated from HepG2 cells cultured under control or hyperinsulinemic conditions. Values are normalized (= 100) to control values. *D*: The graphs depict the expression of SDHA and MCAD in mRNA isolated from HepG2 cells cultured under control and hyperinsulinemic conditions and infected with adenovirus-driving expression of lipin 1β and/or GFP. Values are normalized (= 1.0) to control values. *$P < 0.05$ vs. control culture condition values. **$P < 0.05$ vs. GFP-infected cells. AU, arbitrary units.

trait loci mapping also suggests that single nucleotide polymorphisms in the mouse *lpin1* gene are associated with increased susceptibility to diabetes in intercrossed *db/db* mice (16). *Fld* mice, which lack lipin 1 altogether, are insulin resistant and modestly hyperglycemic (3), whereas mice overexpressing lipin 1 in adipose tissue are insulin sensitive when challenged with high-fat diets (17). Several potential cellular mechanisms could explain how lipin 1 influences insulin action including its PAP activity, coactivation of the PPARα–PGC-1α complex in liver, and activation of PPARγ in adipose tissue (5,17,18). However, further work will be required to define exactly how lipin 1 might be modulating insulin sensitivity.

PGC-1α is a transcriptional coactivator protein that regulates the expression of genes encoding key metabolic steps in fatty acid oxidation, oxidative phosphorylation, triglyceride secretion, and gluconeogenesis (19). Skeletal muscle PGC-1α expression is diminished in individuals who are insulin resistant or have diabetes (20,21). Conversely, hepatic PGC-1α is increased in rodent models of diabetes (22,23). We found that PGC-1α was inversely related to fasting plasma insulin (Table 1) and glucose (data not shown) concentrations, perhaps suggesting different regulatory mechanisms at play in mouse and human subjects. We also detected a direct relationship between lipin 1β, but not lipin 1α, and PGC-1α mRNA levels in human liver samples. Moreover, PGC-1α overexpression in HepG2 cells caused a greater induction of lipin 1β than lipin 1α expression. These data parallel previous work in adipocytes, suggesting a selective enhancement of lipin 1β expression by ligands that activate PPARγ, a transcription factor partner of PGC-1α (12). PGC-1α protein contains an RNA recognition motif in its COOH-terminus, localizes to nuclear speckles, and mediates alternative splicing of transcripts of its other target genes (24). Therefore, selective activation of lipin 1β by PGC-1α could be a critical nodal point of regulatory control for lipin 1 activity. Although the functional significance of the lipin 1β splice variant is not fully understood, the additional amino acids might modulate its subcellular localization (5), elicit a distinct pattern of gene expression (5), or affect the kinetics of its PAP activity (7).

In conclusion, the data from the present study demonstrate that both hepatic and adipose tissue expression of

lipin 1β are dynamically regulated by the metabolic pathophysiology associated with human obesity. However, additional studies are needed to elucidate the molecular mechanisms and the basis for the interaction between insulin signaling and lipin 1β expression, as well as to determine the cause and effect relationship. Understanding how lipin 1 expression and activity are regulated could lead to novel therapies for treating insulin resistance and diabetes.

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