PPARα (Peroxisome Proliferator-activated Receptor α) Activation Reduces Hepatic CEACAM1 Protein Expression to Regulate Fatty Acid Oxidation during Fasting-refeeding Transition*

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Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is expressed at high levels in the hepatocyte, consistent with its role in promoting insulin clearance in liver. CEACAM1 also mediates a negative acute effect of insulin on fatty acid synthase activity. Western blot analysis reveals lower hepatic CEACAM1 expression during fasting. Treating of rat hepatoma FAO cells with Wy14,643, an agonist of peroxisome proliferator-activated receptor α (PPARα), rapidly reduces Ceacam1 mRNA and CEACAM1 protein levels within 1 and 2 h, respectively. Luciferase reporter assay shows a decrease in the promoter activity of both rat and mouse genes by Pparα activation, and 5′-deletion and block substitution analyses reveal that the Pparα response element between nucleotides −557 and −543 is required for regulation of the mouse promoter activity. Chromatin immunoprecipitation analysis demonstrates binding of liganded Pparg to Ceacam1 promoter in liver lysates of Pparα+/−, but not Pparα−/− mice fed a Wy14,643-supplemented chow diet. Consequently, Wy14,643 feeding reduces hepatic Ceacam1 mRNA and CEACAM1 protein levels, thus decreasing insulin clearance to compensate for compromised insulin secretion and maintain glucose homeostasis and insulin sensitivity in wild-type mice. Together, the data show that the low hepatic CEACAM1 expression at fasting is mediated by Pparg-dependent mechanisms. Changes in CEACAM1 expression contribute to the coordination of fatty acid oxidation and insulin action in the fasting-refeeding transition.

Plasma insulin levels are determined by several factors, including insulin secretion from pancreatic β-cells and insulin clearance (1, 2). Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a substrate of the insulin receptor tyrosine kinase (3), promotes insulin clearance by up-regulating receptor-mediated insulin endocytosis and degradation in a phosphorylation-dependent manner (4). Its specific inactivation in liver impairs insulin clearance to cause hyperinsulinemia and ensuing insulin resistance with increased hepatic steatosis (5).

CEACAM1 is ubiquitously produced with a predominant expression in liver by comparison to kidney and a limited expression in white adipose tissue and skeletal muscle (6). Physiologically, this is consistent with the major role of the liver in insulin clearance by comparison to kidney and the insignificant involvement of the other insulin target tissues in insulin extraction. The mechanistic underpinning of this hierarchical expression profile relates to the up-regulation of Ceacam1 promoter activity by insulin (7), the level of which is 2–3-fold higher in the portal relative to the systemic circulation (8).

In addition to CEACAM1, the hepatocyte is home to the highest level of fatty acid synthase, a lipogenic enzyme that catalyzes the conversion of malonyl-CoA to palmitic acid. Similar to CEACAM1, this high level of expression of fatty acid synthase is mediated by transcriptional up-regulation by insulin (9). However, despite the abundance of this enzyme, fatty acid synthase activity in liver is restricted even under stimulating feeding conditions, during which carbohydrates undergo glycolysis and their products are converted to fatty acids to contribute to energy sources at fasting. We have shown (10) that this counter-regulation of fatty acid synthase activity in liver is mediated by increased CEACAM1 phosphorylation and...
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binding to fatty acid synthase in response to transient pulses of insulin (11).

At fasting, when insulin secretion is low, metabolism shifts from glycolysis to lipolysis, during which free fatty acids are released from white adipose tissue into the liver to undergo β-oxidation and supply energy to the heart and brain. This mechanism is largely supported by the activation of peroxisome proliferator-activated receptor α (PPARα), a nuclear transcription factor that induces the expression of genes involved in fatty acid transport to mitochondria to undergo β-oxidation (12–16). Acutely after refeeding (~8 h), PPARα plays a significant role in maintaining fatty acid β-oxidation during the stepwise reversal of the fasting metabolic state by insulin surges until glycolysis stores are replenished (17), at which point the levels of malonyl-CoA are restored, β-oxidation stops, and glycolysis resumes (18).

Because CEACAM1 plays an important regulatory role in insulin and fatty acid homeostasis, we herein examined whether it is itself metabolically regulated during the fasting-refeeding transition and identified the underlying mechanisms.

Experimental Procedures

Animal Husbandry—Male mice were kept in a 12-h dark/light cycle. All procedures were approved by the Institutional Animal Care and Utilization Committee. Wild-type Ppara+/+ and Ppara−/− null mice propagated on the C57BL/6 background were from Taconic Biosciences (Cambridge City, IN). Male mice (2–4 months of age) were fed ad libitum (Harlan Teklad 2016; Harlan, Haslett, MI). In some experiments mice were fed for 3–7 days a chow diet powdered and mixed in a geometric proportion with 0.1% w/w of TCR (Invitrogen) containing 10 mM lactate, 10 nM dexamethasone, 100 nM insulin, 10% FBS, and 1% penicillin-streptomycin, counted and plated onto 12-well cell culture plates at 2.5 × 105/well density, and incubated at 37 °C for 48 h. Medium was changed 24 h after plating. [125I]Insulin (Human [125I]insulin, PerkinElmer Life Sciences) (30,000 cpm) was allowed to bind at 4 °C for 5 h in cold KRP buffer (100 mM HEPES, pH 7.4, 120 mM NaCl, 1.2 mM MgSO4, 1 mM EDTA, 15 mM CH3COONa, 10 mM glucose, 1% BSA) before it was allowed to internalize at 37 °C for 0–90 min, as previously described (21).

Cloning of Mouse Ceacam1 Promoter—Functional mapping of the mouse Ceacam1 promoter revealed three potential peroxisome proliferator response elements (PPRE)/RXR at nucleotides (nts) −1056/−1037, −557/−543, −260/−248. In a PCR reaction, double-stranded genomic DNA spanning nt +30 to −1100 was synthesized and amplified in a polymerase chain reaction (PCR) using 100 ng of mouse genomic DNA as template and 1 μM concentrations of sense forward primer (nt −1100/−1086) containing an XhoI flanking sequence (small letters in italics, 5′-atacctctgagCTTAAGAAGCTTAC-3′) and antisense primer (nt +30/+11) with BglII flanking sequence (small letters in italics, 5′-gaagactttTGTGGAGA-TGTGTGGAG-3′). After the initial DNA denaturation at 94 °C for 5 min, 30 cycles of PCR were carried out as described previously (7). The 5′ deletion mutant was synthesized using the same PCR conditions but with a forward primer spanning nt −467 to −453 (5′-atacctctgagTCAGTGACGATGGAT-3′). Amplified genomic DNA was subsequently purified and subcloned at the KpnI and BglII sites of pGL4.10 [luc2] BASIC promoterless firefly luciferase reporter plasmid (Promega Corp., Madison, WI).

Scanning Mutants of individual PPREs (nts −1056/−1037 (Δ1), nts −557/−543 (Δ2), and nts −260/−248 (Δ3)) were obtained in two sequential PCR reactions using the Quick-Change II XL Site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA). The first PCR reaction utilized 100 ng of the pGL4.10 plasmid containing the genomic DNA sequence between nt −1100 and +30 as the template, with the reverse antisense nt +30/+11 primer and forward primers containing a sequence replacing each of the native PPRE site. The resulting PCR product was then used as the template to insert a mutation replacing the native RXR site downstream of each PPRE to mutate the PPRE/RXR site. The Δ1 mutations were 5′-TAATCGA-3′ (for PPRE alone) and 5′-TAATCGAGCTAGT-3′ (for PPRE/RXR). The Δ2 mutations were 5′-CAATCTCT-3′ (for PPRE alone) and 5′-CAATTCTATGAAATC-3′ (for PPRE/RXR). The Δ3 mutations were 5′-CTTTCGTA-3′ (for PPRE alone) and 5′-CTTTTCGTTAG-3′ (for PPRE/RXR). The resulting individual mutant products were used as templates to...
create any combinational mutations of the three PRE/RXRs following a similar PCR-based strategy.

Cell Culture, Transfection, and Luciferase Assay—FAO rat hepatoma and human embryonic kidney cells (HEK293) were maintained at 5% CO2 in Dulbecco-modified Eagle's medium (DMEM). Human liver carcinoma HepG2 cells were maintained in minimum essential medium α (α-MEM) (Cellgro, Manassas, VA) supplemented with 10% FBS, 1% L-glutamine, 100 units/ml penicillin (Sigma), and 10 mg/ml streptomycin (Sigma). Cells were serum-starved with phenol red-free medium (Invitrogen) before they were treated for 24 h, fixing in 1% formaldehyde, 1 mM Tris-HCl, pH 8.1, 10 mM EDTA, 1% SDS, and proteinase K for 1 h at 4 °C, DNA purified by phenol/chloroform/isoamyl alcohol extraction and PCR-amplified using a proximal (forward, nts −562 to −541, 5′-TCTTGGAATCCAGGTCACCC-3′; reverse, nts −301 to −280, 5′-GCGAGGATCTTCCAGGTCACCC-3′) and a distal primer set (forward, nts +6499 to +6520, 5′-GCTTCATTCCACCTCTTCTCG-3′; reverse, nt +6674 to +6678, 5′-GCTAGGGCCAGAATGTTTG-3′), which yielded a 190-bp and a 265-bp product, respectively. For the malic enzyme control, the primer set yielding a 190-bp was used (forward, 5′-TCTTGGAATCCAGGTCACCC-3′, and reverse, 5′-TCGAGGATCTTCCAGGTCACCC-3′).

Western Blot Analysis—Proteins were analyzed by SDS-PAGE and immunoblotting with custom-made polyclonal antibodies against endogenous mouse CEACAM1 (α-CC1) (10) and rat CEACAM1 (α-rCC1; α-P3(Ex)) raised in rabbit against three peptides (amino acids 49–64, 482–495, and 506–519) and affinity-purified against the extracellular peptide (amino acids 49–64), and phospho-CEACAM1 (α-pCC1) against phosphorylated Tyr-488 and fatty acid synthase (α-Fasn) (10). Also used were antibodies against Ppara and CD36 (Santa Cruz). For normalization, membranes were reprobed with monoclonal antibodies against Actin, GAPDH, and tubulin (Sigma or Santa Cruz). Proteins were detected by enhanced chemiluminescence (ECL; Amersham Biosciences) and quantified by densitometry (Image J software) (National Institutes of Health, Bethesda, MD).

Northern Blot Analysis—mRNA was purified using TRizol (Invitrogen) followed by the MicroPoly (A) Pure kit (Ambion, ThermoFisher Scientific) and analysis by probing with cDNAs for Ppara and Ceacam1 using the Random Primed DNA Labeling kit (Roche Applied Science) before reprobing and normalizing to β-actin.

Semi-quantitative Real-time RT-PCR—Total RNA was isolated with the PerfectPure RNA tissue kit (5 Prime) following the manufacturer’s protocol. cDNA was synthesized with ImProm-II™ reverse transcriptase (Promega) using 1 μg of total RNA and oligo(dT) primers (21). cDNA was evaluated with quantitative real-time-PCR (Step One Plus, Applied Biosystems, Waltham, MA). mRNA was normalized to Gapdh or 18S. Results are expressed in fold-change as the mean ± S.E.

Statistical Analysis—Data were analyzed with SPSS software by two-way analysis of variance or two-tailed Student’s t test with GraphPad Prism 4 software. p < 0.05 was statistically significant.

Results

CEACAM1 Is Regulated by Fasting/Refeeding—After an overnight fast, mice were refed a regular chow diet for up to 24 h. As previously shown (10), insulin surged at 1, 4, and 7 h of refeeding (Fig. 1A). Consistent with its ability to increase Ceacam1 promoter activity (7), hepatic CEACAM1 protein content was induced in parallel to transient insulin surges (Fig. 1B). Insulin surge during refeeding also induced CEACAM1 phosphorylation, as Western blot analysis using an antibody against tyrosyl phosphorylated CEACAM1 (α-pCC1) shows (Fig. 1B). Moreover, the activity of Fasn is diminished in parallel to CEACAM1 phosphorylation (Fig. 1C) as previously reported (10). Of interest, the CEACAM1:Fasn protein ratio begins to increase (23), mice were fed a WY14,643-supplemented diet for 7 days before liver extraction, grinding under liquid nitrogen, and cross-linking in 1% formaldehyde, 1 × PBS at 37 °C for 20 min. Cross-linking was terminated with 125 mM glycine, and the cell pellet was washed with 1 × PBS. Nuclei were lysed in SDS buffer (50 mM Tris-HCl, pH 8.1, 10 mM EDTA, 1% SDS, and protease inhibitors), chromatin was sheared by sonication, and the nuclei lysate was cleared by centrifugation at 50,000 × g for 30 min. The soluble chromatin was diluted 10-fold in 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl and immunoprecipitated with an antibody against Ppara (H-98; Santa Cruz Biotechnology, Inc., Dallas, TX). The antibody-protein-DNA complex was isolated using magnetic beads conjugated with protein A (New England BioLabs, Inc, Ipswich, MA). The protein-DNA complex was washed, eluted in 50 mM NaHCO3, 1% SDS, and left at 65 °C overnight to reverse cross-linking. After protein digestion with proteinase K for 1 h at 45 °C, DNA was purified by phenol/chloroform/isoamyl alcohol extraction and PCR-amplified using a proximal (forward, nts −562 to −541, 5′-TCTTGGAATCCAGGTCACCC-3′; reverse, nts −301 to −280, 5′-GCGAGGATCTTCCAGGTCACCC-3′) and a distal primer set (forward, nts +6499 to +6520, 5′-GCTTCATTCCACCTCTTCTCG-3′; reverse, nt +6674 to +6678, 5′-GCTAGGGCCAGAATGTTTG-3′), which yielded a 190-bp and a 265-bp product, respectively. For the malic enzyme control, the primer set yielding a 190-bp was used (forward, 5′-TCTTGGAATCCAGGTCACCC-3′, and reverse, 5′-TCGAGGATCTTCCAGGTCACCC-3′). This could contribute to the regulation of Fasn substrate, malonyl-CoA, and hence fatty acid transport to mitochondria for β-oxidation, an essential step in glycogen repletion. As has been reported (26), replenishment of glycogen content in liver takes ~8 h of refeeding (Fig. 1D), at which point
CEACAM1 phosphorylation dropped (Fig. 1A) and Fasn enzymatic activity reciprocally increased (Fig. 1C).

Ppara Activation Decreases Ceacam1 Expression—Because PPARα is activated at fasting to increase transcription of genes that are involved in fatty acid β-oxidation in liver (15), we then investigated whether it is implicated in the metabolically driven changes in hepatic CEACAM1 expression. Northern analysis shows a higher hepatic Ppara mRNA levels at fasting than the first few hours of refeeding (Fig. 1E).

To test the hypothesis that CEACAM1 is reduced by Ppara activation, we assessed the effect of PPARα agonist, Wy14,643 (Wy), on rat and mouse Ceacam1 promoter activity as well as on its mRNA and protein levels. Using a luciferase reporter assay (7), we show that Wy14,643 treatment reduced rat Ceacam1 promoter activity by ∼4-fold in rat hepatoma FAO cells (Fig. 2A, Wy-treated versus vehicle (Veh)-treated −1609pLuc). Similarly, Wy14,643 treatment decreased the promoter activity of the rat Ceacam1 promoter (−1609pLuc) in human embryonic kidney (HEK293) cells (not shown), with an expected lower potency than in rat cells (27). Furthermore, Wy14,643 decreased Ceacam1 mRNA and CEACAM1 protein content in rat hepatoma FAO cells beginning at 1 and 2 h, respectively (Fig. 2, B and C).

Like rat promoter, Wy14,643 treatment reduced mouse Ceacam1 promoter activity by ∼2-fold in human HepG2 hepatoma cells (Fig. 3B, Wy-treated versus Veh-treated −1100pLuc). 5'-Deletion analysis indicated that removing the genomic DNA region containing the two potential distal PPREs between nts −1100 and −467 abolished the repressive effect of Ppara activation on mouse Ceacam1 promoter activity (Fig. 3B, Wy-treated versus Veh-treated −467pLuc). To further identify the active PPRE in the mouse Ceacam1 promoter, we then carried out mutational mapping of the three potential PPRE/RXR sites in the promoter. As Fig. 3C indicates, mutating the sequence between nts −557 and −543 either individually (Δ2 construct) or together with the other two (Δ1,2; Δ2,3 and Δ1,2,3 constructs) completely abolished the repressive effect of Wy14,643 treatment on Ceacam1 promoter activity, as opposed to mutating the other two PPRE/RXR motifs alone (Δ1;Δ3 and Δ1,3 constructs). This points to the functional rel-

**FIGURE 1.** Changes in hepatic CEACAM1 protein content at fasting/refeeding and physiologic implications. A, mice were fasted (F) overnight and refed ad libitum for 1–24 h (Rfd) before assessing plasma insulin levels. B, liver lysates were subjected to Western analysis by immunoblotting (ib) with antibodies against α-CEACAM1 (α-CC1) to assess changes in hepatic CEACAM1 protein levels, phospho-CEACAM1 (α-pCC1) to detect phosphorylated CEACAM1, and Fasn to detect protein content of fatty acid synthase. Gels were reimmunoblotted (reb) with an antibody against actin to normalize for protein loading. Liver tissues were assayed for fatty acid synthase activity relative to microgram of proteins (C) and for glycogen content relative to wet tissue weight (D). Assays were carried out in triplicate and on more than three mice per each time point. Data are presented as the mean ± S.E. E, liver tissues were subjected to Northern analysis to assess hepatic Ppara mRNA levels followed by β-actin for normalization. Both Northern and Western gels represent more than three experiments done on more than three mice per each time point.
evance of the PPRE/RXR sequence located between nt −557 and −543 in the regulation of mouse Ceacam1 promoter activity.

A ChIP assay using a proximal primer (PP) set spanning the mouse Ceacam1 promoter between nt −280 and −562 showed that liganded Pparα binds to Ceacam1 gene in liver lysates derived from Wy14,643-fed to a higher extent than Veh-fed Pparα−/− mice or Wy14,643-fed Pparα−/− mice (Fig. 4A). A similar observation was made for the positive control, malic enzyme, a known target of Pparα. The distal primer set amplifying a region between nt +6499 and +6764 in Ceacam1 gene did not detect any binding. Together, the data revealed that activated Pparα binds to Ceacam1 gene to down-regulate its transcription.

Metabolic Consequence of Pparα-dependent Down-regulation of CEACAM1—Feeding 2-month-old male mice a chow supplemented with Wy14,643 activated hepatic Pparα, as assessed by increased mRNA (Fig. 4B) and protein (Fig. 4C) levels of its target gene, CD36/FABP (16), in Pparα+/+, but not Pparα−/− mice. In parallel, this reduced Ceacam1 mRNA (Fig. 4B) and CEACAM1 protein levels (Fig. 4C) in Pparα+/+ but not Pparα−/− mice. Consistently, Pparα activation by Wy14,643 reduced insulin clearance in Pparα+/+ wild-type mice, as assessed by the ~3-fold decrease in C-peptide/insulin molar ratio relative to RD-fed mice (Table 1). This is further supported by a ~2-fold decrease in CEACAM1 protein content (Fig. 5A) and in [125I]insulin internalization (Fig. 5B) in Wy14,643-treated primary hepatocytes derived from Pparα+/+ but not Pparα−/− mice (dashed versus solid lines). Consistent with decreased insulin secretion as a result of enhanced fatty acid oxidation in β-cells upon Pparα activation (28, 29), C-peptide levels were ~4–5-fold lower in Pparα+/+ mice fed with a Wy14,643-supplemented diet compared with Chow-fed (Table 1). In contrast, supplementing chow with Wy14,643 did not affect C-peptide levels in Pparα−/− mutants (Table 1). Furthermore, insulin release in response to glucose is also markedly reduced in Wy14,643-fed by comparison to chow-fed wild-type mice but
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TABLE 1
Effect of Wy14,643 on biochemical parameters
Male mice (n > 8) were fed either RD alone or supplemented with Wy14,643 (Wy) for 7 days. Blood was drawn from overnight-fasted mice to measure blood glucose as well as plasma C-peptide/insulin molar ratio (insulin clearance). Values are expressed as the mean ± S.E.

| Biochemical parameters | Pparaα+/+ | Pparaα−/− |
|------------------------|-----------|-----------|
|                        | RD        | Wy        | RD        | Wy        |
| Plasma insulin (pm)    | 44.2 ± 1.3| 37.2 ± 1.5| 43.2 ± 1.5| 45.0 ± 1.0|
| Plasma C-peptide (pm)  | 47.0 ± 7.5| 106 ± 14* | 337 ± 44  | 391 ± 17  |
| C-peptide/insulin      | 10.2 ± 2.9| 3.5 ± 0.8*| 3.8 ± 1.3 | 9.2 ± 0.7 |
| Fasting glucose (mg/dl)| 90.0 ± 11.0| 82.1 ± 9.9| 85.2 ± 4.2| 83.3 ± 3.2|

*p < 0.05 vs. RD in each mouse group.

A. Western blot

B. [125I] Insulin internalization

FIGURE 5. Effect of PPARα activation on hepatocytic insulin uptake. Primary hepatocytes were isolated from mice and treated with either ethanol (Veh) or Wy14,463 (Wy) as above. A, cell lysates were subjected to Western analysis (ib) with α-CEACAM1 antibody (α-CC1) followed by reimmunoblotting (relb) with α-GAPDH antibody to examine CEACAM1 protein content normalized to GAPDH. AU, arbitrary units. B, [125I]Insulin internalization was determined in primary hepatocytes pretreated with either ethanol (Veh; solid lines) or Wy14,463 (Wy; dashed lines) as a first step in insulin degradation and clearance. After binding, [125I]Insulin was allowed to internalize at 37 °C for 0–90 min (horizontal axis). Internalized ligand was plotted on the vertical axis as the percent of specifically bound ligand. Experiments were done in triplicate. Values are expressed as the mean ± S.D. The graph is representative of more than three experiments.

remained unaffected in the mutants (Fig. 6A; dashed versus solid lines). Given the observed lowering effect of Ppara activation on insulin secretion in response to glucose, it is likely that CEACAM1-dependent insulin clearance is reduced by Ppara activation to compensate for the diminished insulin secretion and limit insulin deficiency, as assessed by mildly reduced plasma insulin levels in Wy14,643-fed wild-type mice (Table 1). This maintains normal glucose homeostasis and insulin sensitivity, as demonstrated by normal fasting glucose levels (Table 1 and Fig. 6B) and by better tolerance to exogenous glucose (Fig. 6B, AUC 19,124 ± 1457 versus 27,290 ± 1113; p < 0.002) and insulin (Fig. 6C, AUC 9,206 ± 559 versus 13,938 ± 403, p < 0.0001) in Wy14,643-fed by comparison to RD-fed wild-type mice (dashed versus solid lines). In contrast, Wy14,643 treatment failed to affect insulin secretion (Fig. 6A), glucose tolerance (Fig. 6B, AUC 16,577 ± 858 versus 16,115 ± 784) and insulin tolerance in Pparaα−/− mice (Fig. 6C, AUC 11,738 ± 1,197 versus 11,528 ± 1,425).

Discussion

The current studies demonstrate that hepatic CEACAM1 expression is lower at fasting than refeeding. The rise in CEACAM1 expression occurs in parallel to insulin surges in the early hours of refeeding, consistent with the ability of insulin to induce Ceacam1 promoter activity (7). Whereas it is possible that minimal basal insulin contributes to low hepatic CEACAM1 levels at fasting, the current studies provide evidence that this is largely mediated by a Ppara-dependent mechanism. That Ppara activation down-regulates CEACAM1 expression at fasting is demonstrated by 1) reduction in the promoter activity of rat and mouse Ceacam1 in response to Wy14,643 (Ppara agonist) in cultured hepatoma cells, 2) rapid decline of Ceacam1 mRNA and CEACAM1 protein content upon treatment of hepatoma cells with Wy14,643, 3) reduction of Ceacam1 mRNA and CEACAM1 protein levels by supplementing the diet with Wy14,643 in Pparaα+/+ but not Pparaα−/− mice, and 4) binding of liganded Ppara to Ceacam1 promoter in liver lysates derived from Pparaα+/+ but not Pparaα−/− mice. Moreover, the rapid down-regulation of...
Ceacam1 mRNA and CEACAM1 protein content by Wy14,643 in hepatoma cells suggests that the effect of Ppara activation on Ceacam1 expression in murine liver can occur independently of confounding metabolic factors. Further studies are needed to delineate the mechanisms and identify co-repressors/co-regulators of Ceacam1 expression by Ppara, but CHIP analysis suggests that liganded Ppara directly regulates Ceacam1 expression. Moreover, our observations are consistent with the reported decrease of Ceacam1 mRNA in mice treated with Ppara-selective piperidine agonists that are potent Ppara activators (30). Although Ppara is more commonly known to increase expression of genes, such as those involved in fatty acid catabolism, it has also been shown to repress the expression of many liver-specific genes involved in glucose metabolism, cell adhesion, the CYP2C family of steroid hydroxylases, and positive acute-phase response genes induced during inflammation (30, 31).

The opposing effect of Ppara and insulin on hepatic CEACAM1 expression is probably related to the well characterized role of CEACAM1 in regulating insulin action by promoting insulin clearance (5, 19, 32). Fasting promotes a shift from glycolytic to lipolytic metabolism and robust Ppara activation (16, 33). In the early hours of refeeding, Ppara maintains fatty acid β-oxidation during the stepwise metabolic recovery by insulin surges until glycogen stores are replenished (17) and glycolysis resumes (18). As summarized in Fig. 7 and reviewed in Hue and Taegtmeyer (34), long chain fatty acyl CoA is transported to the mitochondria at fasting to undergo fatty acid β-oxidation to produce acetyl-CoA followed by citrate. Inhibition of pyruvate dehydrogenase by acetyl-CoA leads to accumulation of citrate in the cytoplasm and its gradual inhibition of 6-phosphofructo-1-kinase. This elevates glucose 6-phosphate (G-6-P) levels and its inhibition of carnitine palmitoyltransferase I (CPT1) to undergo β-oxidation in the fasting-refeeding transition, as mediating by the gradual recovery of malonyl-CoA levels and its inhibition of carnitine palmitoyltransferase I activity. The current studies propose that the pulsatile release of insulin in the early hours of refeeding elevates CEACAM1 expression and its tyrosine phosphorylation (pY) by the insulin receptor (IR) to cause its binding to fatty acid synthase (FAS) and reduction of its activity, thus, contributing to the gradual increase in malonyl-CoA levels and inhibition of carnitine palmitoyltransferase I (dashed lines). In this manner, reduction of CEACAM1 expression by Ppara activation at fasting and its stimulation by insulin positions CEACAM1 to contribute to the regulation of fatty acid β-oxidation in the fasting-refeeding transition, as mediated by the coordinated action of Ppara and pulsatile insulin surges in the early hours of refeeding. LCFAcyl-CoA, long chain fatty acyl CoA.

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By demonstrating changes in CEACAM1 expression during the fasting-refeeding transition, with hepatic CEACAM1 levels being lower during fasting via a Ppara-dependent mechanism, the current studies provide in vivo evidence for the physiologic regulation of hepatic CEACAM1. Moreover, they lend further credence to the critical role of CEACAM1 in promoting normal metabolism. The relevance of this regulation can be exploited
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for therapeutic purposes by targeting this protein to prevent and/or reverse metabolic derangements.

Author Contributions—S. K. R., S. S. K., and Q. Y. A. researched the data, designed and coordinated the experiments, and wrote the manuscript. L. R., S. L. A., P. P. P., G. H., H. T. M., and B. R. M. researched the data. A. M. O. researched and analyzed the data. Y. M. S. researched, analyzed the data, and reviewed the manuscript. E. R. S. analyzed the data and discussed and reviewed the manuscript. S. M. N. was responsible for the study design, conceptualization, data analysis, results interpretation, and review of the manuscript. S. M. N. has full access to all of the data of the study and takes responsibility for the integrity and accuracy of data analysis and for the decision to submit and publish the manuscript.

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