Enhanced De Novo Lipogenesis in the Leptin-Unresponsive Pancreatic Islets of Prediabetic Zucker Diabetic Fatty Rats

Role in the Pathogenesis of Lipotoxic Diabetes

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Overaccumulation of fat in pancreatic islets of obese ZDF *fa/fa* rats is believed to cause β-cell failure and diabetes. Previously, we demonstrated that ZDF islets have an increased capacity to esterify fatty acids imported via the circulation. Here we examine the capacity of ZDF islets to synthesize fatty acids de novo. Compared with age-matched wild-type (+/+) control islets, acetyl CoA carboxylase (ACC) mRNA was fivefold and sixfold higher and fatty acid synthetase (FAS) was fourfold and sevenfold higher in prediabetic and diabetic ZDF islets, respectively. Incorporation of label from [^{14}C]glucose into lipids was 84% higher in ZDF islets and was not suppressed normally by fatty acids. Chronic hyperleptinemia, induced by adenoviral transfer of leptin cDNA, reduced ACC and FAS mRNA in +/+ islets by 93 and 80%, respectively, but did not decrease the high ACC and FAS expression in islets of *fa/fa* rats. Recombinant leptin cultured with islets isolated from +/+ rats lowered ACC and FAS expression by 66 and 47%, respectively, but had no effect in *fa/fa* islets. We conclude that de novo lipogenesis in islets is controlled by leptin and remains low in leptin-responsive islets. It is increased in leptin-insensitive *fa/fa* islets, contributing to the fat overload that leads to β-cell dysfunction and diabetes. *Diabetes* 47:1904–1908, 1998

The development of obesity requires the efficient conversion of ingested nutrients to fat. In one model of rodent obesity linked to the *fa* mutation, the Zucker rat, overexpression of lipogenic enzymes in adipocytes and liver has been reported (1–4). These enzymes include fatty acid synthetase (FAS), which catalyzes the elongation reaction that synthesizes long-chain fatty acids from acetyl CoA, and acetyl CoA carboxylase (ACC), which catalyzes the first committed step in lipogenesis (5). Because the *fa* mutation consists of a Gln 269 → Pro mutation in the extracellular domain of the leptin receptor (OB-R) (6,7), the overexpression of lipogenic enzymes in islets of *fa/fa* rats (8) is presumably the consequence of the loss of leptin action. Indeed, leptin has previously been shown to reduce ACC expression in preadipocytes (9) and islets (10).

In a subgroup of obese Zucker rats with the *fa/fa* genotype, the ZDF-drt rats, overproduction of fat is not limited to adipocytes. Nonadipocytes, such as the pancreatic islet cells, also become overloaded with triglycerides, and their fat content may reach 50 times normal (8,11). Initially, this is accompanied by reversible functional derangements, so-called lipotoxicity, but later in the course of the disease, apoptosis of β-cells occurs (11,12). The *fa/fa* islets exhibit an increased incorporation of [^{3}H]palmitate into triglycerides (8), and this is believed to be a major factor in the overaccumulation of fat given the high levels of circulating fatty acids and triglycerides that characterize obesity. However, the contribution of de novo fatty acid synthesis to the accumulation of triglycerides in the islets of ZDF rats has never been studied.

In normal islets, the expression level of FAS, the enzyme that catalyzes the production of long-chain fatty acids from acetyl CoA, is very low (13,14), but it has never been examined in the fat-laden islets of ZDF rats; ACC, by contrast, is abundantly expressed in normal islets (13), but it, too, has not been studied in ZDF islets. In this report, we compare FAS and ACC expression and activity in islets from obese ZDF *fa/fa* rats and lean wild-type (+/+) control rats. We observe that expression of both enzymes and de novo lipid synthesis are increased in islets of leptin-resistant rats and that leptin, which downregulates FAS and ACC expression in islets of normal (+/+) rats, is without effect in obese *fa/fa* rats. Resistance to the downregulating action of leptin on islet lipogenesis explains the increased de novo fatty acid synthesis and contributes to the excess fat content, the cause of lipotoxic diabetes.

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Received for publication 12 June 1998 and accepted in revised form 31 August 1998.

ACC, acetyl CoA carboxylase; FAS, fatty acid synthetase; FFA, free fatty acid; PCR, polymerase chain reaction.

RESEARCH DESIGN AND METHODS

Animals. Obese homozygous (*fa/fa*) ZDF-drt rats and lean wild-type (+/+) ZDF littermates were bred in our laboratory from [ZDF/Drt-*fa*(F10)] rats obtained from Dr. R. Peterson (University of Indiana School of Medicine, Indianapolis, IN). Genotypes were determined as described by Philips et al. (7).

Hyperleptinemic rat model. Recombinant adenoviruses containing either the rat leptin cDNA (AdCMV-leptin) or the bacterial β-galactosidase gene (AdCMV-β-gal) were prepared as previously described in detail (15). Two milliliters of AdCMV-leptin or AdCMV-β-gal containing a total of 10^{12} plaque-forming units was infused into the jugular vein of homozygous (+/+) or (*fa/fa*) ZDF rats. Animals were studied in individual metabolic cages, and food intake and body weight were measured daily. Plasma leptin was assayed using the Linco leptin assay kit

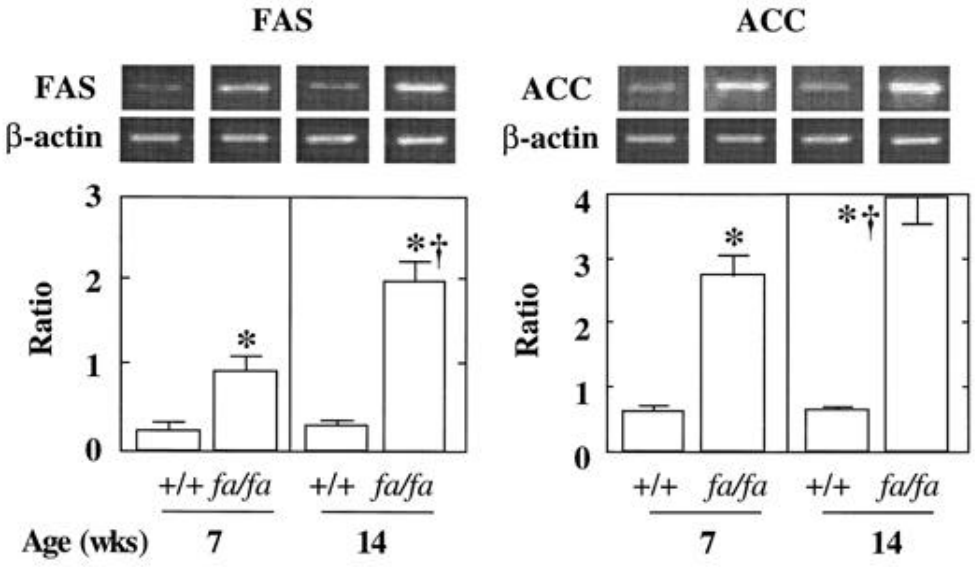

FIG. 1. Comparison of FAS:β-actin and ACC:β-actin mRNA ratios in islets isolated from 20 7-week-old (prediabetic) and 20 14-week-old (diabetic obese) ZDF (*fa/fa*) rats and 20 age-matched lean ZDF (+/+) control rats. Values are means ± SE of five experiments using aliquots from each pool. *P < 0.01 vs. +/+; †P < 0.01 vs. 7-week *fa/fa*.

(Linco Research Immunoassay, St. Charles, MO). It ranged from 18 to over 20 ng/ml (off-scale) in AdCMV-leptin–infused rats but was 3 ng/ml or less in AdCMV-β-gal–infused control rats.

Islet isolation and culture. Pancreatic islets were isolated according to the method of Naber et al. (16) and maintained in suspension culture as previously modified (17). The culture medium consisted of RPMI 1640 supplemented with 8.0 mmol/l glucose, 10% fetal bovine serum, 200 U/ml penicillin, 0.2 mg/ml streptomycin, and 2% bovine serum albumin (fraction V; Miles, St. Louis, MO) either with or without 20 ng/ml of recombinant mouse leptin (provided by Hector Beltrandelrio and Gail Yamamoto, Zymogenetics, Seattle, WA).

U-[^{14}C]glucose incorporation into islets. Incorporation of U-[^{14}C]glucose (14.6 mmol/l; New England Nuclear, Boston, MA) into lipids was measured in islets as previously described in detail (18). About 200 islets were cultured for 3 days in medium containing 8 or 16 mmol/l glucose. After 3 days in culture, lipids were extracted from the islets according to the method of Bligh and Dyer (19), and counts incorporated into total lipid were determined.

Extraction of total RNA and reverse transcriptase–polymerase chain reaction (PCR) quantitation. Total RNA was extracted by the TRIzol isolation method (Life Technologies, Gaithersburg, MD). RNA was treated with RNase-free DNase (Promega, Madison, WI), and first-strand cDNA was generated from 1 µg of RNA in a volume of 20 µl using oligo (dT) as primer and the first-strand cDNA synthesis kit (Clontech, Palo Alto, CA). Primers used to amplify FAS first-strand cDNA were 5′-GTTTGATGGCTCACACACCT-3′ (sense, 15725–15744) and 5′-TCAACCACTC GAGGCTCAG-3′ (antisense, 16602–16621) (GeneBank M84761). Those used to amplify ACC first-strand cDNA were 5′-ACTCCAGGACAGCACAGATC-3′ (sense, 4646–4645) and 5′-TCTGCCAGTCCAATTCTAGC-3′ (antisense, 5161–5180) (GeneBank J03808). As an internal control, β-actin first-strand cDNA was amplified using primers 5′-TTGTAACCAACTGGGACGATATGG-3′ (sense, 1552–1575) and 5′-GATCTTGATCTTCATGGTGCTAGG-3′ (antisense, 2967–2991) (GeneBank J00691). Linearity of the PCR reaction was tested by amplification of 200 ng per reaction from 20 to 50 cycles. The linear range was found to be between 20 and 40 cycles. In no case did the amount of total RNA used for PCR reaction exceed 200 ng per reaction. Two microliters of first-strand cDNA was amplified for 35 cycles (FAS), 28 cycles (ACC), and 25 cycles (β-actin) using the following parameters: 928C for 45 s, 558C for 45 s, and 728C for 1 min. The PCR products were electrophoresed on a 1.5% agarose gel, and Southern blotting was carried out. Radiolabeled probes for amplified fragments (5′-AAGAAGCATATGGCTTCAGCTTCAGCCTCA-3′ for FAS, 5′-ATGACATCTCGGCCATCTGGATATTCAGGGG-3′ for ACC, and 5′-GGTCAGGATCTTCATGAGGTAGTCTGTCAG-3′ for β-actin) were hybridized to the Nylon membrane and quantitated by Molecular Imager (GS-363; Bio-Rad, Hercules, CA). Levels of mRNA expression were expressed as the ratio of signal intensity for ACC and FAS mRNA relative to that for β-actin mRNA.

Statistical analyses. All results are expressed as means ± SE. Statistical significance was evaluated using one-way analysis of variance followed by Bonferroni's multiple comparison test.

RESULTS

ACC and FAS mRNA in islets of *fa/fa* and +/+ ZDF rats. To determine the level of FAS and ACC expression in the fat-laden islets of *fa/fa* ZDF rats, islets were isolated at 7 weeks of age (before the onset of diabetes) and at 14 weeks (after the onset of diabetes), and the ratios of FAS and ACC mRNA to β-actin mRNA were compared with age-matched wild-type control islets. At these age points, the ratio of FAS to β-actin mRNA was, respectively, four and seven times the controls (Fig. 1*A*) and that of ACC was five and six times the controls (Fig. 1*B*). These differences were highly significant (P < 0.01). Expression increased significantly with age in *fa/fa* ZDF rats, but in the wild-type control rats, it remained constant (Fig. 1).

De novo lipid synthesis from glucose in islets of *fa/fa* and +/+ ZDF rats. To determine whether the increased FAS expression was associated with an increase in de novo fatty acid synthesis, we cultured islets from 7-week-old rats in 8 or 16 mmol/l [^{14}C]glucose and measured incorporation of label into total lipids. At an 8 mmol/l glucose concentration, incorporation per islet was 84% higher in islets of *fa/fa* rats (P < 0.01) but was lower when expressed per DNA to correct for differences in islet size (P < 0.05). The increase in incorporation of label induced by 76 mmol/l glucose was 48% per islet and 48% per DNA in +/+ islets and 182% per islet and 183% per DNA in *fa/fa* islets (Fig. 2). These differences were highly significant (P < 0.01).

Effect of hyperleptinemia on ACC and FAS expression. The increase in FAS and ACC expression observed in the *fa/fa* rats with nonfunctioning leptin receptors could reflect a lack of leptin-mediated downregulation of expression of the enzyme. We have previously reported that, in the presence of an intact OB-R, hyperleptinemia reduces ACC expression (10). To determine whether it also reduces FAS expression, lean +/+ ZDF rats were infused with AdCMV-leptin or, as a control, AdCMV-β-gal. Infusion of AdCMV-leptin resulted in hyperleptinemia in all rats, ranging from 18 to over 20 ng/ml, as previously reported (15), whereas in AdCMV-β-gal–infused control

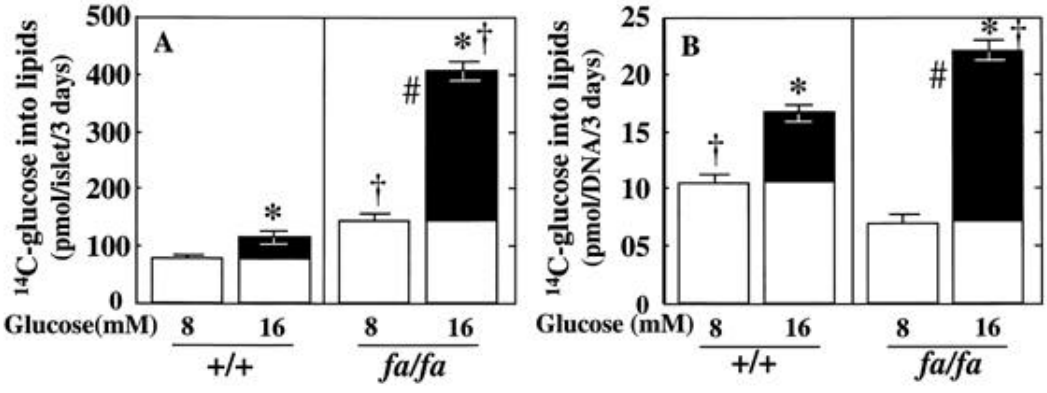

FIG. 2. Comparison of rate of incorporation of label from [^{14}C]glucose into extractable lipids in 7-week-old (prediabetic) ZDF (*fa/fa*) rats and age-matched lean ZDF (+/+) control rats expressed per islet (*A*) and per DNA (*B*). Black area is the increment in incorporation of glucose into lipids caused by the increase from 8 to 16 mmol/l glucose. *P < 0.01 vs. 8 mmol/l glucose; †P < 0.01 vs. islets of +/+ rats; #P < 0.01 vs. 16 mmol/l glucose-induced increment in +/+ islets. Values are means ± SE of three experiments in islet pools from 20 rats.

rats, plasma leptin was <3 ng/ml. In the AdCMV-leptin–infused wild-type rats, the ratios of ACC and FAS to β-actin mRNA fell to 7 and 20% of the β-gal control rats, respectively (P < 0.01) (Fig. 3). However, in obese *fa/fa* rats, whether infused with AdCMV-leptin or with AdCMV-β-gal, the ACC:β-actin and FAS:β-actin mRNA ratios remained elevated at seven and eight times that of wild-type control rats, respectively, (Fig. 3).

Effect of leptin on ACC and FAS expression by ZDF islets in vitro. We have previously reported direct lipopenic effects of leptin on islets in culture (20). The lipopenia was associated with an increase in the enzymes of fatty acid oxidation, acyl CoA oxidase and carnitine palmitoyl transferase-1, and a marked decrease in the lipogenic enzymes ACC and glycerol phosphate acyl transferase (10). To determine whether FAS is also under the direct control of leptin, we measured the ratios of ACC and FAS to β-actin mRNA in wild-type and *fa/fa* islets cultured for 3 days with 20 ng/ml of leptin. As seen in Fig. 4, there was a 66 and 47% reduction in the mRNA ratios of ACC and FAS to β-actin in islets from wild-type rats in the presence of leptin, while leptin elicited no significant reduction in mRNA of either enzyme in the islets of *fa/fa* rats.

Effect of free fatty acids (FFAs) on glucose-induced upregulation of ACC and FAS expression. Glucose stimulates de novo lipogenesis in certain tissues by upregulating ACC and FAS (21,22), while long-chain fatty acids inhibit the expression of these lipogenic enzymes, even in the presence of an elevated glucose level. To determine whether these functions require a normal leptin receptor, we compared the effect of 16 mmol/l glucose in the presence or absence of 2 mmol/l FFA on the mRNA of ACC and FAS. Both enzymes were upregulated by 16 mmol/l glucose in +/+ and

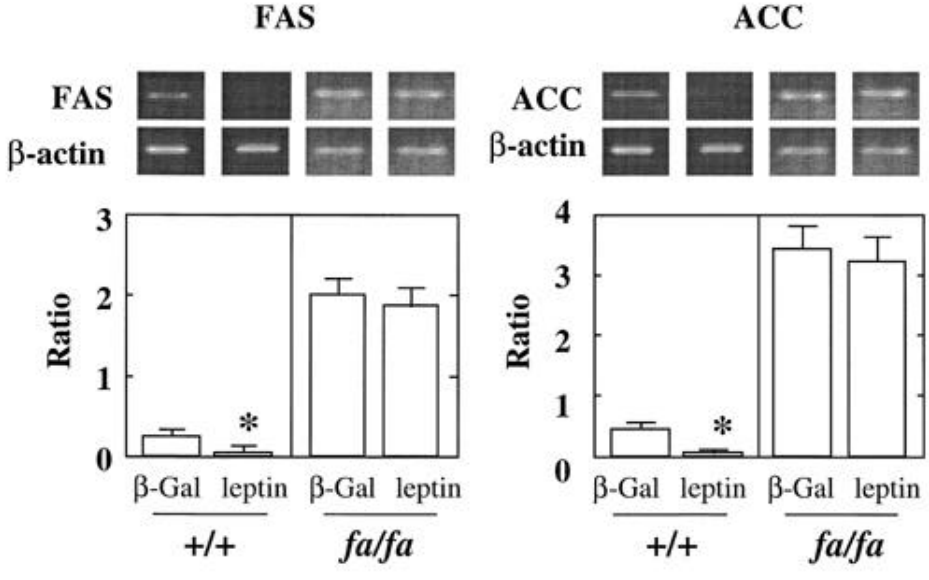

FIG. 3. Comparison of the effect of hyperleptinemia caused by AdCMV-leptin infusion on FAS:β-actin and ACC:β-actin mRNA ratios in five 9-week-old lean ZDF (+/+) rats and five age-matched obese ZDF (*fa/fa*) rats. AdCMV-β-gal–infused rats were used as controls. Values are means ± SE of five islet samples. Each islet sample was obtained from a single rat. *P < 0.01 vs. β-gal.

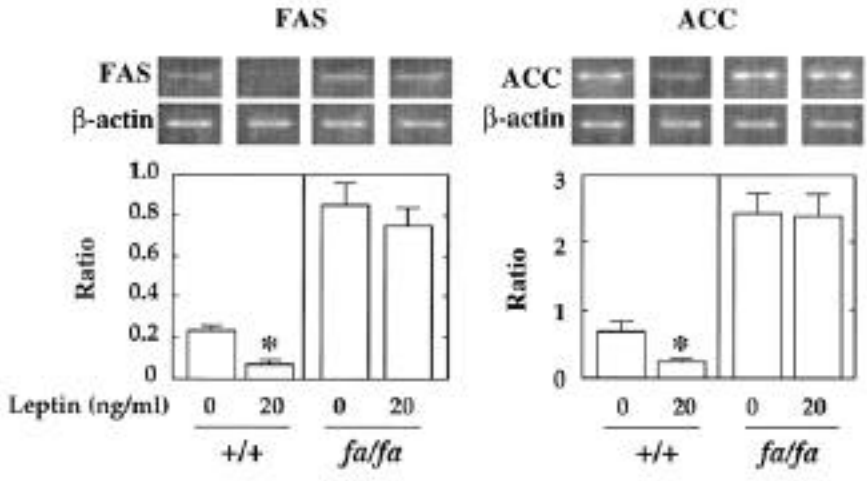

FIG. 4. In vitro effect of recombinant leptin (20 ng/ml) on FAS:β-actin and ACC:β-actin mRNA ratios in cultured islets isolated from 20 7-week-old lean ZDF (+/+) rats and 20 age-matched obese ZDF (*fa/fa*) rats. Values are means ± SE of five experiments using islets from the two pools of islets. *P < 0.05 vs. 0 leptin.

fa/fa islets, but the increase in FAS was 10-fold in the *fa/fa* islets compared with 5-fold in the +/+ islets. Both 1 and 2 mmol/l FFA suppressed the 16 mmol/l glucose-induced rise in FAS mRNA by ~90% in the +/+ islets but only by ~20% in the *fa/fa* islets (Fig. 5). However, 0.5 mmol/l FFA had no significant suppressive effect (data not shown). While the mechanism is unclear, it should be pointed out that the percent increase in intracellular FFA in the normal islets may have been greater than in the fat-laden *fa/fa* islets, perhaps contributing to the greater suppression. Glucose increased ACC mRNA 4.5-fold, and in +/+ islets this was completely suppressed by FFA. In *fa/fa* islets, glucose increased ACC mRNA by more than 3.5-fold, and FFA suppressed it by only 40% (Fig. 5). These findings strongly imply that in *fa/fa* islets, de novo fatty acid synthesis continues inappropriately even though fatty acids are present in abundance.

DISCUSSION

The present results extend our earlier work (8), which demonstrated that the overaccumulation of fat in the islets of obese ZDF rats homozygous for a leptin receptor mutation is associated with an enhanced capacity to esterify long-chain fatty acids. The present study shows that de novo fatty acid synthesis also occurs in islets of ZDF rats and contributes to islet fat content. We attribute this to increased expression of ACC and FAS, the enzymes that catalyze, respectively, the formation of malonyl CoA (5), and the elongation reaction that produces long-chain fatty acids from acetyl CoA. We confirm the earlier work of Brun et al (13) reporting that in normal islets this enzyme is expressed at very low levels. However, in islets from 7-week-old prediabetic and 14-week-old diabetic ZDF rats, the FAS:β-actin mRNA ratio was four and seven times higher, respectively, than it was in islets from +/+

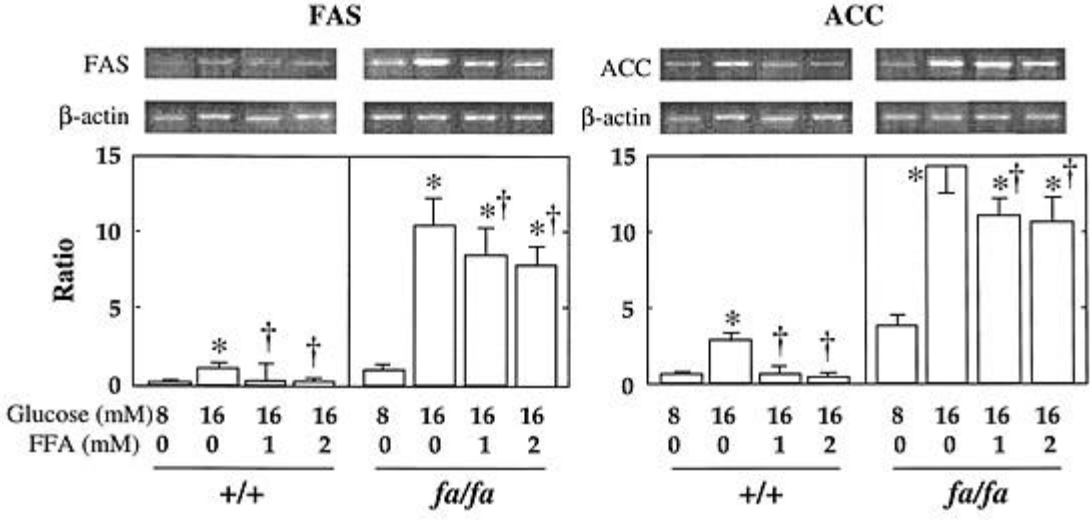

FIG. 5. Effect of a 1 or 2 mmol/l 2:1 oleate:palmitate mixture of FFAs on glucose-induced FAS and ACC expression in cultured islets isolated from 20 lean ZDF (+/+) and 20 age-matched obese ZDF (*fa/fa*) rats determined as incorporation of U-[^{14}C]glucose into lipids. Values are means ± SE of three samples. *P < 0.05 vs. 8 mmol/l glucose; †P < 0.05 vs. 0 mmol/l FFA, 16 mmol/l glucose.

control rats. Additionally, the expression of this enzyme in islets, like other enzymes of fatty acid metabolism (10), is under the direct control of leptin; the presence of 20 ng/ml of leptin in the culture medium reduced both the FAS:β-actin and ACC:β-actin mRNA ratios in islets isolated from normal rats, thus simulating the in vivo effects of hyperleptinemia. By contrast, in islets from *fa/fa* rats homozygous for the mutated leptin receptor, leptin had no in vivo or in vitro effect on the expression of these enzymes. Thus, suppression of the enzymes that synthesize glucose from acetyl CoA requires the downregulatory action of leptin, and these enzymes are high in islets of *fa/fa* rats that do not respond to leptin. It is clearly the result of the leptin unresponsiveness rather than the nonspecific effect of obesity, since FAS expression, de novo fatty triglyceride synthesis (Y.-T.Z., S. Kalra, R.H.U., unpublished observations), and triglyceride content (23) are all normal in islets of rats made obese by ablation of the ventromedial hypothalamus.

The increment in de novo synthesis of lipid from U-[^{14}C]glucose induced by the increase from 8 to 16 mmol/l glucose was significantly greater in *fa/fa* islets than in +/+ islets, whether expressed on a per islet or per DNA basis. However, in this model under the conditions examined, the rate of triglyceride formation from glucose may not have been maximal; the contribution of de novo synthesis might have been even higher had the rats been receiving a high-carbohydrate low-fat diet (24).

The overaccumulation of fat within the islets is associated with a reduced ability of β-cells to secrete insulin in response to both fatty acids and glucose. Normal responsiveness of insulin secretion to a given nutrient signal requires that the β-cells not be overloaded with that nutrient and/or its metabolic products. Just as glycogen excess blunts glucose signaling (25), fat excess blunts signaling by fatty acids. Leptin protects normal β-cells from fat overload by inhibiting lipogenesis and stimulating fatty acid oxidation. In leptin-resistant islets of ZDF rats, this protection is lost, fat accumulates, and β-cells become unresponsive to fatty acids (26). When leptin responsiveness is restored by overexpressing the wild-type leptin receptor in ZDF islets, the stored fat is rapidly metabolized and the insulin response to fatty acids is restored (26). In other words, leptin protects leptin-responsive islets from fat overload.

ACKNOWLEDGMENTS

This work was supported by Veterans Administration Institutional Research Support Grant SMI 821-109, National Institutes of Health (NIH) Grant DK-02700-37, NIH/Juvenile Diabetes Foundation International Diabetes Interdisciplinary Research Program, and grants from Novo Nordisk (Copenhagen) and Sankyo (Tokyo) (to R.H.U.). Animal care was in accordance with institutional guidelines. We would also like to thank Tess Perico for excellent secretarial support and Kay McCorkle for outstanding technical support.

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