Inhibition of ADRP prevents diet-induced insulin resistance

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Varela GM, Antwi DA, Dhir R, Yin X, Singhal NS, Graham MJ, Crooke RM, Ahima RS. Inhibition of ADRP prevents diet-induced insulin resistance. Am J Physiol Gastrointest Liver Physiol 295: G621–G628, 2008. First published July 31, 2008; doi:10.1152/ajpgi.90204.2008.—Diets with high fat content induce steatosis, insulin resistance, and type 2 diabetes. The lipid droplet protein adipose differentiation-related protein (ADRP) mediates hepatic steatosis, but whether this affects insulin action in the liver or peripheral organs in diet-induced obesity is uncertain. We fed C57BL/6J mice a high-fat diet and simultaneously treated them with an antisense oligonucleotide (ASO) against ADRP for 4 wk. Glucose homeostasis was assessed with clamp and tracer techniques. ADRP ASO decreased the levels of triglycerides and diacylglycerol in the liver, but fatty acids, long-chain fatty acyl CoA, ceramides, and cholesterol were unchanged. Insulin action in the liver was enhanced after ADRP ASO treatment, whereas muscle and adipose tissue were not affected. ADRP ASO increased the phosphorylation of insulin receptor substrate 1 (IRS1), IRS2, and Akt, and decreased gluconeogenic enzymes and PKCε, consistent with its insulin-sensitizing action. These results demonstrate an important role for ADRP in the pathogenesis of diet-induced insulin resistance.

THE WORLDWIDE EPIDEMICS of obesity and type 2 diabetes have spurred efforts to develop new drugs (23). Hepatic insulin resistance contributes to fasting and postprandial hyperglycemia (6). Adipokines, inflammation, and endoplasmic reticulum stress have all been implicated in the pathogenesis of hepatic insulin resistance and progression to diabetes (1, 23). Studies have also shown an association between hepatic steatosis and insulin resistance (10, 11). The lipid droplet protein adipose differentiation-related protein (ADRP) is expressed abundantly in the liver (2, 12) and increases with lipid accumulation in hepatocytes (4, 13, 21). Overexpression of ADRP increases the number and size of lipid droplets in hepatocytes, while ablation of the Adpr gene decreases hepatic lipids (3, 11). We have demonstrated (10) that reducing the levels of ADRP with antisense oligonucleotides (ASOs) reversed fatty liver and hypertriglyceridemia in leptin-deficient (Lepob/ob) mice. In the present study, we sought to clarify the role of ADRP in glucose metabolism in a mouse model closely resembling the pathogenesis of type 2 diabetes in humans. When fed a high-fat (Western) diet, C57BL/6J mice become insulin resistant, develop steatosis, and eventually progress to type 2 diabetes (25). Diacylglycerol, fatty acids, and activation of protein kinase C (PKC) have been suggested as putative mediators of insulin resistance associated with excessive lipid accumulation in the liver and various organs (18). The aim of this study was to determine whether reducing steatosis by preventing the expression of ADRP in diet-induced obese mice would benefit insulin action in the liver and peripheral organs.

MATERIALS AND METHODS

Animals and ASO treatment. Experiments were performed according to protocols reviewed and approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania School of Medicine. Eight-week-old wild-type male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were housed (n = 5 per cage) under a 12:12-h light-dark cycle (light on at 0700) and an ambient temperature of 22°C and allowed free access to water and food. The mice were fed a high-fat diet (Research Diets, New Brunswick, NJ; catalog no. D12451, containing 45% fat, 35% carbohydrate, 20% protein, 4.7 kcal/g) (27) at the same time the ASO treatment was initiated and remained on the diet throughout the duration of the study. The specificity of ADRP and control ASOs has been described previously (10). ASOs were injected intraperitoneally with sterile-filtered saline (50 mg/kg ip twice weekly) for 4 wk. We have demonstrated (10) that this dose decreases hepatic triglycerides without inducing toxicity. The control ASO has no effect on hepatic lipids, similar to saline vehicle (10). Control mice on regular chow diet (LabDiet, Richmond, IN, catalog no. 5001, containing 4.5% fat, 49.9% carbohydrate, 23.4% protein; 4 kcal/g) were also studied. Food intake was measured weekly and body weight twice weekly. Tail blood glucose was measured weekly with a glucometer (One Touch Ultra, Johnson & Johnson) (10, 15, 27).

To assess very low-density lipoprotein (VLDL) secretion from the liver, the mice were fasted for 4 h (0800–1200) after the third week of ASO treatment and received 1 g/kg Poloxamer-407 intraperitoneally. Tail blood was drawn at time 0 and 1 and 4 h later, and serum was prepared for triglyceride measurement. The detergent Poloxamer-407 inhibits lipases and prevents clearance of triglycerides (10, 13). Body composition was measured in conscious mice with magnetic resonance (NMR) (Echo Medical Systems, Houston, TX) after the fourth week of treatment. The mice were then deprived of food for 3 h (0900–1300) and euthanized via carbon dioxide inhalation, and tissues were harvested for chemistry and analysis of genes involved in lipid metabolism.

Energy homeostasis. The effects of ADRP ASO versus control ASO treatment were assessed in another cohort of mice on a high-fat diet with a Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH) (27, 28). After the seventh dose of ADRP ASO or control ASO, the mice were habituated in single cages for 3 days in 12:12-h light-dark cycles and ambient temperature of 22°C and allowed free access to high-fat diet and

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water, after which oxygen consumption ($V_{O_2}$) and carbon dioxide production ($V_{CO_2}$) were measured for 2 days with an airflow of 500 ml/min and a sample flow of 400 ml/min. The respiratory quotient ($RQ = V_{CO_2}/V_{O_2}$) is an index of fuel oxidation. Food intake and locomotor activity (photobeam breaks) were measured simultaneously. Body composition was measured after metabolic testing.

**Hyperinsulinemic-euglycemic clamp.** Glucose homeostasis was examined in mice treated with ADRP ASO or control ASO for 4 wk with hyperinsulinemic-euglycemic clamp (14). An indwelling catheter was inserted in the right internal jugular vein and extended to the right atrium. Four days after recovery, the mice were fasted for 4 h, placed in restrainers, and administered a bolus injection of 5 $\mu$g of [3-$^3$H]glucose followed by continuous intravenous infusion at 0.05 $\mu$Ci/min. Baseline glucose kinetics was measured for 60 min, followed by hyperinsulinemic clamp for 120 min. A priming dose of regular insulin (16 $\mu$g/kg, Humulin; Eli Lilly, Indianapolis, IN) was given intravenously, followed by continuous infusion at 2.5 $\mu$g $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$. Blood glucose was maintained at 120–140 mg/dl via a variable infusion rate of 20% glucose. 2-Deoxy-D-$[1-14C]$glucose (10 $\mu$Ci) was injected 45 min before the end of the clamp, and blood samples were collected to estimate glucose uptake. The mice were euthanized, and liver, perigonadal white adipose tissue (WAT), brown adipose tissue (BAT), and soleus muscle were excised, frozen immediately in liquid nitrogen, and stored at $-80^\circ$C for subsequent analysis of glucose uptake (14, 16). Liver samples were processed for immunoblotting of Akt, phospho-Akt, and PKC$\varepsilon$ and for immunoprecipitation of insulin receptor substrate (IRS1) and IRS2. RNA was also extracted from liver samples for measurement of glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), and PKC$\varepsilon$ by real-time PCR.

**Tissue chemistry.** Mice on normal chow or high-fat diet were euthanized via CO$_2$ inhalation after ASO treatment, blood was obtained via cardiac puncture, and liver samples were rapidly dissected, frozen in liquid nitrogen, and stored at $-80^\circ$C. Serum triglyceride, nonesterified fatty acids (NEFA), cholesterol, and $\beta$-hydroxybutyric acid levels were measured by enzymatic assays (15, 28). Insulin, leptin, and adiponectin were measured with immunoassays (Linco Research, St. Charles, MO) (10). Hepatic lipids were extracted and analyzed by the Mouse Metabolic Phenotyping Center (MMPC) at Vanderbilt University School of Medicine. Ceramides were measured by Dr. Scott Summers at the University of Utah (24), and long-chain fatty acyl-CoAs (LCFA-CoAs) were measured by the Yale Medical School MMPC (29). Other liver samples were processed for RNA extraction, and the expression of ADRP, TIP-47, and genes involved in hepatic lipid metabolism was measured with quantitative real-time PCR as previously described (10). Epididymal WAT was excised and weighed, RNA was extracted, and the expression of ADRP, perilipin, and TIP-47 mRNA levels was measured with quantitative real-time PCR (10).

**Immunoblotting.** Liver samples were homogenized in lysis buffer containing (in mM) 50 Tris-HCl (pH 7.4), 250 mannitol, 50 NaF, 1 sodium pyrophosphate, 1 benzamidine, and 1 phenylmethylsulfonyl fluoride with 0.5% (wt/vol) Triton X-100, supplemented with complete protein inhibition cocktail tablet from Roche (Penzberg, Germany). Protein extracts were separated by 4–12% NuPAGE Bis-Tris gel (Invitrogen) and transferred to nitrocellulose membranes with semidry transfer cells (Bio-Rad Laboratories, Hercules, CA). After 1 h of blocking with Tris-buffered saline with 0.1% (vol/vol) Tween 20 containing 3% (wt/vol) nonfat dried milk, membranes were incubated with a polyclonal antibody against ADRP (Fitzgerald, Concord, MA), TIP-47 (Progen, Germany), PKC$\varepsilon$ (Santa Cruz Biotechnology), or glyceralddehyde-3-phosphate dehydrogenase (GAPDH) (Cell Signaling). Akt and phospho-Akt were blotted as described previously (15). The signals were detected with enhanced chemiluminescence (ECL, Amersham), and film autoradiograms were analyzed with laser densitometry and Image J (National Institutes of Health).

Liver lysates from mice subjected to insulin clamp were immunoprecipitated with antibodies against insulin receptor substrates (IRS1 and IRS2) (Santa Cruz Biotechnology) for 2 h at $4^\circ$C. The immune complex was incubated overnight with protein A Sepharose beads (GE Healthcare Biosciences), washed twice with washing buffer (1% Triton X-100, 0.1% SDS, 50 mM HEPES pH 7.8, 150 mM NaCl), and boiled for 5 min with 5% $\beta$-mercaptoethanol in Laemmli buffer (Bio-Rad Laboratories). Twenty-five microliters of solubilized proteins was loaded into a 12% SDS-HEPES gel (Pierce Biotechnology). The immunoblot was performed with antibodies against phospho-IRS1, phosphatidylinositol 3-kinase (PI3-kinase) p85 subunit (Santa Cruz Biotechnology), and pY-4G10 (Upstate Biotechnology) to detect IRS2.

**Histology.** Liver samples were fixed in 10% buffered formalin overnight, washed with 1× PBS, incubated with 30% sucrose, and frozen with Optimal Control Temperature compound. Liver sections were stained with hematoxylin and eosin (H&E) and analyzed with an ImageScope system (Aperio Technologies, Inc.)

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![Fig. 1. Effects of adipose differentiation-related protein (ADRP) antisense oligonucleotide (ASO) vs. control ASO (C-ASO) on hepatic ADRP mRNA (A) and protein (B) levels. Data are means ± SE; n = 5, *P < 0.001 vs. normal chow (NC).](image-url)
were stained with Oil Red O to visualize neutral lipids. Other liver sections were stained for ADRP immunofluorescence (10). The slides were examined under brightfield and fluorescence microscopy (Nikon E600), and images were captured with a Cool Snap CF digital camera (BD Biosciences Bioimaging, Rockville, MD).

Statistics. Changes in various parameters were analyzed with ANOVA, and differences between treatment groups were determined with Fisher paired least significant difference test; \( P < 0.05 \) was considered significant.

RESULTS

ADRP ASO treatment decreases hepatic triglyceride and diacylglycerol levels. ADRP ASO treatment prevented the rise in hepatic ADRP mRNA and protein levels associated with a high-fat diet (Fig. 1). As we previously reported (10), ADRP ASO did not affect the mRNA or protein levels of TIP-47 in liver (data not shown). Histological examination revealed a marked reduction in hepatic steatosis after ADRP ASO treatment (Fig. 2, A–C). This was associated with significant decreases in the levels of triglycerides and diacylglycerol (Fig. 2, D and E). On the other hand, ADRP ASO did not affect the levels of free fatty acids, LCFACoAs, ceramide, or cholesterol in the liver (Fig. 2, F–I). We assessed the effect of ADRP ASO treatment on genes involved in hepatic lipid metabolism. The mRNA expression of lipogenic genes, i.e., sterol regulatory element-binding protein (SREBP)1, acyl-CoA carboxylase (ACC), stearoyl-CoA desaturase-1 (SCD1), fatty acid synthase

A

B

C

D

E

F

G

H

I

Fig. 2. Effects of ADRP ASO vs. control ASO on hepatic lipids. A–C: comparison of Oil Red O staining on liver sections from NC (A), HF-Control ASO (B), and HF+ADRP ASO mice (C). Scale bar, 50 \( \mu \)m. D–I: ADRP ASO treatment decreased hepatic triglyceride content (D) but not diacylglycerol (E), free fatty acids (F), long-chain fatty acyl-CoAs (LCFACoAs; G), ceramide (H), and cholesterol (I). Data are means \( \pm \) SE; \( n = 5. *P < 0.01 \) vs. NC.
Table 1. Effects of ADRP ASO treatment on metabolic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC + Control ASO</th>
<th>HF + Control ASO</th>
<th>HF + ADRP-ASO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>27.66 ± 0.20</td>
<td>29.6 ± 0.92</td>
<td>27.9 ± 0.56</td>
</tr>
<tr>
<td>% Body fat</td>
<td>16.7 ± 1.06</td>
<td>26.86 ± 1.28</td>
<td>17.96 ± 1.5</td>
</tr>
<tr>
<td>Food intake, kcal/day</td>
<td>16.1 ± 2.8</td>
<td>12.4 ± 1.3</td>
<td>11.6 ± 1.9</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>139.6 ± 5.4</td>
<td>176.8 ± 5.5</td>
<td>129.8 ± 4.7</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>69.32 ± 4.1</td>
<td>62.56 ± 7.5</td>
<td>51.62 ± 2.7</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>0.5 ± 0.03</td>
<td>0.73 ± 0.07</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>β-Hydroxybutyric acid, mg/dl</td>
<td>1.38 ± 0.05</td>
<td>3.47 ± 0.45</td>
<td>2.54 ± 0.29</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>120.06 ± 9.23</td>
<td>156.8 ± 7.96</td>
<td>167.19 ± 4.03</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>8.32 ± 2.15</td>
<td>11.17 ± 1.47</td>
<td>2.23 ± 1.09</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.76 ± 0.68</td>
<td>2.25 ± 0.44</td>
<td>0.96 ± 0.92</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>11.31 ± 1.02</td>
<td>12.02 ± 0.44</td>
<td>12.78 ± 0.35</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>3.46 ± 2.68</td>
<td>7.48 ± 1.09</td>
<td>4.24 ± 0.89</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7–10. ADRP, adipose differentiation-related protein; ASO, antisense oligonucleotide; NC, normal chow; HF, high-fat diet; NEFA, nonesterified fatty acids; ALT, alanine aminotransferase. *P < 0.05 vs. NC; †P < 0.05 vs. HF + Control ASO.

(PPARα), carnitine palmitoyl acyl transferase 1α (CPT1α), and, to a lesser extent, hormone-stimulated lipase (HSL) (Fig. 3B). In contrast, the level of uncoupling protein 2 (UCP2) increased in response to ADRP ASO treatment (Fig. 3B).

ADRP ASO treatment decreases body fat and triglyceride production. ADRP ASO decreased body fat (Table 1). This was associated with reduction in leptin levels (Table 1). The serum levels of adiponectin, triglycerides, β-hydroxybutyric acid, and cholesterol did not change after ADRP ASO treatment (Table 1). However, the accumulation of serum triglycerides in response to Poloxamer was significantly attenuated by ADRP ASO treatment (Fig. 4). Serum alanine aminotransferase (ALT) activity fell after ADRP ASO treatment, arguing against hepatic toxicity (7). We further examined the effects of ADRP ASO on adipose tissue and energy balance (Table 2). ADRP ASO decreased epididymal WAT in parallel with whole body fat assessed by NMR (Table 2). We did not detect changes in the levels of ADRP, perilipin, or TIP-47 in epididymal WAT (Table 2).

Table 2. Effects of ADRP ASO treatment on energy homeostasis in mice on a high-fat diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HF + Control ASO</th>
<th>HF + ADRP-ASO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>29.6 ± 0.5</td>
<td>29.7 ± 0.6</td>
</tr>
<tr>
<td>% Body fat</td>
<td>15.9 ± 0.7</td>
<td>12.8 ± 0.14*</td>
</tr>
<tr>
<td>Epididymal WAT, g</td>
<td>0.69 ± 0.01</td>
<td>0.49 ± 0.01†</td>
</tr>
<tr>
<td>Food intake, kcal</td>
<td>4.02 ± 0.32</td>
<td>3.51 ± 1.02</td>
</tr>
<tr>
<td>Respiratory quotient</td>
<td>9.7 ± 0.7</td>
<td>10.8 ± 1.03</td>
</tr>
<tr>
<td>V̇O₂, ml·kg⁻¹·h⁻¹</td>
<td>3.708 ± 0.54</td>
<td>3.247 ± 0.57†</td>
</tr>
<tr>
<td>Locomotor activity, counts</td>
<td>4.130 ± 0.29</td>
<td>3.870 ± 0.19</td>
</tr>
<tr>
<td>mRNAs expression in WAT, relative units</td>
<td>0.79 ± 0.01</td>
<td>0.81 ± 0.01*</td>
</tr>
<tr>
<td>ADRP</td>
<td>0.01 0.7</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>Perilipin</td>
<td>619 ± 74</td>
<td>430 ± 37*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 5. WAT, white adipose tissue; V̇O₂, oxygen consumption. *P < 0.05 vs. HF + Control ASO; †P < 0.001 vs. HF + Control ASO.
ADRP ASO did not affect the total caloric intake or food consumption during the light and dark cycles (Tables 1 and 2). However, $\dot{V}O_2$ and locomotor activity fell and RQ increased during the light cycle in response to ADRP ASO treatment (Table 2).

ADRP ASO preserves hepatic insulin sensitivity in diet-induced obese mice. ADRP ASO decreased serum glucose and insulin in mice on a high-fat diet, suggesting enhanced insulin sensitivity (Table 1). Thus we performed insulin clamp and radioactive tracer kinetic studies to ascertain the effects on glucose homeostasis. Compared with a normal chow diet, the high-fat diet increased basal glucose production, but not significantly (Fig. 5A), decreased the glucose infusion rate (GIR) (Fig. 5B), and increased hepatic glucose production (HGP) (Fig. 5C). The reduced ability of insulin to suppress HGP is consistent with hepatic insulin resistance (Fig. 5D). ADRP

![Graphs and images](G625ADRP EFFECT ON HEPATIC INSULIN SENSITIVITY AJP-Gastrointest Liver Physiol • VOL 295 • SEPTEMBER 2008 • www.ajpgi.org)

Fig. 5. Effects of ADRP ASO vs. control ASO on basal glucose production (A), glucose infusion rate (GIR; B), hepatic glucose production (HGP; C), % HGP suppression (D), glucose disposal rate (Rd; E), and glucose uptake by muscle (F), white adipose tissue (WAT; G), and brown adipose tissue (BAT; H). Data are means ± SE; n = 5. *P < 0.01 vs. NC.
ASO treatment enhanced insulin sensitivity manifested by an increase in GIR and a reduction in HGP (Fig. 5, B and C). Compared with basal glucose production, the HGP suppression in response to insulin clamp was 26.3 ± 6.2% in mice on a high-fat diet, 63.1 ± 3.09% in mice on a high-fat diet treated with ADRP ASO, and 73.5 ± 4.2% in normal chow-fed mice (Fig. 5D). This demonstrates a complete prevention of hepatic insulin resistance by ADRP ASO treatment. In contrast, the glucose disposal rate (Rd) did not change after ADRP ASO treatment (Fig. 5E). This paralleled the inability of ADRP ASO treatment to increase glucose uptake in muscle, WAT, and BAT (Fig. 5, F–H).

We analyzed the changes in molecular mediators of glucose metabolism in the liver. ADRP ASO decreased the levels of key gluconeogenic enzymes, G6Pase, and, to a lesser extent, PEPCK (Fig. 6, A and B). ADRP ASO increased the tyrosine phosphorylation of IRS1 and IRS2 (Fig. 6, C and D), p85 subunit of PI3-kinase (Fig. 6E), and Akt phosphorylation (Fig. 6F). On the other hand, ADRP ASO suppressed the protein and mRNA levels of PKCε in the liver (Fig. 6, G and H).

DISCUSSION

Hepatic steatosis is strongly linked to insulin resistance and type 2 diabetes in diet-induced obesity; however, the underlying mechanisms are unclear (1). Studies have shown that a high-fat diet disrupts the ability of insulin to phosphorylate IRS1 and IRS2, PI3-kinase, and Akt (18, 22, 29), thereby stimulating gluconeogenesis and increasing hepatic glucose output (18, 20, 29). Factors thought to mediate diet-induced insulin resistance include diacylglycerol, LCFA-CoAs, ceramides, inflammatory cytokines, and PKC (18–20).

For the first time, we show that inhibition of a member of the family of lipid droplet proteins, ADRP, prevented hepatic insulin resistance in C57BL/6J mice on a high-fat diet. ADRP ASO decreased body fat and leptin, but these changes did not result in an increase in glucose uptake in muscle or fat. We showed previously (10) that ADRP ASO decreased hepatic steatosis and increased insulin sensitivity in leptin-deficient Lepob/ob mice, implying that leptin is not required for the improvement in glucose homeostasis. In the present study, we found that the effect of ADRP ASO to enhance insulin sensi-
tivity was related to reductions in the levels of triglycerides and diacylglycerol in the liver. On the other hand, the levels of cholesterol, fatty acids, LCFACoAs, and ceramides in the liver were unchanged by ADRP ASO treatment. Hence, the insulin-sensitizing action of ADRP ASO in the liver cannot be attributed to LCFACoAs or ceramides, as suggested in other studies (9, 18).

ADRP ASO treatment resulted in suppression of lipogenic genes, i.e., SREBP1, ACC, FAS, SCD1, and DGAT2. Importantly, ADRP ASO decreased the expression of MTP concomitant with its ability to suppress VLDL production. The specific mechanism by which ADRP ASO controls hepatic lipogenesis is unknown. It is possible that ADRP ASO decreases the expression of key enzymes by interacting with SREBP1. ADRP ASO could also affect the feedback regulation of hepatic triglycerides by reducing the storage capacity of lipid droplets. ADRP ASO had discordant effects on the expression of genes implicated in fatty acid oxidation (10). PPARα and CPT1α expression were reduced while UCP2 expression was increased in response to ADRP ASO. Nonetheless, these changes were not associated with an increase in β-hydroxybutyric acid or a decrease in RQ, suggesting that fatty acid oxidation plays a minor role, if any, in the antisteatotic action of ADRP ASO.

ADRP ASO treatment decreased total body fat and the weight of epididymal WAT. Studies have shown that ASOs are capable of acting on adipose tissue after intraperitoneal administration (30), but we did not detect any changes in ADRP or other lipid droplet proteins, i.e., perilipin or TIP-47. Thus it appears that ADRP ASO mainly targeted the liver in our present model. Although we did not detect obvious effects of ADRP ASO on food intake, diurnal VO₂ and locomotor activity were reduced and RQ was increased. Thus the reduction in fat after ADRP ASO treatment is unlikely to be the result of fatty acid oxidation. A potential explanation is that ADRP ASO attenuates VLDL export from the liver, leading to a decrease in the availability of triglycerides delivered to adipose tissue.

There are important differences between our results and those of Chan and colleagues (3) in Adrp-knockout mice. In the latter, hepatic steatosis was reduced but there was no change in body fat (3). This discrepancy may be attributed in part to developmental compensation. We did not detect changes in PAT (perilipin, ADRP, TIP-47) proteins in the liver or WAT. In contrast, TIP-47 was found to be increased in Adrp-null adipocytes (26). Moreover, Adrp-null mice express an NH₂-terminally truncated but fully functional form of ADRP capable of maintaining normal milk production (17). A similar effect of truncated ADRP in the liver may account for the mild changes in lipid metabolism described previously (3).

The major finding of the present study is that reducing the levels of hepatic triglyceride and diacylglycerol via ADRP ASO treatment prevents the development of hepatic insulin resistance. Using clamp and radiotopic tracer techniques, we found that ADRP ASO specifically improved hepatic insulin response, resulting in suppression of glucose production. In agreement, ADRP ASO decreased G6Pase and PEPCK, enhanced the ability of insulin to increase the phosphorylation of IRS1, IRS2, and Akt, and stimulated the levels of p85, consistent with an increase in PI3-kinase activity (25). ADRP ASO suppressed lipogenic enzymes, e.g., SCD1, which has been linked to insulin resistance by Ntambi and colleagues (5). Conversely, ADRP ASO prevented the accumulation of PKCe in the liver. Studies by Shulman and colleagues (20) have implicated PKCe activation by diacylglycerol as a major cause of hepatic insulin resistance. Plasma membrane content of PKCe is increased in response to a high-fat diet and interferes with insulin signal transduction (18). In our model, ADRP ASO treatment decreased both PKCe protein and mRNA levels, suggesting an effect on transcription.

Together, our results demonstrate an important link between the lipid droplet protein ADRP, hepatic steatosis, and insulin resistance. We propose that a high-fat diet increases hepatic ADRP, leading to expansion of the number and size of lipid droplets in the liver. The net effect of accumulation of triglycerides and in particular diacylglycerol is to inhibit insulin signaling and stimulate hepatic glucose production via gluconeogenesis. Our data suggest that ADRP could be a target for the treatment of insulin resistance in patients with hepatic steatosis and type 2 diabetes.

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GRANTS

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