

# Overexpression of APOC1 in *obob* mice leads to hepatic steatosis and severe hepatic insulin resistance

Martin Muurling,<sup>\*,†</sup> Anita M. van den Hoek,<sup>\*,§</sup> Ronald P. Mensink,<sup>†</sup> Hanno Pijl,<sup>§</sup> Johannes A. Romijn,<sup>\*\*</sup> Louis M. Havekes,<sup>\*,§</sup> and Peter J. Voshol<sup>1,\*,\*\*</sup>

Netherlands Organization for Applied Scientific Research-Prevention and Health,<sup>\*</sup> Gaubius Laboratory, Leiden, The Netherlands; the Department of Human Biology/NUTRIM,<sup>†</sup> Maastricht University, Maastricht, The Netherlands; and the Departments of Cardiology and Internal Medicine,<sup>§</sup> and the Department of Endocrinology and Diabetes,<sup>\*\*</sup> Leiden University Medical Center, Leiden, The Netherlands

**Abstract** Obese *obob* mice with strong overexpression of the human apolipoprotein C1 (APOC1) exhibit excessive free fatty acid (FFA) and triglyceride (TG) levels and severely reduced body weight (due to the absence of subcutaneous adipose tissue) and skin abnormalities. To evaluate the effects of APOC1 overexpression on hepatic and peripheral insulin sensitivity in a less-extreme model, we generated *obob* mice with mild overexpression of APOC1 (*obob*/APOC1<sup>+/-</sup>) and performed hyperinsulinemic clamp analysis. Compared with *obob* littermates, *obob*/APOC1<sup>+/-</sup> mice showed reduced body weight (-25%) and increased plasma levels of TG (+632%), total cholesterol (+134%), FFA (+65%), glucose (+73%), and insulin (+49%). Hyperinsulinemic clamp analysis revealed severe whole-body and hepatic insulin resistance in *obob*/APOC1<sup>+/-</sup> mice and, in addition, increased hepatic uptake of FFA and hepatic TG content. Treatment of *obob*/APOC1<sup>+/-</sup> mice with rosiglitazone strongly improved whole-body insulin sensitivity as well as hepatic insulin sensitivity, despite a further increase of hepatic fatty acid (FA) uptake and a panlobular increase of hepatic TG accumulation. We conclude that overexpression of APOC1 prevents rosiglitazone-induced peripheral FA uptake leading to severe hepatic steatosis. Interestingly, despite rosiglitazone-induced hepatic steatosis, hepatic insulin sensitivity improves dramatically. We hypothesize that the different hepatic fat accumulation and/or decrease in FA intermediates has a major effect on the insulin sensitivity of the liver.—Muurling, M., A. M. van den Hoek, R. P. Mensink, H. Pijl, J. A. Romijn, L. M. Havekes, and P. J. Voshol. Overexpression of APOC1 in *obob* mice leads to hepatic steatosis and severe hepatic insulin resistance. *J. Lipid Res.* 2004. 45: 9–16.

**Supplementary key words** free fatty acid metabolism • lipid metabolism • hepatic fat accumulation • rosiglitazone • peroxisome proliferator-activated receptor- $\gamma$

The human apolipoprotein C1 (APOC1) gene is predominantly expressed in the liver and adipose tissue (1).

APOC1 is secreted as a 6.6 kDa protein in plasma, where it resides on chylomicrons, VLDLs, and HDLs (2). To elucidate the role of APOC1 in lipid metabolism, we have previously generated transgenic mice overexpressing human APOC1 (3). Overexpression of human APOC1 in these mice is found predominantly in the liver and to a lesser degree in the skin and adipose tissue (4). Homozygous overexpressing APOC1 mice have strongly elevated levels of plasma total cholesterol (TC) and triglyceride (TG) due to the inhibitory action of APOC1 on VLDL uptake via hepatic receptors, in particular the LDL receptor-related protein (LRP) (3). In addition, homozygous APOC1 mice exhibit elevated plasma free fatty acid (FFA) concentrations (4). Another striking observation was the strong reduction in body weight and adipose tissue mass in these APOC1-overexpressing mice on an *obob* background, due to diminished net uptake of FFA into adipose tissue (5).

Thus, homozygous APOC1 overexpression in mice impairs peripheral FFA metabolism and adipose tissue development, and, as a consequence, APOC1 may be involved in the pathophysiology of insulin resistance. However, these homozygous APOC1 mice are an extreme model, because subcutaneous fat is totally absent and, in addition, they exhibit severe skin abnormalities, e.g., scaly skin and hair loss (4). Therefore, to study the effect of APOC1 overexpression on tissue-specific insulin sensitivity in a less-extreme model, we used mildly APOC1-overexpressing (heterozygous) mice on an *obob* background (*obob*/APOC1<sup>+/-</sup>). In *obob* mice, mild overexpression of human APOC1 results in slightly reduced body weight. Concomitantly with increased hepatic FFA uptake in *obob*/APOC1<sup>+/-</sup> mice, we observed hepatic steatosis and severe hepatic insulin resistance. In these mice, rosiglitazone treatment restored hepatic insulin sensitivity, despite a further increase in hepatic FFA uptake and increased steatosis. Rosiglitazone

Manuscript received 6 June 2003 and in revised form 21 September 2003.

Published, JLR Papers in Press, October 1, 2003.  
DOI 10.1194/jlr.M300240JLR200

<sup>1</sup> To whom correspondence should be addressed.  
e-mail: pj.voshol@pg.tno.nl

induced a different localization of the hepatic steatosis. We hypothesize that the different hepatic fat accumulation and/or decrease in fatty acid (FA) intermediates has a major effect on the insulin sensitivity of the liver.

## MATERIALS AND METHODS

### Animals

Transgenic mice with high expression of human APOC1 in the liver (line 11/1) were previously generated in our laboratory (3, 4) and further bred on a C57BL/6J background. Heterozygous *ob/ob* mice on a C57BL/6J background, obtained from the Jackson Laboratories, were intercrossed with heterozygous APOC1 transgenic mice (APOC1<sup>+/-</sup>) to obtain wild-type and APOC1<sup>+/-</sup> mice on an *obob* background (*obob* and *obob/APOC1<sup>+/-</sup>*, respectively). Mice were genotyped by PCR procedure and housed in a temperature-controlled room on a 12 h light/dark cycle with free access to water and standard mouse/rat chow [7.2 wt/wt % crude fat (corn oil), 24.4 wt/wt % crude protein, and 41.8% wt/wt carbohydrates (corn starch)] diet. At age 3–4 months, mice were housed individually. Body weight was measured weekly during the study. The study was approved by the institution's animal welfare committee, following Dutch guidelines for using laboratory animals.

### Pair-feeding experiment

Three-month-old *obob* and *obob/APOC1<sup>+/-</sup>* mice were subjected to a pair-feeding regime. During the pair-feeding period, mice received 4.0 g of chow diet during the first 4 weeks, followed by 4.5 g for 8 weeks. During the pair-feeding period, body weight was measured weekly.

### Plasma analysis

To measure plasma parameters, blood was taken from the mice by tail bleeding after a 4 h fast. The blood was collected in paroxinized tubes (to prevent hydrolysis of TGs) (6) and kept on ice. Subsequently, the samples were spun (13,000 rpm) at 4°C for 3 min, and the separated plasma was immediately assayed for TG, FFAs, ketone bodies, TC, and glucose. The remaining plasma was frozen in liquid nitrogen and stored at -20°C for later measurement of insulin.

Levels of TG (corrected for free glycerol) and TC were determined by using commercially available enzymatic kits (#2336691, Boehringer Mannheim GmbH, Mannheim, Germany; and GPO-trinder kit 337-B, Sigma, St. Louis, MO). FFA was measured enzymatically with a NEFA-C kit (Wako Chemicals GmbH, Germany). Ketone bodies were determined by measuring  $\beta$ -hydroxybutyrate, using a commercially available enzymatic kit (#310-A, Sigma Diagnostics, Inc., St. Louis, MO). Plasma glucose was determined by a commercially available kit (#315-500, Sigma Diagnostics, Inc.). Insulin levels were measured by using a radioimmunoassay kit (Sensitive Rat Insulin Assay, Linco Research Inc., St. Charles, MO).

### Hyperinsulinemic clamp analysis

Whole-body insulin sensitivity was measured by hyperinsulinemic clamp analysis. During the clamp analysis, whole-body glucose uptake and hepatic glucose production (HGP) were determined using [<sup>3</sup>H]D-glucose, (Amersham, Little Chalfont, UK). The clamp experiments were performed as described earlier (7). At the end of the hyperinsulinemic clamp analysis period (insulin infusion of 7.0 mU/kg/min), a bolus (100  $\mu$ l) of [<sup>14</sup>C]palmitate (3  $\mu$ Ci, Amersham) was given to measure tissue-specific uptake of FA. One minute after administering the [<sup>14</sup>C]palmitate bolus, blood was collected and the animal was sacrificed. Liver

and white adipose tissue (WAT) and reproductive (visceral) and subcutaneous fat pads were rapidly collected, snap frozen in liquid nitrogen, and kept at -20°C for analysis. The collected blood was used to measure plasma insulin, glucose, and FFA.

### Calculations

Glucose uptake and HGP were calculated as described by Voshol et al. (7). In short, under steady-state conditions, the rate of glucose disappearance equals the rate of glucose appearance. The latter was calculated as the ratio of the infusion rate of [<sup>3</sup>H]glucose (dpm) to the steady-state plasma [<sup>3</sup>H]glucose-specific activity (dpm/ $\mu$ mol glucose). The HGP ( $\mu$ mol/kg/min) was calculated as the difference between the rate of glucose disappearance and the rate of glucose infusion. The whole-body insulin sensitivity index was calculated as the ratio of the change in whole-body glucose uptake to the change in plasma insulin levels from basal to hyperinsulinemic conditions. The hepatic insulin sensitivity index was calculated as the ratio of the suppression of HGP during the hyperinsulinemic condition to the change in plasma insulin levels.

### Tissue homogenates

To determine the uptake of palmitate by the various tissues, tissue samples ( $\pm$ 250 mg) were homogenized in 1 ml demineralized water (demi-water). Tissue protein was measured according to the method of Lowry et al. (8), using BSA (Sigma, Deisenhofen, Germany) as standard. To determine the uptake of [<sup>14</sup>C]palmitate in liver, muscle, and WAT, lipids were extracted by a modification of the method of Bligh and Dyer (9). TLC analyses revealed that 90% of the label was in the FFA fraction. Uptake of palmitate was calculated as percent uptake by the tissue of total administered <sup>14</sup>C activity per gram tissue protein and subsequently corrected for plasma FFA levels by multiplying the uptake of palmitate by the plasma FFA levels measured during the clamp experiment.

### Tissue lipid content

Total TG and diacylglycerol (DAG) content was determined in homogenates of liver, muscle, and WAT retrieved from the mice. Lipids were extracted and separated by high-performance TLC as described previously (10). Quantification of the amounts was performed by scanning the plates and integrating the density areas using TINA<sup>®</sup> version 2.09 software (Raytest, Straubenhardt, Germany). To confirm hepatic steatosis, frozen sections (7  $\mu$ m) were made and hepatic morphology was visualized by Oil Red O staining.

### Rosiglitazone treatment

*Obob/APOC1<sup>+/-</sup>* littermates were divided into two groups that were matched for body weight. One group received normal chow, the other chow containing rosiglitazone maleate (Avandia; SmithKline Beecham plc, Brentford, UK), achieving a daily dose of  $\sim$ 3 mg/kg per mouse. During treatment, body weight was measured every week.

Plasma levels of glucose, insulin, TG, TC, FFA, and ketone bodies were measured after 5 weeks of treatment. Furthermore, whole-body glucose uptake, HGP, and tissue-specific uptake of FA were measured under hyperinsulinemic clamp conditions in mice that were treated for 5 weeks. All analytical procedures were performed as mentioned above.

### Statistical analysis

For statistical analysis, SPSS version 11 was used. The Mann-Whitney nonparametric test for two independent samples was used to define differences between the groups of mice. The criterion for significance was set at  $P < 0.05$ .

## RESULTS

### Hyperlipidemia, severe hyperglycemia, and hyperinsulinemia, but decreased body weight in *obob/APOC1* overexpressing mice

*Obob/APOC1*<sup>+/-</sup> mice were hyperlipidemic (Table 1), showing significantly increased plasma TG, TC, and FFA levels, compared with their *obob* littermates. In addition, plasma levels of glucose and insulin were significantly elevated in *obob/APOC1*<sup>+/-</sup> mice, suggesting, indeed, alterations in insulin sensitivity. As expected, rosiglitazone treatment in *obob/APOC1*<sup>+/-</sup> mice resulted in significantly decreased plasma TG levels. Rosiglitazone treatment in *obob/APOC1*<sup>+/-</sup> mice showed a dramatic normalization of the hyperglycemia, concomitant with decreased plasma insulin levels, compared with untreated littermates.

At 4 to 5 months of age, *obob/APOC1*<sup>+/-</sup> mice showed significantly lower body weight and visceral adipose tissue content compared with their *obob* littermates (Table 2). No differences were observed in subcutaneous fat pad weight between *obob/APOC1*<sup>+/-</sup> mice and their *obob* littermates. The difference in body weight between *obob* and *obob/APOC1*<sup>+/-</sup> mice was not due to altered food intake, because forced food restriction (daily intake of 4.5 g chow per day for a period of 12 weeks) resulted in the same absolute difference in body weight between *obob* and *obob/APOC1*<sup>+/-</sup> mice (Fig. 1). Rosiglitazone treatment in *obob/APOC1*<sup>+/-</sup> mice resulted in significantly increased body weights (+ 18.6 ± 2.0 g, Table 2) in 5-month-old mice.

### Severe whole-body and hepatic insulin resistance in *obob/APOC1*<sup>+/-</sup> mice

To investigate whether the severe hyperglycemia and hyperinsulinemia were due to insulin resistance, a hyperinsulinemic clamp analysis was performed. Table 3 shows plasma glucose, insulin, and FA levels determined at the end of the clamp period. Plasma glucose and insulin levels were not different between *obob/APOC1*<sup>+/-</sup> and *obob* mice during the clamp period. Rosiglitazone-treated *obob/APOC1*<sup>+/-</sup> mice showed significantly lower plasma glucose and insulin levels during the clamp period compared with untreated *obob/APOC1*<sup>+/-</sup> and *obob* control mice (Table 3). Plasma FFA concentration remained increased in untreated and rosiglitazone-treated *obob/APOC1*<sup>+/-</sup> mice

TABLE 1. Plasma levels of TG, TC, FFA, glucose, and insulin of *obob* mice, untreated *obob/APOC1*<sup>+/-</sup> mice, and ROSI-treated *obob/APOC1*<sup>+/-</sup> mice

	<i>obob</i>	<i>obob/APOC1</i>	<i>obob/APOC1</i> + ROSI
TG (mM)	0.28 ± 0.11	7.6 ± 2.6 <sup>a</sup>	3.1 ± 2.0 <sup>b</sup>
TC (mM)	4.1 ± 1.0	9.6 ± 2.9 <sup>a</sup>	10.7 ± 2.5
FFA (mM)	0.85 ± 0.13	1.4 ± 0.2 <sup>a</sup>	1.1 ± 0.1
Glucose (mM)	10.2 ± 2.8	17.6 ± 5.4 <sup>a</sup>	8.4 ± 1.0 <sup>b</sup>
Insulin (ng/ml)	19.9 ± 5.7	29.6 ± 14.9 <sup>a</sup>	5.8 ± 0.3 <sup>b</sup>

TG, triglyceride; TC, total cholesterol; FFA, free fatty acid; ROSI, rosiglitazone. Values represent mean ± SD.

<sup>a</sup> *P* < 0.05 *obob/APOC1* mice versus *obob* mice.

<sup>b</sup> *P* < 0.05 *obob/APOC1* mice versus *obob/APOC1* + ROSI mice.

TABLE 2. Body weight and subcutaneous and visceral adipose tissue content of *obob* mice, untreated *obob/APOC1*<sup>+/-</sup> mice, and ROSI-treated *obob/APOC1*<sup>+/-</sup> mice

	<i>obob</i>	<i>obob/APOC1</i>	<i>obob/APOC1</i> + ROSI
Body weight (g)	61.6 ± 6.0	46.0 ± 5.4 <sup>a</sup>	64.6 ± 5.5 <sup>b</sup>
Subcutaneous adipose tissue content (% of body weight)	11.3 ± 1.8	11.6 ± 0.9	ND
Visceral adipose tissue content (% of body weight)	12.0 ± 3.9	8.6 ± 0.9 <sup>a</sup>	ND

ND, not determined. Values represent mean ± SD.

<sup>a</sup> *P* < 0.05 *obob/APOC1* mice versus *obob* mice.

<sup>b</sup> *P* < 0.05 *obob/APOC1* + ROSI mice versus *obob/APOC1* mice.

compared with *obob* littermates. *Obob/APOC1*<sup>+/-</sup> mice showed severe whole-body insulin resistance, as indicated by the insulin sensitivity index (Fig. 2A), compared with *obob* littermates. The results of wild-type mice shown are given to confirm that *obob* and *obob/APOC1*<sup>+/-</sup> mice are insulin resistant in our hands using a similar insulin infusion protocol. Whole-body glucose uptake did not increase during hyperinsulinemia in *obob/APOC1*<sup>+/-</sup> mice compared with a 30% increase in *obob* littermates. Furthermore, *obob/APOC1*<sup>+/-</sup> mice showed complete hepatic insulin resistance, because there was no suppression of the HGP during the clamp conditions (Fig. 2B). The insulin-sensitizer rosiglitazone improved both whole-body (Fig. 2A) as well as hepatic insulin sensitivity (Fig. 2B) in *obob/APOC1*<sup>+/-</sup> mice, compared with untreated littermates.

### Increased hepatic FA uptake and hepatic lipid content in *obob/APOC1*<sup>+/-</sup> mice

To obtain more insight into a possible cause of the severe hepatic insulin resistance in *obob/APOC1*<sup>+/-</sup> mice, we measured hepatic FA uptake during the hyperinsulinemic clamp and determined hepatic TG content (Fig. 3). *Obob/APOC1*<sup>+/-</sup> mice showed a 3-fold increased hepatic FA uptake (Fig. 3A) compared with *obob* controls. This increased hepatic FA flux in *obob/APOC1*<sup>+/-</sup> mice was associated with an increased hepatic TG accumulation (Fig.

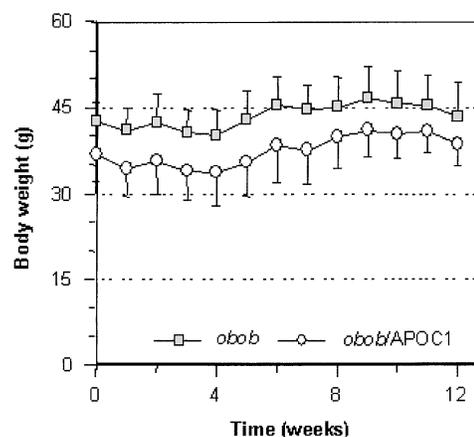


Fig. 1. Body weights of *obob* and *obob/APOC1*<sup>+/-</sup> mice during pair feeding. Both *obob* and *obob/APOC1*<sup>+/-</sup> mice received equal amounts of food for 12 weeks.

TABLE 3. Plasma insulin, glucose, and FFA levels measured at the end of the clamp period

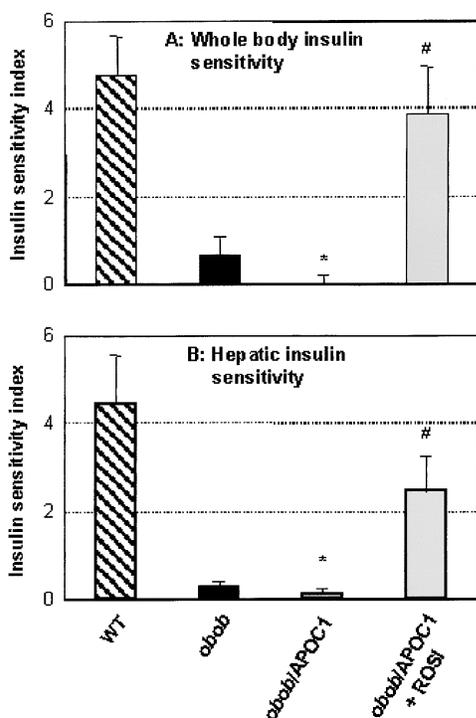
	<i>obob</i>		<i>obob/APOC1</i>	
	Chow	Chow	Chow	ROSI
Insulin (ng/ml)	41.8 ± 10.7	35.7 ± 4.2	17.5 ± 3.5 <sup>b</sup>	
Glucose (mM)	9.0 ± 2.6	17.7 ± 1.9 <sup>a</sup>	9.6 ± 1.4 <sup>b</sup>	
FFA (mM)	0.78 ± 0.24	2.0 ± 1.1 <sup>a</sup>	2.2 ± 0.16 <sup>a</sup>	

Values represent mean ± SD.

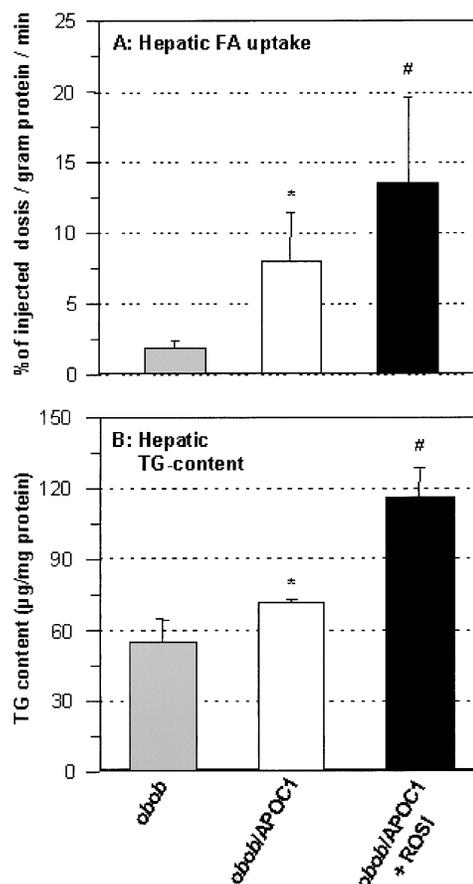
<sup>a</sup>  $P < 0.05$  *obob/APOC1* mice versus *obob* mice.

<sup>b</sup>  $P < 0.05$  ROSI versus chow.

3B), compared with *obob* littermates. DAG levels were also significantly increased in *obob/APOC1*<sup>+/-</sup> mice compared with *obob* controls ( $0.7 \pm 0.1$  µg/mg cell protein vs.  $0.5 \pm 0.1$  µg/mg cell protein, respectively;  $P < 0.05$ ). Surprisingly, although rosiglitazone treatment increased hepatic insulin sensitivity in *obob/APOC1*<sup>+/-</sup> mice, hepatic FA uptake and hepatic TG content were significantly increased, compared with untreated littermates. Hepatic DAG content did not significantly decrease with rosiglitazone treatment in *obob/APOC1*<sup>+/-</sup> mice ( $0.9 \pm 0.3$  µg/mg cell pro-



**Fig. 2.** Insulin sensitivity index. Whole-body insulin sensitivity index (A) and hepatic insulin sensitivity index (B) of overnight-fasted *obob* mice, untreated *obob/APOC1*<sup>+/-</sup> mice, and rosiglitazone (ROSI)-treated *obob/APOC1*<sup>+/-</sup> mice. Values represent mean ± SD. \*  $P < 0.05$  *obob* mice versus *obob/APOC1* mice; #  $P < 0.05$  *obob/APOC1* mice versus *obob/APOC1* + ROSI mice using the Mann-Whitney nonparametric test for two independent samples. Due to this severe whole-body and hepatic insulin resistance in the *obob/APOC1*<sup>+/-</sup> mice, no exogenous glucose infusion was needed; in comparison, glucose infusion rates in *obob* littermates were  $32.1 \pm 13.4$  µmol/kg/min versus  $66.1 \pm 11.5$  µmol/kg/min in ROSI-treated *obob/APOC1*<sup>+/-</sup> mice,  $P < 0.05$ .



**Fig. 3.** Hepatic uptake of fatty acid (FA) and triglyceride (TG) content. A: Mice were fasted overnight, and tissue-specific <sup>14</sup>C-labeled palmitate was measured under hyperinsulinemic conditions after a 4-week period during which *obob/APOC1*<sup>+/-</sup> mice were treated with ROSI. Data are means ± SD for  $n = 4$  mice per group. \*  $P < 0.05$  *obob* versus *obob/APOC1*; #  $P < 0.05$  *obob/APOC1* versus *obob/APOC1* + ROSI, using the Mann-Whitney nonparametric test for two independent samples. B: TG content was determined after a 10-week period during which *obob/APOC1*<sup>+/-</sup> mice were treated with ROSI. Data are means ± SD for  $n = 5$  per group. \*  $P < 0.05$  *obob* versus *obob/APOC1*<sup>+/-</sup>; #  $P < 0.05$  *obob/APOC1* versus *obob/APOC1* + ROSI, using the Mann-Whitney nonparametric test for two independent samples.

tein vs.  $0.7 \pm 0.1$  µg/mg cell protein). In addition, we found that plasma ketone bodies were significantly increased in *obob/APOC1*<sup>+/-</sup> mice receiving rosiglitazone treatment ( $0.65 \pm 0.17$  vs.  $0.39 \pm 0.16$  mM, respectively;  $P < 0.05$ ), compared with untreated littermates, concomitant with the increased hepatic FA flux.

## DISCUSSION

FFA and TG are involved in the pathophysiology of insulin resistance in nonadipose tissues. Overexpression of human APOC1 considerably affects whole-body FA and TG metabolism and adipose tissue formation. Strong APOC1 overexpression, as in homozygous APOC1 mice,

however, is an extreme model with complete loss of subcutaneous adipose tissue mass and severe skin complications. Thus, to study the effect of APOC1 on the pathophysiology of insulin resistance under more physiological circumstances, we studied the effects of mild, (heterozygous) overexpression of human APOC1 in *obob* mice (*obob/APOC1<sup>+/-</sup>* mice).

Compared with *obob* mice, *obob/APOC1<sup>+/-</sup>* mice show strongly elevated levels of plasma TG, cholesterol, and FFA. These observations are in line with those reported previously for homozygous APOC1-overexpressing mice on an *obob* background (5). The observed hypertriglyceridemia can be explained by APOC1-mediated inhibition of the uptake of TG-rich particles by the liver via the LDL and LRP receptors (3). In addition, Jong et al. (3) have postulated that strong APOC1 overexpression completely blocks binding of VLDL particles to the VLDL receptor, which is thought to function as a docking protein for efficient TG-rich lipoprotein lipolysis (11) and subsequent delivery of FFA to underlying tissue, such as adipose tissue, thus leading to less adipose tissue mass. The molecular mechanisms underlying the impaired FFA uptake in APOC1-overexpressing mice remains unknown at the present time. In addition to the VLDL receptor, the action of FA transporters (such as CD36 and FATP) may also be affected by APOC1. Our data indicate that APOC1 is not likely to inhibit FFA tissue uptake through interference with the FA transporter CD36. Recent studies with CD36 knockout mice showed reduced uptake of FA in heart, skeletal muscle, and adipose tissue, whereas APOC1 appears to inhibit FA uptake in WAT only (5). In addition, it is possible that APOC1, either bound to VLDL or present in a free form in plasma, is able to bind FAs, thereby preventing rapid uptake by peripheral tissues.

In the current study, we show that mild overexpression of APOC1 (*obob/APOC1<sup>+/-</sup>* mice) leads to mildly reduced body weights compared with their *obob* littermates, which is also in line with our previous study (5). In accordance with this previous study, the lower body weight observed in *obob/APOC1<sup>+/-</sup>* mice was due mainly to reduced fat pad weight. To exclude a possible interfering role of food intake in body weight control in these *obob/APOC1<sup>+/-</sup>* mice, we applied feeding restrictions to our *obob/APOC1<sup>+/-</sup>* mice and their *obob* littermates (4.5 g of chow diet per day). During the 12 weeks of feeding restrictions, the absolute body weight differences between the two genotypes remained unchanged, indicating that human APOC1 overexpression has direct effects on adipose tissue formation, independent of food intake, most probably by blocking the VLDL receptor. This hypothesis is sustained by two previous studies: *i*) Goudriaan et al. (12) showed less adipose tissue formation with high-fat feeding in VLDL receptor-deficient mice, resulting in decreased body weight compared with wild-type mice; and *ii*) net FFA uptake in adipose tissue was decreased in APOC1-overexpressing mice (5).

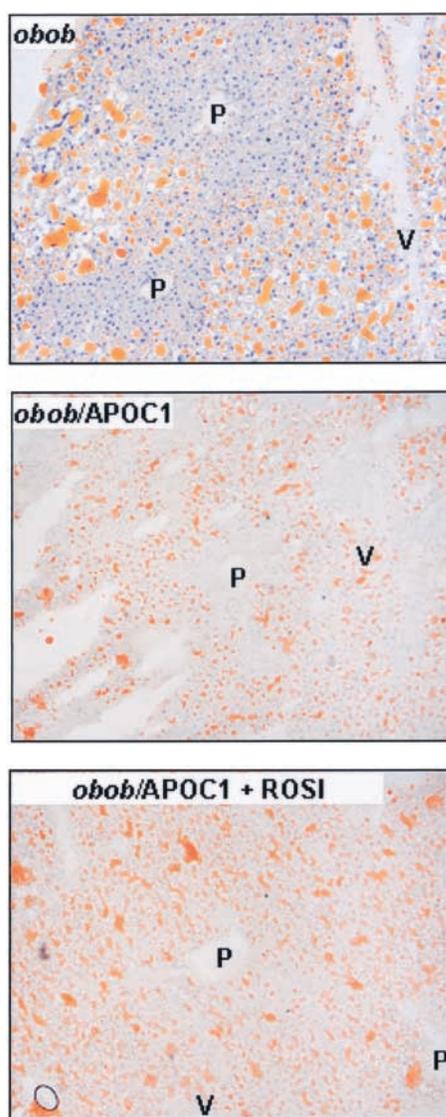
Interestingly, *obob/APOC1<sup>+/-</sup>* mice revealed severe hyperglycemia concomitant with hyperinsulinemia, two phenotypic features of severe insulin resistance and type 2 diabetes

mellitus. Hyperinsulinemic clamp studies using [<sup>3</sup>H]glucose as a tracer showed indeed severe whole-body and hepatic insulin resistance in *obob/APOC1<sup>+/-</sup>* mice compared with *obob* controls. After an overnight fast, HGP was ~45% increased in *obob/APOC1<sup>+/-</sup>* mice, compared with their *obob* littermates. Thus, both the increased HGP and the inability of insulin to suppress this HGP, in combination with the whole-body insulin resistance, fully explain the observed severe hyperglycemia in the *obob/APOC1<sup>+/-</sup>* mice. Several studies have shown that HGP is the main regulator of plasma glucose concentrations during fasting (13–15). We can only speculate on a possible mechanism underlying the link between decreased peripheral FA uptake and whole-body insulin resistance. The mild overexpression of APOC1 may have effects only on adipose tissue FA uptake ( $4 \pm 2\%$  doses vs.  $8 \pm 3\%$  doses, in *obob/APOC1<sup>+/-</sup>* mice and *obob* littermates, respectively), which seems to be confirmed by the observation that only visceral fat pad weight was decreased in *obob/APOC1<sup>+/-</sup>* mice. In fact, muscle FA uptake might not be decreased at all in these mildly overexpressing *obob/APOC1<sup>+/-</sup>* mice.

Several studies using different animal models have shown an association between hepatic insulin resistance and increased hepatic FA uptake and hepatic steatosis (16–18). Previously, we showed that APOC1 overexpression was associated with increased plasma albumin-bound FAs and diminished adipose tissue FA uptake (5). Interestingly, in the current study, we show that this reduction in adipose tissue FFA uptake leads to a significantly higher FFA flux to the liver in *obob/APOC1<sup>+/-</sup>* mice, leading to hepatic TG storage. This observation is in line with our previous finding that albumin-bound FFA is the main source of FAs for hepatic TG storage (19).

Rosiglitazone, a peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) agonist identified as being an insulin sensitizer (20), indeed strongly reduced plasma glucose levels to normoglycemic levels in *obob/APOC1<sup>+/-</sup>* mice. Furthermore, as shown in other studies using different animal models (21–23), plasma insulin and TG levels decreased upon rosiglitazone treatment in *obob/APOC1<sup>+/-</sup>* mice (22, 23). Because rosiglitazone treatment has been shown to lower hepatic TG content and increase insulin sensitivity (21), we examined whether rosiglitazone treatment could affect these parameters in *obob/APOC1<sup>+/-</sup>* mice. Despite the increased body weight in rosiglitazone-treated *obob/APOC1<sup>+/-</sup>* mice, whole-body insulin sensitivity was profoundly enhanced by rosiglitazone. Furthermore, basal HGP was reduced by ~50% in rosiglitazone-treated *obob/APOC1<sup>+/-</sup>* mice versus their untreated littermates, concomitant with the decreased plasma glucose levels in rosiglitazone-treated *obob/APOC1<sup>+/-</sup>* mice. In addition to the reduction in basal HGP, rosiglitazone also enhanced the hepatic insulin sensitivity in *obob/APOC1<sup>+/-</sup>* mice. Insulin was able to suppress the HGP in *obob/APOC1<sup>+/-</sup>* mice up to 75%, whereas untreated mice showed no suppression. This improvement in whole-body and liver insulin sensitivity in rosiglitazone-treated *obob/APOC1<sup>+/-</sup>* mice is in line with earlier observations made by others (20, 23, 24).

Surprisingly, rosiglitazone treatment in *obob*/APOC1<sup>+/-</sup> mice led to a further increase in hepatic TG storage. Because peripheral FA uptake is still impaired due to APOC1 overexpression in rosiglitazone-treated animals, an increased FA flux to the liver was seen in rosiglitazone-treated *obob*/APOC1<sup>+/-</sup> mice. The rosiglitazone-induced increase in TG content was associated with a widespread, periportal to perivenous, lipid accumulation within the liver (Fig. 4). Paradoxically, this increased steatosis was accompanied by increased hepatic insulin sensitivity, reflected by the ability of insulin to suppress HGP during the hyperinsulinemic clamp analysis. These data may imply that the rosiglitazone-induced altered hepatic zonation of TG accumulation is not associated with insulin resistance. In fact, rosiglitazone induces FA utilization, both esterification and oxidation (25), probably leading to a reduction in metabolically active FA/TG intermediates (26).



**Fig. 4.** Lipid Oil Red O staining in livers obtained from *obob* mice (upper panel), untreated *obob*/APOC1<sup>+/-</sup> mice (middle panel), and ROSI-treated *obob*/APOC1<sup>+/-</sup> (bottom panel) mice. P, periportal zone; V, perivenous zone.

Interestingly, we observed no significant decrease in hepatic DAG content in rosiglitazone-treated *obob*/APOC1<sup>+/-</sup> mice, compared with untreated animals. These data lead us to hypothesize that decreased levels of fatty acylCoAs or ceramides probably underlie the increased hepatic insulin sensitivity in rosiglitazone-treated *obob*/APOC1<sup>+/-</sup> mice.

The increase in hepatic TG content seems not to be in concordance with observations made by others in wild-type mice (23, 27). However, in line with our observations, it has been shown that rosiglitazone treatment in murine models with liver steatosis leads to further increase of the hepatic TG content (27, 28). Compared with wild-type mice, A-ZIP/F-1 mice have markedly increased hepatic PPAR- $\gamma$  mRNA levels, which increased even further upon rosiglitazone treatment (27). Furthermore, rosiglitazone treatment increased the TG content of the steatotic livers of A-ZIP/F-1 mice (27). In addition, Memon et al. (29) showed that rosiglitazone treatment leads to increased expression of adipose tissue-specific genes such as aP2 and FAT/CD36 in the liver. These effects of rosiglitazone in mouse models with steatotic livers indicate that rosiglitazone treatment elicits PPAR- $\gamma$  effects, which result in exacerbated hepatic steatosis. Whether this increase in TG content is caused by increased lipogenesis and/or lipid uptake is not clear for A-ZIP/F1 mice (27). We showed in our study that hepatic FFA uptake is increased in rosiglitazone-treated *obob*/APOC1<sup>+/-</sup> mice. Because rosiglitazone treatment in A-ZIP/F1 mice did not result in increased expression of sterol-regulatory element binding protein-1c and FAS (27), it is unlikely that lipogenesis is increased upon rosiglitazone treatment. Thus, the observed increase in hepatic TG content in rosiglitazone-treated *obob*/APOC1<sup>+/-</sup> mice is very likely due to increased FFA uptake. This is sustained by observations by Chao et al. (27) and Memon et al. (29) showing that rosiglitazone increases gene expression of aP2 and FAT/CD36, which are involved in hepatic FFA uptake.

We observed effects on insulin sensitivity in *obob*/APOC1<sup>+/-</sup> mice receiving rosiglitazone treatment, in contrast with rosiglitazone treatment in A-ZIP/F1 mice (27). The absence of effects on insulin sensitivity in A-ZIP/F1 mice might be caused by the total absence of adipose tissue. *Obob*/APOC1<sup>+/-</sup> mice have adipose tissue, although visceral adipose tissue mass was diminished as compared with their *obob* littermates. In this, *obob*/APOC1<sup>+/-</sup> mice share similarities with aP2/DTA mice, which have strongly reduced WAT. Troglitazone treatment in aP2/DTA mice results in improved insulin sensitivity (30). Therefore, Burant et al. (30) concluded that troglitazone action is independent of adipose tissue. The discrepancy in the effect of troglitazone on A-ZIP/F1 and aP2/DTA mice is caused by the small amount of adipose tissue that is present in aP2/DTA mice. This small amount of adipose tissue is apparently enough to elicit the effects of troglitazone. This line of reasoning explains the positive effect of rosiglitazone treatment on insulin sensitivity in *obob*/APOC1<sup>+/-</sup> mice. We cannot exclude that alterations in adipocyte-derived cytokines (adiponectin, resistin, leptin) might play an important role in the rosiglitazone-induced im-

provement of hepatic insulin sensitivity despite increased hepatic steatosis in *obob/APOC1<sup>+/-</sup>* mice. Plasma adiponectin levels are positively correlated with hepatic insulin sensitivity, and rosiglitazone treatment is known to increase plasma adiponectin levels (31, 32). However, recently Voshol et al. (33) showed improved hepatic insulin sensitivity even with decreased plasma adiponectin levels in hormone-sensitive lipase knockout mice.

In summary, we showed that human APOC1 overexpression in mice decreases FA flux to adipose tissue and leads to increased FA flux to the liver. This increase in liver FA flux correlates with hepatic steatosis accompanied by severe hepatic insulin resistance in the human APOC1-overexpressing mice. Interestingly, rosiglitazone, although improving hepatic insulin sensitivity, increased FA flux toward the liver, concomitant with increased hepatic steatosis. Because rosiglitazone induced a different localization of the hepatic steatosis, we hypothesize that the different hepatic fat accumulation and/or decrease in FA intermediates has a major effect on the insulin sensitivity of the liver. **FIG**

The authors are grateful to Erik Offermans for his excellent technical contribution to the hepatic histology. The research described in this paper was supported by the Netherlands Organization for Scientific Research (NWO) project 980-10-006.

## REFERENCES

- Lauer, S. J., D. Walker, N. A. Elshourbagy, C. A. Reardon, B. Levy-Wilson, and J. M. Taylor. 1988. Two copies of the human apolipoprotein C-I gene are linked closely to the apolipoprotein E gene. *J. Biol. Chem.* **263**: 7277–7286.
- Shulman, R. S., P. N. Herbert, K. Wehrly, and D. S. Fredrickson. 1975. The complete amino acid sequence of C-I (apoLp-Ser), an apolipoprotein from human very low density lipoproteins. *J. Biol. Chem.* **250**: 182–190.
- Jong, M. C., V. E. Dahlmans, P. J. van Gorp, K. W. van Dijk, M. L. Breuer, M. H. Hofker, and L. M. Havekes. 1996. In the absence of the low density lipoprotein receptor, human apolipoprotein C1 overexpression in transgenic mice inhibits the hepatic uptake of very low density lipoproteins via a receptor-associated protein-sensitive pathway. *J. Clin. Invest.* **98**: 2259–2267.
- Jong, M. C., M. J. Gijbels, V. E. Dahlmans, P. J. Gorp, S. J. Koopman, M. Ponc, M. H. Hofker, and L. M. Havekes. 1998. Hyperlipidemia and cutaneous abnormalities in transgenic mice overexpressing human apolipoprotein C1. *J. Clin. Invest.* **101**: 145–152.
- Jong, M. C., P. J. Voshol, M. Muurling, V. E. Dahlmans, J. A. Romijn, H. Pijl, and L. M. Havekes. 2001. Protection from obesity and insulin resistance in mice overexpressing human apolipoprotein C1. *Diabetes*. **50**: 2779–2785.
- Zambon, A., S. I. Hashimoto, and J. D. Brunzell. 1993. Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. *J. Lipid Res.* **34**: 1021–1028.
- Voshol, P. J., M. C. Jong, V. E. Dahlmans, D. Kratyk, S. Levak-Frank, R. Zechner, J. A. Romijn, and L. M. Havekes. 2001. In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. *Diabetes*. **50**: 2585–2590.
- Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin reagent. *J. Biol. Chem.* **193**: 265–275.
- Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Med. Sci.* **37**: 911–917.
- Havekes, L. M., E. C. de Wit, and H. M. Princen. 1987. Cellular free cholesterol in Hep G2 cells is only partially available for down-regulation of low-density-lipoprotein receptor activity. *Biochem. J.* **247**: 739–746.
- Yamamoto, T., A. Hoshino, S. Takahashi, Y. Kawarabayasi, H. Iijima, and J. Sakai. 1995. The role of the very low density lipoprotein receptor in the metabolism of plasma lipoproteins containing ApoE. *Ann. N.Y. Acad. Sci.* **748**: 217–224.
- Goudriaan, J. R., P. J. Tacke, V. E. Dahlmans, M. J. Gijbels, K. W. van Dijk, L. M. Havekes, and M. C. Jong. 2001. Protection from obesity in mice lacking the VLDL receptor. *Arterioscler. Thromb. Vasc. Biol.* **21**: 1488–1493.
- Owen, O. E., G. A. J. Reichard, M. S. Patel, and G. Boden. 1979. Energy metabolism in feasting and fasting. *Adv. Exp. Med. Biol.* **111**: 169–188.
- Tayek, J. A. 1995. Low-dose oral glyburide reduces fasting blood glucose by decreasing hepatic glucose production in healthy volunteers without increasing carbohydrate oxidation. *Am. J. Med. Sci.* **309**: 134–139.
- Moore, M. C., C. C. Connolly, and A. D. Cherrington. 1998. Auto-regulation of hepatic glucose production. *Eur. J. Endocrinol.* **138**: 240–248.
- Lam, T. K., H. Yoshii, C. A. Haber, E. Bogdanovic, L. Lam, I. G. Fantus, and A. Giacca. 2002. Free fatty acid-induced hepatic insulin resistance: a potential role for protein kinase C-delta. *Am. J. Physiol. Endocrinol. Metab.* **283**: E682–E691.
- Boden, G., P. Cheung, T. P. Stein, K. Kresge, and M. Mozzoli. 2002. FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. *Am. J. Physiol. Endocrinol. Metab.* **283**: E12–E19.
- Seppala-Lindroos, A., S. Vehkavaara, A. M. Hakkinen, T. Goto, J. Westerbacka, A. Sovijarvi, J. Halavaara, and H. Yki-Jarvinen. 2002. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J. Clin. Endocrinol. Metab.* **87**: 3023–3028.
- Teusink, B., P. J. Voshol, V. E. Dahlmans, P. C. Rensen, H. Pijl, J. A. Romijn, and L. M. Havekes. 2003. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. *Diabetes*. **52**: 614–620.
- Werner, A. L., and M. T. Travaglini. 2001. A review of rosiglitazone in type 2 diabetes mellitus. *Pharmacotherapy*. **21**: 1082–1099.
- Martin, G., K. Schoonjans, B. Staels, and J. Auwerx. 1998. PPAR-gamma activators improve glucose homeostasis by stimulating fatty acid uptake in the adipocytes. *Atherosclerosis*. **137** (Suppl): 75–80.
- Miyazaki, Y., L. Glass, C. Triplitt, M. Matsuda, K. Cusi, A. Mahankali, S. Mahankali, L. J. Mandarino, and R. A. DeFronzo. 2001. Effect of rosiglitazone on glucose and non-esterified fatty acid metabolism in Type II diabetic patients. *Diabetologia*. **44**: 2210–2219.
- Oakes, N. D., P. G. Thalen, S. M. Jacinto, and B. Ljung. 2001. Thiazolidinediones increase plasma-adipose tissue FFA exchange capacity and enhance insulin-mediated control of systemic FFA availability. *Diabetes*. **50**: 1158–1165.
- Kim, J. K., M. D. Michael, S. F. Previs, O. D. Peroni, F. Mauvais-Jarvis, S. Neschen, B. B. Kahn, C. R. Kahn, and G. I. Shulman. 2000. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. *J. Clin. Invest.* **105**: 1791–1797.
- Edvardsson, U., M. Bergstrom, M. Alexandersson, K. Bamberg, B. Ljung, and B. Dahllof. 1999. Rosiglitazone (BRL49653), a PPAR-gamma-selective agonist, causes peroxisome proliferator-like liver effects in obese mice. *J. Lipid Res.* **40**: 1177–1184.
- Ye, J. M., M. A. Iglesias, D. G. Watson, B. Ellis, L. Wood, P. B. Jensen, R. V. Sorensen, P. J. Larsen, G. J. Cooney, K. Wassermann, and E. W. Kraegen. 2003. PPARalpha/gamma ragaglitazar eliminates fatty liver and enhances insulin action in fat-fed rats in the absence of hepatomegaly. *Am. J. Physiol. Endocrinol. Metab.* **284**: E531–E540.
- Chao, L., B. Marcus-Samuels, M. M. Mason, J. Moitra, C. Vinson, E. Arioglu, O. Gavrilova, and M. L. Reitman. 2000. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J. Clin. Invest.* **106**: 1221–1228.
- Bedoucha, M., E. Atzpodien, and U. A. Boelsterli. 2001. Diabetic KKAY mice exhibit increased hepatic PPARgamma gene expression and develop hepatic steatosis upon chronic treatment with antidiabetic thiazolidinediones. *J. Hepatol.* **35**: 17–23.

29. Memon, R. A., L. H. Tecott, K. Nonogaki, A. Beigneux, A. H. Moser, C. Grunfeld, and K. R. Feingold. 2000. Up-regulation of peroxisome proliferator-activated receptors (PPAR-alpha) and PPAR-gamma messenger ribonucleic acid expression in the liver in murine obesity: troglitazone induces expression of PPAR-gamma-responsive adipose tissue-specific genes in the liver of obese diabetic mice. *Endocrinology*. **141**: 4021–4031.
30. Burant, C. F., S. Sreenan, K. Hirano, T. A. Tai, J. Lohmiller, J. Lukens, N. O. Davidson, S. Ross, and R. A. Graves. 1997. Troglitazone action is independent of adipose tissue. *J. Clin. Invest.* **100**: 2900–2908.
31. Combs, T. P., J. A. Wagner, J. Berger, T. Doebber, W. J. Wang, B. B. Zhang, M. Tanen, A. H. Berg, S. O'Rahilly, D. B. Savage, K. Chatterjee, S. Weiss, P. J. Larson, K. M. Gottesdiener, B. J. Gertz, M. J. Charron, P. E. Scherer, and D. E. Moller. 2002. Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. *Endocrinology*. **143**: 998–1007.
32. Yang, W. S., C. Y. Jeng, T. J. Wu, S. Tanaka, T. Funahashi, Y. Matsuzawa, J. P. Wang, C. L. Chen, T. Y. Tai, and L. M. Chuang. 2002. Synthetic peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. *Diabetes Care*. **25**: 376–380.
33. Voshol, P. J., G. Haemmerle, D. M. Ouwens, R. Zimmermann, R. Zechner, B. Teusink, J. A. Maassen, L. M. Havekes, and J. A. Romijn. 2003. Increased hepatic insulin sensitivity together with decreased hepatic triglyceride stores in hormone-sensitive lipase-deficient mice. *Endocrinology*. **144**: 3456–3462.