PPARα deficiency increases secretion and serum levels of apolipoprotein B-containing lipoproteins

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Abstract **This study investigates the importance of peroxisome proliferator activated receptor α (PPARα) for serum apolipoprotein B (apoB) levels and hepatic secretion of apoB-containing lipoproteins. Total serum apoB and VLDL-apoB levels were higher in female PPARα-null mice compared with female wild-type mice, but no difference was seen in male mice. Furthermore, hepatic triglyceride secretion rate, determined in vivo after Triton WR1339 injection, was 2.4-fold higher in female PPARα-null mice compared with female wild-type mice, but no difference was observed in male mice. However, when fed a high fat diet, male PPARα-null mice displayed 2-fold higher serum levels of apoB and LDL cholesterol compared with male wild-type mice, but triglyceride levels were not affected. Hepatic LDL receptor protein levels were not influenced by PPARα deficiency, gender, or the fat diet. Hepatocyte cultures from female PPARα-null mice (cultured for 4 days in serum free medium) showed 2-fold higher total apoB secretion and increased secretion of apoB-48 VLDL, as well as 2.7-fold larger accumulation of VLDL-triglycerides in the medium compared with wild-type cultures. In conclusion, PPARα-deficient female mice, but not males, display high serum apoB associated with VLDL and increased hepatic triglyceride secretion. Moreover, male PPARα-null mice show increased susceptibility to high fat diet in terms of serum apoB levels.**—Lindén, D., M. Alsterholm, H. Wennbo, and J. Oscarsson. **PPARα deficiency increases secretion and serum levels of apolipoprotein B-containing lipoproteins.** ***J. Lipid Res.*** **2001.** 42: **1831–1840.**

Supplementary key words VLDL • LDL • apolipoprotein B-48 • apolipoprotein B-100 • triglycerides • LDL receptor • hepatocytes • dietary fat • gender • cholesterol

Fibrates are widely used drugs in the treatment of hypertriglyceridemia and combined hyperlipidemia, which are conditions associated with type II diabetes. Fibrates belong to the structurally diverse group of peroxisome proliferators that induce peroxisome proliferation and hepatomegaly in rodents and up-regulate several genes involved in peroxisomal as well as mitochondrial β-oxidation and microsomal ω-oxidation of fatty acids [as reviewed in refs. (1–3)]. Fibrates have been shown to elicit their effects through peroxisome proliferator activated receptor α (PPARα) (4), a member of the steroid nuclear receptor super family. PPARα is mostly expressed in tissues that have a high ratio of fatty acid oxidation, such as liver, brown adipose tissue, skeletal muscle, heart, and kidney (5, 6). The endogenous ligands for PPARα are mono- and polyunsaturated fatty acids as well as eicosanoids (7).

The PPARα-null mouse has proved to be a valuable model for the study of the importance of PPARα for various functions in the intact animal (8). When fasted, the PPARα-null mice developed hypoglycemia, hypoketonemia, hypothermia, elevated plasma free fatty acid levels, and liver steatosis (9, 10), indicating the importance of PPARα in glucose and lipid homeostasis during fasting. The hypoglycemia was shown to be due to reduced gluconeogenesis secondary to reduced fatty acid oxidation (11). Apart from decreased gene expression of several enzymes involved in fatty acid oxidation (8, 12), PPARα-null mice have been shown to have higher serum cholesterol levels than wild-type mice (13). With age, PPARα-null mice developed elevated serum triglycerides and late onset obesity despite stabile caloric intake. The obesity and increase in plasma triglycerides were shown to be more pronounced in female PPARα-null mice, while the hepatic accumulation of triglycerides and cholesterol were more pronounced in male PPARα-null mice (14). The mechanisms for the sexually dimorphic obesity and liver steatosis are not known, but sex differences in lipoprotein metabolism may contribute.

PPARα activation by fibrates influences metabolism of both HDL and apolipoprotein B (apoB)-containing lipoproteins in rodents and man (2, 13, 15, 16). Fibrates decrease apoB-containing lipoproteins, especially large VLDL particles, but also other apoB-containing lipoproteins as well as total serum apoB levels (16). It has been suggested that an increased catabolism of VLDL and chylomicrons is impor-

Abbreviations: apoB, apolipoprotein B; apoB-48, apolipoprotein B-48; apoB-100, apolipoprotein B-100; FPLC, fast protein liquid chromatography; MTP, microsomal triglyceride transfer protein; PPARα, peroxisome proliferator activated receptor α.

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tant for the triglyceride-lowering effect of fibrates (1, 3). This effect of fibrates may be via both increased lipoprotein lipase expression and decreased hepatic apoC-III expression (1, 3, 13). However, a decreased production of apoB-containing lipoproteins by fibrates may also contribute (17). PPARα activation has been shown to decrease triglyceride synthesis and secretion from cultured rat hepatocytes (18, 19), indicating a decreased VLDL secretion. However, the importance of changed PPARα expression for the effects of endogenous ligands on serum apoB levels and apoB production is not known.

In humans, the liver secretes only apoB-100-containing VLDL, and apoB-48 secretion occurs exclusively from the intestine (20). VLDL secreted from the rodent liver contains either apoB-48 or apoB-100 as structural protein [as reviewed in refs. (21) and (22)]. Assembly and secretion of apoB-containing lipoproteins is mainly regulated via co- or post-translational degradation of the apoB. Correct folding of apoB and subsequent assembly of VLDL has been shown to be highly dependent on the availability of fatty acids and lipid biosynthesis (21–24). Thus, PPARα expression may play a role for apoB secretion although gene expression of apoB, apoE, and microsomal triglyceride transfer protein (MTP) was not influenced by PPARα deficiency (9). The aim of the present study was to investigate the role of PPARα for apoB metabolism and VLDL secretion in vivo and in vitro. We found that lack of PPARα results in hyperapobetalipoproteinemia in female mice and in fat fed male mice. Moreover, the increased VLDL secretion in female PPARα-null mice could be reproduced in vitro, indicating that this effect of PPARα deficiency was not a result of changed flux of metabolites in vivo.

METHODS

The Ethics Committee of Göteborg University has approved this study. All chemicals used were from Sigma Chemical Co. (St. Louis, MO, USA) if not stated otherwise.

Animals, diet, and serum analyses

Homozygous PPARα-null mice on pure Sv/129 genetic background and corresponding wild-type Sv/129 control mice were kindly provided by Dr. F. J. Gonzalez (National Institute of Health, Bethesda, MD, USA) and kept on the Sv/129 genetic background (8). In one high fat diet experiment, the PPARα-null mice were backcrossed to the C57BL/6 genetic background for 2 generations and littermate wild-type mice were used as controls. A PCR-based screening was used to determine the genotypes of the mice (14). In most experiments, the mice had free access to standard laboratory chow (Rat and mouse standard diet, B&K Universal Ltd, Sollentuna, Sweden). This chow contained (w/w) 2.5% fat (33% saturated fatty acids), 61% carbohydrates, including 4% fibers and 18% protein. In some experiments, the mice had free access to a high fat diet containing 57.6 energy percent (%) fat, 26.6% carbohydrates, and 15.8% protein (Bio-Serv Inc., Frenchtown, NJ, USA). The fatty acid profile of the high fat diet was as follows: oleic acid ($C_{18:1}$), 153.80 g/kg; palmitic acid (C_{16}), 93.93 g/kg; stearic acid (C_{18}), 48.39 g/kg; linoleic acid ($C_{18:2}$), 32.27 g/kg; *cis*-9-hexadecanoeic acid ($C_{16:1}$), 14.34 g/kg; myristic acid (C_{14}), 6.09 g/kg; eicosanoeic acid (C_{20}), 2.87 g/kg; and heptadecanoic acid (C_{17}), 1.793 g/kg. The mice were anesthetized with isofluran inhalation (Forene®, Abbot Scandinavia AB, Sweden) and sacrificed between 0900 and 1100 h. Blood was collected through open-heart puncture and serum was assayed for triglycerides, cholesterol, and glucose as described earlier (25). Serum apoB levels were measured with an elecro-immunoassay as previously described (26). Standardization of apoB measurements was made by isolation of a narrow density cut of LDL (d = 1.030–1.055 g/ml) containing only apoB as a protein component. The protein concentration of the narrow density cut of LDL was determined by the BIO-RAD DC protein assay (BIO-RAD, Hercules, CA). The intra- and inter-assay CV for the apoB determinations were 5% and 8%, respectively. Serum β-hydroxybutyrate was analyzed using an enzyme colorimetric assay (Sigma Chemical Co.). A rat insulin RIA with 100% cross-reactivity to mouse insulin was used for serum insulin measurements (Linco Research Inc., St. Louis, MO, USA).

Size distribution of serum lipoproteins

The size distribution profiles of lipoproteins were measured by using either fast protein liquid chromatography (FPLC) of pooled serum samples or high performance liquid chromatography system, SMART, of serum samples from individual mice. In the FPLC separation, serum from 6 to 8 mice were pooled to a total volume of 900 μl and the density adjusted to 1.215 g/ml with KBr in 0.9% NaCl. After ultracentrifugation (35,000 *g*, 4°C, 24 h), the total lipoprotein fraction was loaded on a 25 ml Superose 6B column (Pharmacia Upjohn, Uppsala, Sweden) using a constant flow rate of 0.35 ml/min. Triglyceride and cholesterol concentrations were determined in the collected 0.5 ml fractions. In the SMART separation, 10 μl serum sample from a single mouse was loaded on a Superose 6 PC 3.2/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden) and the chromatographic system was linked to an air segmented continuous flow system for on-line post-derivatization analysis of total cholesterol. The SMART-system was connected to a sample injector (Gina 50, Gynkotek HPLC, Germering, GmbH), and the elution buffer (0.01 M Tris, 0.03 M NaCl, pH 7.4) had the flow rate of 35 μl/min. The integrated area of the fractions was expressed in molar concentration. For both the FPLC and SMART systems, various peaks in the profiles are designated VLDL (0.98–1.006 g/ml), IDL + LDL (mentioned as LDL in the text) (1.006–1.063 g/ml), and HDL (1.063–1.215 g/ml) based on the elution profile of density fractions obtained by ultracentrifugations.

In vivo hepatic triglyceride secretion using Triton WR1339

Triglyceride secretion rate in vivo was measured by intravenous administration of Triton WR1339 (27). After a 4 h period without access to food (0700–1200 h), anesthetized mice were injected intravenously with Triton WR1339 diluted in saline (200 mg/ml) via the tail vein (500 mg/ kg body weight). Blood samples were taken before the injection (0 min) and 45 and 90 min after Triton WR1339 injection. The triglyceride accumulation was linear during this time period. Serum triglyceride levels were analyzed as described above, and micromole of triglycerides was calculated using published plasma volume in normal male (0.071 ml/g bodyweight) and female mice (0.09 ml/g body weight) (27). Hepatic triglyceride secretion rate was calculated from the slope of the curve and expressed as micromoles per hour per gram body weight.

ApoB mRNA quantification

Liver RNA was isolated with the Tri Reagent system (Sigma Chemical Co.). First strand cDNA was synthesized from total RNA using TaqMan® reverse transcription reagents (Applied Biosystems). Real time PCR was performed with ABI Prism 7700

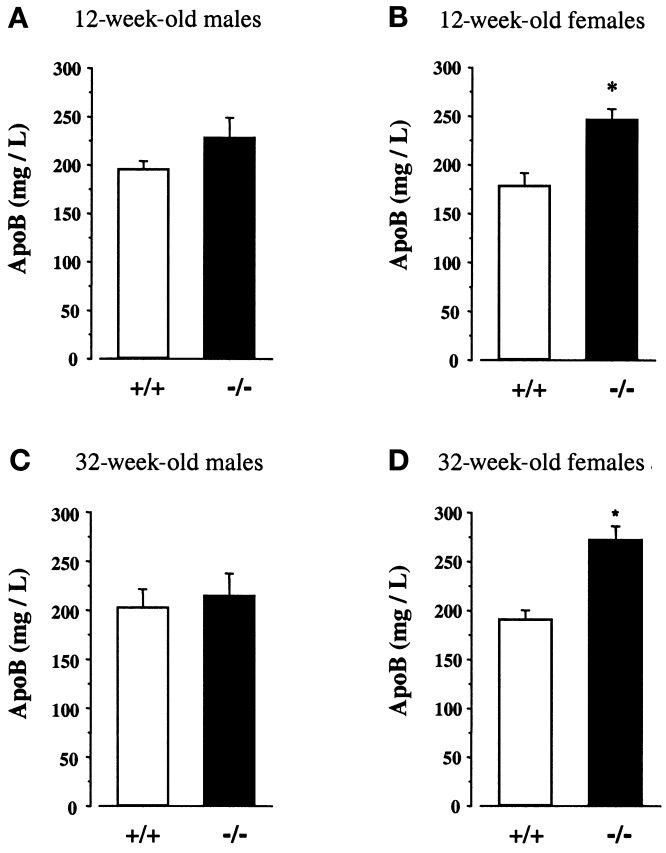

Fig. 1. Serum apoB levels in female and male PPARα-null mice and corresponding wild-type mice on Sv/129 genetic background at 12 and 32 weeks of age. Serum apoB levels were determined by an electro-immunoassay as described in Methods. There were 10 mice in each group at 12 weeks of age and 5 mice in each group at 32 weeks of age. +/+, Wild-type mice; −/−, PPARα-null mice. Values are means ± SEM (* $P < 0.05$, Student's *t*-test).

Sequence Detection System (Applied Biosystems) using FAM or VIC labeled fluorogenic probes. The apoB-specific primers amplified an 81 bp long fragment of apoB mRNA (nt 175-256, Acc. No. X15191.5), and the expression data were normalized against ribosomal 18S RNA (No. 4310843E, Applied Biosystems). The mean quantity of apoB and 18S in a sample were calculated from their respective standard curves, and data are expressed as apoB/18S.

Western blot analysis

Western blotting was performed using enhanced chemiluminescence (ECL) protocol (Amersham Pharmacia Biotech, Buckinghamshire, UK) and quantified using Fluor-S Multi imager and Quantity one software (BIO-RAD).

ApoB. In each lane, adjacent FPLC fractions were pooled in pairs (9 μl each) and separated by SDS-PAGE. After electrophoresis, the proteins were transferred to Hybond™-P Polyvinylidene difluoride (PVDF) transfer membrane (Amersham Pharmacia Biotech). The membrane was blocked overnight at 4°C in PBS containing 0.1% Tween-20 (PBS-T) and 5% nonfat milk prior to incubation with polyclonal rabbit anti-rat apoB antiserum (26) diluted 1:1,000 in PBS-T. The membrane was washed and incubated with diluted (1:2,000) horseradish peroxidase-linked donkey anti-rabbit Ig (Amersham Life Science, UK) and developed.

LDL receptor. In order to quantify the LDL receptor, livers were homogenized as described earlier (28) in the presence of 4 mM pefablock, 20 nM leupeptin, 15 nM pepstatin, and 0.15 nM aprotenin (Boehringer Mannheim, Germany). Proteins (30 μg/lane) were separated and transferred as described above, but under non-reducing conditions. Primary rabbit anti-bovine LDL receptor antibodies (a generous gift from Professor Joacim Herz, Dept. of Molecular Genetics, University of Texas, Dallas, TX, USA) and secondary horseradish peroxidase-linked donkey anti-rabbit Ig were both diluted 1:2,000 in blocking buffer.

Primary hepatocyte cultures and estimation of secreted apoB and triglycerides

Hepatocytes were prepared by a non-recirculating collagenase perfusion through the portal vein of age- and sex-matched PPARα-null and wild-type mice on pure Sv/129 genetic background as described earlier (24, 29). The cells were seeded at a density of approximately 135,000 cells/cm^2 on laminin-rich matrigel (Collaborative Research, Medical Products, Bedford, MA, USA) coated plastic 100 mm dishes (Falcon, Plymouth, England), and plated during the first 16 to 18 h in Williams E medium supplemented as described previously (29). The medium, given the following 3 days of culture, was supplemented with 1 nM dexamethasone and 3 nM insulin (Actrapid®, Novo Nordisk A/S, Denmark), and changed every day. The secretion of newly synthesized apoB-48 and apoB-100 into the medium was estimated by labeling the cells with a [^{35}S]methionine-cysteine mix (Amersham) for 2 h followed by a 4 h chase period using

TABLE 1. Serum levels of lipids and β-hydroxybutyrate in male and female PPARα-null mice

Sex	Genotype	Age	Triglycerides	Cholesterol	β-hydroxybutyrate
		Weeks	*mM*	*mM*	*mM*
Male	+/+	12	1.33 ± 0.12	3.06 ± 0.06	0.42 ± 0.07
	−/−	12	1.17 ± 0.07	3.33 ± 0.10[a]	0.25 ± 0.01[a]
	+/+	32	1.00 ± 0.07	2.68 ± 0.15	ND
	−/−	32	0.93 ± 0.05	3.10 ± 0.15	ND
Female	+/+	12	0.95 ± 0.10	2.91 ± 0.26	0.36 ± 0.07
	−/−	12	1.65 ± 0.09[a]	3.05 ± 0.20	0.37 ± 0.08
	+/+	32	1.04 ± 0.17	2.70 ± 0.29	ND
	−/−	32	2.46 ± 0.30[a]	3.20 ± 0.26	ND

The serum analyses were performed using enzymatic kits as described in Methods. The mice were on Sv/129 genetic background. For serum triglyceride and cholesterol measurements, 10 mice in each group at 12 weeks of age and 5 mice in each group at 32 weeks of age were used. For serum β-hydroxybutyrate measurements, 8 PPARα-null mice and 6 wild-type mice were used. ND = not determined. Values are means ± SEM.

[a] $P < 0.05$ vs. corresponding wild type, Student's *t*-test.

culture medium supplemented with 10 mM methionine, as described earlier (24, 30). In order to isolate the VLDL fraction of the medium, sucrose gradient ultracentrifugation was used as previously described [gradient 2, (24)]. Labeled apoB-48 and apoB-100 were isolated by immunoprecipitation as previously described (24, 30) and quantified using a Phosphoimager and Image Quant software (Molecular Dynamics, Inc., Sunnyvale, CA, USA). The obtained STORM units of apoB-48 and apoB-100 were related to the DNA content of the cells in the culture dish.

The mass of triglycerides in the VLDL fraction ($d < 1.006$ g/ml) of 24 h culture medium was determined as previously described (24, 31). The mass of triglycerides was related to the DNA content of the cells in the culture dish. The DNA content in each culture dish was determined according to the method of Labarca and Paigen (32).

Statistical analysis

Values are expressed as means ± SEM. In some experiments the mean value of the control group was set as 100%. Comparisons between groups in vivo were made by Student's *t*-test or one-way analysis of variance (ANOVA), followed by Bonferroni test. The values were transformed to logarithms when appropriate. Comparison between groups in vitro was made by Mann-Whitney U test.

RESULTS

Serum levels of apoB, triglycerides, and cholesterol

Serum apoB levels were determined in male and female PPARα-null mice and matched wild-type controls on Sv/129 genetic background given standard laboratory chow (**Fig. 1**). The serum apoB levels were not different between 12-week-old male PPARα-null mice and age-matched male wild-type mice (Fig. 1A). However, 12-week-old female PPARα-null mice had higher serum levels of apoB (+38%) compared with age-matched wild-type mice (Fig. 1B). ApoB mRNA levels were measured by real time PCR in 12-week-old male and female mice of both genotypes. No differences in apoB mRNA levels were observed between male PPARα-null and wild-type mice (apoB/18S; KO, 10.4 ± 1.5, wt, 11.1 ± 2.2, n = 5 in each group, NS, Student's *t*-test) or between female PPARα-null mice and respective wild-type mice (apoB/18S; KO, 11.7 ± 2.5, wt, 9.3 ± 1.0, n = 4–5 in each group, NS, Student's *t*-test). Because a significant difference in body weight has been observed between female PPARα-null mice and female wild-type mice at ~10 weeks of age (14), we analyzed the body fat depot weights in 12-week-old male and female mice of both genetic backgrounds. No differences in absolute or relative weights of retroperitoneal or gonadal (epididymal or parametrial) fat depots were observed between PPARα-null mice and their controls (n = 10, NS, Student's *t*-test, data not shown). At 8 months of age, PPARα-null mice have been shown to be more obese than their controls in terms of fat depot weights (14). Therefore, serum apoB levels were also measured in male and female PPARα-null mice at 32 weeks of age. At this age, there was still no difference in serum apoB levels between male PPARα-null mice and corresponding wild-type mice, but the female PPARα-null mice had higher serum apoB levels (+42%) than did their controls (Fig. 1C and D). In the same groups presented in Fig. 1, serum triglyceride and cholesterol levels were analyzed (**Table 1**). The serum triglyceride level was elevated in 12-week-old female PPARα-null mice (+74%) as compared with wild-type mice, and the difference was even larger at 32 weeks of age (+137%). However, no difference in serum triglyceride levels was observed between male PPARα-null and wild-type mice. In spite of a tendency toward higher serum cholesterol levels in all the PPARα-null mice compared with the respective control mice, a statistical difference was only obtained in young male mice (Table 1). Serum levels of β-hydroxybutyrate were measured as an indication of differences in ketone

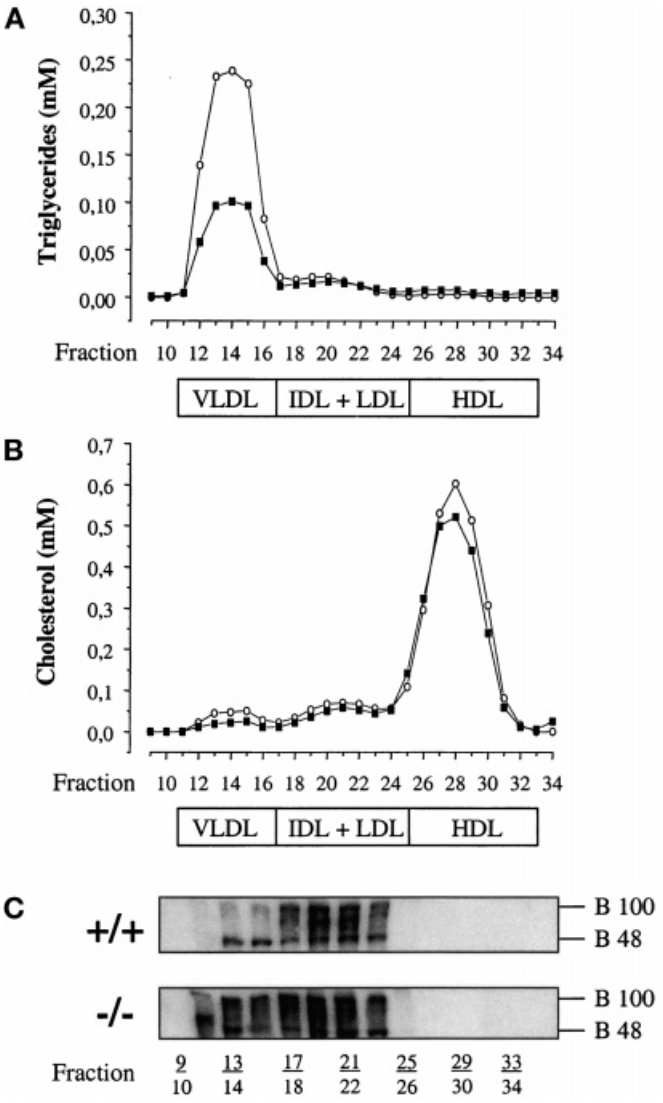

Fig. 2. Size distribution of serum lipoproteins in female PPARα-null mice and female wild-type mice. Total lipoprotein fractions ($d < 1.215$ g/ml) obtained from pooled serum from 8 PPARα-null and 6 wild-type mice on Sv/129 genetic background were subjected to FPLC as described in Methods. Panel A shows the distribution of triglycerides and panel B distribution of cholesterol in serum from PPARα-null mice (open circles) and wild-type mice (filled squares). VLDL, LDL, and HDL are indicated based on the elution profile of density fractions obtained by ultracentrifugations. Panel C shows the distribution of apoB-48 and apoB-100 as determined with Western blot analysis of the FPLC fractions.

production. Male PPARα-null mice had lower levels of β-hydroxybutyrate than did wild-type males, but no difference between the genotypes was observed in female mice (Table 1). Changes in size distribution of lipoproteins were determined by FPLC analysis of total lipoproteins ($d < 1.215$ g/ml). Female PPARα-null mice had markedly higher levels of VLDL triglycerides and slightly higher cholesterol levels in all fractions compared with female wild-type mice (**Fig. 2**). Male PPARα-null mice had slightly higher levels of VLDL triglycerides and cholesterol compared with male wild-type mice (data not shown). Female PPARα-null mice had increased levels of both apoB-100 and apoB-48 in the VLDL fraction compared with female wild-type mice (Fig. 2C). Male PPARα-null mice did not differ in apoB distribution compared with male wild-type mice (data not shown).

In vivo hepatic triglyceride secretion rate

The hepatic triglyceride secretion rate was markedly higher in female PPARα-null mice (+136%) compared with female wild-type mice. In contrast, hepatic triglyceride secretion rate was not different between male PPARα-null mice and male wild-type mice (**Fig. 3**). Female wild-type mice tended to have a higher hepatic triglyceride secretion rate than did wild-type male mice (+63%), but in the absence of PPARα, female mice had markedly higher triglyceride secretion rate than did male mice (+195%).

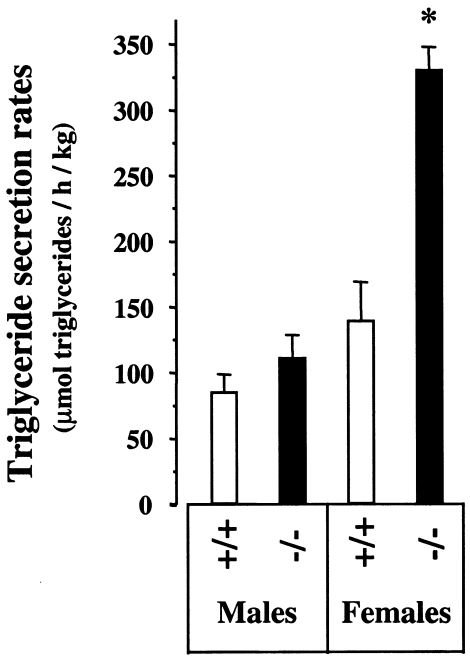

Fig. 3. In vivo hepatic triglyceride secretion rates in 32-week-old mice on Sv/129 genetic background. The triglyceride secretion rate was measured by injecting the mice with Triton WR1339 (500 mg/kg). Serum triglycerides were determined before injection (0 min) and at 45 and 90 min after Triton injection. Hepatic triglyceride secretion rate was calculated from the slope of the curve. There were 4 to 6 mice in each group, and values are means ± SEM (* $P < 0.05$, versus all other groups, one-way ANOVA followed by Bonferroni test).

High fat feeding of male PPARα-null mice

In order to see if hyperapobetalipoproteinemia could be induced in male PPARα-null mice by changing the diet, 9-week-old male PPARα-null and wild-type mice on Sv/129 genetic background were fed a high fat diet for 3 weeks. Fat fed male PPARα-null mice had significantly higher serum apoB levels than did fat fed male wild-type mice (+49%, $P < 0.05$, Student's *t*-test, data not shown). In addition, PPARα-null mice were back-crossed toward the C57BL/6 genetic background for two generations. The C57BL/6 strain is a common background to several obese phenotypes and highly sensitive to fat diet in terms of weight gain and hyperinsulinemia (33, 34). Using this genetic background, 13-week-old male PPARα-null mice and corresponding littermate controls were fed the high

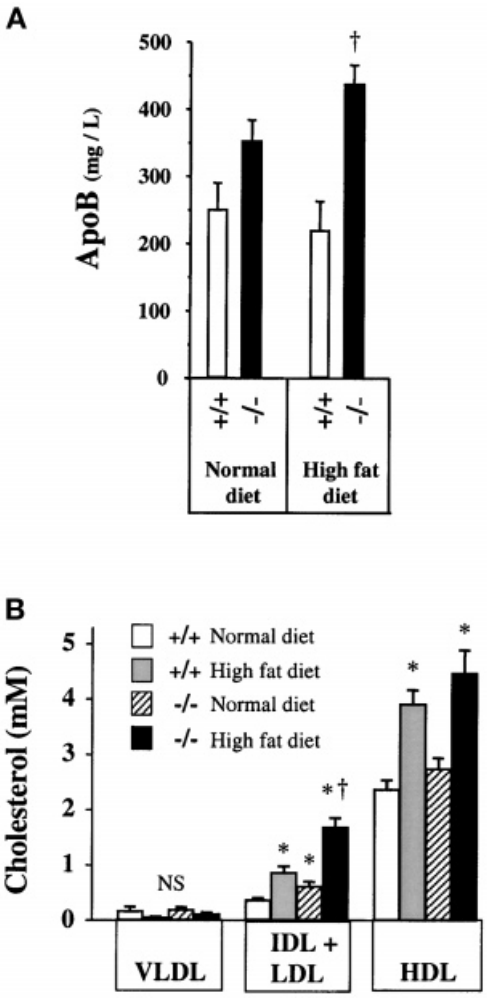

Fig. 4. Serum apoB levels (A) and cholesterol profile (B) in PPARα-null mice fed a high fat diet. Thirteen-week-old littermate male PPARα-null mice and wild-type mice (F2 Sv/129 × C57BL/6) were fed normal or a high fat diet for 3 weeks. Serum apoB was determined by an electro-immunoassay and the cholesterol distribution profiles were measured on individual mice by using a size exclusion high performance liquid chromatography system, SMART. The cholesterol levels were then determined in the different lipoprotein classes. There were 5 mice in each group. Values are means ± SEM (* $P < 0.05$ vs. wild-type normal diet, † $P < 0.05$ vs. wild-type high fat diet, one-way ANOVA followed by Bonferroni test).

TABLE 2. Effects of high fat diet on tissue weights, insulin, glucose, and serum lipids in male PPARα-null mice

	Wt Normal diet	KO Normal diet	Wt High fat diet	KO High fat diet
Weight gain (g)	0.86 ± 0.58	1.00 ± 0.41	6.44 ± 1.21[a]	5.88 ± 0.99[a]
Liver weight (g)	4.00 ± 0.24	3.95 ± 0.24	3.45 ± 0.24	4.39 ± 0.26
Epididymal WAT (%body)	1.85 ± 0.42	2.22 ± 0.27	4.00 ± 0.52[a]	4.28 ± 0.53[a]
Retroperitoneal WAT (%body)	0.60 ± 0.11	0.64 ± 0.09	1.47 ± 0.15[a]	1.43 ± 0.13[a]
Insulin (nM)	0.28 ± 0.06	0.26 ± 0.05	0.50 ± 0.09	0.64 ± 0.17
Glucose (mM)	9.80 ± 0.50	9.00 ± 0.31	11.80 ± 0.70[a]	9.96 ± 0.14
Triglycerides (mM)	1.22 ± 0.33	1.52 ± 0.27	1.04 ± 0.23	1.22 ± 0.20
Cholesterol (mM)	2.85 ± 0.24	3.47 ± 0.24	4.91 ± 0.35[a]	6.37 ± 0.44[a,b]

Thirteen-week-old littermate male PPARα-null (KO) mice and wild-type (Wt) mice (F2 Sv/129 × C57BL/6) were given a normal diet or a high fat diet for 3 weeks. The mice were sacrificed between 0900–1100 h. Liver and fat pads from different regions were dissected, blotted, and weighed. The different serum analyses were performed as described in Methods. There were 5 mice in each group. Values are means ± SEM.

[a] $P < 0.05$ vs. wild-type normal diet.

[b] $P < 0.05$ vs. wild-type high fat diet, one-way ANOVA followed by Bonferroni test.

fat diet for 3 weeks. The serum levels of apoB were significantly higher (+98%) in fat fed PPARα-null mice compared with fat fed wild-type mice, whereas no difference between male PPARα-null mice and wild-type mice was observed when the mice were fed a normal diet (**Fig. 4A**). The high fat diet increased body weight gain to a similar degree in both PPARα-null mice and wild-type mice (**Table 2**). There was a tendency toward higher liver weight in PPARα-null mice fed a high fat diet and macroscopically the livers were clearly paler as compared with the rest of the groups, indicating lipid accumulation. The weights of the epididymal and retroperitoneal fat depots were similarly increased by the high fat diet in the PPARα-null and wild-type mice (Table 2). Serum levels of insulin tended to be higher in the two groups of mice given the fat diet ($P = 0.059$, one-way ANOVA followed by Bonferroni test), and 20% higher serum glucose levels were observed in fat fed wild-type mice compared with wild-type mice given normal diet (Table 2). There were no significant differences in serum levels of triglycerides between the groups. The serum cholesterol levels were not significantly different between PPARα-null and wild-type mice fed the normal diet (Table 2). However, the serum cholesterol levels in the fat fed PPARα-null mice were 30% higher than in the fat-fed wild-type mice (Table 2).

The size distribution of lipoproteins was measured in individual mice by using high performance liquid chromatography system, SMART, followed by determination of cholesterol levels in the different fractions (Fig. 4B). VLDL cholesterol levels tended to be lower in the fat fed groups, but no significant differences were observed. The LDL cholesterol levels were significantly higher in PPARα-null mice fed either normal or high fat diet compared with the corresponding wild-type mice. HDL cholesterol levels were increased by the high fat diet in both PPARα-null and wild-type mice, but the effect of the high fat diet was not different between PPARα-null and wild-type mice (Fig. 4B).

LDL receptor measurements

In order to investigate if the changed serum levels of apoB and LDL cholesterol were partly due to decreased expression of the LDL receptor (35), the LDL receptor protein expression was determined with Western blot (**Fig. 5**). Both the 130 kDa and the 220–240 kDa [corresponding to a dimer of the LDL receptor (36)] were quantified and added to a total expression value. The LDL receptor protein levels did not differ between female PPARα-null and wild-type mice or between fat fed male PPARα-null and wild-type mice, respectively (n = 5 in each group, NS, Student's *t*-test, data not shown).

Secretion of total and VLDL-associated apoB and triglycerides from primary cultures of hepatocytes

The increased triglyceride secretion in female PPARα-null mice may be dependent on the metabolic changes in

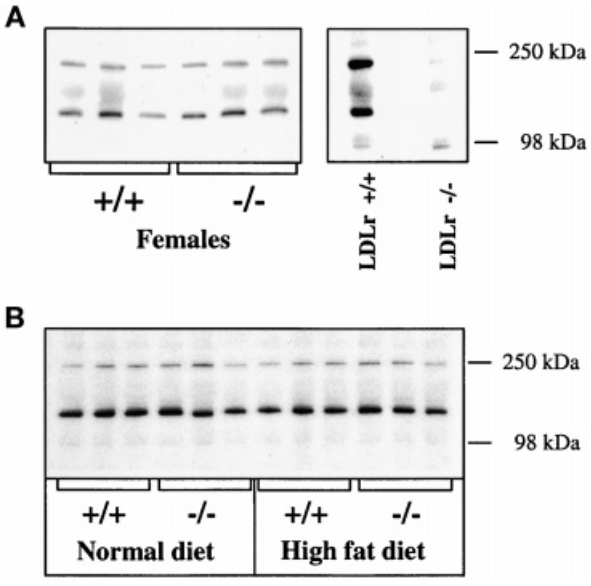

Fig. 5. Hepatic LDL receptor protein levels in 32-week-old female PPARα-null mice on Sv/129 genetic background (A) and in 16-week-old male PPARα-null mice (F2 Sv129 × C57/BL/6) fed normal diet or high fat diet for 3 weeks (B). The LDL receptor protein levels were identified by Western blot. Three of five mice in each group are presented and as a negative control, liver protein preparations from an LDL receptor-deficient mouse and corresponding wild-type mouse are shown (A).

the intact animal, such as changed flux of fatty acids to the liver. In order to investigate if there is a difference in the capacity for apoB and triglyceride secretion between hepatocytes from PPARα-null and wild-type mice, primary hepatocyte cultures were used. Hepatocytes from 32-week-old female PPARα-null mice and wild-type mice on Sv/129 genetic background were prepared through collagenase perfusion. The cells were cultured for 4 days on laminin-rich matrigel in serum free medium supplemented with 1 nM dexamethasone and 3 nM insulin (24). In an initial experiment, the intracellular accumulation of labeled apoB-48 and apoB-100 was found to be similar after 1, 2, 3, and 4 h of labeling in hepatocytes from PPARα-null mice and wild-type mice. No further increase in labeling of these proteins occurred after 2 h (data not shown). Therefore, the cells were labeled for 2 h with [^{35}S]methionine-cysteine mix and chased for 4 h in excess of unlabeled methionine followed by immunoprecipitation of apoB. Neither apoB-48 nor apoB-100 could be detected in the cells after the 4 h chase period. The accumulation of labeled apoB-100 and apoB-48 in the culture medium was higher in cultures from the PPARα-null mice as compared with wild-type cells (**Fig. 6A** and **B**). There was no difference in the proportion of apoB-48 of total apoB [calculated as apoB-48/(apoB-48 + apoB-100)] in the medium between PPARα-null cultures, 92.7 ± 2.6%, and wild-type cultures, 93.3 ± 2.0%. In the experiments presented, the chase medium was subjected to sucrose gradient ultracentrifugation in order to isolate the VLDL fraction (24) (Fig. 6C). The accumulation of apoB-100-containing VLDL particles in the chase medium was found to be very low in both PPARα-null and wild-type hepatocyte cultures. The accumulation of apoB-48-containing VLDL particles in the chase medium was higher in cultures from PPARα-null mice compared with wild-type cells (Fig. 6C). The mean individual experimental values in apoB-48-VLDL in

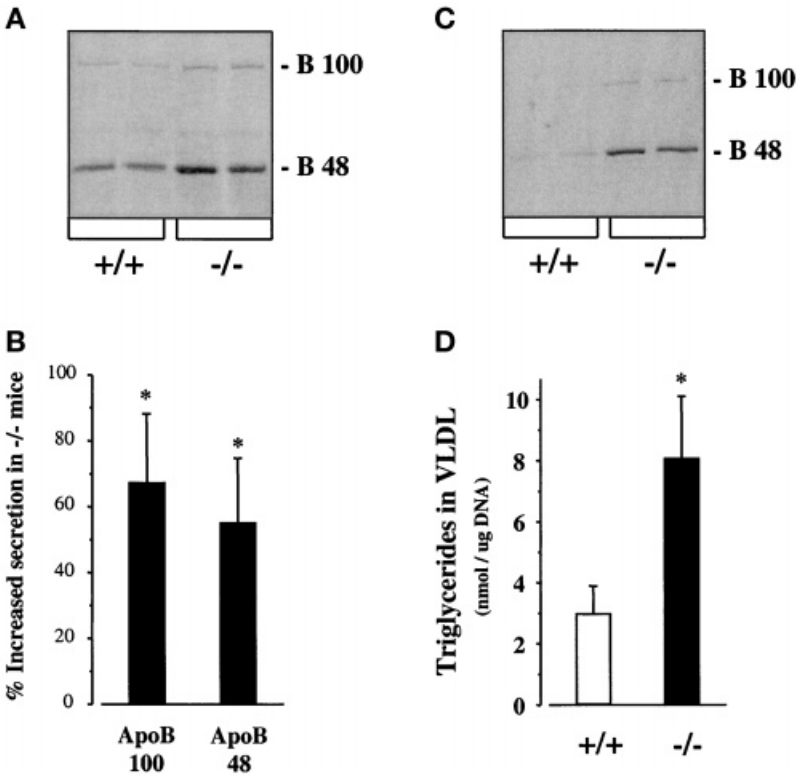

Fig. 6. Accumulation of apoB-100 and apoB-48 in culture medium (A, B), in the VLDL fraction (C), and VLDL triglycerides in hepatic culture medium (D) from 32-week-old female PPARα-null (n = 3–4) and wild-type mice (n = 3–4) on Sv/129 genetic background. The cells were plated with 16 nM insulin during the first 16–18 h followed by incubation with 3 nM insulin and 1 nM dexamethasone during the last 3 days of culture. Cells were labeled for 2 h with a [^{35}S]methionine-cysteine mix, washed and cultured for 4 h in a medium containing 10 mM unlabeled methionine (chase medium). VLDL was isolated from the chase medium using sucrose gradient ultracentrifugation. Amounts of labeled apoB-100 and apoB-48 in the chase medium (total amount) (A) and in the VLDL fraction (C) were measured after immunoprecipitation and separation by SDS-PAGE as described in Methods. Representative autoradiograms are given (A, C). In the experiments, the quantified STORM units (Molecular Dynamics) of each band are divided with the DNA content in the culture dish. The data for total apoB secretion (B) is given as percentage of the increased accumulation of labeled apoB-100 and apoB-48 in the culture medium from PPARα-null cells compared with wild-type cells in three different experiments. The mass of triglycerides in VLDL fraction of 24 h culture medium ($d < 1.006$) was determined after extraction of total lipids as described in Methods. The result on VLDL associated triglycerides (D) is based on 4 liver perfusions of mice from each genotype. Values are means ± SEM (* $P <$ 0.05, Mann-Whitney U test).

the three experiments (wild-type; PPARα-null) were 1081; 1765, 15; 687 and 12; 1179 STORM units/μg DNA. In addition, the mass of triglycerides in the VLDL fraction of overnight culture medium (last 24 h incubation) was measured. The mass of triglycerides in the VLDL fraction of the medium was higher (+170%) in cultures from PPARα-null mice compared with the medium of wild-type cultures (Fig. 6D).

DISCUSSION

This study demonstrates that functional PPARα expression is of importance for serum apoB levels. PPARα deficiency was shown to result in higher serum levels of apoB in female mice but not in male mice, indicating that gonadal steroids and PPARα could interact in the regulation of apoB levels. Moreover, when fed a high fat diet, male PPARα-null mice displayed markedly elevated serum levels of apoB compared with male wild-type mice, indicating that the expression of PPARα also influences the effect of dietary fat on apoB levels. PPARα deficiency had no effect on LDL receptor protein level. However, the hepatic triglyceride production rate in vivo was markedly higher in female PPARα-null mice compared with female wild-type mice, whereas no difference was observed in male mice. The increased secretion of triglyceride-rich lipoproteins may therefore explain the higher apoB and VLDL triglyceride levels in females. In order to study the effect of PPARα deficiency on apoB and VLDL secretion from hepatocytes without the influence of the in vivo milieu, hepatocytes were cultured for 4 days. Secretion of total and VLDL-associated apoB and triglycerides were found to be higher in hepatic cultures from female PPARα-null mice as compared with cultures from wild-type mice. These results indicate that PPARα-deficient hepatocytes have an increased capacity for apoB and VLDL secretion.

Recently, a point mutation in the human PPARα gene (L162V) was shown to result in hyperapobetalipoproteinemia and hypercholesterolemia (37–39). Our results indicate that in addition to the effects of mutations in the PPARα gene, the level of PPARα expression might be of importance for serum levels of apoB and production of apoB-containing lipoproteins, but not LDL receptor expression. Interestingly, Tugwood et al. (40) demonstrated a great inter-individual variation in PPARα expression in human liver. Thus, individual variation in the expression of PPARα could be of importance for serum levels of apoB.

The ovarian and retroperitoneal adipose tissue depot weights were similar between the genotypes in both males and females at 12 weeks of age. Thus, the increased serum levels of apoB and triglycerides seen in female PPARα-null mice were probably not primarily due to obesity and associated insulin resistance. The gender differences in serum apoB and hepatic triglyceride secretion rate might be due to different actions of the sex hormones. Female rats have been shown to have a higher VLDL secretory rate than male rats (41, 42), and our results indicate that a difference between the sexes in VLDL secretion rate may exist also in mice. Furthermore, it has been shown that gonadectomy of female rats decreases triglyceride secretion, while estrogen replacement therapy increases triglyceride secretion (42). Testosterone may have the opposite effect (43). Thus, it is concluded from the present results that lack of PPARα unmasks a gender-specific influence on VLDL secretion. A cross-talk between PPARα and estrogen receptors has been suggested and may be involved in the regulation of apoB and triglyceride secretion (44, 45). In this context, it is interesting to note that livers from PPARα-null females accumulate less triglyceride than male mice (14), which may at least partly be explained by the larger export of triglycerides from the liver of female PPARα-null mice. Furthermore, female PPARα-null mice have been shown to be less susceptible to the action of etomoxir than male PPARα-null mice (46). Etomoxir inhibits mitochondrial β-oxidation and results in severe hypoglycemia and accumulation of fatty acids in liver and heart, causing death among male, but not to the same extent among female, PPARα-null mice given this drug (46). Our results indicate that a possible cause of better tolerance against etomoxir treatment among female PPARα-null mice could be a larger export of fatty acids from the liver as VLDL particles. Our finding that female PPARα-null mice had higher serum levels of β-hydroxybutyrate than male PPARα-null mice indicates that female PPARα-null mice also have a larger capacity for β-oxidation than male PPARα-null mice. In line with our study, it has been reported that male PPARα-null mice have lower β-hydroxybutyrate levels than wild-type males in the fed state (9).

The 4 day cultures of hepatocytes indicated that the secretion of apoB and VLDL triglycerides is higher from female PPARα-deficient cells independent of the flux of metabolites in vivo. The secretion of both apoB-48 and apoB-100 was increased to a similar degree from PPARα-null hepatocytes, indicating that there was no effect on editing of apoB mRNA. The low total secretion of apoB-100 explains the very low and nearly undetectable apoB-100-VLDL secretion. We were therefore not able to show if the apoB-100-VLDL secretion was influenced. The increased VLDL triglyceride accumulation in the medium from PPARα-null hepatocytes is in line with the increased apoB-48-VLDL secretion and strengthens the importance of PPARα for normal apoB-VLDL secretion from hepatocytes.

We tested the effect of high fat diet in mouse on pure Sv129 background and in mice on Sv/129 × C57BL/6 genetic background because different mouse strains have been found to respond differently to a high fat diet (33, 34). The response to a high fat diet on serum apoB levels was similar between the genotypes, but the magnitude of the increase in serum apoB differed. This finding indicates that the genetic background of the mice does not determine the effect of PPARα deficiency on serum apoB levels after fat feeding. We also showed that the increased total serum cholesterol after fat feeding of PPARα-null mice was due to an increased LDL cholesterol level because LDL, but not VLDL or HDL, cholesterol differed between the PPARα-null mice and wild-type mice after fat feeding. In a recent paper by Tordjman et al., fat fed male

PPARα-null mice on an apoE-deficient background showed an increased hepatic triglyceride secretion rate and unchanged VLDL clearance compared with fat fed wild-type mice on apoE-deficient background (47). Our data extends these findings by showing that PPARα-deficient mice are more sensitive to high fat diet in terms of serum apoB and LDL cholesterol levels in the presence of apoE expression.

We showed that variation in the expression of PPARα does not influence hepatic LDL receptor expression in males or females or during manipulation of the dietary intake of fat. These results indicate that changed expression of the LDL receptor is not responsible for the effect of PPARα deficiency on serum apoB levels. In line with our results, male PPARα-null mice have been shown to have similar LDL receptor mRNA levels as wild-type mice (48). Moreover, fibrate treatment of rat hepatocytes did not influence LDL receptor mRNA levels (49).

In summary, these results indicate that PPARα expression is of large importance for serum levels of apoB. The increase in serum levels of apoB was associated with increased VLDL triglyceride levels in female mice on ordinary chow and associated with increased LDL cholesterol levels in fat fed male mice. Hepatocytes from female PPARα-deficient mice showed higher apoB and VLDL secretion, indicating that the increased secretion of triglycerides in female mice lacking PPARα is not a result of increased flux of fatty acids to the liver.

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