EXPRESSION OF APOLIPOPROTEIN B IN THE KIDNEY ATTENUATES RENAL LIPID ACCUMULATION

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Running head: Renal lipoprotein secretion

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The ability to produce apolipoprotein (apo)B-containing lipoproteins enables hepatocytes, enterocytes and cardiomyocytes to export triglycerides. In this study, we examined secretion of apoB-containing lipoproteins from mouse kidney and its putative impact on triglyceride accumulation in the tubular epithelium. Mouse kidney expressed both the apoB and microsomal triglyceride transfer protein genes, which permit lipoprotein formation. To examine de novo lipoprotein secretion, kidneys from human apoB-transgenic mice were minced and placed in medium with 35S-amino acids. Upon sucrose gradient ultracentrifugation of the labeled medium, fractions were analyzed by apoB-immunoprecipitation. 35S-labeled apoB100 was recovered in ~1.03-1.04 g/ml lipoproteins (i.e. similar to the density of plasma low density lipoproteins). Immunohistochemistry of kidney sections suggested that apoB mainly is produced by tubular epithelial cells. ApoB expression in the kidney cortex was reduced ~90% in vivo by treating wild type mice with apoB-antisense LNA oligonucleotide. Inhibition of apoB expression increased fasting-induced triglyceride accumulation in the kidney cortex by 20-25% (P=0.008). Cholesterol stores were unaffected. Treatment with control oligonucleotides with 1 or 4 mismatching base pairs affected neither the triglyceride nor the cholesterol content of the kidney cortex. The results suggest that mammalian kidney secretes apoB100-containing lipoproteins. One biological effect may be to dampen excess storage of triglycerides in proximal tubule cells.

INTRODUCTION

Apolipoprotein (apo) B is the principal structural protein in triglyceride-rich lipoproteins when secreted from the liver and intestine. The human liver produces the full-length apoB100 protein whereas the intestine mainly secretes a truncated version of the apoB protein, i.e. apoB48 (1). The formation of apoB-containing lipoproteins depends on microsomal triglyceride transfer protein (MTP), which transfers lipids on to the newly synthesized apoB polypeptide during its translation and translocation into the endoplasmatic reticulum (2). Formation of apoB-containing lipoproteins provides cells with a capacity to efficiently export triglycerides and other lipids including cholesterol. For instance, the human intestine packs and secretes ~70 g/day of fat in apoB-containing chylomicrons. Export of triglycerides in the form of apoB-containing lipoproteins also affects lipid homeostasis in other tissues than liver and intestine. Thus, apoB100-containing particles are made in the placenta, where they might participate in lipid transport between mother and fetus (3;4). The heart produces apoB100-containing lipoproteins that prevent deleterious lipid accumulation, e.g. as induced by type I diabetes or obesity (5-8). Recent data also suggests a role of apoB in formation of age-related macular degeneration of the eye (9).

The chicken kidney proximal tubule cells produce apoB100-containing lipoproteins. Thus, ultrastructural studies demonstrated very low density lipoprotein (VLDL) and low density lipoprotein (LDL)-sized lipoproteins in secretory granulae and suggested that these are secreted to the baso-lateral lumen of proximal tubule cells (10). In other studies, slices of chicken kidney were incubated with 35S-labeled amino acids and secretion of newly formed apoB100 in VLDL, LDL, IDL and even HDL-like lipoproteins was seen (11). Even though the lipoprotein metabolism is markedly different in birds and mammals, indications that the mammalian kidney might also produce apoB-containing lipoproteins came from early studies showing...
triglyceride transfer activity in microsomal extracts of rat kidney (12).

Lipid accumulation in the kidney is associated with damage to proximal tubule cells, e.g. following ischemia or rhabdomyolysis (13). Also, cisplatin-induced tubular damage involves free fatty acid and triglyceride accumulation in the proximal tubules (14). Cisplatin deactivates the transcription factor peroxisome proliferator-activated receptor (PPAR)-α (15), which controls fatty acid oxidation and lipid and lipoprotein metabolism (16). Interestingly, deleterious effects of cisplatin and ischemia-reperfusion were alleviated by treating mice with PPARα agonists (14;17). These seminal studies have supported the hypothesis that lipid accumulation during acute kidney injury might involve blockade of fatty acid oxidation with deleterious lipid accumulation as one result. Acute renal failure affects as much as 5 % of hospitalized patients and is often fatal (18). Irrespective of the mechanism, proximal tubule lipid accumulation may be important for the tubular damage in the critically ill patients.

In the present study, we used mice to examine whether the mammalian kidney has capacity to secrete apoB-containing lipoproteins. Inhibition of lipoprotein formation in vivo was achieved with an apoB-antisense oligonucleotide to test the hypothesis that lipoprotein formation affects triglyceride storage in proximal tubule cells.

**EXPERIMENTAL PROCEDURES**

**Mice**

C57BL/6 mice (M&B, Ry, Denmark) and human apoB-transgenic mice (Taconic Europe) were fed a standard mouse chow (Altromin no. 1314; Altromin, Rugarden, Denmark) and housed at the Panum Institute, University of Copenhagen in a temperature-controlled (21—23 °C) facility with a 12-hour light/dark cycle for at least 5 days before any treatment. The animal experiments were performed according to the principles of the Danish law on animal experiments and approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

**mRNA quantification**

Frozen tissue biopsies (10–50 mg) were homogenized in TRIzol reagent (Life Technologies, Taastrup Denmark) using a tissue-lyzer (Retsch/Qiagen cat nr 85220, Haan, Germany). Total RNA was extracted according to the manufacturer’s instructions and suspended in RNase-free H2O. The RNA concentration was calculated from the absorbance at 260 nm (A260) using a Nanodrop (Thermo Scientific, Denmark, www.swab.dk). The RNA integrity was assured with a Standard 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

cDNA was made with M-MULV reverse transcriptase (Roche, Hvidovre, Denmark) from 1 µg of total RNA as described (19). Real-time PCR was performed with a Lightcycler (7) or using 384 well clear plates, Fast SYBR Green Master Mix and the ABI 7900 HT Sequence Detection System (Applied Biosystems, Foster City, USA). Reaction volumes were 20 µl using 2 µl template cDNA. The specificity and sequences of primers have been reported previously (7;20-22). All analyses were done in duplicate and gene expression data were normalized with the amount of HPRT in the same cDNA preparation. Similar results were obtained using 18S as housekeeping gene.

**Histological analyses**

ApoB was detected with immunohistochemistry in sections of formalin-perfusion-fixed and paraffin-embedded kidneys from human apoB transgenic mice as described (23). Lipid accumulation in the kidney was visualized with Oil-red O staining of formalin-fixed and frozen 10-µm sections (8).

**Secretion of apoB-containing lipoproteins from mouse kidney**

Neoformation of apoB-containing lipoproteins in the kidney were examined as previously described (4). Briefly, human apoB transgenic mice were anaesthetized with Hypnorm/Dormicum (Boehringer Ingelheim, Ingelheim, Germany) and perfused with 0.9 % saline. The kidney was minced into pieces of ~0.5 mm3 and incubated with 35S-Met and -Cys at 37 °C for ~16 hours. Lipoproteins in the medium were separated by sucrose gradient ultracentrifugation at 35000 rpm for 71 hours. Fractions (the upper 1 ml and then 1.5 ml each) were collected from the top and weighed to determine the density. ApoB-containing lipoproteins in the collected fractions were immunoprecipitated with a polyclonal anti-human apoB antibody (DAKO A/S, Glostrup, Denmark). The immunoprecipitates were separated in 4-20 % polyacrylamide gels under denaturing and reducing conditions. Human LDL
was always included on the gels to identify the migration of the apoB100 band. $^{35}$S-labeled apoB was visualized with a phosphor-imager.

**In vivo knock down of apolipoprotein B expression**

To knock down apoB expression, mice were injected intraperitoneally (i.p.) with a single dose of a 14-mer locked nucleic acid antisense oligonucleotide against apoB (antisense apoB LNA; 5'- AG$^{m}$CattggtatT$^{m}$CA - 3' where upper case letters denote LNA monomers, lower case letters DNA monomers and superscript ‘m’ indicates methylation, e.g., $^{35}$C stands for LNA-5-methyl-cytidine. For controls, we used a 13-mer LNA with four mismatching bases (antisense control-4; 5'- G$^{m}$CataggaTaTCA-3'), and a 14-mer oligonucleotide with one mismatching base (antisense control-1; 5'- AG$^{m}$CataggtatT$^{m}$CA-3'). Each mouse received 5 mg/kg body weight of oligonucleotide dissolved in ~100 µl 0.9 % NaCl, or 0.9 % NaCl (100 µl). The LNA oligonucleotides were provided by Santaris Pharma, Horsholm, Denmark. Four days after the injection of LNA, a blood sample was collected from the eye before the mice were anaesthetized with Hypnorm/Dormicum (Boehringer Ingelheim, Ingelheim, Germany) and perfused with ice-cold 0.9 % NaCl trough the left ventricle of the heart prior to removal of kidney, heart, and liver biopsies. The kidney cortex was carefully dissected from the medulla. Tissues were immediately frozen in liquid N$_2$ and stored at –80 °C. In some experiments the mice were fasted for 12 hours before sacrifice.

**Statistical analyses**

Statistical analyses were performed with GraphPad Prism 4.00 (GraphPad software Inc., San Diego, CA, USA). Two-group comparisons were done with Student’s $t$-test. $P < 0.05$ was considered significant.

**RESULTS**

Mouse kidney secretes apoB100-containing lipoproteins

Both apoB and MTP mRNA were easily detected in the mouse kidney. On real-time PCR analyses, the kidney expression of the mouse apoB and MTP genes were ~3-5 % of that in the liver. We also examined the kidney expression of a human apoB mRNA in human apoB-transgenic mice generated with an ~80-kb genomic transgene with ~19 kb of 5'-flanking sequences (25) and found robust amounts of human (and mouse) apoB mRNA in the kidney (Fig. 1). Immunohistochemistry showed human apoB protein staining in the kidney tubular epithelial cells in the cortex, whereas there was no apoB staining in glomerular cells (Fig. 2A and 2B).

To examine kidney lipoprotein secretion in mice, we incubated minced kidney tissue from human apoB-transgenic mice with $^{35}$S-labeled amino acids, isolated lipoprotein fractions from the conditioned medium with density gradient ultracentrifugation, immunoprecipitated human apoB-containing particles, and separated them with SDS-PAGE. We saw apoB100-sized $^{35}$S-labeled bands in lipoproteins with a density of ~1.030-1.040 g/l, i.e. similar to plasma LDL particles (Fig. 2C). These data suggest that
mouse kidney secretes apoB-containing lipoproteins.

**Knock-down of apoB expression augments fasting induced lipid accumulation in the kidney**

To determine the impact of kidney lipoprotein formation in vivo, we inhibited apoB expression in the kidney of wild type mice with an LNA antisense oligonucleotide (antisense apoB-LNA). Four days after an i.p. injection of antisense apoB-LNA (5 mg/kg), the kidney cortex apoB mRNA expression was ~9 % of that in saline-injected control mice (Fig. 3). The antisense apoB-LNA also effectively reduced apoB mRNA expression in the liver (Fig. 3), but we did not see effects on intestinal or cardiac apoB mRNA expression (data not shown). In accordance with the marked effect on hepatic apoB mRNA expression, plasma triglyceride and cholesterol concentrations were reduced by ~82 % (P < 0.0001) and ~56 % (P < 0.0001), respectively, in antisense apoB-LNA treated mice (Fig. 4A and 4B). The effect of antisense apoB-LNA on plasma triglycerides and cholesterol was similar in fasted and fed mice (Fig. 4A and 4B). Plasma free fatty acids increased during fasting (P < 0.0001) but were unaffected by antisense apoB-LNA treatment (Fig. 4C).

Antisense apoB-LNA treatment did not affect total kidney cortex triglyceride or cholesterol stores in fed mice (Fig. 5A and 5B). However, when deposition of lipids in the kidney was induced by fasting mice for 12 hours, apoB knock down further increased kidney triglyceride accumulation. Fasting of saline-treated control mice on average increased kidney cortex triglycerides ~10 fold (Fig. 5A) whereas the free cholesterol content only increased marginally by ~6 % (P = 0.04) (Fig. 5B). The fasting-induced increase of triglyceride stores in tissues such as kidney, skeletal muscle and heart probably to some extent reflects the fasting-induced increase of plasma free fatty acids (Fig. 4A). Oil-red-O staining of neutral lipids showed that the fasting-induced triglyceride accumulation in the kidney cortex mainly occurred in tubular epithelial cells (Fig. 6). In three independent experiments, antisense-apoB LNA treatment increased kidney cortex triglycerides by ~20-25% (P = 0.008) in fasted mice but did not consistently affect the cholesterol content (Fig. 5A and 5B). Notably, the effect of apoB-antisense LNA treatment on kidney cortex apoB mRNA expression was similar in fasted and fed mice (data not shown).

As expected, antisense-apoB LNA treatment increased liver triglyceride stores both in fed and in fasted mice (Fig. 5C). Interestingly, apoB-LNA treatment slightly reduced the cardiac triglyceride stores in fasted mice (Fig. 5E).

To ensure that the effect of apoB-antisense LNA did not result from off-target or non-specific effects in the kidney cortex, we compared the antisense apoB-LNA with two control LNA oligonucleotides (antisense control-1 and -4). Although antisense control-1 reduced apoB mRNA kidney and liver expression marginally, it was not statistically significant (Supplemental Figure). Antisense control-4 also did not affect apoB mRNA expression (Supplemental Figure). Neither of the two control oligonucleotides increased kidney triglycerides (Supplemental Figure).

**Effect of reducing apoB expression on kidney expression of genes involved with lipid metabolism**

Antisense apoB-LNA treatment both reduced lipid substrate availability (i.e. plasma triglycerides) and lipoprotein secretion capacity in the kidney cortex. To further examine the combined effect of inhibiting apoB expression on lipid metabolism in the kidney, we measured the kidney cortex expression of genes controlling lipid metabolism. Fasting decreased the expression of lipoprotein lipase (LPL) mRNA. It increased the expression of cytosolic acyl-CoA thioesterase 1 (CTE1), fatty acid transport protein 1 and 4 (FATP1, FATP4) and heart-type fatty acid binding protein (H-FABP) all involved with intracellular fatty acid transport, and the expression of long chain acyl-CoA dehydrogenase (LCAD), which affects fatty acid oxidation (Fig. 7). Antisense apoB-LNA treatment attenuated the fasting-induced increases of FATP1, FATP4, H-FABP, and CTE1 mRNA in the kidney cortex (Fig. 7). In fed mice, antisense apoB-LNA treatment significantly decreased CTE1 mRNA expression whereas the other genes measured were unaffected (Fig 7).
DISCUSSION

The present study demonstrates that the mouse kidney produces apoB-containing lipoproteins and that inhibition of apoB expression increases fasting-induced lipid accumulation in the kidney cortex.

We detected newly formed apoB100-containing particles in the supernatant after incubation of minced kidney tissue in cell culture medium. This result was obtained in two separate experiments where labeled amino acids were incubated ex vivo with minced kidney tissue. Both experiments yielded relatively faint apoB bands, which might reflect that the kidney proximal tubules are notoriously sensitive to ischemic or toxic insults. As such, the results shown in Fig 2C cannot be used to judge the quantity of lipoproteins secreted from the kidney in vivo. Immunohistochemistry studies suggested that the lipoproteins might be produced by tubular epithelial cells rather than glomerular or vascular cells. However, it should be kept in mind that the apoB staining seen in the proximal tubules might at least to some extent reflect plasma-derived apoB rather than local synthesis. The secreted apoB100-containing particles had a density similar to plasma LDL. We cannot exclude that the kidney actually secretes more buoyant triglyceride-enriched particles and that our observations reflect the effect of residual lipoprotein lipase activity in the minced kidney biopsies. Nevertheless, the density of the apoB100-containing particles was similar to that of lipoproteins secreted by the heart. In the avian kidney, Walzem et al. saw intracellular lipoproteins of varying size along the tubular epithelium (10) and Tarugi et al. detected secretion of apoB-containing particles from chicken kidney slices that varied from dense HDL to buoyant VLDL (11). Thus, Walzem et al. suggested that the density of the apoB-derived apoB-containing lipoproteins may depend on the lipid availability which may be higher in the proximal than in the more distal tubule cells of the kidney (10).

Treatment of mice with an apoB-antisense LNA oligonucleotide effectively reduced apoB mRNA expression by ~90% in the kidney cortex. Whilst this did not affect the triglyceride stores in the kidney cortex in fed mice (indicating that inhibition of apoB in the kidney is not lipotoxic in itself), the fasting-induced lipid accumulation in the tubular epithelium was augmented by the inhibition of apoB expression. This result supports the idea that apoB-containing lipoproteins can export excess triglycerides from the tubular epithelium when the supplies exceed the utilization in β-oxidation.

The apoB-antisense LNA treatment also reduced apoB expression in the liver and consequently decreased plasma cholesterol and triglycerides. Thus, in addition to inhibiting local lipoprotein formation in the kidney, the apoB-antisense LNA treatment at the same time reduced the availability of plasma triglycerides for peripheral tissues. Indeed, in the heart, where apoB expression was not affected by the apoB-antisense LNA, the lowering of plasma triglycerides was associated with a decrease in cardiac triglyceride stores in fasted mice. Also, the fasting-induced changes in kidney cortex expression of genes involved with lipid metabolism tend to be attenuated by apoB-antisense LNA treatments. Therefore, it is possible that the triglyceride-increasing effect of inhibited local lipoprotein secretion in the kidney at the same time was counteracted by a decreased delivery of plasma triglycerides to the kidney. As such, the present studies may have underestimated the importance of local secretion of apoB-containing lipoproteins from the kidney.

The kidney cortex triglyceride content increased ~10-fold, whereas kidney cortex cholesterol essentially was unchanged upon overnight fasting of the mice. Interestingly, fasting also induced the kidney cortex expression of genes involved in the intracellular metabolism of fatty acids. The changes in gene expression are similar to those associated with increased delivery of free fatty acids and accumulation of triglycerides in the heart of obese mice (8). Thus, we suspect that the fasting-induced triglyceride accumulation reflects increased delivery of fatty acids to the kidney, but cannot exclude that decreased fuel utilization also plays a role. Plasma free fatty acids, which were doubled by fasting, are bound to albumin, which is filtered in the glomeruli and taken up by the tubular epithelium (26). Indeed, oil-red-O staining showed that the fasting-induced lipid accumulation in the kidney cortex was confined to the tubule cells. Thus, the fasting-induced triglyceride accumulation may reflect increased glomerular filtration and tubular re-uptake of albumin-bound fatty acids. It remains to be explored to what extent the fasting-induced triglyceride accumulation in the kidney also reflects increased uptake of lipoprotein-bound triglycerides. Fatty acids can be delivered to
tissues via the blood and by lipoprotein lipase acting on triglyceride-rich lipoproteins. Whereas fasting decreases the activity of LPL in adipose tissue, the LPL activity is increased in cardiac and skeletal muscle in rodents (27-29). It is unknown how fasting affects LPL activity in the mouse kidney.

Why has the kidney maintained a capacity to secrete lipoproteins in evolutionary distant species such as mammals and birds? Birds need large amounts of cholesterol and lipids for yolk production and the kidney is integrated with avian whole body lipid metabolism (10). In mammals, however, the kidney contribution to the total plasma lipoprotein pool likely is negligible. Perhaps lipoprotein formation by the kidney epithelium instead helps re-secrete fatty acids and perhaps other essential lipid molecules that are taken up from the pre-urine after filtration in glomeruli. For instance, retinol binding protein is filtered into the pre-urine from where it is normally taken up, and endocytosed by the tubule epithelial cells, thus, preventing the loss of retinol in the urine. The endocytosed retinol binding protein likely is degraded in lysosomes (30). ApoB-containing lipoproteins contain retinol and play a role in the transport of newly ingested retinol from the intestine to the liver (30). Thus, the re-secretion to plasma of retinol or other essential lipid molecules could involve formation of apoB-containing lipoproteins, but further studies are obviously needed to explore this possibility. Secretion of apoB-containing lipoproteins could also serve to attenuate triglyceride accumulation as suggested by the present results. Lipid accumulation has detrimental effects on cellular function, e.g. by inducing oxidative stress and promoting apoptosis (31;32). Thus lipotoxicity can induce insulin resistance in skeletal muscle, decrease contractile function in the heart and impair insulin secretion from pancreatic β-cells (33-35). The mechanisms are not completely resolved but may relate to the accumulation of ceramides and increased fluxes of free fatty acids causing oxidative stress (31;34). Recent data suggest that the kidney proximal tubular epithelium suffers from excess triglyceride accumulation in association with tubular damage induced, e.g., by reperfusion after ischemia, rhabdomyolysis, and treatment with cisplatin (13;30). Indeed, studies by Portilla et al have mechanistically linked cisplatin-induced kidney damage with a decrease in the fatty acid oxidation (15). It remains to be explored whether the capacity of the kidney to secrete apoB-containing lipoproteins plays a role in protecting the kidney from deleterious triglyceride accumulation in humans. The lipoprotein secretion capacity from the human liver and heart is affected by a polymorphism in the promoter region of the MTP gene (36). The present data suggest that it may be worthwhile to explore whether the polymorphism affects the risk of tubular affection and acute kidney injury in critically ill patients.

REFERENCES


**FOOTNOTES**

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**FIGURE LEGENDS**

Fig. 1. Expression of mouse apoB, human apoB, and MTP mRNA in the kidney cortex, liver, duodenum, and heart of human apoB-transgenic mice. Data are expressed as % of the value in the liver and are mean ± SEM, n=4-5. Note that the human apoB transgene as previously described does not confer apoB mRNA expression in the intestine (23) but robust expression in the kidney.

Fig. 2. ApoB100-containing lipoproteins are produced by mouse kidney. A. Laser confocal microscopy (combined flourescent and differential interference contrast micrograph) showing human apoB protein staining (red) in kidney tubular epithelial cells in a human apoB-transgenic mouse. B. Control-stained section without primary antibody. C. Kidney tissue from a human apo-B transgenic
mouse was incubated with $^{35}$S-labeled amino acids. The medium was subsequently subjected to sucrose-density-gradient ultracentrifugation. ApoB was isolated from each density fraction by immunoprecipitation and analyzed by SDS-PAGE and filmless autoradiographic analysis. $^{35}$S-ApoB100 was seen in the d ~ 1.03-1.04 g/ml lipoprotein fractions.

**Fig. 3.** ApoB-antisense LNA oligonucleotide reduces apoB mRNA expression in kidney and liver. C57Bl/6 mice received an i.p. injection of apoB-antisense LNA oligonucleotide (filled bars) or saline (open bars). Four days later, the RNA was isolated from the kidney cortex and a liver biopsy and used for mouse apoB mRNA quantification. Data are expressed as % of the average value in the saline group and are mean ± SEM. The numbers of mice in each group are shown in the figure. *** P < 0.0001.

**Fig.4.** ApoB-antisense LNA oligonucleotide reduces plasma triglycerides and cholesterol. Plasma triglycerides (A), cholesterol (B), and free fatty acids (C) were determined in C57Bl/6 mice 4 days after an i.p. injection of apoB-antisense LNA oligonucleotide (filled bars) or saline (open bars). Mice were fasted for 12 hours or allowed free access to chow prior to collecting blood for plasma analyses in the morning. Values are mean ± SEM. The numbers of mice in each group are shown in the figure. *** P < 0.0001.

**Fig. 5.** ApoB-antisense LNA oligonucleotide reduces kidney triglyceride stores in fasted mice. Triglycerides (A, C, and E) and cholesterol (B, D, and F) were determined in kidney cortex (A and B), liver (C and D) and heart (E and F) 4 days after an i.p. injection of apoB-antisense LNA oligonucleotide (filled bars) or saline (open bars). Mice were fasted for 12 hours or allowed free access to chow prior to removal of tissues. Values are mean ± SEM. The numbers of mice in each group are shown in the figure. * P < 0.05, ** P < 0.01, *** P < 0.0001.

**Fig. 6.** Fasting induces neutral lipid accumulation in kidney tubule epithelial cells. Histological sections of kidneys from a 12-hour fasted (A) and a fed (B) mice were stained with oil-red-O visualizing neutral lipids (red) in tubular epithelial cells (white arrow), but not in glomeruli (black arrow) of fasted mice.

**Fig. 7.** Effect of apoB-antisense LNA oligonucleotide and fasting on kidney expression of genes involved in fatty acid metabolism. Kidney cortex mRNA expression of lipoprotein lipase (LPL) (A), cytosolic acyl-CoA thioesterase 1 (CTE1) (B), fatty acid transport protein (FATP)1 (C), FATP4 (D), heart-type fatty acid binding protein (H-FABP) (E), and long chain acyl-CoA dehydrogenase (LCAD) (F) was measured 4 days after an i.p. injection of apoB-antisense LNA oligonucleotide (filled bars) or saline (open bars). Mice were fasted for 12 hours or allowed free access to chow prior to removal of tissues. Values are mean ± SEM. The numbers of mice in each group are shown in the figure. * P < 0.05, ** P < 0.01, *** P < 0.0001.
FIGURE 1

Mouse apoB mRNA (% of liver expression)

Liver
Duodenum
Kidney
Heart

Human apoB mRNA (% of liver expression)

Liver
Duodenum
Kidney
Heart

MTP mRNA (% of liver expression)

Liver
Duodenum
Kidney
Heart

Moore apoB mRNA (% of liver expression)

Liver
Duodenum
Kidney
Heart
FIGURE 3

ApoB mRNA [%] by organ and treatment group.

- Kidney
  - Saline (n=15)
  - Anti-ApoB LNA (n=10)

- Liver
  - Saline (n=6)
  - Anti-ApoB LNA (n=5)

Significance levels: **p < 0.01, ***p < 0.001.
**FIGURE 4**

**A**

 Plasma Triglycerides [mmol/L]

- Non-fasted
- Fasted

- Saline (n=4)
- Anti-ApoB LNA (n=5)

- Saline (n=9)
- Anti-ApoB LNA (n=9)

**B**

 Plasma Cholesterol [mmol/L]

- Non-fasted
- Fasted

- Saline (n=4)
- Anti-ApoB LNA (n=5)

- Saline (n=9)
- Anti-ApoB LNA (n=9)

**C**

 Plasma Free Fatty Acids [mmol/L]

- Non-fasted
- Fasted

- Saline (n=4)
- Anti-ApoB LNA (n=5)

- Saline (n=9)
- Anti-ApoB LNA (n=9)
**FIGURE 5**

(A) Kidney Triglycerides (nmol/mg ww)

(B) Kidney Cholesterol (nmol/mg ww)

(C) Liver Triglycerides (nmol/mg ww)

(D) Liver Cholesterol (nmol/mg ww)

(E) Heart Triglycerides (nmol/mg ww)

(F) Heart Cholesterol (nmol/mg ww)
FIGURE 7

A) Non-fasted  Fasted

B) Non-fasted  Fasted

C) Non-fasted  Fasted

D) Non-fasted  Fasted

E) Non-fasted  Fasted

F) Non-fasted  Fasted

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