

Silencing of Hepatic Fatty Acid Transporter Protein 5 *in Vivo* Reverses Diet-induced Non-alcoholic Fatty Liver Disease and Improves Hyperglycemia*^[5]

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Non-alcoholic fatty liver disease is a serious health problem linked to obesity and type 2 diabetes. To investigate the biological outcome and therapeutic potential of hepatic fatty acid uptake inhibition, we utilized an adeno-associated virus-mediated RNA interference technique to knock down the expression of hepatic fatty acid transport protein 5 *in vivo* prior to or after establishing non-alcoholic fatty liver disease in mice. Using this approach, we demonstrate here the ability to achieve specific, non-toxic, and persistent knockdown of fatty acid transport protein 5 in mouse livers from a single adeno-associated virus injection, resulting in a marked reduction of hepatic dietary fatty acid uptake, reduced caloric uptake, and concomitant protection from diet-induced non-alcoholic fatty liver disease. Importantly, knockdown of fatty acid transport protein 5 was also able to reverse already established non-alcoholic fatty liver disease, resulting in significantly improved whole-body glucose homeostasis. Thus, continued activity of hepatic fatty acid transport protein 5 is required to sustain caloric uptake and fatty acid flux into the liver during high fat feeding and may present a novel avenue for the treatment of non-alcoholic fatty liver disease.

The worldwide prevalence of non-alcoholic fatty liver disease (NAFLD)² is presently estimated at 30% of the general population and affects a majority of patients with obesity and type 2 diabetes (1, 2). In obese individuals, chronically elevated serum free fatty acids (FFAs) and high insulin levels lead to both

increased FFA uptake by the liver and increased synthesis of lipids, resulting in hepatic triglycerides (TG) accumulation, typically accompanied by hepatic insulin desensitization (1, 3) involving protein kinase C ϵ (3). Current pharmacological treatment strategies for NAFLD focus principally on increasing hepatic fatty acid oxidation (4) and improving extrahepatic insulin sensitivity (5). However, none of these treatment methods reduce hepatic uptake of dietary fats, and novel therapeutics that specifically aim at reversing NAFLD in the context of obesity would be highly desirable.

Based on the premises that obesity-associated NAFLD is primarily driven by the continuous protein-mediated uptake of fatty acids by the liver and that NAFLD is a contributing factor to whole-body insulin desensitization, we argued that blocking proteins responsible for hepatic fatty acid uptake should prevent or reverse hepatic steatosis, thus improving insulin sensitivity and glucose homeostasis. Two members of the fatty acid transport protein (FATP) family, FATP2 and FATP5, are robustly expressed in liver (6) and are thought to be involved in the early steps of long-chain fatty acid uptake/activation (7, 8). We recently demonstrated the importance of FATP5 in hepatic lipid metabolism by showing that deletion of FATP5 partially protected mice from developing high fat diet-induced obesity and improved insulin-sensitivity (9, 10).

To explore the consequences of hepatic FATP5 ablation in the context of NAFLD for hepatic fatty acid uptake and whole-body lipid fluxes, we took advantage of a recently developed stabilized double-stranded (sds) adeno-associated virus (AAV) 8-mediated delivery method for liver-directed small hairpin (sh) RNA expression (11). Using this approach, we could achieve specific silencing of endogenous FATP5 in mice after a single delivery of sdsAAV-shRNA expression constructs, resulting in the protection/reversal of NAFLD and hyperglycemia in diet-induced obesity mice.

MATERIALS AND METHODS

Antibodies and Reagents—BODIPY fatty acid (C1-BODIPY-C12) was obtained from Molecular Probes (Eugene, OR). [¹⁴C]Oleic acid was purchased from ARC, Inc. (St. Louis, MO). Polyclonal antisera against the C termini of FATP2, -4, and -5 were raised as described previously (9, 12). Anti- β -tubulin and anti-insulin-degrading enzyme antibodies were purchased from BD Biosciences and BD Transduction Laboratories,

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² The abbreviations used are: NAFLD, non-alcoholic fatty liver disease; AAV, adeno-associated virus; sdsAAV, stabilized double-stranded AAV; FATP, fatty acid transport protein; FFA, free fatty acid; TG, triglycerides; shRNA, short hairpin RNA; PBS, phosphate-buffered saline; v.p., viral particles; a.i., after injection; SCR, scrambled control; HF, high fat; ND, normal diet; SREBP-1c, sterol regulatory element-binding protein 1c; KO, knockout.

respectively. Immunoblot analysis was performed as reported previously (13). All other chemicals were obtained from Sigma.

AAV-shRNA Constructs—Oligonucleotides against FATP5 were designed as suggested (14) and did not share any significant homology with other genes in the mouse genome. All shRNAs used *in vitro* were expressed from the human H1 promoter (pSUPERIOR-based expression construct; OligoEngine, Seattle, WA). Loop sequence was 5'-TTCAAGAGA-3'. Stabilized double-stranded (sds) AAV vectors for persistent and efficient expression of shRNAs in the liver were derived from elements from AAV serotypes 2, 4, and 8 (11). Viral particles were generated, purified, and titered as described by Grimm *et al.* (15).

Fatty Acid Uptake Assay in HEK293 Cells—Fatty acid uptake assays were performed as described previously (12).

General Animal Procedures—C57BL/6 or Swiss Webster mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and acclimated for 1 week after arrival before they were used for experiments. Animals were maintained on regular lab chow (5P75, LabDiet, Richmond, IN) or on a special diet (see below), receiving food and water *ad libitum*, and kept at 22 °C on a 12-h/12-h light/dark cycle. FATP5 knock-out mice were generated and maintained as described previously (9). All study groups had comparable initial ages and weights. Intravenous sdsAAV8 deliveries (5×10^{10} or 3×10^{11} viral particles (v.p.), total volume 250 μ l in PBS) were performed by tail vein injections using established methods (16). For diet studies, separately housed 8-week-old male mice were fed *ad libitum* a high fat diet containing 60% fat (D12492, Research Diets, NJ) or a low fat diet (D12450) containing 10% fat. Weight was measured weekly, and food intake was measured twice a week. Standard glucose tolerance tests and insulin tolerance tests as well as lipid gavages were performed as described previously (17). All procedures were approved by the Animal Care Committee at the Palo Alto Medical Foundation Research Institute and the University of California Berkeley Institutional Animal Care and Use Committee.

Hepatocyte Preparation—Mouse livers were cannulated through the portal vein and an incision was made in the inferior vena cava. Liver perfusion with digestion and perfusion media and isolation of hepatocytes was carried out according to the manufacturer's instructions (Invitrogen) followed by long-chain fatty acid uptake assays as described (9).

Tissue Lipid Analysis—Samples from various tissues were powdered in liquid nitrogen, and total lipids were extracted by the method of Folch *et al.* (18). Total triglycerides were assayed using a colorimetric kit (Sigma Diagnostics).

Liver Morphology—Cryosections obtained from livers of FATP knockdown or knock-out animals and the respective controls were stained with either Masson's trichrome reagent or hematoxylin and eosin. Neutral lipid droplets were stained with BODIPY^{493/503} (Molecular Probes).

Statistical Analysis—Statistical analysis of FATP5 knockdown or FATP5 knock-out phenotypes *versus* scrambled control (SCR) or wild-type controls was done by Student's *t* test or analysis of variance test as appropriate. S.E. values are shown. $p < 0.05$ was considered significant.

RESULTS

shRNA-mediated Knockdown of Hepatic Fatty Acid Transporters in Vitro—We generated (14) eight FATP5 shRNA expression construct, and an SCR with no complementarities to any known gene sequence in the mouse genome (supplemental Fig. 1) and assessed the impact of the shRNA constructs on FATP expression and FFA uptake by co-transfecting them into HEK293 cells with FATP5 expression plasmids. Two days following transfection, FFA uptake and FATP protein levels were determined using fluorescence-activated cell sorter-based assays (12) (Fig. 1A) and immunoblots (Fig. 1B), respectively. Both assays identified two constructs (FATP5-2, FATP5-3) capable of triggering a robust RNA interference effect. We did not observe any effect for the other shRNA constructs or the SCR control when compared with cells transfected with the respective FATP expression vectors only.

Specific and Non-toxic Knockdown of Hepatic Fatty Acid Transport Protein 5 in Vivo—To translate our *in vitro* results to an *in vivo* system of diet-induced NAFLD, we used optimized viral vehicles (11) to package and express our *in vitro* validated target sequences for FATP5 and the SCR control, resulting in the sdsAAV vectors sdsAAVFATP5-2, sdsAAVFATP5-3, and sdsAAVSCR. Two different mouse strains, C57BL/6 and Swiss Webster mice, were injected via the tail vein with the different viral constructs at two different concentrations (5×10^{10} and 3×10^{11} v.p.). Four weeks after injection (a.i.), hepatic protein expression of FATP5 was analyzed by Western blotting. FATP5 expression was considerably blunted (>95% inhibition) in the sdsAAVFATP5-2-injected animals, but not in the sdsAAVFATP5-3-injected animals (Fig. 1C). Clearly, more research is needed to determine the factors that ultimately determine *in vivo* efficacy of shRNA constructs. The functional construct will be referred to from hereon as "AAVFATP5." Robust FATP5 knockdown was achieved at the lowest viral dose tested (5×10^{10} v.p.), which is considerably less than in previous studies using 1×10^{12} v.p. (11). Importantly, we did not detect any nonspecific effects of FATP5 knockdown on the expression levels of other liver proteins (Fig. 1C), including the highly related FATP2 and FATP4 (43%/33% identity with FATP2/FATP4). Based on serum aspartate and alanine aminotransferase levels and liver histology, shRNA expression and FATP knockdown caused no hepatotoxicity after viral transduction (data not shown). Thus, using this sdsAAV-based *in vivo* RNA interference approach, we were able to achieve specific and non-toxic protein knockdown of endogenous FATP5. To quantify the magnitude by which loss of FATP5 would reduce FFA uptake, we performed *ex vivo* uptake assays with freshly isolated hepatocytes from *ad libitum* chow-fed mice 4 weeks after viral transduction as described before (9). Although there was no difference in the fatty acid uptake capacity between hepatocytes from the AAVSCR and PBS group, our experiments demonstrated a robust (40%) reduction in long-chain fatty acid uptake after knockdown of FATP5 (Fig. 1D). The remaining uptake activity either may be due to other hepatic FATPs such as FATP2 and -4 and/or may represent protein-independent uptake.

Loss of Hepatic FATPs Redirects Dietary Lipid Fluxes—To characterize changes in the postprandial clearance of lipids in

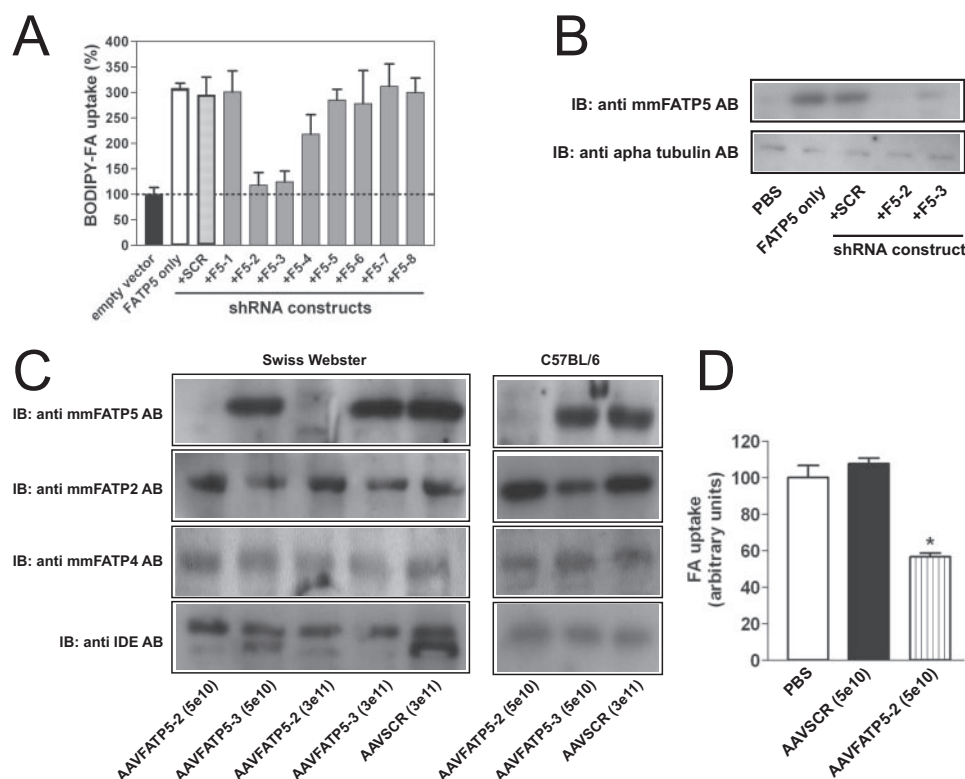


FIGURE 1. *In vitro* and *in vivo* knockdown of FATP2 and FATP5. *A*, HEK293 cells were co-transfected with empty expression vector (*black bar*) or FATP5 expression plasmids alone (*white bar*) or in combination with the indicated shRNA constructs. Uptake of fluorescent fatty acid (FA) (C1-BODIPY-C12) was determined 2 days after transfection by flow cytometry. Mean fluorescence normalized to empty vector. *Error bars* indicate standard deviation. *B*, Western blot analysis of cell lysates from co-transfections. α -Tubulin served as loading control. *IB*, immunoblot. *C*, Western blot analysis of sdsAAV-shRNA-mediated knockdown of FATP5 protein *in vivo*. Proteins were extracted from liver tissue homogenates of different mouse strains (C57BL/6 and Swiss Webster) 4 weeks after injection with the indicated constructs and viral titers and probed with antisera specific for FATP5, FATP2, FATP4, and insulin-degrading enzyme. *AB*, antibody. *D*, fatty acid uptake by hepatocytes isolated 4 weeks after virus injection. FFA uptake was determined *ex vivo* using a fluorescent fatty acid uptake assay.

AAVSCR and AAVFATP5 mice, we performed oil gavages containing [¹⁴C]oleate tracer after an overnight fast using C57BL/6 mice fed a normal chow 4 weeks a.i. Initial appearance of ¹⁴C in serum was comparable among all groups (Fig. 2*A*), indicative of normal absorption in all animals. However, ¹⁴C counts (Fig. 2*A*) as well as serum TG and FFA (data not shown) were elevated for prolonged periods in the FATP5 knockdown group, hinting at impaired lipid clearance from the circulation, presumably due to loss of hepatic FATP function. Four hours after gavage, we found decreased absorption by the livers of FATP5 knockdown animals with increased lipid deposition in heart, skeletal muscle, and fat (Fig. 2*B*). These data are consistent with the observed changes of liver total TG content following chronic high fat feeding (see Fig. 4*B*) and suggest that loss of hepatic FATPs causes a redirection of lipids away from the liver to tissues relying on other FATP paralogues, such as FATP6, -1, and -4. Taken together, these findings strongly support the notion that FATP5 plays a critical role in hepatic fatty acid uptake and in the compartmentalization of postprandial lipids.

Comparability of postprandial lipids.

Comparable Phenotypes of FATP5 Knockdown and Knock-out Animals—Next we compared the phenotypes of FATP5KO mice with AAVFATP5-injected wild-type mice (both in a C57BL/6 genetic background) placed on a 5-week HF diet. Weight gain and food consumption by FATP5KO and AAVFATP5-injected animals started to deviate significantly from the wild-type and AAVSCR-injected controls 2 weeks a.i. to similar extents. This resulted by the end of the study in a 40% weight reduction and significantly improved serum TG levels for both hypomorphic groups when compared with their respective controls (Fig. 3, *A* and *B*). Although livers of PBS- and AAVSCR-injected control mice showed typical characteristics of macrovascular steatosis, both AAVFATP5-injected mice and FATP5KO mice (9, 10) displayed a significant protection with a marked decrease in lipid infiltration (Fig. 3*C*) and significant reduction in total liver TG content (AAVSCR, 35.4 ± 1.1 μmol/g; FATP5KO, 21.9 ± 4.2 μmol/g). In summary, these results demonstrate that a single sdsAAV-mediated delivery of shRNA expression constructs results in phenotypes that are highly comparable with that of genetically engineered knock-out animals and that AAV-mediated loss of FATP5 protects animals from the development of diet-induced hepatic steatosis.

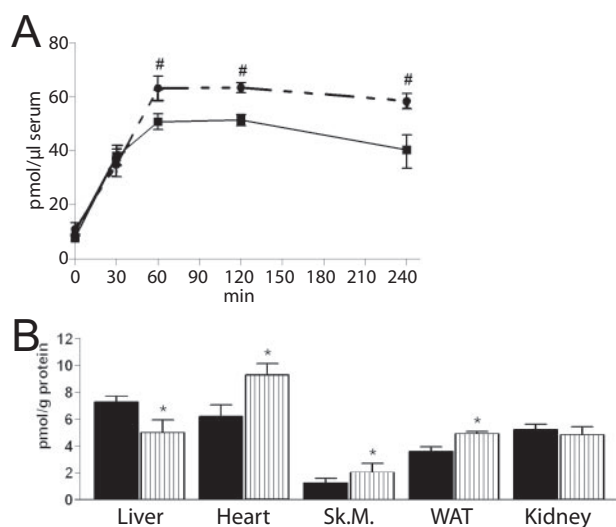


FIGURE 2. Effects of FATP knockdown on postprandial lipid flows. Four weeks after viral transduction, mice were gavaged with 250 μl of olive oil spiked with 3.5 μCi of [¹⁴C]oleic acid. *A*, ¹⁴C counts were determined in serum samples drawn at 0, 30, 60, 120, and 240 min after gavage. *B*, 240 min after gavage, mice were euthanized, and ¹⁴C counts normalized to protein content were determined for liver, heart, skeletal muscle (*Sk.M.*), white adipose tissue (*WAT*), and kidney lysates. *Error bars* indicate standard deviation, and *asterisks* indicate *p* < 0.05 in Student's *t* test.

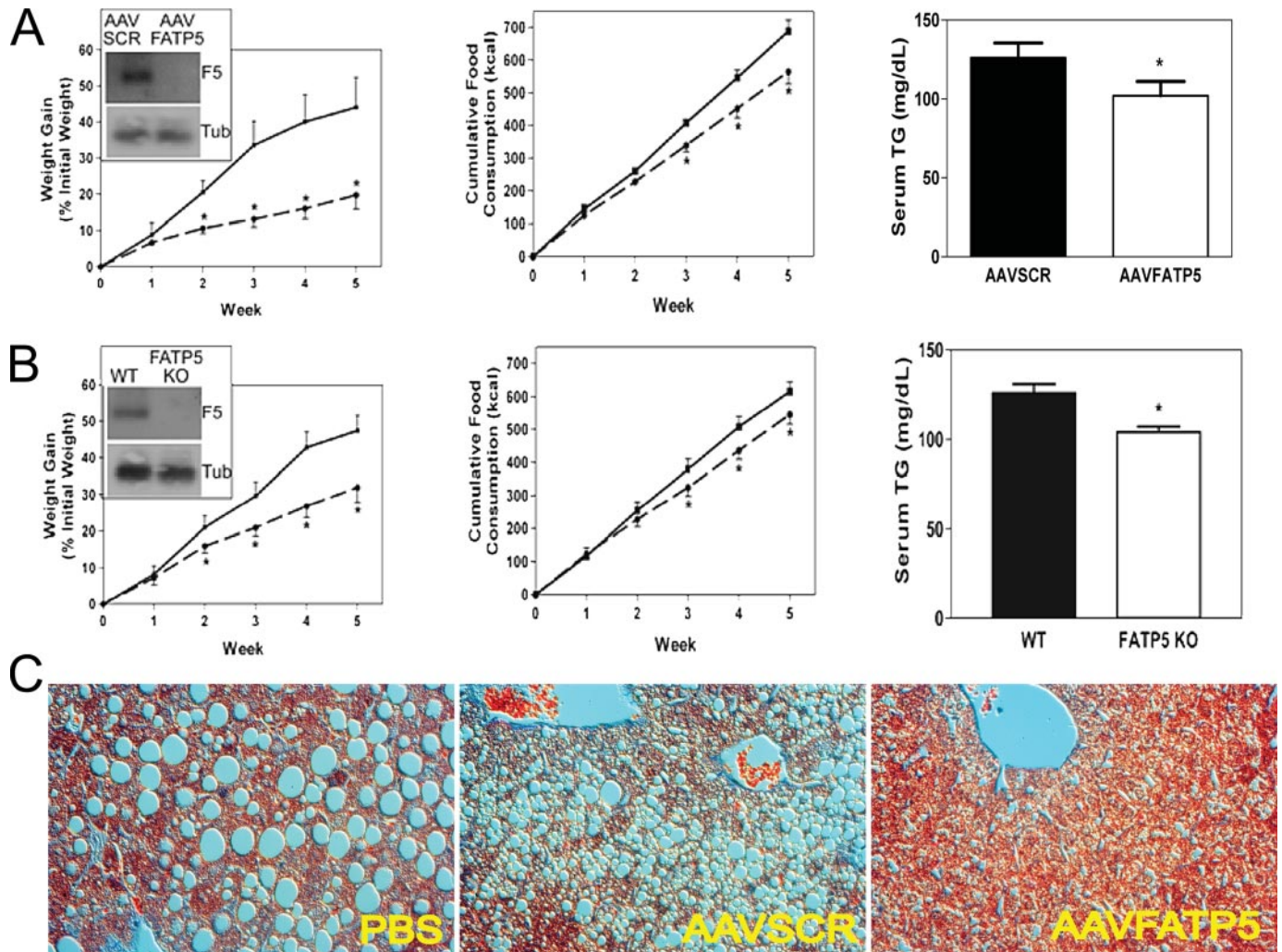


FIGURE 3. Comparison of AAVFATP5 knockdown and FATP5 gene knock-out phenotypes. *A* and *B*, weight gain (left panels) and caloric consumption (middle panels) of AAVFATP5 (solid line) versus AAVSCR (broken line) animals (*A*) and FATP5KO (broken line) versus wild-type (WT, solid line) animals (*B*) (all in a C57BL/6 background) fed HF diet (60% fat) for 5 weeks. Insets show Western blots for FATP5 and a loading control (tubulin (*Tub*)). Serum TG levels (right panels) were determined at the end of the 5-week study for controls (black bars) and hypomorphs (white bars). *C*, Masson's trichrome staining of liver sections from PBS-, AAVSCR-, and AAVFATP5-injected mice at the end of the 5-week HF feeding study. Error bars indicate standard deviation, and asterisks indicate $p < 0.05$ in Student's *t* test.

Knockdown of FATP5 Reverses Hepatic Steatosis—To test the feasibility of hepatic shRNA-mediated FATP knockdown to reverse already established NAFLD, C57BL/6 mice were fed for 6 weeks a HF diet or a nutrient-matched low fat diet (ND) followed by AAVshRNA injection and were then kept on the respective diets for an additional 7 weeks. Mice were injected with either PBS or 5×10^{10} v.p. of AAVSCR or AAVFATP5 to achieve *in vivo* knockdown. We chose a 6-week pretreatment period as preliminary studies showed that 6 weeks of HF diet feeding induce hepatic steatosis with marked lipid droplet accumulation inside of hepatocytes and a significant increase in liver TG (supplemental Fig. 2). However, even after 13 weeks of HF feeding, liver enzymes remained at normal levels (supplemental Fig. 3), indicating that this model more closely resembles mild forms of NAFLD. AAV-injected and control groups kept on the ND did not show any signs of abnormalities throughout the study (not shown). Although transduction of animals with the AAVSCR control resulted in phenotypes indistinguishable from PBS-injected mice

(not shown), we found that the single injection of AAV-FATP5 resulted in complete, specific, and persistent ablation of hepatic FATP5 in both HF-fed (Fig. 4A) and ND-fed animals (data not shown). As before, basic liver function was unimpaired by the shRNA-mediated knockdowns as serum aspartate aminotransferase, alanine aminotransferase, and bilirubin levels were similar to control animals (supplemental Fig. 3). Importantly, when compared with controls (PBS or AAVSCR injections), livers from AAVFATP5-transduced animals showed a significant improvement of NAFLD symptoms, including normalized morphology lacking signs of macrovesicular steatosis (Masson's trichrome, Fig. 4C), diminished neutral lipid droplet deposition (as assessed by BODIPY^{493/503} staining, Fig. 4D), and reduced total hepatic TG content (SCR, $80.9 \pm 10.1 \mu\text{mol/g}$; FATP5, $28.1 \pm 2.99 \mu\text{mol/g}$; Fig. 4B). Taken together, our results show that targeting hepatic FATPs via sdsAAV-shRNA-mediated RNA interference can significantly improve established obesity-associated hepatic steatosis even in the continued presence of high caloric diets.

Knockdown of FATP5 Reverses NAFLD

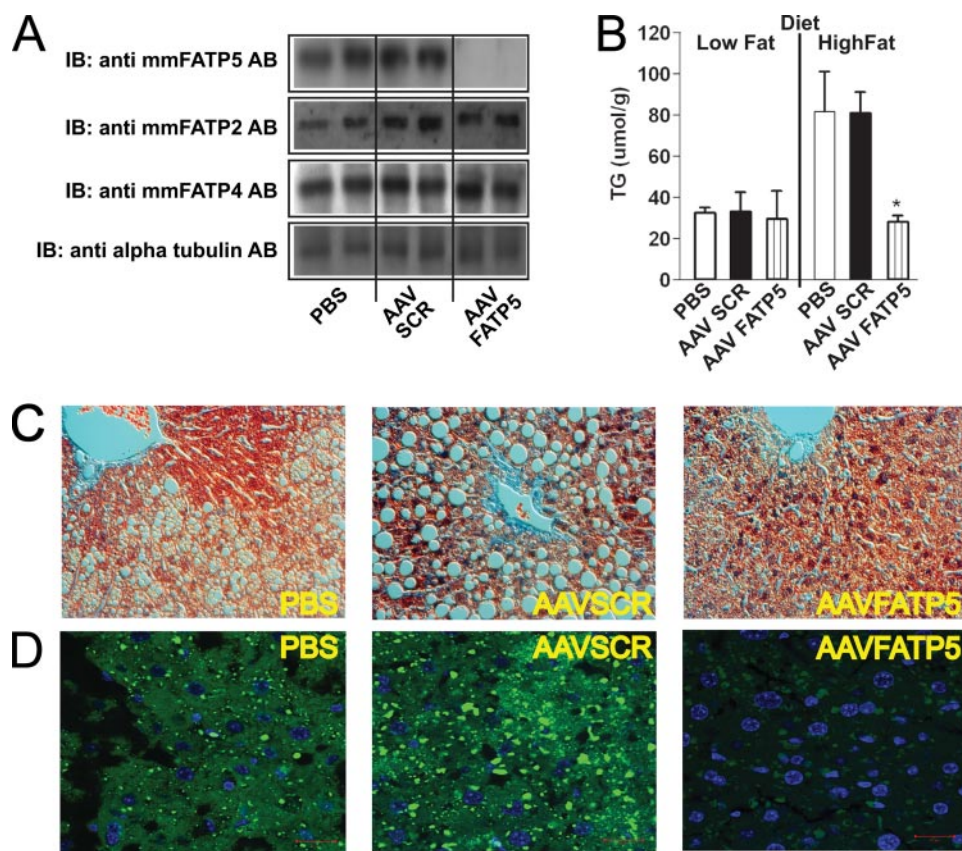


FIGURE 4. Reversal of obesity-induced NAFLD *in vivo*. Six-week-old C57BL/6J mice were fed HF or ND chows for 6 weeks followed by tail vein injections of PBS or the indicated AAV-shRNA constructs and then maintained for an additional 7 weeks on their respective diets. *A*, Western blot showing expression of FATP5, FATP2, FATP4, or tubulin in liver lysates from C57BL/6 mice transduced with the indicated viruses. *B*, immunoblot; *AB*, antibody. *B*, total hepatic TG content normalized to protein of mice fed low or high fat diet for a total of 13 weeks determined 7 weeks a.i. of the indicated AAVs. *C*, Masson's trichrome staining. *D*, BODIPY493/503 fluorophore (green)/4',6-diamidino-2-phenylindole (blue) staining for neutral lipids and nuclei of liver sections at the end of the diet study.

shRNA-mediated Reversal of NAFLD Improves Whole-body Energy Homeostasis—HF-fed AAVFATP5-injected mice showed a significant decrease in body weight when compared with controls (area under curve, 293 versus 401; $p = 0.047$) starting at week 3 a.i. and approaching the values for the ND study groups by week 5 a.i. The reduced weight gain observed in the AAVFATP5-injected HF groups was, at least in part, due to a reduced caloric intake, which significantly deviated from the control group at 3 weeks a.i. (Fig. 5*B*). A similar reduction of HF chow consumption was observed in FATP5KO mice (9). Differences in body weight were reflected by significantly smaller epididymal fat pads in FATP5 knockdown animals (71% reduction, when compared with SCR control); in contrast, both heart (SCR, 0.5 ± 0.1 g versus FATP5, 0.7 ± 0.1 g) and livers (SCR, 3.5 ± 1.8 g versus FATP5, 6.9 ± 1.6 g) were significantly enlarged. Organs from PBS-injected animals remained indistinguishable from the AAVSCR control group throughout the study. Following the initial 6 weeks of HF feeding, all mice developed hyperglycemia. Knockdown of FATP5 led to a rapid (2–3 weeks a.i.) reduction in serum glucose levels, which reached normoglycemia by week 5 a.i. (Fig. 5*C*). This improvement in glucose homeostasis was also reflected in glucose tolerance test and insulin tolerance test profiles 7 weeks a.i. showing profiles for HF-fed FATP5 knockdown mice (insulin

tolerance test HF FATP5 versus HF SCR area under curve mean difference 12004, $p < 0.001$) that were indistinguishable from ND-fed animals (Fig. 5, *D* and *E*).

DISCUSSION

A prior study of AAV8-shRNA vectors that differed in shRNA length and loop sequence, directed against transgenes, revealed that 36 out of 49 shRNA/vector combinations resulted in dose-dependent liver injury caused by perturbation of microRNA biosynthesis (11). Importantly, optimized construct sequences, promoters, and transduction conditions that proved to be non-toxic were also identified (11) and adapted by us for the stable, non-toxic, and liver-directed *in vivo* knockdown of FATP5. A single injection of sdsAAV-shRNA viruses sufficed to suppress expression of FATP5, resulting in phenotypes that were largely comparable with those of classical knock-out models while being substantially less time- and work-consuming. Further, major advantages of AAV-mediated gene knockdown over previously reported methods aiming at gene hypomorphism, such as antisense oligonucleotide injections (19), different double-stranded small RNA injections (20), or other viral approaches (e.g. adenoviral (21) or retroviral (22)), include robust and liver-directed transduction, lack of inherent pathogenicity, and potential for long term persistence of shRNA expression (11).

Investigation of the sources of hepatic TG in patients with NAFLD using stable isotope labeling techniques showed that 74% were derived from exogenous FFAs and 26% from *de novo* synthesis (23). Dietary lipids have been shown to rapidly influence liver fat content (24), and high fat diets cause NAFLD in humans and animals (25). Although diet-derived lipids are likely to significantly contribute to the etiology of obesity-associated NAFLD, *de novo* synthesis can also play an important role as overexpression of SREBP-1c results in hepatic steatosis (26). In contrast, a liver-specific knock-out of SREBP-1c in obese ob/ob mice resulted in a marked decrease in liver TG (27). The role of proteins in hepatic fatty acid uptake has been appreciated for over a decade (28), whereas the identity of the components of this uptake system has only recently been addressed genetically, demonstrating an important role of FATP5 (7).

We argued that if obesity-associated NAFLD is primarily driven and maintained by the continuous protein-mediated uptake of fatty acids by the liver, suppression of FATP5 function should at least partially revert hepatic steatosis. Indeed, we

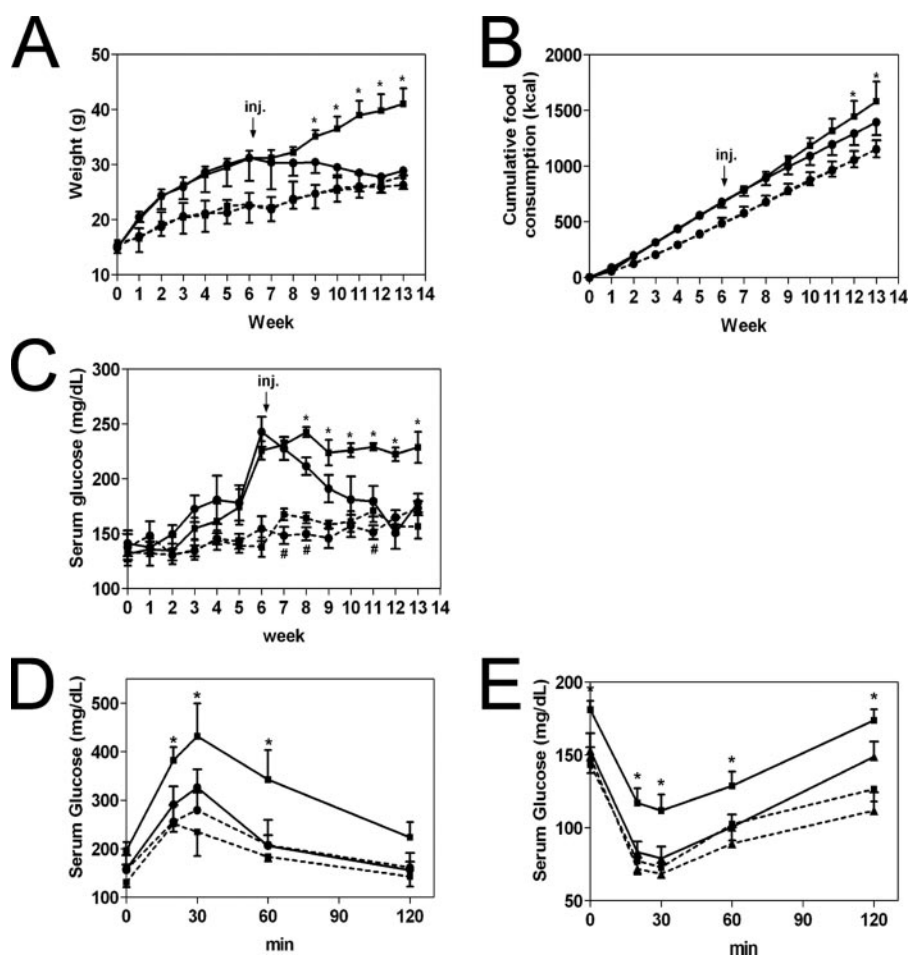


FIGURE 5. **Metabolic consequences of FATP5 knockdown in animals with NAFLD.** A–C, mice from Fig. 3 were analyzed throughout the study for weight gain (A), food consumption (B), and ND-fed serum glucose (C). D and E, glucose tolerance test (D) and insulin tolerance test (E) were performed at the end of the study with fasted animals. Circles, AAVFATP5; squares, AAVSCR. Solid lines, HF diet; dotted lines, nutrient-matched low fat diet. Error bars indicate standard deviation, and asterisks indicate $p < 0.05$ in Student's *t* test.

found that knockdown of FATP5 reduced established hepatic steatosis and hyperglycemia of mice fed a high fat diet. We identified two mechanisms that may underlie these beneficial effects. Firstly, loss of FATP5 reduces hepatic fatty acid uptake rates, as shown with isolated hepatocytes, and decreases hepatic postprandial fatty acid deposition following oil gavages. Decreasing hepatic lipid load is likely to improve insulin sensitivity in this organ (3). Interestingly, the inverse lipid distribution pattern was observed in previous studies with another FATP family member expressed by extrahepatic tissues showing that loss of FATP1 activity led to a decrease in fat pad and muscle TG and increase in liver lipids (17). Thus, we propose that the activity and tissue distribution of FATPs play a critical role in determining the metabolic fate and organ-specific impact of dietary fatty acids. Importantly, exogenous dietary fatty acids not only contribute directly to hepatic TG accumulation but can also stimulate endogenous fatty acid and cholesterol synthesis. Particularly, saturated fatty acids have been found to increase hepatic levels of the transcriptional co-activator PGC-1 β , which in turn leads to increased SREBP- and liver X receptor-dependent transcriptional activity, resulting in elevated TG, cholesterol, and very low density lipoprotein synthe-

sis (29). Thus, targeting of hepatic FATPs may be preferable over downstream targets in lipid metabolism such as DGAT2. Indeed, although suppression of hepatic DGAT2 via antisense oligonucleotides led to reduced hepatosteatosis in obese mice, it neither improved insulin sensitivity nor body weight (19), and hepatic DGAT2 overexpression resulted in steatosis but not insulin resistance (30). This is in clear contrast to our findings here for FATP5 knockdown, which leads to a reversal of obesity-induced NAFLD and improves whole-body glucose homeostasis.

A likely second contributor to the improved hepatic steatosis and glucose homeostasis following FATP5 knockdown is the reduction in caloric uptake and body weight. The mechanism behind this interesting phenotype is poorly understood. As reduced food uptake upon FATP5 knockdown was only observed when animals were fed a high fat diet (Fig. 5B) and postprandial clearance of FFA from the circulation was delayed in FATP5 KO animals (9, 10), one possible explanation could be that the elevated postprandial serum fatty acid levels themselves serve as a satiety signal (31).

Taken together, we have made three important observations. Firstly, we demonstrate that AAV-mediated

shRNA expression is an efficient tool for the suppression of hepatic genes in normal and steatotic livers. This is an important finding as this is the first report of non-toxic, liver-specific transduction and RNA interference induction utilizing stabilized double-stranded serotype 8 AAV particles and should allow for future experiments to dissect the molecular components underlying hepatic lipid fluxes under physiological and pathophysiological conditions. Secondly, we show that AAV-mediated knockdown of FATP5 protects from high fat diet-induced hepatic steatosis and redirects postprandial lipid fluxes away from the liver. Lastly, suppression of FATP5 is sufficient to reverse established NAFLD in our diet-induced obesity model and to improve whole body glucose homeostasis. Thus, reduction of hepatic FATPs either via gene therapy or via small molecular inhibitors is a novel tool to dynamically redirect lipid fluxes and may provide novel approaches for the treatment of NAFLD and insulin resistance.

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