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Shp deletion prevents hepatic steatosis and when combined with Fxr loss protects against type 2 diabetes

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Abstract

Nuclear receptors Farnesoid X Receptor (FXR) and Small Heterodimer Partner (SHP) are important regulators of bile acid, lipid and glucose homeostasis. Here we show that global *Fxr*^{-/-} *Shp*^{-/-} double knockout (DKO) mice are refractory to weight gain, glucose intolerance and hepatic steatosis when challenged with high-fat diet. DKO mice display an inherently increased capacity to burn fat and suppress *de novo* hepatic lipid synthesis. Moreover, DKO mice are also very active and that correlates well with the observed increase in *Pepck* expression, type IA fibers and mitochondrial function in the skeletal muscle. Mechanistically, we demonstrate that liver-specific *Shp* deletion protects against fatty liver development by suppressing the expression of *Pparγ2* and lipid-droplet protein *Fsp27β*. Conclusions: These data suggest that *Fxr* and *Shp* inactivation may be beneficial to combat diet-induced obesity and uncover that hepatic SHP is necessary to promote fatty liver disease.

Keywords

Nuclear Receptors; Fatty liver disease; Farnesoid X Receptor; Small Heterodimer Partner and Fat-Specific protein 27β

INTRODUCTION

Obesity is a major epidemic with a stark prevalence of about 35% in adults, as estimated by the Center of Disease Control and Prevention in the United States. Importantly, obese individuals have an increased risk for developing a variety of metabolic diseases, including

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fatty liver (hepatic steatosis), abnormal lipid levels in the blood (dyslipidemia), and most commonly type II diabetes (1–3). Fat accumulation in the liver can promote insulin resistance, (3, 4) which in turn results in increased morbidity and mortality rates. Thus, understanding the molecular machinery that causes fatty liver will provide insight into approaches that are amenable for therapeutic options.

Nuclear receptors, Farnesoid X Receptor (FXR) and Small Heterodimer Partner (SHP) in addition to regulating bile acid levels also control energy metabolism and glucose homeostasis (5–10). Several human SNPs in *Fxr* gene have been correlated with fasting hyperglycemia and free fatty acids (6) while mutations in the *Shp* gene are associated with obesity (11, 12) and susceptibility to type 2 diabetes (13). Global *FxrKO* mice are protected against diet-induced obesity; however, there is also evidence that *FxrKO* mice are glucose intolerant and insulin insensitive (14–16). *ShpKO* mice do not exhibit obesity but develop insulin resistance when challenged with western high carbohydrate/high fat diet (17). These results demonstrate that FXR- and SHP- mediated control of glucose is multi-layered.

In addition to glucose control, FXR and SHP are known to play a critical role in regulating cholesterol, and lipid levels (18, 19) and thus can directly affect fat content in the liver. For instance, FXR via activation of *Shp* has been shown to suppress *Srebp1-c*, an important regulator of lipogenesis in the liver (20). Thus, FXR and SHP are central for metabolic homeostasis since they not only regulate glucose levels but also hepatic fat content. These two tasks are subtly regulated to maintain a beneficial metabolic state. But when this balance is perturbed, it can trigger a vicious cycle whereby hepatic steatosis can cause insulin resistance which in turn can promote more hepatic steatosis (21).

We previously discovered a synergistic role for FXR and SHP in controlling bile acid levels (22), which led us to investigate if these receptors cooperate to maintain energy balance and to carefully dissect their liver-specific contribution towards metabolic homeostasis.

Here we show that combined loss of *Fxr* and *Shp* in mice (*DKO*) results in reduced body weight gain, and better glucose control both under chow as well as high-fat fed conditions. Further, DKO mice displayed increase in fat burning capacity, mitochondrial function, and type IA fibers along with higher physical activity. This was substantiated by the observed increase in *Pepck* and *Pparγ* transcript levels in the DKO skeletal muscle compared to the WT mice on high-fat diet. Notably, DKO animals did not develop fatty liver when challenged with a high fat diet. Mechanistically, we find that fatty acid synthesis and metabolism is down regulated in the livers of the DKO mice. Importantly, protection against hepatic steatosis upon high fat feeding was recapitulated when *Shp* was specifically deleted in the liver (*L-ShpKO*). These findings indicate that antagonizing SHP may be beneficial to treat hepatic steatosis.

METHODS

Animals

The generation of *Fxr* and *Shp* double knockout mice has been previously described²². Mice were bred and maintained in a temperature-controlled (23°C) facility on a standard 12-hour-

light/dark cycles. Adult wild type (WT) C57BL/6, DKO, *Fxr*KO, *Shp*KO, *Albumin-Cre*, *Flox/Flox Fxr*, *Flox/Flox Shp*, *L-Fxr*KO, and *L-Shp*KO mice were used in this study. *L-Fxr*KO and *L-Shp*KO mice were generated by breeding the respective homozygous floxed alleles with *Albumin-Cre* animals. At 8–10 weeks, mice were fed normal chow diet (11% fat) or 45% high fat diet (HFD) from Harlan Teklad for 8 weeks to mimic obese conditions. *Flox/Flox* control, and *L-Shp*KO mice were also challenged with control or western diet (WD) from Harlan Teklad for 21 weeks (23). Mice were sacrificed at the end of the experimental regimen. Liver, intestine, and skeletal muscle tissues were collected for gene expression and histology. National Institutes of Health (NIH) guidelines for use and care of laboratory animals was followed and all the experiments were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign, Baylor College of Medicine and at Northeast Ohio Medical University. Body fat composition was evaluated by dual energy x-ray absorptiometry.

Indirect calorimetry was performed using the Comprehensive Lab Animals Monitoring System system (Oxymax, Columbus Instruments) as previously described (24). Animals were acclimated to the CLAMS cages and data were collected over the subsequent 72 hr time period. Food intake and time spent to access food was recorded *ad libitum*. Feces were collected after 24 hours of acclimatization. Pair-fed mice were utilized to measure the rate of oxygen consumption (VO₂), carbon dioxide production (VCO₂) as well as ambulatory activity. Data was analyzed using Oxymax software (Columbus Instruments).

RNA Analysis

Total RNA from mice livers were isolated using TRIzol (Invitrogen) reagent and prepared according to manufacturers instructions. Upon DNase treatment (NEBioLabs), RNA (~2ug) was reverse transcribed using random primers and Maxima reverse transcriptase kit (Thermo Scientific). The cDNA was diluted to 12.5ng/uL and 50 ng was used for each SYBR green-based qPCR reaction.

Serum Chemistry

Blood was collected via retro-orbital bleeding from mice and centrifuged at 8000×g for 10 min. Serum triglycerides and cholesterol were performed using Thermo Scientific Total cholesterol or triglyceride kits. Additionally, Insulin ELISA kit (AbNova) was used to determine serum Insulin levels. Serum glucose was measured using one-touch glucose strips.

Glucose and Insulin tolerance tests

Glucose tolerance test (GTT) and Insulin Tolerance Test (ITT) were performed as previously described (17, 25). Briefly, mice were fasted overnight and injected with D-glucose at 2g/kg intraperitoneally to perform GTT. Blood glucose concentrations were measured at 0, 15, 30, 60 and 120 min after glucose injections. For ITT, the mice were fasted for 4 hours and injected intraperitoneally with insulin (Humulin, Eli Lilly) 1.0U/kg body weight. Blood glucose concentrations were measured at 15, 30, 60, and 90 min after insulin injection.

Histology and Imaging

Liver and skeletal muscle tissues were fixed in 10% neutral-buffered formalin or embedded in optimum cutting temperature (OCT) fixative and processed. Frozen sections were stained with either oil red O or succinate dehydrogenase as previously described (26). Paraffin embedded sections were used to perform H & E staining. After a 4-hour fast, mice were gavaged with BODIPY 500/510 and olive oil (10 μ l/g body weight). Liver and intestines were excised and frozen in OCT medium 4 hours post-gavage. Ten-micrometer sections were cut, mounted with Antifade reagent with DAPI, and examined under fluorescence microscope and quantitated using adiposoft in Image J (27).

Mitochondrial Activity

The electron transport chain enzymes were assayed using a temperature-controlled spectrophotometer (Ultraspec 6300 pro, Biochrom Ltd., Cambridge, England) as previously described (28). All the assays were performed in duplicates. The activities of complex I (NADH:ferricyanide reductase), complex II (succinate dehydrogenase), and complex IV (cytochrome c oxidase) were measured. Citrate synthase (CS) was used as a marker for mitochondrial content. Enzyme activities are expressed in nmol/min/mg protein.

Statistical Analysis

Data are expressed as mean $\pm$ SEM, unless otherwise specified. Statistics were performed using Graph Pad Prism 6 software. Student's t-test was used to compare two groups, and One-way ANOVA Bonferroni test for multiple group comparisons. Data is significant if p-value $\leq$ 0.05, with * to denote p $\leq$ 0.05, ** denotes p $\leq$ 0.01, *** denotes p $\leq$ 0.001, and **** denotes p $\leq$ 0.0001.

RESULTS

***Fxr*, *Shp* DKO mice are resistant to diet induced obesity and are protected against developing hepatic steatosis**

To determine if there is a synergistic role for FXR and SHP in maintaining glucose and energy metabolism, we challenged the *Fxr* and *Shp* double knockout (DKO) mice (22) with either a chow diet or a high fat diet. Compared to WT, the DKO mice had a lower body weight even on the normal chow diet (Figure 1a–b). Despite the reduction in body weight, the DKO mice exhibited an increase in lean mass with a striking reduction in total fat mass (Figure 1c). We next examined if the difference in weight gain is due to alterations in food intake by CLAMS. We found that the DKO mice ate less than WT under normal chow condition but this reduction was lost when normalized to their respective body weights (Figure 1d). To evaluate the decrease in absolute value of food consumed in DKO mice we examined serum leptin levels, which inhibits feeding (29, 30), and found lower leptin levels in DKO mice indicating that these mice did not have a suppressed appetite (data not shown). However the basal glucose levels were lower and upon glucose and insulin challenge the DKO animals displayed better glucose homeostasis and insulin sensitivity (Figure 1e–f, Supplementary figure 1a–b) than the WT mice. The DKO mice also showed inflammation and cytoplasmic swelling of hepatocytes along with no change in circulating triglycerides or

cholesterol levels (Supplementary figure 1c–e). Furthermore, urine amount, fecal weight and feces color of the DKO mice were similar to that of the WT mice. This data suggests that the DKO mice may not excrete excess fat in their feces (Supplementary figure 1f). We conclude that FXR and SHP are necessary for maintaining a normal body weight, and in their absence animals have less body fat and better glucose homeostasis.

We next wanted to determine how the mice responded when challenged with a high fat diet (HFD). The DKO mice were resistant to gaining weight (Figure 2a), had reduced fat mass (Figure 2b) despite similar food intake, did not develop insulin resistance and maintained an excellent glucose tolerance (Figure 2c–f, Supplementary figure 2a) compared to WT animals. Further, HFD fed DKO animals displayed similar circulating levels of insulin, triglyceride and cholesterol except significantly reduced levels of hepatic triglycerides compared to HFD fed WT mice (Supplementary figure 2a–c). We then questioned whether DKO mice had a defect in fat uptake in the gut. To address this, we oral gavaged BODIPY together with olive oil and analyzed the enterocytes with confocal microscopy. We found abundant labeled lipid droplets in both the WT and DKO mice indicating no defect in absorption except that DKO mice showed smaller droplet size (Supplementary figure 2d–e).

DKO mice burn more fat and exhibit increased activity compared to WT mice

We then examined if energy expenditure and fat burning were contributing towards the lean metabolic phenotype in the DKO mice by measuring oxygen consumption (VO₂) and carbon dioxide production (VCO₂) (Figure 3a–b). Our analysis revealed that the DKO mice have lower Respiratory Exchange Rate (RER) both during the day and night compared to WT mice indicating preferential fat burning (Figure 3c). The time point line graphs clearly demonstrate that both WT and DKO mice exhibit increased carbohydrate burning during the night as they eat nocturnally (Supplementary figure 3a–c). Despite this shift, the DKO mice maintain an overall lower RER throughout day and night under HFD that is more pronounced under normal chow fed state (Supplementary figure 3c).

We next analyzed their physical activity and found that DKO mice displayed increased activity (Figure 4a & c). Since the DKO mice were more mobile and active, we examined if the skeletal muscle was supporting efficient fat utilization. We carefully analyzed the mitochondrial respiratory chain complexes I through IV in the skeletal muscle and identified a significant increase in complex I activity along with the complementary increase in complex IV activity. Additionally, the Krebs cycle enzyme citrate synthase, which indicates mitochondrial function, was also considerably up regulated in the DKO mice (Figure 4b). These data clearly show enhanced mitochondrial function in the DKO skeletal muscle compared to the WT mice. Substantiating these functional biochemical data, we also demonstrate that the mitochondria-rich type I slow-twitch myofibers were significantly increased in the quadriceps muscle of DKO mice (Supplementary figure 4a & c). Importantly, these high oxidative capacity type I fibers were more pronounced when challenged with HFD in the DKO than the WT mice (Supplementary figure 4b & d). Coherent with the increased oxidative capacity, the muscles expressed high levels of β -oxidation gene *Cpt1*, *Ppar γ* and *Pepck* gene in the DKO mice (Figure 4d). Previously it has been shown that increase in skeletal muscle *Ppar γ* levels increases oxidative muscle fibers

(31) whereas increase in *Pepck* is associated with elevated citric acid cycle flux and a preference for fatty acids as a fuel source (32). Similarly, the up regulation of muscle *Cpt1* is directly linked with increased fatty acid oxidation and improved insulin sensitivity (33). Taken together, these results reveal that the skeletal muscle of the DKO mice promotes enhanced fat usage in these animals.

DKO mice do not develop fatty liver, and deletion of hepatic *Fxr* does not recapitulate this effect

We found that liver expression of a glucose sensing and lipid metabolic gene, Carbohydrate-responsive element-binding protein β (*Chrebp*- β) and a gluconeogenic gene, *Pepck*, were maintained lower when *Fxr* and *Shp* were deleted (Figure 5a). Interestingly, overexpression of *Chrebp*- β has been shown to promote hepatic steatosis and *Pepck* overexpression causes insulin resistance (34, 35). Thus, the hepatic *Chrebp*- β and *Pepck* expression are consistent with the beneficial metabolic profile of the DKO mice. Further, *Cpt-1*, the rate-limiting enzyme for fatty acid β -oxidation, was up regulated both in the liver of chow and HFD fed DKO animals (Figure 5b). Also, we found that the master regulators of fatty acid and triglyceride synthetic machinery in the liver, *Srebp1c*, *Fasn*, *Acc1* and *Dgat2*, were significantly downregulated in the absence of *Fxr* and *Shp* upon HFD (Figure 5c). Further histological analyses demonstrate an overall resistance to fat accumulation in the liver even when fed with excess calories (Figure 5d–e). These data indicate that decreased *de novo* lipid synthesis coupled with increased oxidation of fat protects the DKO livers from developing hepatic steatosis. The reduction in hepatic steatosis thus may contribute towards the beneficial glucose and insulin tolerance seen in these DKO animals.

It is known that FXR can regulate hepatic *de novo* lipogenesis and thus control triglyceride content in the liver (20). Therefore to precisely determine the role for hepatic FXR in mediating the beneficial effects observed in the DKO model, we generated the liver-specific *FxrKO* mice (*L-FxrKO*). In contrast to the DKO mice, the *L-FxrKO* animals gained weight, developed fatty liver, and became glucose intolerant on HFD similar to controls (Supplementary figure 5a–b, d). Our results reveal that the *L-FxrKO* mice accumulated more cholesterol and triglyceride levels in serum and that their liver triglyceride content was comparable to that of *f/f Fxr* mice fed HFD (Supplementary figure 5c). The glucose intolerance observed in *L-FxrKO* mice is in line with previously published data (15).

At the molecular level, we were intrigued to find suppression of *Chrebp*- β , *Srebp1c* and *Fasn* gene expression in the livers of HFD fed *L-FxrKO* compared to *f/f Fxr* mice (Supplementary figure 5e–f), but this down regulation was not to the same extent of the DKO mice (Figure 5a–b). Despite suppression of several *de novo* lipogenic genes, *L-FxrKO* mice exhibit accumulation of hepatic triglycerides and development of steatosis. Further the expression profile of *Pepck*, *Cpt1* and *Fsp27* β , a crucial lipid droplet stability protein were regulated similarly between *L-FxrKO* and WT mice (Supplementary figure 5e–f). This can potentially be attributed to the marginal decrease in fatty acid oxidation and pronounced increase in lipid droplet stability as measured by *Cpt1* and *Fsp27* β respectively. Taken together these findings imply that deletion of hepatic FXR is not responsible for the protection against hepatic steatosis and glucose tolerance observed in the DKO mice.

Liver-specific *Shp* deletion is sufficient to protect mice from developing hepatic steatosis

To determine if liver *Shp* was responsible for the observed beneficial phenotype in the DKO mice, we generated and characterized liver-specific *ShpKO* (*L-ShpKO*) mice (Supplementary figure 6a). These mice gained ~25–30% less weight, accumulated significantly lower levels of visceral fat and showed lower cholesterol and hepatic triglycerides when challenged with the HFD (Figure 6a–b). Consequently, the *L-ShpKO* mice exhibited better glucose tolerance (Figure 6c) with significantly decreased accumulation of hepatic fat (Figure 6d) compared to control *f/fShp* mice. These beneficial effects on glucose and weight gain were restricted to only HFD or WD conditions and were comparable between the two genotypes in chow fed conditions (Supplementary figure 6b–c). However, loss of *Shp* resulted in lower serum triglycerides and cholesterol even under chow fed state (Supplementary Figure 6d). Finally, we examined whether this protection against fatty liver was maintained under western diet (WD) condition comprising of increased cholesterol, high fat and high sucrose content. In line with HFD data, *L-ShpKO* mice were also protected against WD induced weight gain and hepatic steatosis (Supplementary figure 7a–e) highlighting the importance of liver *Shp* in regulating the cholesterol and triglyceride levels. We investigated further and found down regulation of important lipogenic regulators *Srebp1c*, *Pparγ2* and lipid droplet protein *Fsp27*, which corroborates well with the reduction in hepatic triglycerides level in *L-ShpKO* mice (Figure 6e, Supplementary figure 7f). It has been recently shown that *Fsp27* not only promotes droplet stability but also inhibits lipolysis (36, 37). Importantly, increased expression of *Fsp27* is sufficient to promote hepatic steatosis (38). Furthermore, when *Shp* is deleted, NCOR as well as HNF4α are no longer repressed and thus are able to mediate suppression of *Pparγ2* and subsequently down regulate *Fsp27* (23, 39). Overall, these results provide a mechanistic insight by which hepatic SHP can direct fat accumulation in the liver (Figure 6f).

DISCUSSION

There are several reports on the role for FXR and SHP in regulating glucose homeostasis (7, 10, 40). In this study we examined their combined metabolic role in maintaining energy homeostasis and found that DKO mice exhibit improved glucose handling, increased fatty acid oxidation and are protected against hepatic steatosis, a marker for insulin resistance(3). This implied that reducing both FXR and SHP function may be beneficial to achieving energy homeostasis in chow and HFD conditions.

To identify the mechanisms underlying this positive effect, we systematically examined the energy intake and expenditure. We found that increased utilization of fat rather than differences in food intake or lipid absorption contributed to the observed metabolic effect in the DKO mice. The increased physical activity and enhanced mitochondrial capacity of DKO skeletal muscle supports the premise that the muscle may actually contribute towards utilizing excess fat (Figure 4). Several studies have demonstrated that fiber-type distribution is directly correlated with glucose tolerance (41). Histological analysis in adult DKO skeletal muscle revealed an increase in SDH positive fibers indicating increased mitochondrial competence. Consequently, we also found increases in genes responsible for fat metabolism and gluconeogenesis that prevent insulin resistance and protect against

metabolic defects (32, 42). Moreover, we found increased *Ppar-γ* along with *Pepck* expression in the DKO muscle, both of which have been previously shown to increase type I fibers and be protective against diet-induced insulin-resistance (31, 32). These data reveal an important role for the skeletal muscle in protecting the DKO mice against diet-induced obesity.

One intriguing finding is the tissue-specific misregulation of *Pepck* leading to its decrease in the liver but increase in the muscle (Figure 4 and 5). This dichotomy of tissue-specific *Pepck* levels however is consistent with the observed beneficial metabolic phenotype in DKO mice since hepatic *Pepck* overexpression causes insulin resistance (35) whereas muscle overexpression promotes insulin sensitivity (32). Insulin signaling has been shown to down regulate hepatic *Pepck* levels (43), which is in line with increased insulin sensitivity observed in the DKO mice under both chow and high fat fed conditions. However, transcriptional regulation of *Pepck* expression in the skeletal muscle expression remains to be determined.

Apart from the skeletal muscle, the liver expression profile of glucose and fatty acid metabolic genes of the DKO animals also demonstrate a favorable pattern of expression (Figure 5). For instance, the lack of lipid accumulation in the liver appears to be a direct effect of decreased lipogenesis coupled with increased fatty acid β -oxidation. Since the DKO mice are global knockouts, in order to tease apart the individual contribution of *Fxr* and *SHP* to the observed beneficial phenotype, we generated the liver-specific *FxrKO* as well as liver-specific *ShpKO* (Supplementary Figure 5 & 6). Our data show that deletion of hepatic *Fxr* did not protect mice against diet-induced steatosis and these findings are in line with earlier studies, which also revealed little role for liver *Fxr* in ameliorating diabetes subsequent to obesity (15). However, intestinal *Fxr* agonism and antagonism have been implicated in protecting against obesity by two separate mechanisms (44, 45). Therefore intestinal *Fxr* rather than hepatic *Fxr* seems to be crucial in regulating metabolic outcome during obesity.

Next we analyzed the liver-specific *ShpKO* mice and found that loss of *Shp* in the liver contributes to the protection against hepatic steatosis and glucose tolerance phenotype when challenged with HFD. The protection against accumulation of hepatic triglycerides was dramatic and was maintained under both HFD and WD conditions thus underscoring the importance of *SHP* in regulating this process. Importantly, this data is substantiated by the previous finding which showed that *Shp* overexpression was sufficient to promote the development of fatty liver in mice (40). We found that despite calorie excess, *L-ShpKO* mice were not able to promote hepatic *de novo* lipogenesis to the extent of the control mice. This was apparent as *Srebp1c* and *Ppar γ* levels were significantly lower in *L-ShpKO* mice. In addition *Fsp27 β* , which is necessary for lipid droplet growth and fat storage (46) was also robustly downregulated in the absence of *Shp*. Furthermore, it has been recently shown that *Fsp27 β* is sufficient to cause fat accumulation in the liver (37). *SHP* deletion thus effectively combats hepatic steatosis by reducing lipid synthesis as well as lipid droplet stability (Figure 6).

Lastly, as we know that the DKO mice accumulate high bile acid levels (22), we assessed whether the beneficial metabolic effects are secondary to the observed increase in bile acids. Thyroid hormone activation has been implicated in mediating the ability of bile acids to burn fat (47). Therefore we analyzed thyroid levels in DKO mice but did not find activation or increase in thyroid levels in these animals (Supplementary Figure 8). However, it was recently shown that both increase (48) as well as decrease in bile acid pool was found to be protective against metabolic dysfunction subsequent to obesity (49). These conflicting findings reveal that bile composition rather than the total pool may be crucial to regulating metabolic homeostasis. Importantly, if bile acids were contributing to the DKO phenotype, then they would have to be functioning independently of FXR-SHP axis. Some of the beneficial effects such as effective glucose homeostasis could be achieved by activating TGR5 (50).

However, we know from our analysis of the individual *L-FxrKO* and *L-ShpKO* that the deletion of SHP is sufficient to protect against steatosis. This effect is bile acid independent since *L-ShpKO* mice do not have elevated hepatic bile acid levels (data not shown). These findings demonstrate that SHP regulates triglyceride levels in the liver independent of FXR (Figure 6e).

As discussed earlier, the profound metabolic protection of the DKO mice may be attributed to a combinatorial effect of elevated bile acids, intestinal deletion of *Fxr* along with liver-specific deletion of *Shp*. The individual tissue-specific knockout studies have allowed us to disentangle the complexities and precisely identify the beneficial effects. Based on this study we know that strong protection against HFD-induced steatosis can be achieved by inhibiting hepatic SHP levels. Since SHP is required for both lipogenesis and to maintain intact lipid droplets, thus inhibiting hepatic SHP levels could be a potential therapeutic modality to treat fatty liver disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Loss of *Fxr* and *Shp* in mice results in better glucose handling

(a) DKO mice exhibit lower body weight on normal chow diet. (n= 6–7 mice per group). (b) Gross pictures of WT and DKO mice on normal chow diet. (c) DKO mice have increased lean mass and decreased fat mass compared to WT, as measured by DEXA scan. (n=6 mice per group). (d) Difference in food intake between DKO and WT mice. (n=6 mice per group). (e–f) DKO mice are more glucose tolerant and insulin sensitive as determined by glucose and insulin tolerance tests. (n>9 per group). Student t-test was used to compare two groups. Mean± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ compared to WT mice.

Figure 2. DKO mice exhibit resistance to diet-induced obesity

(a) DKO mice gain less weight compared to WT mice and gross mice pictures depict this reduced weight gain in DKO mice after 8 weeks HFD. (b) DKO mice accumulate less fat mass than the WT mice when challenged with HFD. (c) Basal serum glucose levels are decreased in DKO (n=16) mice compared to WT (n=12) despite similar food intake (d) between DKO and WT mice (n=6/group). DKO mice show robust glucose tolerance (e) and insulin sensitivity (f) when maintained on HFD (n>12/group). Student's t-test was used to compare two groups. Mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to WT mice.

Figure 3. *Fxr* and *Shp* deficiency increases overall energy expenditure

(a) Oxygen consumption (VO₂) and (b) carbon dioxide production (CO₂) at rest. (c) Lower respiratory exchange ratio (RER) in DKO on normal chow suggests preference for fat whereas WT mice exhibit preference for glucose as their fuel source. On HFD, WT mice shift to fat metabolism and DKO further increase their fat utilization. Mean ± SEM.

p*<0.05, *p*<0.01, ****p*<0.001 compared to WT mice.

Figure 4. DKO mice have increased physical activity and fatty acid oxidation. (a–c)

There is increased physical activity in DKO mice on normal chow and high fat diet compared to WT. Increased mitochondrial complexes of the electron transport in DKO mice on normal chow indicating increased supply of energy compared to WT. **(d)** Gene expression as determined by RT-qPCR shows increase in genes involved in fatty acid oxidation and protection against insulin resistance. (n=4–6 mice per group) Mean ± SEM.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to WT mice.

Figure 5. DKO liver display decreased glucose and fatty acid metabolism and increased oxidative metabolism

Expression of genes involved in (a) glycolytic metabolism, (b) oxidative metabolism and (c) fatty acid metabolism (n= 6 mice per group). (d) H&E staining of liver tissues shows hepatic steatosis is less severe in DKO mice compared to WT animals fed HFD. (e) Oil red O staining confirms the reduction in hepatic steatosis in DKO compared to WT. Mean $\pm$ SEM.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ compared to WT mice.

Figure 6. Loss of *Shp* in the liver protects against HFD-induced hepatic steatosis

(a) *L-ShpKO* and *f/f Shp* mice on HFD exhibit similar weight gain. Liver to body weight ratio did not change but a modest decrease in white adipose tissue to body weight ratio was observed in *L-ShpKO* compared to *f/f Shp* control. (b) Despite HFD challenge, improved lipid metabolic profile was seen in *L-ShpKO* mice compared to *f/f Shp* control. (c) *L-ShpKO* mice exhibit improved glucose tolerance but did not show enhanced insulin sensitivity on HFD. (d) The neutral lipid staining revealed a striking reduction in hepatic fat in the *L-ShpKO* mice when compared to *f/f Shp* mice. (e) Hepatic gene expression showed reduction in lipogenesis and lipid droplet stability when *Shp* is deleted (n= 3–5 mice per group) Mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to *f/f Shp* mice. (f)

Schematic describing the underlying mechanism(s) that protect DKO and *L-ShpKO* mice from high-fat diet induced steatosis.

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