Aldo-keto reductase 1B7 is a target gene of FXR and regulates lipid and glucose homeostasis[S]

Xuemei Ge, Liya Yin, Huiyan Ma, Tiangang Li, John Y. L. Chiang, and Yanqiao Zhang[1]

Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH

Abstract **Aldo-keto reductase 1B7 (AKR1B7) is proposed to play a role in detoxification of by-products of lipid peroxidation. In this article, we show that activation of the nuclear receptor farnesoid X receptor (FXR) induces AKR1B7 expression in the liver and intestine, and reduces the levels of malondialdehyde (MDA), the end product of lipid peroxidation, in the intestine but not in the liver. To determine whether AKR1B7 regulates MDA levels in vivo, we overexpressed AKR1B7 in the liver. Overexpression of AKR1B7 in the liver had no effect on hepatic or plasma MDA levels. Interestingly, hepatic expression of AKR1B7 significantly lowered plasma glucose levels in both wild-type and diabetic db/db mice, which was associated with reduced hepatic gluconeogenesis. Hepatic expression of AKR1B7 also significantly lowered hepatic triglyceride and cholesterol levels in db/db mice. These data reveal a novel function for AKR1B7 in lipid and glucose metabolism and suggest that AKR1B7 may not play a role in detoxification of lipid peroxides in the liver.[jlr] AKR1B7 may be a therapeutic target for treatment of fatty liver disease associated with diabetes mellitus.—**Ge, X., L. Yin, H. Ma, T. Li, J. Y. L. Chiang, and Y. Zhang. **Aldo-keto reductase 1B7 is a target gene of FXR and regulates lipid and glucose homeostasis. *J. Lipid Res.* 2011.** 52: **1561–1568.**

Supplementary key words AKR1B7 • triglyceride • cholesterol • farnesoid X receptor

Lipid peroxidation is a process in which free radicals attack lipids in cell membranes, resulting in cell damage. Lipid peroxidation is used as an indicator of oxidative stress in cells and tissues. The end products of lipid peroxidation, such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), are reactive and toxic compounds. MDA is a highly reactive aldehyde that can form covalent protein adducts, referred to as advanced lipoxidation endproducts (1).

Aldo-keto reductase 1B7 (AKR1B7) is a member of AKR1B family and is highly expressed in vas deferens, adrenal gland, eye, intestine, and to a lesser extent, in the liver, kidney and testis (2). Biochemical analysis identified isocaproaldehyde (3), a product of side chain cleavage of cholesterol, and 4-HNE (4), a lipid peroxidation product, as preferred substrates for AKR1B7. However, it is unclear whether AKR1B7 has an effect on lipid peroxide levels in vivo. In addition, the role of AKR1B7 in metabolism is largely unknown.

Farnesoid X receptor (FXR) is a nuclear hormone receptor that plays an important role in maintaining bile acid, lipid, and glucose homeostasis. FXR regulates gene transcription by forming heterodimers with retinoid X receptor (RXR). Activation of FXR has been shown to lower blood triglyceride and cholesterol levels and improve insulin sensitivity in diabetic mouse models (reviewed in Ref. 5). Activation of FXR by synthetic agonists is also shown to protect against atherosclerosis in $Ldlr^{-/-}$ and $Apoe^{-/-}$ mice (6–8), and to protect against nonalcoholic fatty liver disease (NASH) in animal models (9, 10). In addition, FXR also plays a role in protection against bacterial infection in the intestine (11), in preventing liver or intestine from

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Abbreviations: 4-HNE, 4-hydroxynonenal; Abcg5, ATP-binding cassette, sub-family G, member 5; Acc, acetyl-CoA carboxylase; Akr1b7, aldo-keto reductase 1B7; Akt, protein kinase B; Apob, apolipoprotein B; Cd36, cluster of differentiation; ChIP, chromatin immunoprecipitation; Cyp7a1, cholesterol 7α-hydroxylase; Dgat, diacylglycerol acyltransferase; EMSA, electrophoretic mobility shift assay; Foxo1, forkhead box protein O1; FXR, farnesoid X receptor; G6pase, glucose 6-phosphatase; Gck, glucokinase; GSK3β, glycogen synthase kinase 3β; IR, inverted repeat; LCA, lithocholic acid; MDA, malondialdehyde; Mtp, microsomal triglyceride transfer protein; Pdk4, pyruvate dehydrogenase kinase 4; Pepck, phosphoenolpyruvate carboxykinase; Pgc-1α, peroxisome proliferator-activated receptor γ coactivator 1α; RXR, retinoid X receptor; Shp, small heterodimer partner; Sr-b1, scavenger receptor class B type 1; Srebp, sterol regulatory binding protein.

[1]To whom correspondence should be addressed.
e-mail: yzhang@neoucom.edu

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of three figures and two tables.

carcinogenesis (12–14), and in liver regeneration (15). Therefore, FXR is a multipurpose nuclear receptor.

Very recently, AKR1B7 was shown to convert 3-keto lithocholic acid (LCA) to less toxic 3β-hydroxy LCA and was induced in enterohepatic tissues when FXR was activated following acute treatment with an FXR agonist (16). In this report, we show that activation of FXR induces AKR1B7 mRNA and protein levels in both the intestine and liver. Activation of FXR significantly reduces MDA levels in the intestine but not in the liver. Overexpression of AKR1B7 in the liver has no effect on hepatic or plasma MDA levels. In addition, we demonstrate that AKR1B7 has striking effects on lowering blood glucose levels and reducing hepatic lipid accumulation in diabetic mice. These effects are associated with reduced expression of hepatic gluconeogenic genes and increased very low-density lipoprotein (VLDL) secretion. Our data suggest that AKR1b7 may be a therapeutic target for treatment of diabetes mellitus.

MATERIALS AND METHODS

Mice

C57BL/6J mice, ob/ob mice, and db/db mice on a C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, ME) and fed a standard chow diet. All experiments were approved by the Institutional Animal Care and Use Committee at the Northeast Ohio Medical University.

Adenovirus

Ad-Akr1b7 was generated by cloning mouse Akr1b7 cDNA to pEnter-TOPO/D vector, and recombinant adenoviruses were generated following the manufacturer's instructions (Invitrogen, CA). Adenoviruses were grown in 293A cells and purified by cesium chloride density gradient ultracentrifugation. To overexpress genes in mice, 10^9 plaque formation units (pfu) of adenoviruses were transfused into each mouse via intravenous injection.

Real-time PCR

RNA was isolated using TRIzol Reagent (Invitrogen), and mRNA levels were determined by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) using SYBR Green Supermix and a real-time PCR machine from Applied Biosystems (Foster City, CA). Results were normalized to *36b4* mRNA. The primer sequences for qRT-PCR were described previously (17, 18), except for Akr1b7 (forward primer, 5′-AAGCGGGAGGATCTCTTCAT-3′ and reverse primer, 5′-TCAGATCCGAGAGGGTGTTC-3′).

Western blot assay

Whole liver lysates (19) were prepared, and Western blot assays were performed as described previously (19). β-actin antibody and Akr1b7 antibody were from Novus Biologicals (CO) and Santa Cruz Biotechnology (CA), respectively. ApoB antibody was from Biodesign (ME). p-AKT(ser473) and p-GSK3β(ser9) antibodies were from Cell Signaling (MA).

ChIP assay

Wild-type mice were treated with either vehicle or GW4064 (30 mg/kg, twice a day) for 7 days. Livers were homogenized in cold PBS containing protease inhibitor cocktail (Roche, NJ), 2 μg/ml PMSF, 1 mM EDTA and 1 mM EGTA. Chromatin immunoprecipitation was carried out using a ChIP assay kit (Millipore, MA) according to the manufacturer's protocol with minor modifications. Briefly, the cell lysates were fixed with formaldehyde at a final concentration of 1%, sonicated, and precleared with Protein A beads. Aliquots of the precleared, sheared chromatin were then immunoprecipitated using mouse IgG or anti-FXR antibody (Santa Cruz Biotechnology). After elution, the resulting DNA was used for qPCR analysis.

Electrophoretic mobility shift assay

Oligonucleotides containing the putative inverted repeat (IR)-1 element are shown in Fig. 2A. Electrophoretic mobility shift assays (EMSA) and competition studies were performed as previously described (20). For supershift assays, in vitro transcribed FXR proteins were pre-incubated with FXR antibody for 30 min prior to addition of double-stranded, oligonucleotide probes.

Mutagenesis and transient transfection

The mutant pGL3 promoter-luciferase construct was generated using a QuickChange Site-directed Mtagenesis kit from Agilent (CA). HepG2 cells were plated in a 48-well plate and cultured in DMEM containing 10% FBS. Transient transfections were performed as described (21). Briefly, pGL3-Akr1b7 luciferase reporter constructs were cotransfected into HepG2 cells with plasmids expressing FXR or RXR, followed by treatment with either vehicle or GW4064. After 36 h, luciferase activities were determined and normalized to β-galactosidase activity.

MDA measurement

Malondialdehyde levels in the intestine, liver and plasma were determined as described (22).

Lipid and lipoprotein analysis

Approximately 100 mg liver was homogenized in methanol, and lipids were extracted in chloroform/methanol (2:1 v/v) as described (23). Hepatic triglyceride and cholesterol levels were then quantified using kits from Wako Chemicals (Richmond, VA). Plasma lipid levels were determined using kits from Wako Chemicals. Plasma lipoprotein profile was analyzed by fast protein liquid chromatography (FPLC) as described (18).

VLDL secretion

C57BL/6J mice were injected with either Ad-null or Ad-Akr1b7 via tail vein injection. On day 6, these mice were fasted overnight, followed by intravenous injection of Tyloxapol (500 mg/kg). Blood was taken at indicated time points, and plasma triglyceride levels were determined (18).

Glucose/insulin/pyruvate tolerance tests and hepatic glycogen levels

The db/db mice were fasted overnight and then injected intraperitoneally with D-glucose (2 g/kg). Glucose tolerance test was performed as described previously (19). For pyruvate tolerance test, mice were fasted overnight and then injected intraperitoneally with sodium pyruvate (2 g/kg). Plasma glucose levels were determined at indicated time points using a glucometer. For insulin tolerance test, mice were fasted for 6 h, followed by bolus injection of insulin (0.75 unit/kg) as described (19). To determine hepatic insulin signaling, mice were fasted for 6 h and then given bolus injection of insulin (5 unit/kg) via vena cava. Tissues were collected 5 min after insulin injection, as described (19). Hepatic glycogen levels were determined as described (19).

Statistical analysis

Statistical significance was analyzed using unpaired Student *t*-test or one-way ANOVA (GraphPad InStat3 software). All values are expressed as mean ± SEM. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Activation of FXR induces AKR1B7 expression

To identify novel genes regulated by FXR, we treated C57BL/6 mice with either vehicle or the synthetic FXR agonist GW4064 for 7 days. We then did microarray assays. One of the highest induced genes by GW4064 in the intestine and liver was Akr1b7 (supplemental Tables I and II). Analysis of mRNA levels by real-time PCR indicated that GW4064 treatment significantly induced the mRNA levels of the small heterodimer partner (*Shp*) (**Fig. 1A**), a well-characterized FXR target gene (24), and *Akr1b7* (Fig. 1B) in both the intestine and liver. GW4064 treatment also significantly induced the protein levels of Akr1b7 in both the intestine (~9.8-fold; Fig. 1C) and the liver (~4.9-fold; Fig. 1D) ($P < 0.05$). Bile acids are the endogenous ligands for FXR. Consistent with the increased FXR expression (19) and the increased bile acid pool size under diabetic conditions (25–28), *Akr1b7* mRNA levels in the livers of diabetic db/db mice were also significantly increased. In db/db mice, hepatic *Shp* mRNA levels tended to increase ($P = 0.06$; data not shown). These data indicate that activation of FXR induces Akr1b7 expression in the liver and intestine and that hepatic Akr1b7 expression is induced under diabetic conditions.

FXR regulates AKR1B7 expression through binding to an IR-1 element

To understand the mechanism by which FXR regulates Akr1b7 expression, we investigated *Akr1b7* promoter activity using pGL3 promoter-reporter constructs with a series of 5′-deletions (**Fig. 2A**). As shown in Fig. 2B, GW4064 treatment highly induced *Akr1b7* promoter activity when longer promoter-reporter constructs were used; 5′-deletion of the *Akr1b7* promoter from −447 bp to −230 bp completely abolished the promoter activity induced by FXR, suggesting that the FXR response element in the *Ark1b7* promoter is located between −447 bp and −230 bp. FXR is known to often bind to an IR-1 element to regulate gene transcription. In *Akr1b7* promoter, there is a candidate IR-1 element located at −291 bp (Fig. 2A). Mutation of the IR-1 element resulted in ~15-fold reduction in FXR-induced *Akr1b7* promoter activity (Fig. 2C). EMSA data showed that the FXR/RXR complex bound to the wild-type, but not

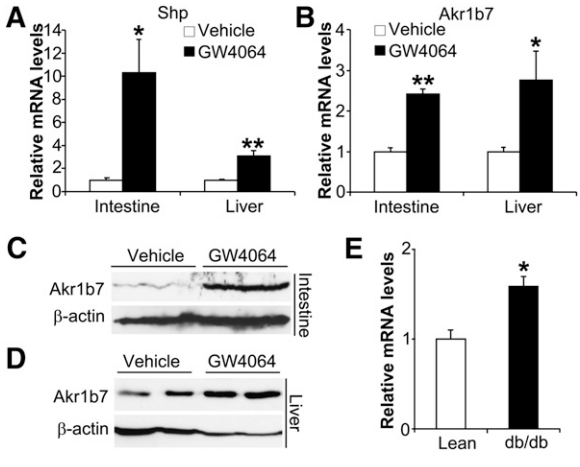

Fig. 1. Activation of FXR induces mRNA and protein levels of AKR1B7 in the intestine and liver. A–D: C57BL/6 mice were gavaged with either vehicle or GW4064 (30 mg/kg, twice a day) for 7 days (n = 7 per group). The mRNA levels of *Shp* (A) and *Akr1b7* (B) were determined by real-time PCR. The protein levels of Akr1b7 in the intestine (C) and liver (D) were determined by Western blot assays. E: *Akr1b7* mRNA levels in the livers of lean mice and db/db mice were determined by real-time PCR (n = 4 per group). *$P < 0.05$, **$P < 0.01$.

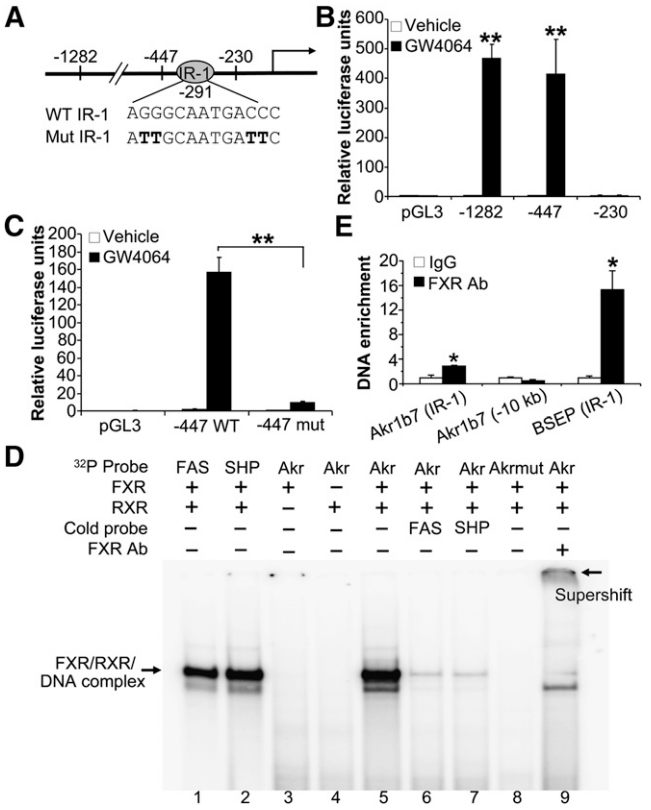

Fig. 2. Identification of AKR1B7 as a direct target gene of FXR. A: The potential FXR response element (IR-1) is shown in the diagram. B, C: HepG2 cells were transiently transfected with various Akr1b7 promoter constructs [pGL3-Akr1b7(−1282, −447, or −230 to +41] and plasmids expressing FXR or RXR, followed by treatment with either DMSO or GW4064 (1 μM) for 36 h. Luciferase activity was determined and normalized to β-galactosidase activity. In panel C, the pGL3-Akr1b7(−447 mut to +41) construct (−447 mut) contained a mutant IR-1 element at −291 as shown in panel A. D: EMSA was performed. FAS and SHP served as positive controls (lanes 1 and 2). Only the wild-type IR-1 element of Akr1b7 (lane 5) bound to the FXR/RXR complex (lane 5), and such a binding was competed away by the IR-1 element of FAS (lane 6) or SHP (lane 7). The mutant IR-1 element of Akr1b7 did not bind to the FXR/RXR complex (lane 8). The Akr1b7 DNA/FXR/RXR complex was supershifted in the presence of an FXR antibody (lane 9). E: ChIP assay was performed using whole liver lysates. The IR-1 element in BSEP served as a positive control, whereas the −10 kb region in the Akr1b7 promoter served as a negative control. *$P < 0.05$, **$P < 0.01$.

mutant, IR-1 element of *Akr1b7* (Fig. 2D). This binding could be competed away by a wild-type IR-1 element of fatty acid synthase (*Fas*) or *Shp*, two known FXR target genes (24, 29) (Fig. 2D). In addition, the FXR/RXR/DNA complex was supershifted in the presence of an FXR antibody (Fig. 2D).

Finally, ChIP assays were performed using liver lysates. FXR protein was enriched in the *Akr1b7* promoter that contained the IR-1 element (Fig. 2E). The −10 kb region of the *Akr1b7* promoter and the bile salt export protein (BSEP) containing a known IR-1 element served as negative and positive controls, respectively (Fig. 2E). Together, the data in Fig. 2 demonstrate that FXR regulates *Akr1b7* promoter activity through binding to the IR-1 element located at −291 bp upstream of the transcription start site (Fig. 2A). Such observations are consistent with a recent report by Schmidt et al. (16).

FXR but not AKR1B7 regulates MDA levels in vivo

Data from in vitro biochemical assays have suggested that Akr1b7 may play a role in detoxification of lipid peroxidation by-products (3). However, whether Akr1b7 also plays a role in lipid peroxidation in vivo is unknown. As FXR highly induced Akr1b7 expression in both the liver and intestine (Fig. 1), we first determined the effect of FXR activation on lipid peroxidation. Treatment with GW4064 for 7 days significantly reduced the MDA levels in the intestine (**Fig. 3A**) but not in the liver (Fig. 3B) or plasma (data not shown). Next, we determined the effect of overexpression of Akr1b7 on MDA levels in vivo. Adenoviruses expressing Akr1b7 were delivered intravenously to wild-type mice. Adenovirus-mediated Akr1b7 expression was limited to the liver (supplemental Fig. IA), with ∼3.0-fold increase in hepatic Ark1b7 protein levels (supplemental Fig. IB). Overexpression of Akr1b7 did not affect the MDA levels in the liver (Fig. 3C) or plasma (data not shown). These data indicate that activation of FXR reduces MDA levels in the intestine and that hepatic AKR1B7 does not regulate hepatic or plasma MDA levels.

Hepatic expression of AKR1B7 regulates plasma glucose levels and ApoB secretion in wild-type mice

In addition to determining the effect of AKR1B7 on MDA levels, we also investigated the role of AKR1B7 in lipid and glucose homeostasis. Overexpression of Akr1b7 in the liver increased plasma cholesterol levels, but it had no effect on plasma triglyceride levels (**Fig. 4A**) or hepatic levels of triglycerides or cholesterol (Fig. 4B). Analysis of plasma by FPLC showed that hepatic expression of Akr1b7 increased VLDL triglyceride levels (supplemental Fig. II). Interestingly, overexpression of hepatic Akr1b7 significantly reduced plasma glucose levels by ∼33% (Fig. 4C). Analysis of hepatic gene expression by real-time PCR indicated that overexpression of hepatic Akr1b7 significantly reduced hepatic mRNA levels of gluconeogenic genes phosphoenolpyruvate carboxykinase (*Pepck*) and glucose 6-phosphatase (*G6pase*) by ∼50% and of peroxisome proliferator-activated receptor γ coactivator 1α (*Pgc-1α*), but it significantly increased hepatic *Shp* mRNA levels (Fig. 4D). Hepatic PGC-1α (30) and SHP (31) are known to induce and inhibit hepatic gluconeogenic genes, respectively. Thus, the reduction in *Pgc-1α* and induction of *Shp* are consistent with the inhibition of hepatic gluconeogenic genes following hepatic expression of Akr1b7.

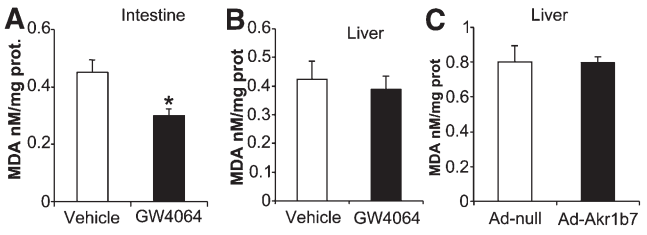

Fig. 3. FXR but not AKR1B7 reduces MDA levels in vivo. A, B: C57BL/6 mice were treated with either vehicle or GW4064 for 7 days (n = 7 per group). The MDA levels in the intestine (A) and the liver (B) were determined. C: C57BL/6 mice were injected intravenously with either the control adenovirus (Ad-null) or adenovirus expressing Akr1b7 (Ad-Akr1b7) (n = 7 per group). After 7 days, hepatic MDA levels were determined. *$P < 0.05$.

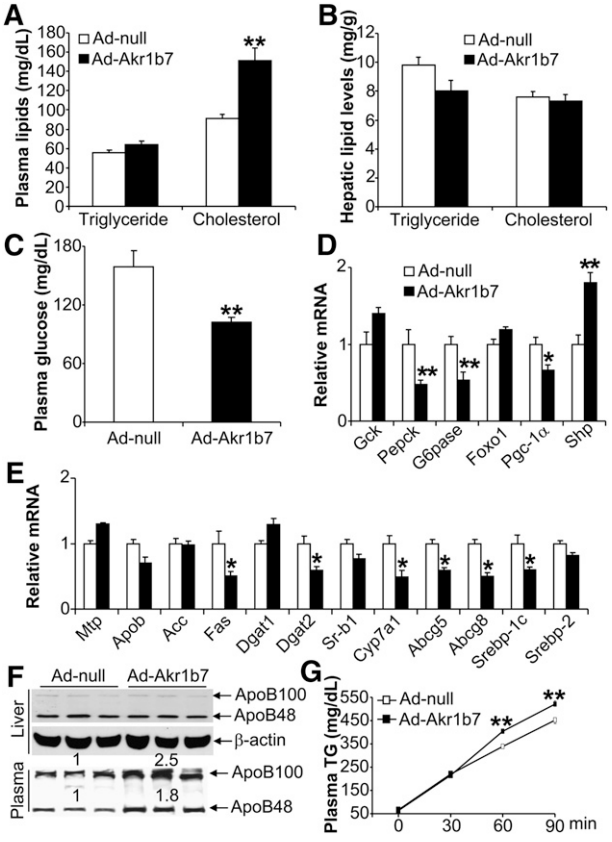

Fig. 4. Overexpression of AKR1B7 lowers plasma glucose levels and increases VLDL secretion in wild-type mice. A–F: C57BL/6 mice were injected intravenously with Ad-null (control) or Ad-Akr1b7 (n = 7 per group). After 7 days, mice were fasted for 6 h prior to euthanasia. A, B: Plasma (A) and hepatic (B) triglyceride and cholesterol levels were determined. Plasma glucose levels (C) were determined. Hepatic mRNA levels (D, E) were determined by real-time PCR. Hepatic protein levels (F) in the liver (top panel) or plasma (lower panel) were determined by Western blot assays. G: Hepatic VLDL secretion in C57BL/6 mice was determined (n = 7 mice per group). *$P < 0.05$, **$P < 0.01$.

Hepatic genes involved in lipid metabolism were also determined. Hepatic mRNA levels of fatty acid synthase (*Fas*), diacylglycerol acyltransferase (*Dgat*) 2, cholesterol 7α-hydroxylase (*Cyp7a1*), ATP binding cassette G5 (*Abcg5*), *Abcg8*, and sterol regulatory element binding protein 1c (*Srebp-1c*) were also significantly reduced, whereas the mRNA levels of microsomal triglyceride transfer protein (*Mtp*), apolipoprotein B (*Apob*), acetyl-CoA carboxylase (*Acc*), *Dgat1*, scavenger receptor class B type I (*Sr-b1*), and *Srebp-2* remained unchanged (Fig. 4E). These data suggest that overexpression of Akr1b7 in the liver selectively regulates hepatic gene expression.

Consistent with the unchanged *Apob* mRNA levels, hepatic ApoB-100 or ApoB-48 protein levels were unchanged (Fig. 4F, top panel). Interestingly, plasma ApoB-100 or ApoB-48 protein levels in Akr1b7-overexpressing mice were 2.5-fold and 1.8-fold higher than those in control mice ($P < 0.05$) (Fig. 4F, bottom panel). In agreement with the latter data, hepatic Akr1b7 expression significantly increased triglyceride accumulation in the plasma after intravenous injection of tyloxapol (Fig. 4G), a lipoprotein lipase inhibitor, indicating that Akr1b7 increases VLDL secretion. Thus, the increased plasma cholesterol levels in wild-type mice following hepatic expression of Akr1b7 (Fig. 4A) may result from reduced hepatic expression of Cyp7A1 and/or increased VLDL secretion. On the other hand, the unchanged hepatic cholesterol levels may be a net effect of decreased hepatic expression of Abcg5/g8 and Cyp7a1 and increased VLDL secretion.

Hepatic expression of AKR1B7 ameliorates hepatic lipid accumulation in db/db mice

The striking effect of AKR1B7 on glucose metabolism in wild-type mice led us to investigate whether AKR1B7 also has similar effects in diabetic mice. Ad-null or Ad-Akr1b7 was delivered to db/db mice intravenously (supplemental Fig. II). Interestingly, overexpression of Ark1b7 in diabetic db/db mice significantly reduced hepatic cholesterol (**Fig. 5A**) and triglyceride (Fig. 5B) levels by more than 40% and 50%, respectively. Oil Red-O staining showed that overexpression of Akr1b7 significantly reduced hepatic neutral lipid accumulation (Fig. 5C).

Akr1b7 expression also increased plasma triglyceride levels, but it did not alter plasma cholesterol levels (Fig. 5D). Analysis of plasma by FPLC indicated that Akr1b7 increased VLDL triglyceride (Fig. 5E) and cholesterol (Fig. 5F) levels. These data are consistent with the role of Akr1b7 in promoting hepatic VLDL secretion (Fig. 4G).

Analysis of hepatic gene expression by real-time PCR indicated that Akr1b7 reduced hepatic mRNA levels of *Fas*, *Cyp7a1*, and *Srebp-2* (Fig. 5G). Overexpression of Akr1b7 had no effect on hepatic Apob protein levels (Fig. 5H), but it significantly increased plasma ApoB-100 and ApoB-48 protein levels ($P < 0.05$) (Fig. 5I), suggesting that Akr1b7 also increases hepatic VLDL secretion in db/db mice. Taken together, the data in Fig. 5 indicate that hepatic expression of Akr1b7 markedly reduces hepatic lipid accumulation in diabetic db/db mice. Such effects of

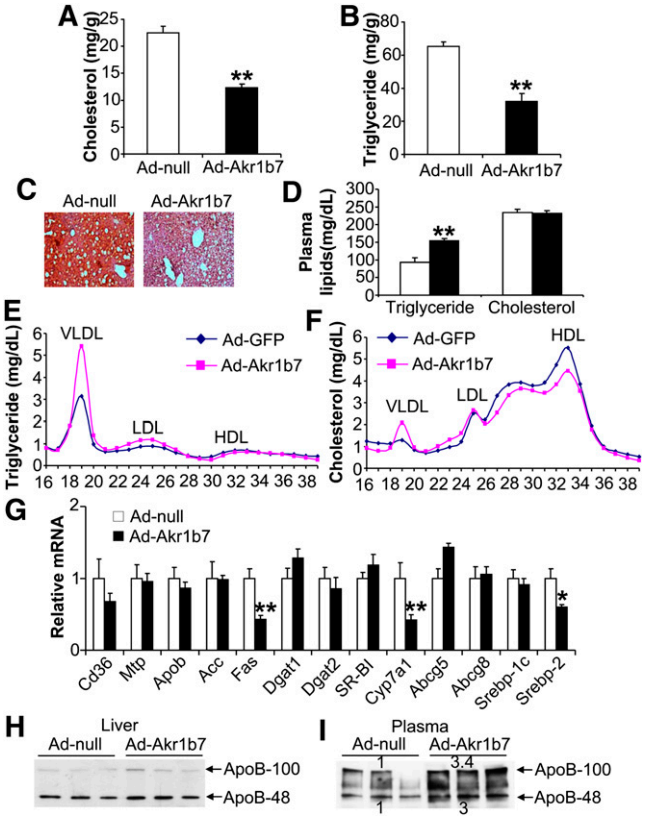

Fig. 5. Overexpression of AKR1B7 markedly reduces hepatic lipid accumulation in db/db mice. The db/db mice were injected intravenously with either Ad-null or Akr1b7 (n = 7 mice per group). After 7 days, mice were fasted 6 h prior to euthanasia. A, B: Hepatic levels of cholesterol (A) and triglycerides (B) were determined. C: Representative Oil Red-O staining from each group was shown. D: Plasma levels of triglyceride and cholesterol were determined. E, F: Plasma triglyceride (E) and cholesterol (F) lipoprotein profile was determined. G: Hepatic mRNA levels were determined by real-time PCR. H, I: Hepatic (H) or plasma (I) ApoB levels were determined by Western blot assays. $*P < 0.05$, $**P < 0.01$.

Akr1b7 are associated with reduced FAS expression and increased ApoB secretion.

Overexpression of AKR1B7 markedly improves hyperglycemia in db/db mice

As shown in **Fig. 6A**, overexpression of Akr1b7 in db/db mice significantly reduced hepatic *Pepck, G6pase*, and *Pgc-1α* expression. Consistent with the changes in hepatic gene expression, overexpression of Akr1b7 in db/db mice had a pronounced effect on preventing the increase in blood glucose levels at each indicated time point after bolus injection of glucose (Fig. 6B). These data indicate that hepatic Akr1b7 markedly improves glucose tolerance in diabetic db/db mice. Interestingly, Akr1b7 overexpression had no effect on hepatic glycogen levels (Fig. 6C). In addition, Akr1b7 did not affect hepatic phosphorylated levels of AKT or GSK3β after bolus injection of insulin (Fig. 6D). These data suggest that hepatic Akr1b7 expression lowers blood glucose levels without improving hepatic insulin signaling in db/db mice.

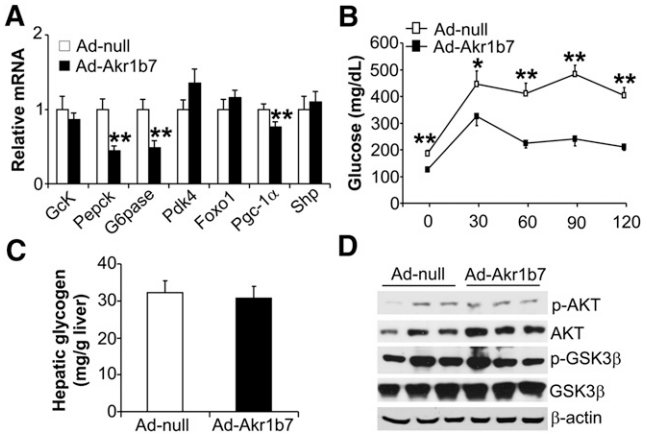

Fig. 6. Overexpression of AKR1B7 markedly ameliorates glucose tolerance in db/db mice. db/db mice were injected with either Ad-null or Ad-Akr1b7 (n = 7 per group). A: Hepatic mRNA levels were determined by real-time PCR. B: Glucose tolerance test was performed after an over-night fast. C: Hepatic glycogen levels were determined. D: db/db mice were given bolus injection of insulin (5 units/kg). After 5 min, hepatic levels of phosphorylated AKT (p-AKT), total AKT, phosphorylated GSK3β (p-GSK3β) and total GSK3β were determined by Western blot assays. $*P < 0.05$, $**P < 0.01$.

Overexpression of AkR1B7 improves pyruvate tolerance

To determine whether AKR1B7 overexpression affects whole body insulin sensitivity, an insulin tolerance test was performed. As shown in **Fig. 7A**, hepatic expression of Akr1b7 had no effect on insulin sensitivity, consistent with unchanged hepatic insulin signaling in db/db mice (Fig. 6D).

The finding that hepatic Akr1b7 expression significantly reduces hepatic gluconeogenic genes (Figs. 4D and 6A) in both wild-type and db/db mice led us to determine whether Akr1b7 affects gluconeogenesis in vivo. After bolus injection of pyruvate, mice infected with Akr1b7 had significantly reduced plasma glucose levels at 30, 60, 90, and 120 min postpyruvate injection (Fig. 7B). These data, together with the reduced hepatic expression of gluconeogenic genes (Figs. 4 and 6), indicate that hepatic expression of Akr1b7 inhibits gluconeogenesis.

DISCUSSION

In this article, we demonstrated that activation of FXR induces Akr1b7 mRNA and protein levels in the liver and intestine and that Akr1b7 is a direct target gene of FXR (Figs. 1 and 2). We also demonstrated that activation of FXR lowers MDA levels in the intestine, whereas overexpression of Akr1b7 in the liver has no effect on hepatic or plasma MDA levels (Fig. 3 and data not shown). In addition, we provided evidence to show that overexpression of hepatic Akr1b7 has a pronounced effect on lipid and glucose homeostasis (Figs. 4–7).

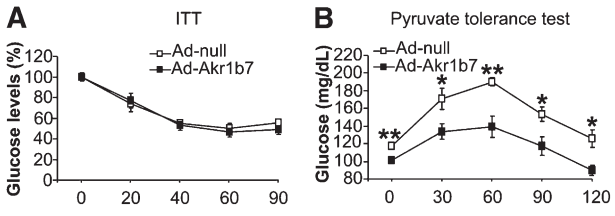

Fig. 7. AKR1B7 overexpression improves pyruvate tolerance. Wild-type mice were injected with Ad-null or Ad-Akr1b7 (n = 7 per group). A: Insulin tolerance test was performed on day 6 postinfection. B: Pyruvate tolerance test was performed on day 8 postinfection. $*P < 0.05$, $**P < 0.01$.

Several groups have identified Akr1b7 as a target for nuclear receptors, such as liver X receptor (LXR) (32) and xenobiotic receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (22). We noted that while this article was in preparation, Schmidt et al. reported that treatment of mice with GW4064 for 4 or 12 h induced *Akr1b7* mRNA levels in the liver and intestine (16). Consistent with these data, we demonstrated that oral gavage of C57BL/6 mice with GW4064 for 7 days significantly induces both mRNA and protein levels of *Akr1b7*. Such observations led us to first demonstrate that activation of FXR reduces MDA levels in the intestine (Fig. 3). MDA is highly reactive and may cause DNA mutagenesis. FXR has been shown to be important in protection against carcinogenesis in the intestine (13). The finding that activation of FXR reduces intestinal MDA levels may partly account for the protective role of FXR in intestinal carcinogenesis. Interestingly, hepatic expression of Akr1b7 has no effect on MDA levels in the liver or plasma, thus raising a question regarding whether Akr1b7 regulates lipid peroxidation in vivo. The finding that Akr1b7 has no effect on hepatic MDA levels is consistent with the data from *Akr1b7*$^{-/-}$ mice, in which no obvious changes in morphology or defects in reproduction have been observed (33).

Despite the unchanged MDA levels, hepatic overexpression of Akr1b7 has pronounced effects on regulating lipid and glucose metabolism. Overexpression of Akr1b7 in the liver markedly reduces plasma glucose levels in both wild-type mice and diabetic db/db mice (Figs. 4 and 6), and it significantly improves glucose tolerance in db/db mice (Fig. 6). The reduced glucose levels are associated with reduced expression of gluconeogenic genes in the liver (Figs. 4 and 6) and increased pyruvate tolerance (Fig. 7), suggesting that hepatic expression of AKR1B7 inhibits hepatic gluconeogenesis.

Interestingly, the hypoglycemic effect of Akr1b7 is not associated with increased insulin sensitivity. First, overexpression of hepatic Akr1b7 does not increase hepatic glycogen levels (Fig. 6C). Second, Hepatic p-AKT or p-GSK3β levels are unchanged following bolus injection of insulin (Fig. 6D). Third, Akr1b7 does not improve insulin sensitivity in an insulin tolerance test (Fig. 7A). Thus, the inhibition of hepatic gluconeogenesis may account, at least in part, for Akr1b7-induced decrease in plasma glucose levels.

In addition to lowering blood glucose levels, hepatic expression of Akr1b7 has a striking effect on ameliorating hepatic lipid accumulation in db/db mice (Fig. 5). Such an effect may be partly due to reduced expression of lipogenic gene FAS and increased hepatic VLDL secretion (Figs. 4 and 6). We also noted differential effects of Akr1b7 on plasma triglyceride and cholesterol levels in wild-type

versus db/db mice (Figs. 4 and 5). Compared with wild-type mice, db/db mice are known to have high levels of triglycerides and cholesterol in the liver and plasma. The high blood cholesterol levels in db/db mice may prevent further increase in plasma cholesterol levels following hepatic expression of Akr1b7. The unchanged plasma triglyceride levels in wild-type mice may be due to low hepatic triglyceride levels in these mice (Fig. 4).

Although Akr1b7 has pronounced effects on lipid and glucose metabolism, the exact mechanism leading to these effects remains to the further explored. Recent data suggest that certain bile acids are substrates for Akr1b7 (16). Bile acids are known to activate FXR, a nuclear receptor that has both hypoglycemic and hypolipidimic effects. Overexpression of hepatic Akr1b7 induces SHP, but does not induce other FXR target genes, such as bile salt export protein (BSEP) or multidrug resistant protein 2 (Mdr2) (data not shown), suggesting that FXR is not activated following Akr1b7 overexpression. One possibility is that the catalytic activity of Akr1b7 may lead to glucose cleavage or production of new metabolite(s) responsible for the metabolic changes following hepatic overexpression of Akr1b7. Our in vitro biochemical assays, using D-glucose as substrate and cell lysates from AML12 cells (a mouse liver cell line) infected with Ad-null or Ad-Akr1b7, suggest that Akr1b7 does not have glucose cleavage activity. One of our future directions is to identify the metabolites of Akr1b7 that are responsible for the changes in lipid and glucose metabolism.

The *AKR1B7* gene is not present in humans. The closest human ortholog of murine Akr1b7 is AKR1B10, which is also expressed in the liver and intestine. Human AKR1B10 shares 89% amino acid homology with murine Akr1b7. It will be interesting to investigate whether human AKR1B10 also regulates glucose and lipid metabolism.

Hyperglycemia is often associated with fatty liver disease. The finding that expression of Ark1b7 in the liver markedly ameliorates both hyperglycemia and lipid accumulation in diabetic mice suggests that human Akr1b isoforms may be therapeutic targets for treatment of fatty liver disease associated with diabetes mellitus.

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REFERENCES

1. Farmer, E. E., and C. Davoine. 2007. Reactive electrophile species. *Curr. Opin. Plant Biol.* **10:** 380–386.
2. Lau, E. T., D. Cao, C. Lin, S. K. Chung, and S. S. Chung. 1995. Tissue-specific expression of two aldose reductase-like genes in mice: abundant expression of mouse vas deferens protein and fibroblast growth factor-regulated protein in the adrenal gland. *Biochem. J.* **312:** 609–615.
3. Lefrancois-Martinez, A. M., C. Tournaire, A. Martinez, M. Berger, S. Daoudal, D. Tritsch, G. Veyssiere, and C. Jean. 1999. Product of side-chain cleavage of cholesterol, isocaproaldehyde, is an endogenous specific substrate of mouse vas deferens protein, an aldose reductase-like protein in adrenocortical cells. *J. Biol. Chem.* **274:** 32875–32880.
4. Schneider, C., N. A. Porter, and A. R. Brash. 2008. Routes to 4-hydroxynonenal: fundamental issues in the mechanisms of lipid peroxidation. *J. Biol. Chem.* **283:** 15539–15543.
5. Zhang, Y., and P. A. Edwards. 2008. FXR signaling in metabolic disease. *FEBS Lett.* **582:** 10–18.
6. Mencarelli, A., B. Renga, E. Distrutti, and S. Fiorucci. 2009. Antiatherosclerotic effect of farnesoid X receptor. *Am. J. Physiol. Heart Circ. Physiol.* **296:** H272–H281.
7. Hartman, H. B., S. J. Gardell, C. J. Petucci, S. Wang, J. A. Krueger, and M. J. Evans. 2009. Activation of farnesoid X receptor prevents atherosclerotic lesion formation in $LDLR^{-/-}$ and $apoE^{-/-}$ mice. *J. Lipid Res.* **50:** 1090–1100.
8. Flatt, B., R. Martin, T. L. Wang, P. Mahaney, B. Murphy, X. H. Gu, P. Foster, J. Li, P. Pircher, M. Petrowski, et al. 2009. Discovery of XL335 (WAY-362450), a highly potent, selective, and orally active agonist of the farnesoid X receptor (FXR). *J. Med. Chem.* **52:** 904–907.
9. Zhang, S., J. Wang, Q. Liu, and D. C. Harnish. 2009. Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis. *J. Hepatol.* **51:** 380–388.
10. Fiorucci, S., G. Rizzo, E. Antonelli, B. Renga, A. Mencarelli, L. Riccardi, S. Orlandi, M. Pruzanski, A. Morelli, and R. Pellicciari. 2005. A farnesoid X receptor-small heterodimer partner regulatory cascade modulates tissue metalloproteinase inhibitor-1 and matrix metalloprotease expression in hepatic stellate cells and promotes resolution of liver fibrosis. *J. Pharmacol. Exp. Ther.* **314:** 584–595.
11. Inagaki, T., A. Moschetta, Y. K. Lee, L. Peng, G. Zhao, M. Downes, R. T. Yu, J. M. Shelton, J. A. Richardson, J. J. Repa, et al. 2006. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc. Natl. Acad. Sci. USA.* **103:** 3920–3925.
12. Yang, F., X. Huang, T. Yi, Y. Yen, D. D. Moore, and W. Huang. 2007. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. *Cancer Res.* **67:** 863–867.
13. Maran, R. R., A. Thomas, M. Roth, Z. Sheng, N. Esterly, D. Pinson, X. Gao, Y. Zhang, V. Ganapathy, F. J. Gonzalez, et al. 2009. Farnesoid X receptor deficiency in mice leads to increased intestinal epithelial cell proliferation and tumor development. *J. Pharmacol. Exp. Ther.* **328:** 469–477.
14. Kim, I., K. Morimura, Y. Shah, Q. Yang, J. M. Ward, and F. J. Gonzalez. 2007. Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. *Carcinogenesis.* **28:** 940–946.
15. Huang, W., K. Ma, J. Zhang, M. Qatanani, J. Cuvillier, J. Liu, B. Dong, X. Huang, and D. D. Moore. 2006. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science.* **312:** 233–236.
16. Schmidt, D. R., S. Schmidt, S. R. Holmstrom, M. Makishima, R. T. Yu, C. L. Cummins, D. J. Mangelsdorf, and S. A. Kliewer. 2011. AKR1B7 is induced by the farnesoid X receptor and metabolizes bile acids. *J. Biol. Chem.* **286:** 2425–2432.
17. Zhang, Y., L. Yin, J. Anderson, H. Ma, F. J. Gonzalez, T. M. Willson, and P. A. Edwards. 2010. Identification of novel pathways that control farnesoid X receptor-mediated hypocholesterolemia. *J. Biol. Chem.* **285:** 3035–3043.
18. Yin, L., H. Ma, X. Ge, P. A. Edwards, and Y. Zhang. 2011. Hepatic hepatocyte nuclear factor 4alpha is essential for maintaining triglyceride and cholesterol homeostasis. *Arterioscler. Thromb. Vasc. Biol.* **31:** 328–336.
19. Zhang, Y., F. Y. Lee, G. Barrera, H. Lee, C. Vales, F. J. Gonzalez, T. M. Willson, and P. A. Edwards. 2006. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc. Natl. Acad. Sci. USA.* **103:** 1006–1011.
20. Lee, H., Y. Zhang, F. Y. Lee, S. F. Nelson, F. J. Gonzalez, and P. A. Edwards. 2006. FXR regulates organic solute transporters alpha and beta in the adrenal gland, kidney, and intestine. *J. Lipid Res.* **47:** 201–214.
21. Zhang, Y., H. R. Kast-Woelbern, and P. A. Edwards. 2003. Natural structural variants of the nuclear receptor farnesoid X receptor affect transcriptional activation. *J. Biol. Chem.* **278:** 104–110.
22. Liu, M. J., Y. Takahashi, T. Wada, J. He, J. Gao, Y. Tian, S. Li, and W. Xie. 2009. The aldo-keto reductase Akr1b7 gene is a common transcriptional target of xenobiotic receptors pregnane X receptor and constitutive androstane receptor. *Mol. Pharmacol.* **76:** 604–611.
23. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
24. Lu, T. T., M. Makishima, J. J. Repa, K. Schoonjans, T. A. Kerr, J. Auwerx, and D. J. Mangelsdorf. 2000. Molecular basis for feedback

regulation of bile acid synthesis by nuclear receptors. *Mol. Cell.* **6:** 507–515.
25. Hassan, A. S., M. T. Subbiah, and P. Thiebert. 1980. Specific changes of bile acid metabolism in spontaneously diabetic Wistar rats. *Proc. Soc. Exp. Biol. Med.* **164:** 449–452.
26. Nervi, F. O., C. H. Severin, and V. D. Valdivieso. 1978. Bile acid pool changes and regulation of cholate synthesis in experimental diabetes. *Biochim. Biophys. Acta.* **529:** 212–223.
27. Uchida, K., S. Makino, and T. Akiyoshi. 1985. Altered bile acid metabolism in nonobese, spontaneously diabetic (NOD) mice. *Diabetes.* **34:** 79–83.
28. Andersen, E., G. Karlaganis, and J. Sjovall. 1988. Altered bile acid profiles in duodenal bile and urine in diabetic subjects. *Eur. J. Clin. Invest.* **18:** 166–172.
29. Matsukuma, K. E., M. K. Bennett, J. Huang, L. Wang, G. Gil, and T. F. Osborne. 2006. Coordinated control of bile acids and lipogenesis through FXR-dependent regulation of fatty acid synthase. *J. Lipid Res.* **47:** 2754–2761.
30. Yoon, J. C., P. Puigserver, G. Chen, J. Donovan, Z. Wu, J. Rhee, G. Adelmant, J. Stafford, C. R. Kahn, D. K. Granner, et al. 2001. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature.* **413:** 131–138.
31. Ma, K., P. K. Saha, L. Chan, and D. D. Moore. 2006. Farnesoid X receptor is essential for normal glucose homeostasis. *J. Clin. Invest.* **116:** 1102–1109.
32. Volle, D. H., J. J. Repa, A. Mazur, C. L. Cummins, P. Val, J. Henry-Berger, F. Caira, G. Veyssiere, D. J. Mangelsdorf, and J. M. Lobaccaro. 2004. Regulation of the aldo-keto reductase gene akr1b7 by the nuclear oxysterol receptor LXRalpha (liver X receptor-alpha) in the mouse intestine: putative role of LXRs in lipid detoxification processes. *Mol. Endocrinol.* **18:** 888–898.
33. Baumann, C., B. Davies, M. Peters, U. Kaufmann-Reiche, M. Lessl, and F. Theuring. 2007. AKR1B7 (mouse vas deferens protein) is dispensable for mouse development and reproductive success. *Reproduction.* **134:** 97–109.