

Regulation of Plasma Fructose and Mortality in Mice by the Aldose Reductase Inhibitor Lidorestat

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ABSTRACT

Aldose reductase (AR), an enzyme widely believed to be involved in the aberrant metabolism of glucose and development of diabetic complications, is expressed at low levels in the mouse. We studied whether expression of human AR (hAR), its inhibition with lidorestat, which is an AR inhibitor (ARI), and the presence of streptozotocin (STZ)-induced diabetes altered plasma fructose, mortality, and/or vascular lesions in low-density lipoprotein (LDL) receptor-deficient [*Ldlr*($-/-$)] mice. Mice were made diabetic at 12 weeks of age with low-dose STZ treatment. Four weeks later, the diabetic animals (glucose > 20 mM) were blindly assigned to a 0.15% cholesterol diet with or without ARI. After 4 and 6 weeks, there were no significant differences in body weights or plasma cholesterol, triglyceride,

and glucose levels between the groups. Diabetic *Ldlr*($-/-$) mice receiving ARI had plasma fructose levels of 5.2 ± 2.3 $\mu\text{g/ml}$; placebo-treated mice had plasma fructose levels of 12.08 ± 7.4 $\mu\text{g/ml}$, $p < 0.01$, despite the induction of fructose-metabolizing enzymes, fructose kinase and adolase B. After 6 weeks, hAR/*Ldlr*($-/-$) mice on the placebo-containing diet had greater mortality (31%, $n = 9/26$ versus 6%, $n = 1/21$, $p < 0.05$). The mortality rate in the ARI-treated group was similar to that in non-hAR-expressing mice. Therefore, diabetic hAR-expressing mice had increased fructose and greater mortality that was corrected by inclusion of lidorestat, an ARI, in the diet. If similar effects are found in humans, such treatment could improve clinical outcome in diabetic patients.

Although the relationship between hyperglycemia and a number of vascular disorders is well established, responsible pathways are still unclear. Methods to define these pathways have been hindered by the difficulty of reproducing human diabetic complications in animal models. This has especially been the case for macrovascular disease (Goldberg and Dansky, 2006). In part, this is because of the difficulty of controlling other risk factors in diabetic setting; many atherosclerosis-prone diabetic mice become severely hyperlipidemic. Thus, severe hypercholesterolemia in the mouse might obscure the vascular-toxic effects of hyperglycemia (Kanter et al., 2007).

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Several pathways have been implicated in glucose-induced cellular toxicity (Reusch, 2003). One of these, the polyol pathway, is mediated by the enzyme aldose reductase (AR), an enzyme whose activity is markedly lower in mice than in humans (Hwang et al., 2002; Vikramadithyan et al., 2005). Perhaps, for this reason, by expressing human AR (hAR) in mice, atherosclerosis was increased in the presence of streptozotocin (STZ)-induced diabetes (Vikramadithyan et al., 2005).

To determine whether pharmacologic inhibition of AR altered complications in diabetic hAR-expressing LDL receptor knockout [*Ldlr*($-/-$)] mice, a blinded study was performed. We first showed that hAR expression and its inhibition alter plasma levels of fructose, a product of the polyol pathway. Lidorestat, a potent AR inhibitor (ARI), reduced mortality rates in hAR transgenic mice. Although all causes of death were not apparent, several placebo-treated animals developed what seemed to be vascular complications. In contrast, mice receiving lidorestat had similar survival rates as non-hAR-expressing diabetic mice.

ABBREVIATIONS: AR, aldose reductase; hAR, human AR; STZ, streptozotocin; LDL, low-density lipoprotein; *Ldlr*($-/-$), LDL receptor-deficient; ARI, aldose reductase inhibitor; CCD, cholesterol-containing diet; PCR, polymerase chain reaction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; TSP, thrombospondin; MMP, matrix metalloproteinase; FK, fructose kinase.

Materials and Methods

Mice Breeding and Diets. These experiments were reviewed and approved by the Columbia University Institutional Animal Care and Use Committee. Mice transgenic for hAR were obtained from M. Itakura (University of Tokushima, Tokushima, Japan; Yamaoka et al., 1995), and a colony was established at Columbia University. This line of mice expressing hAR via a histocompatibility antigen class I promoter crossed onto the *Ldlr*($-/-$) background was maintained on a chow diet (Research Diets, Inc., New Brunswick, NJ). Some mice were made diabetic at age 12 weeks by intraperitoneal administration of 50 mg/kg body weight STZ for 5 days. Four weeks later, the diabetic and control animals (glucose > 20 mM) were blindly assigned to semisynthetic modified AIN76 diet containing a 0.15% cholesterol-containing diet (CCD) (Teupser et al., 2003) with or without lidorestat (25 mg/kg/day; Alinea Pharmaceutical, Cambridge, MA) for 6 weeks.

Glucose, Triglyceride, and Cholesterol Measurements. Plasma samples were obtained from 6-h fasted mice. Glucose was measured directly from the tail tip of unanesthetized mice with a glucometer. Total cholesterol and triglyceride levels were measured enzymatically using kits from Infinity (Thermo Fisher Scientific, Waltham, MA).

Measurement of Plasma Lidorestat Levels. Two 1 mg/ml stock solutions of lidorestat were prepared in methanol. Two working solutions of 10 μ g/ml were prepared by diluting 10 μ l of each stock solution to 1 ml with control mouse plasma. The first working solution was serially diluted with control mouse plasma to produce calibration standards ranging from 0.1 to 5000 ng/ml. The second working solution was serially diluted with control mouse plasma to produce quality control standards of 2, 20, 200, and 2000 μ g/ml. Plasma samples and standards (100 μ l) were aliquoted into 96-well plates (1-ml well volume) along with 500 μ l of methanol containing 0.1 μ g/ml of the internal standard. Because of low sample volumes, all samples were diluted 4-fold in control mouse plasma by adding 75 μ l of control plasma to 25 μ l of in vivo sample plasma. Mixtures were vortexed and centrifuged at approximately 3000 rpm. A 10- μ l aliquot of each sample and standard supernatant was injected for liquid chromatography-tandem mass spectrometry analysis (PE Sciex API 4000; Agilent Technologies, Santa Clara, CA).

Analysis of Fructose Formation. Plasma and tissue fructose concentrations were measured using the enzymatic fluorometric assay (Siegel et al., 2000). Fructose was oxidized to 5-keto-fructose by the enzyme fructose dehydrogenase, and the redox dye resazurin was reduced to fluorescent compound resorufin. The fluorescence of resorufin was measured by fluorescence plate reader (Fluostar Optima; BMG Labtech, Winooski, VT) using 560-nm excitation and 580-nm emission filters and was stoichiometric with the amount of fructose.

Analysis of Heart Tissue Sorbitol Content. The sorbitol concentration in the heart tissue samples was determined using the following method (Nakano et al., 2003). The tissue lysates were deproteinated through addition of ice-cold 1 M perchloric acid followed by neutralization. A 30- μ l aliquot of sample was combined with 66.7 μ l of buffer (0.1 M sodium pyrophosphate, pH 9.5), 3.3 μ l of NAD (5 mg/ml), and 1.7 μ l of sorbitol dehydrogenase (30 mg/ml). The absorbance at 340 nm was measured before addition of the sorbitol dehydrogenase and 25 min after addition when the reaction had consumed all substrate.

TABLE 1

Effects of diets on weight, glucose, and lipid profiles

Data are expressed mean \pm S.D.

Diabetes Genotype	Diet	<i>n</i>	Glucose	TC	TG	Body Weight
				<i>mM</i>		<i>g</i>
<i>Ldlr</i> ($-/-$)	Placebo	16	31.4 \pm 3.9	80.4 \pm 30.7	4.6 \pm 2.2	21.7 \pm 2.6
	+ARI	21	29.5 \pm 4.1	67.5 \pm 32.2	2.9 \pm 1.6†	23.6 \pm 2.8†
hAR/ <i>Ldlr</i> ($-/-$)	Placebo	25	30.1 \pm 3.7	80.2 \pm 38.0	3.4 \pm 2.0	19.5 \pm 2.9
	+ARI	22	30.1 \pm 3.8	79.4 \pm 23.8	3.8 \pm 1.7	20.8 \pm 3.4

† $P < 0.05$ relative to placebo in the same genotype.

Quantitative Real-Time PCR for Heart Gene Expression.

Total RNA was isolated from hearts using TRIzol reagent (Invitrogen, Carlsbad, CA) and RNeasy Mini kit (QIAGEN, Valencia, CA). The mRNA levels were determined by SYBR Green (Applied Biosystems, Foster City, CA) real-time PCR using 10 or 100 μ g of total RNA. The primer sequences were hAR, sense, 5'-AGTCGGGCAATGTGGTTCCC-3' and antisense, 5' GGATTAACCTCTCCTGAGTG-3'; common AR, sense, 5' TTCTCTCCTGGAG GATCCCA GGAT-3' and antisense, 5'-TCTGGTGT CACAGACTTG-3'; atrial natriuretic peptide (ANP), sense, 5'-CTTG TGGTGTG TCACG-3' and antisense, 5'-CCTTGGT GCTGAAGTT-3'; brain natriuretic peptide (BNP), sense 5'-AGG TTT GCT ATC TGG CA-3' and antisense, 5'-ATG TCG AAG TTT AAG GCT CTG GA-3'; thrombospondin (TSP)-1, sense, 5'-GGGGCAGGAAGACTATGACA-3' and antisense, 5'-CT CCCCCTTTTGTCTGTGT-3'; matrix metalloproteinase (MMP)-9, sense, 5'-CATGCACTGGGGGCTTAGATC-3' and antisense, 5'-CGAGGGTAGCTATACAGCGGG-3'; fructokinase (FK), sense, 5'-TCCAACCTCTGCACTGT-3' and antisense, 5'-TCGACCTTCT CAAAGTCC-3'; aldolase A, sense, 5'-CCTTAGTCCTTTCG CC-3' and antisense, 5'-GGGA TTCACACGGTCG-3'; aldolase B, sense, 5'-AGCATGTC ACCCAATAG-3' and antisense, 5'-GTACCAACGGAAGGTTTC-3'; and aldolase C, sense, 5'-ATCCAGCGCCAAGTCATCTC-3' and antisense, 5'-AGTCAGGGCTGGAGTTCACG-3'.

Echocardiography. Two-dimensional echocardiography was performed on conscious 20- to 22-week-old mice using techniques described previously (Noh et al., 2006). Two-dimensional echocardiographic images were obtained and recorded. Images were then analyzed off-line by a single observer blinded to the murine genotype (Takuma et al., 2001).

Histology. Hearts from 20- to 22-week-old male mice were perfused with 10% buffered formalin solution. Hearts were subsequently immersed in 10% buffered formalin for 24 h, embedded in paraffin, and 5- μ m sections of the ventricles were cut. Sections were stained with Masson's Trichrome to assess interstitial fibrosis.

Statistical Analysis. Survival curves were plotted using the Kaplan and Meier method with 95% confidence levels for fractional survival. Statistical analyses were calculated by an unpaired one-tailed Student's *t* test. All data are expressed as mean \pm S.E.M., with a statistically significant difference defined as a value of $p < 0.05$.

Results

Effects of Diets and Diabetes on Weight, Glucose, and Lipoproteins. The effects of diabetes, hAR expression, and ARI on glucose, lipids, and body weight were determined (Table 1). As expected, STZ treatment increased glucose in all mice from 8.5 \pm 0.7 to 31.4 \pm 3.9 mM; there were no differences between control and hAR-expressing mice. Diabetic animals eating CCD had cholesterol levels of 80.2 \pm 38.0 mM; nondiabetic mice eating CCD had cholesterol levels of 23.5 \pm 5.6 mM. This marked increase in cholesterol in diabetic *Ldlr*($-/-$) mice has been noted in other studies (Renard et al., 2004; Berti et al., 2005; Vikramadithyan et al., 2005). Triglyceride levels in CCD-consuming nondiabetic

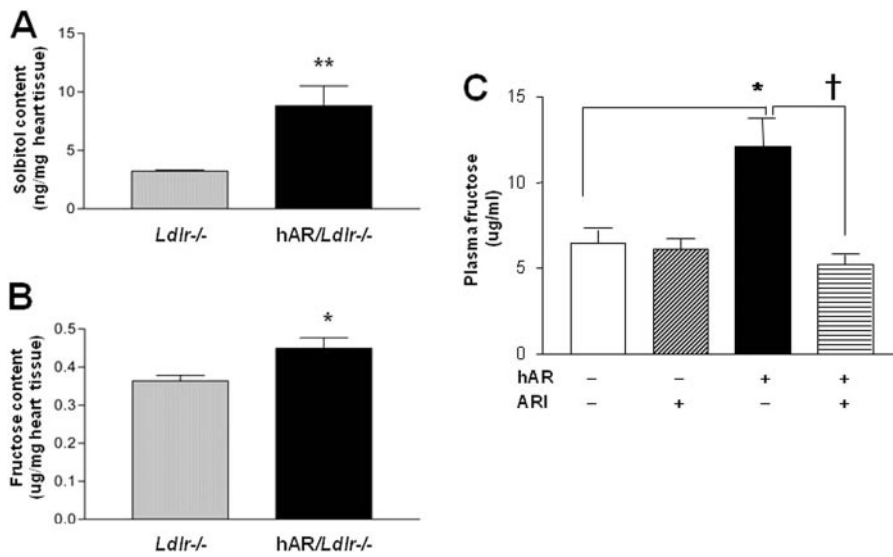


Fig. 1. CCD-fed hAR-expressing mice had a greater concentration of fructose. **A**, sorbitol concentration in the hearts was measured in STZ-treated diabetic mice with CCD diet. Data show n are means \pm S.E.; $n = 3$ to 5 in each group. **B**, fructose concentration in the heart was measured in STZ-treated diabetic mice with CCD diet, with or without hAR transgene. **C**, plasma fructose levels were measured after 6 weeks of CCD in *hAR/Ldlr*^{-/-} with placebo ($n = 20$), *hAR/Ldlr*^{-/-} with ARI ($n = 21$), *Ldlr*^{-/-} with placebo ($n = 11$), and *Ldlr*^{-/-} with ARI ($n = 12$). The mice were all STZ-treated diabetic. †, $p < 0.05$ versus placebo; *, $p < 0.05$ versus *Ldlr*^{-/-}.

mice averaged 1.9 ± 1.1 mM. With diabetes, triglyceride approximately doubled.

Inclusion of the hAR transgene or lidorestat containing CCD did not significantly alter glucose or cholesterol levels of control or STZ-treated mice. In one group, the diabetic *Ldlr*^{-/-} mice, lidorestat was associated with a modest decrease in triglyceride levels from an average of 4.6 to 2.9 mM, $p < 0.05$. However, a similar decline in triglyceride was not noted in the nondiabetic or hAR-expressing groups.

All mice with diabetes had reduced weight at the conclusion of the 10-week study. Nondiabetic mice weighed 28.2 to 36.8 g, and diabetic mice weighed 19.5 to 21.7 g. Diabetic *Ldlr*^{-/-} and hAR mice had identical weights and lidorestat did not affect weight.

hAR Effects on Plasma and Heart Fructose. Because sorbitol and fructose are produced downstream of AR actions, we measured sorbitol and fructose in hearts of control and hAR-expressing diabetic mice (Fig. 1, A and B). CCD-fed diabetic hAR-expressing mice had higher sorbitol and fructose levels in the hearts. Plasma from CCD-fed diabetic hAR-expressing mice also had a greater concentration of fructose than did non-hAR-expressing mice (12.1 ± 7.4 versus $6.4 \pm$

2.4 μ g/ml, $p < 0.05$). Lidorestat reduced plasma fructose levels by 56% in diabetic hAR mice.

AR Gene Expression in the Hearts. Because of the wide variation in fructose, we questioned whether expression of hAR varied in the transgenic mice. To assess this, we determined hAR mRNA levels using reverse transcriptase-PCR (Fig. 2, A and B). Human gene expression was noted, there was a wide variation in this expression. Using common primers, the level of AR expression (mouse plus human) in the control and hAR-expressing mice was compared. Although AR expression was greater in the transgenic animals, the increase was only 1.4-fold, significantly less than that found in a prior colony of hAR-transgenic mice (Vikramadithyan et al., 2005).

Lidorestat Concentration in the Plasma. We measured lidorestat concentration in plasma after 6 weeks of the CCD diet with lidorestat. hAR-expressing mice and control mice had the same plasma lidorestat concentrations (13.2 ± 7.9 versus 12.4 ± 7.4 μ M).

FK and Aldolase B Expression. One possible modulator of AR effects on plasma fructose is the expression of the enzymes that modulate fructose metabolism. For this reason, we measured expression of FK and aldolase A, B, and C (Fig.

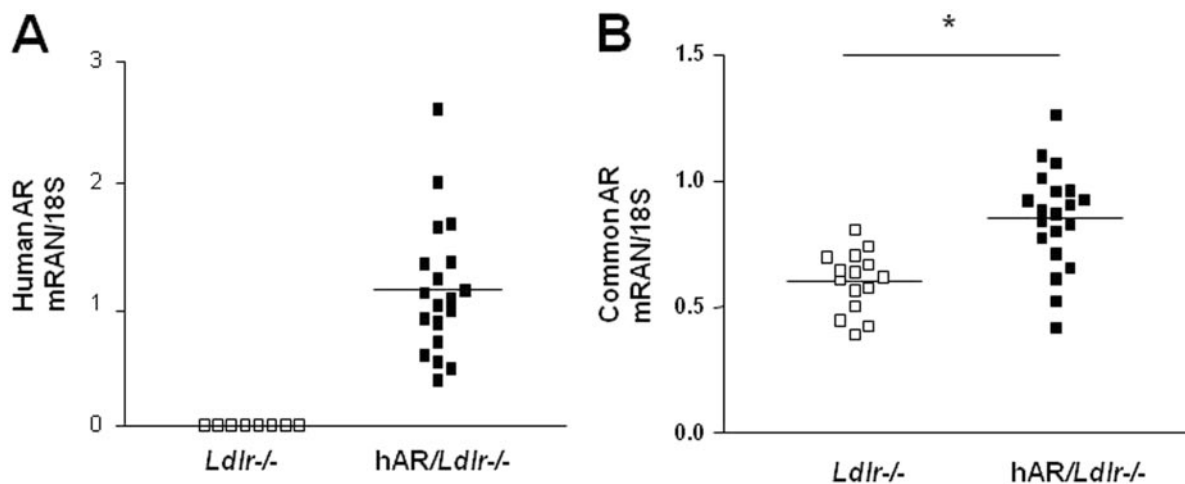


Fig. 2. AR gene expression in the hearts. **A**, hAR gene expression was detected only in the *hAR/Ldlr*^{-/-} mice. **B**, with the common primers, the level of AR expression (mouse plus human) was compared in the *hAR/Ldlr*^{-/-} ($n = 20$) and *Ldlr*^{-/-} mice ($n = 19$).

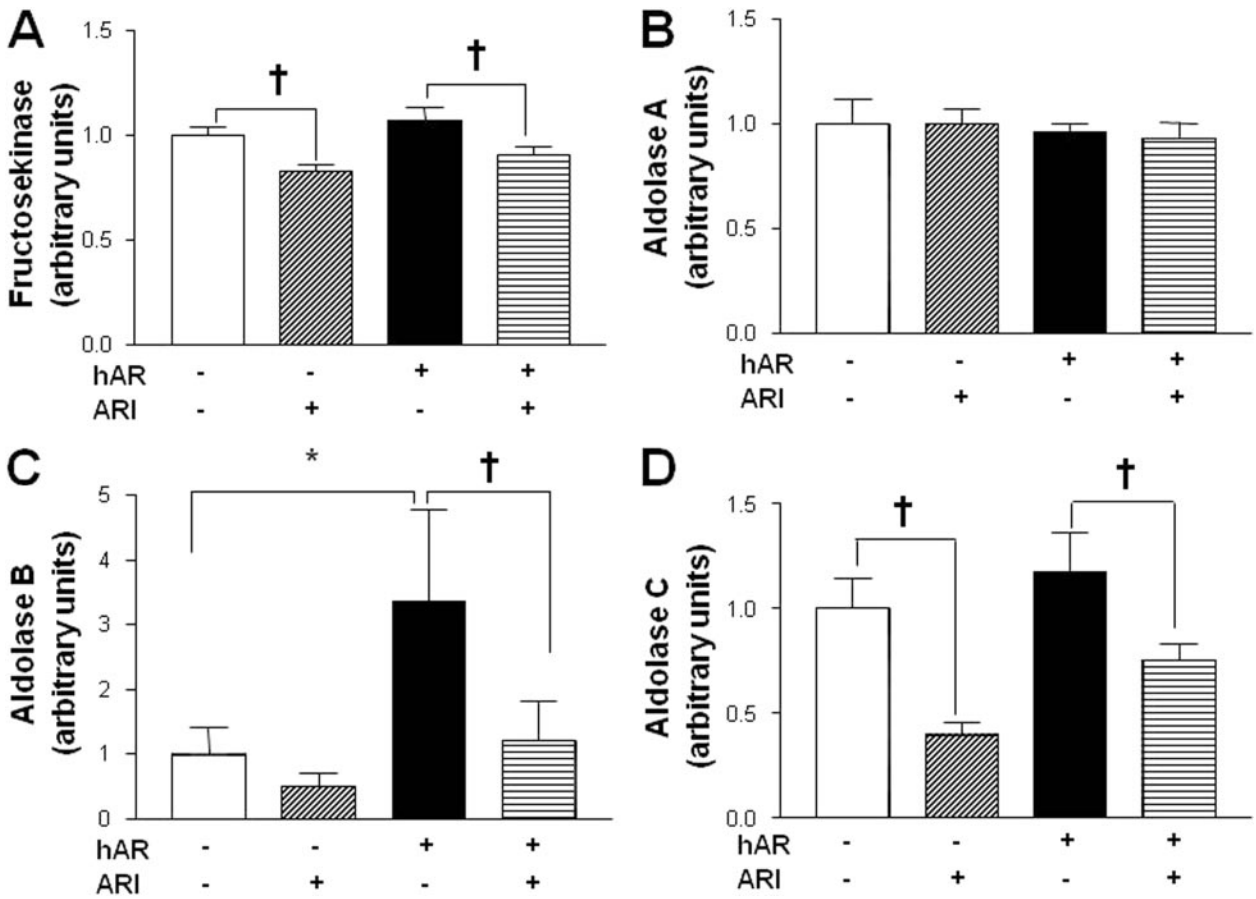


Fig. 3. Fructose metabolic enzymes gene expressions. A, fructosekinase; B, aldolase A; C, aldolase B; D, aldolase C. Gene expression was assessed by real-time PCR as described under *Materials and Methods*. Data are normalized to *Ldlr*(*-/-*) with placebo. $n = 7-8$ in each group. †, $p < 0.05$ versus placebo. *, $p < 0.05$ versus *Ldlr*(*-/-*).

3). FK mRNA levels were elevated in diabetic mice but not altered by expression of hAR. Aldolase A mRNA was not altered under the four conditions. In contrast, aldolase B mRNA was increased by expression of the hAR transgene and reduced by the lidorestat. Aldolase C was reduced by 2- to 3-fold in both strains of mice with ARI. Aldolase B is involved in fructose catabolism and might be a reason that plasma fructose levels were not even greater in the hAR-expressing transgenic mice.

Lidorestat-Containing Diets Reduced Mortality. During the 6-week trial we assessed the effects of AR inhibition on mortality. Although diabetic *Ldlr*(*-/-*) mice tolerated the di-

abetes and the CCD diet, hAR/*Ldlr*(*-/-*) had a 36% mortality rate (Fig. 4). The lidorestat-containing diet reduced mortality rates to levels of the *Ldlr*(*-/-*) group.

We were unable to determine the cause of excess mortality in many of the STZ-treated diabetic hAR/*Ldlr*(*-/-*) mice; however, in several animals, the causes of death were apparent. One mouse developed a bilateral lower extremity hemiplegia and a second an upper and lower extremity paralysis on one side. Another animal had a necrotic-appearing gut (Fig. 4B).

In the hAR/*Ldlr*(*-/-*) mice, areas of intracardiac fibrosis were evident (Fig. 5, A and B). Although these findings were occasionally noted in the lidorestat-treated group, their pres-

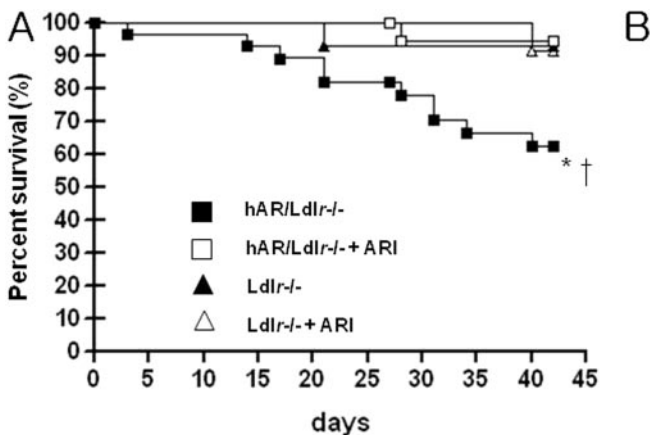


Fig. 4. Effect of lidorestat on the mice mortality. A, six-week survival rate was improved by ARI in the hAR/*Ldlr*(*-/-*) group. B, one animal had a necrotic-appearing gut. $n = 18$ to 21 in each group. †, $p < 0.05$ versus placebo; *, $p < 0.05$ versus *Ldlr*(*-/-*).

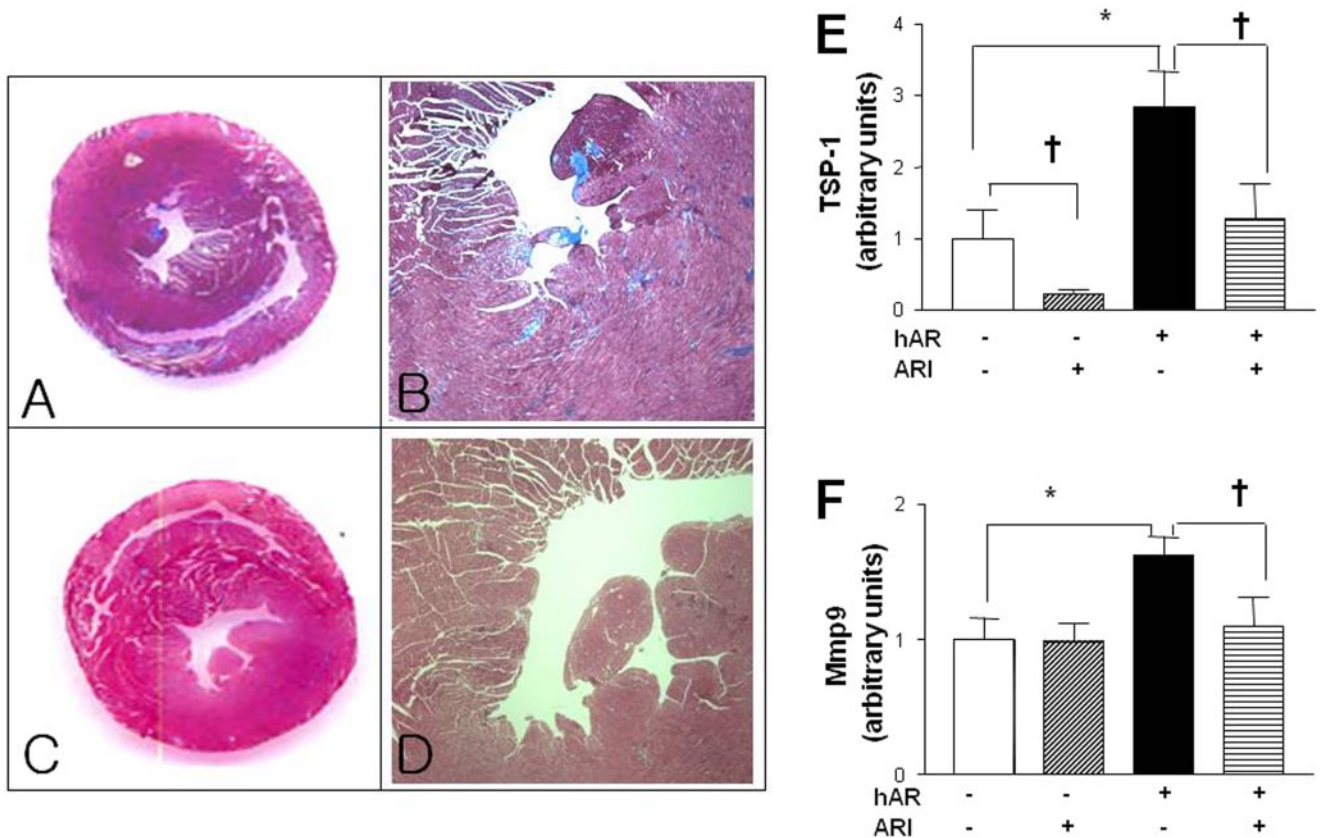


Fig. 5. The fibrosis in hearts of the hAR/*Ldlr*($-/-$) mice. A to D, sections were stained with Masson's trichrome after 6 weeks of CCD diet with or without ARI from three different mice in each group: hAR/*Ldlr*($-/-$) with placebo (A and B) and hAR/*Ldlr*($-/-$) with ARI (C and D). A and C, photomicrographs from histological sections in each group. B and D, 10 \times magnification. E and F, TSP-1 (E) and MMP9 (F) gene expression was assessed after 6 weeks of CCD with ARI or placebo by real-time PCR as described under *Materials and Methods*. Data are normalized to *Ldlr*($-/-$) with placebo. $n = 7$ to 8 in each group. †, $p < 0.05$ versus placebo; *, $p < 0.05$ versus *Ldlr*($-/-$).

ence was much less (Fig. 5, C and D). Cardiac fibrosis was associated with increased heart expression of TSP1 and MMP9 (Fig. 5, E and F). TGF- β was also increased in STZ-treated hAR mice (1.4-fold, $p < 0.05$) but was not reduced by ARI (1.3-fold) (data not shown).

Heart Function in hAR/*Ldlr*($-/-$) Mice. Cardiac function was assessed by ultrasonography. At the end of the 6 weeks of CCD, diabetic hAR mice showed reduced fractional shortening compared with lidorestat-treated mice (Fig. 6A), but there were no significant differences in heart function between hAR and non-hAR mice. However, when the hearts were assessed for the failure markers, BNP and ANP (Fig. 6, C and D), the highest expression levels were found in the hAR mice not treated with the ARI; ARI treatment reduced ANP expression in hAR- and non-hAR-expressing hearts.

To adjust for survivor bias, a study was performed that terminated after only 4 weeks of CCD feeding. The STZ-treated hAR mice had no excess mortality in this short study. Although most mice had normal heart function at 4 weeks, hAR mice had significantly reduced fractional shortening compared with non-hAR mice and lidorestat-treated mice groups; FS was 45% in hAR mice, 48% in hAR lidorestat-treated mice, 49% in non-hAR mice, and 51% in non-hAR mice treated with lidorestat (Fig. 6B).

Discussion

Unlike microvascular disease, which has a pathology that is unique to patients with diabetes, atherosclerosis of me-

dium and large vessels does not have a diabetes-specific fingerprint. To understand the factors that accelerate macrovascular disease, we and others have employed a number of animal models (Goldberg and Dansky, 2006). Diabetic mice have been created recently that develop plaque hemorrhage and increased aortic root lesion areas on chow diets (Renard et al., 2004). In addition, our laboratory was able to increase aortic atherosclerosis by using mice that express human AR (Vikramadithyan et al., 2005). We attempted to understand how AR affects in vivo metabolism by studying one of its downstream products, fructose. In addition, we tested whether a new and potent pharmacologic inhibitor of AR would decrease atherosclerosis and improve the survival of diabetic hAR-expressing mice. Our data show the following. 1) Fructose levels were increased with hAR expression. 2) Aldolase B, a gene that increases fructose metabolism, was increased in diabetic hAR-expressing mice. 3) Lidorestat treatment did not affect plasma lipids, glucose, or weights of diabetic mice. 4) Drug-treated mice had improved survival; hAR-expressing *Ldlr*($-/-$) diabetic mice have greater mortality than diabetic *Ldlr*($-/-$) mice. 5) This was associated with evidence of cardiac dysfunction.

Because the objective of the study was to determine whether AR inhibition improved the health of the mice, unidentified samples of lidorestat and a placebo were sent to the investigators. The drug did not alter plasma metabolic parameters. These compounds were incorporated into cholest-

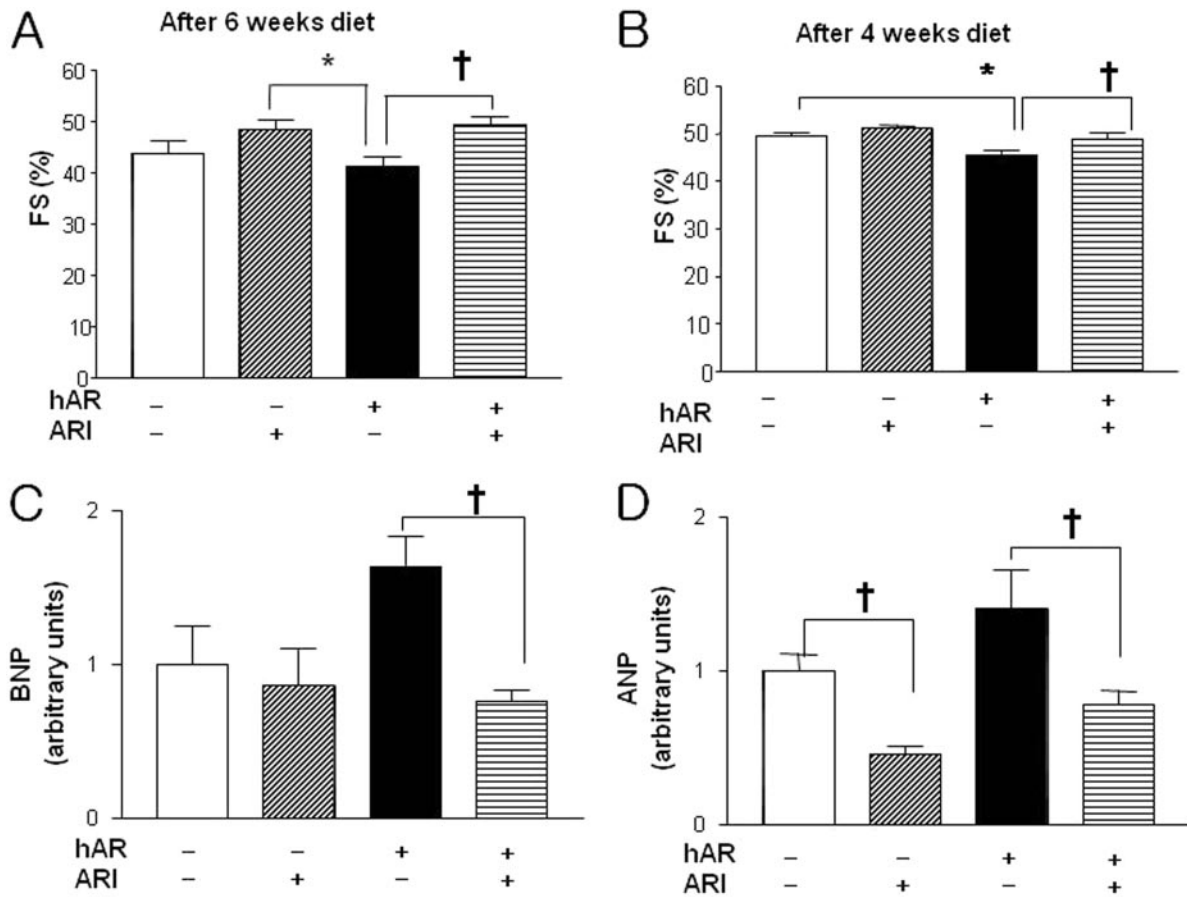


Fig. 6. Heart echocardiography and gene expression for heart failure markers. The echocardiograms were performed after CCD with ARI or placebo for each mouse group. A, fractional shortening (percentage) after 6 weeks of CCD with ARI or placebo. $n = 7$ to 10 in each group. B, fractional shortening (percentage) after 4 weeks of CCD with ARI or placebo. $n = 6$ to 9 in each group. C and D, BNP (C) and ANP (D) gene expression was assessed after 6 weeks of CCD with ARI or placebo by real-time PCR as described under *Materials and Methods*. Data normalized to *Ldlr*($-/-$) with placebo. $n = 7$ to 8 in each group. †, $p < 0.05$ versus placebo. *, $p < 0.05$ versus *Ldlr*($-/-$).

terol-containing diets and fed to two groups of diabetic hAR/*Ldlr*($-/-$) mice.

As reported by Van Zandt et al. (2005) and summarized below, lidorestat is a highly potent and selective aldose reductase inhibitor with good oral bioavailability that is reported to improve nerve conduction and reduce cataract formation (Van Zandt et al., 2005). From in vitro experiments, lidorestat has a reported IC_{50} against recombinant human aldose reductase (*h*-ALR2) of $5 \mu\text{M}$. Against recombinant human aldehyde reductase (*h*-ALR1), lidorestat has a reported IC_{50} of $27,000 \mu\text{M}$, yielding a selectivity of *h*-ALR1//*h*-ALR2 of 5400:1. In vivo efficacy of lidorestat was assessed in a number of studies utilizing the STZ-induced diabetic rat. In our study, reduction in plasma fructose, a downstream product of AR, was in vivo proof that the lidorestat was effective in the hAR/*Ldlr*($-/-$) mice.

Kawasaki et al. (2002) found that diabetic humans have increased circulating levels of fructose, but other studies reported that comparable serum fructose concentrations are found in nondiabetic and diabetic patients (Pitkänen, 1996; Yoshii et al., 2001). In our study, we did not find a marked increase in fructose when samples from nondiabetic control and hAR-expressing mice were assayed (data not shown). Although there may be many differences in fructose metabolism between mice and humans, in part, these differences might reflect the lower AR expression in the mouse. On the

CCD, STZ treatment increased fructose levels in the hAR-expressing mice but not control *Ldlr*($-/-$) mice. It is surprising that fructose was lower and not increased by either STZ treatment or hAR when the mice consumed a chow diet.

AR converts glucose to sorbitol that is then converted to fructose. Fructose is more reactive and has a greater propensity to produce advanced glycation end products (Suárez et al., 1989; Ruderman et al., 1992; Ganea and Harding, 1995, 2005). Because the rate of glycation depends on the percentage of sugar in the open-chain form, fructose, which exists in the open-chain form more often than glucose, has a 300-fold faster glycation rate than glucose (Bunn and Higgins, 1981; Ruderman et al., 1992). Mouse ingestion of an advanced glycation end product-enriched diet increases atherosclerosis (Lin et al., 2003). In addition, a recent study has shown that metabolism of glucose via the hexosamine pathway up-regulates TSP1, an atherogenic protein (Raman et al., 2007). TSP1 was also increased in the hAR/*Ldlr*($-/-$) mouse hearts that we studied.

Although AR expression increased plasma fructose levels, the relationship, although significant, suggested that other factors were playing a role. We considered whether fructose metabolism was such a factor. There are several pathways involved in elimination of plasma fructose: 1) It can be internalized by cells via glucose transporters 2 and 5 (Cui et al., 2003). 2) Fructose can be phosphorylated via phosphofruc-

tokinase and enter the hexosamine pathway. 3) Fructose is also metabolized by FK to fructose-1-phosphate. Fructose-1-phosphate is cleaved by aldolase B to form dihydroxyacetone phosphate and glyceraldehydes, which can be further metabolized in the glycolytic pathway (de Walque et al., 1999; Berg and Stryer, 2001). The expression levels of these two enzymes, FK and aldolase B, were studied in the hearts. The highest levels of aldolase B mRNA were in hearts from diabetic hAR-expressing mice. Increased expression of aldolase B might have increased metabolism of fructose. It is noteworthy that lidorestat treatment reduced both aldolase B and FK; these changes might have been secondary to reduced fructose production. Such a conclusion is obviously speculative because there are limited data on genetic regulation of aldolase B. Nonetheless, induction of fructose-metabolizing enzymes might have dampened the effects of hAR expression on plasma fructose levels.

Diabetic hAR/*Ldl(-/-)* mice receiving the placebo died more often than did hAR/*Ldlr(-/-)* mice consuming lidorestat. Almost 31% of the diabetic hAR-expressing mice did not survive the 6-week study. In contrast, diabetic hAR/*Ldlr(-/-)* mice receiving lidorestat had a similar mortality rate to that of non-hAR-expressing mice. All causes of the excess mortality in the placebo group could not be determined because mice were found dead in their cages, often in the morning. However, one mouse seemed to die with a bowel infarction and two mice developed a hemiplegia.

A relationship between fructose and greater vascular disease in mice has been found by others. Other investigators (Merat et al., 1999; Collins et al., 2001) have reported that fructose-rich diets increase mouse atherosclerosis via a manner that is exclusive of plasma cholesterol or glucose concentrations. Cardiac function seemed to differ between the two groups of animals. Ejection fraction was better in the lidorestat-treated group. When the hearts were analyzed, several mice in the placebo group had a patchy area of fibrosis in the myocardium. In addition, we identified several smaller vessels that seemed to be occluded. Whether this pathology represented small myocardial infarctions is unconfirmed.

Ramasamy and co-workers suggested that cardiac AR expression leads to greater heart dysfunction especially during ischemia-reperfusion (Hwang et al., 2004). Moreover, Hwang et al. (2002) showed that ARI-treatment improved cardiac function in isolated perfused hearts. An ARI was reported more recently to protect hearts from toxic effects of lipopolysaccharide (Ramana et al., 2006). Our data further show a role for AR-inhibition; ARI improves heart function in diabetic, hypercholesterolemic hAR-expressing mice.

How does diabetes and hAR expression lead to cardiac dysfunction? There are several postulated mechanisms for diabetic cardiomyopathy including increased production of reactive oxygen species leading to apoptosis (Cai et al., 2002, 2006), mitochondrial dysfunction (Tanaka et al., 1992; Flarsheim et al., 1996; Russell et al., 2005), and lipotoxicity associated with greater fatty acid and reduced glucose oxidation (Finck et al., 2003).

The most significant cause of mortality in patients with diabetes are diseases of the cardiovascular system. This includes peripheral vascular disease that can lead to intestinal ischemia, a clinical condition that might be similar to that found in the diabetic hAR/*Ldlr(-/-)* mouse shown in Fig. 4B. Coronary artery disease is more common in patients with

diabetes. In addition, the incidence of heart failure is greater even with a similar level of vascular disease. Elevated glucose levels are a marker for worse outcome in patients admitted to the hospital with congestive heart failure (Held et al., 2007). Our data provide evidence that during diabetes, a metabolic pathway mediated by AR is harmful. This may be because of increased fructose formation; as noted above, two studies have found greater atherosclerosis in fructose-fed than high fat-fed mice (Merat et al., 1999; Collins et al., 2001). This suggests that fructose might be especially toxic to blood vessels. Perhaps drugs such as lidorestat will improve human health by reducing the production of toxic products of the polyol pathway.

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