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Endothelin antagonism improves hepatic insulin sensitivity associated with insulin signaling in Zucker fatty rats

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Abstract

In the present study, we investigated the effects of long-term treatment with the endothelin (ET) antagonist atrasentan, an ET_A-selective antagonist, on whole body glucose metabolism and insulin signaling in a commonly used model of insulin resistance, the Zucker fatty rat. Zucker lean and fatty rats were maintained for 6 weeks on either control or atrasentan-treated water. Euglycemic-hyperinsulinemic clamps (4 mU/kg per minute) were performed at the end of the 6-week treatment on a subset of rats (n = 10/treatment). In another subset (n = 5/treatment), an insulin tolerance test was performed; liver and muscle tissues were harvested 10 minutes following the challenge for further analysis. Results of the clamps demonstrated that long-term atrasentan treatment significantly increased whole body glucose metabolism in fatty rats compared with vehicle control subjects. Insulin-induced insulin receptor substrate 1 tyrosine and protein kinase B serine phosphorylation were significantly reduced in the liver and muscle of fatty animals compared with their lean littermates. This reduction was overcome with atrasentan treatment in the liver but not in the muscle. There was no difference between lean and fatty animals, however, in insulin receptor substrate 1 and protein kinase B protein expression in the liver and muscle and no effect by atrasentan. In contrast, expression of the regulatory subunit of PI-3 kinase (p85α) was significantly increased in the liver but not in the muscle of fatty animals compared with their lean littermates and this was normalized to levels of lean animals with atrasentan treatment. These findings indicate that long-standing ET antagonism improves whole body glucose metabolism in Zucker fatty rats through improvements in insulin signaling in the liver. These results indicate that therapeutic ET antagonism may assist in correcting the insulin-resistant state.

1. Introduction

Endothelin (ET) is one of the most potent vasoconstrictors known [1]. In addition to its well-known effects on the cardiovascular system [2], this peptide may be involved in pathologies such as insulin resistance and diabetes and/or their well-known cardiovascular complications [3,4].

Elevated production of endothelin-1 (ET-1) has been reported in mesenteric arteries from streptozotocin-induced diabetic rats, a well-known model of type 1 diabetes mellitus [5]. Our group has recently demonstrated that as insulin-resistant Zucker fatty rats age and are submitted to meal challenge conditions, plasma ET-1 levels are elevated compared with their lean littermates [6]. Elevated plasma ET has also been reported in patients with type 2 diabetes mellitus and in those with metabolic syndrome [7,8].

Ferri et al [9] demonstrated that circulating ET-1 levels increased during a euglycemic-hyperinsulinemic clamp in lean type 2 diabetic men. Furthermore, they observed a negative correlation between the glucose infusion rate (GINFR) and circulating ET-1 levels, suggesting that this peptide might exert negative effects on the insulin sensitivity of target tissues. Others have demonstrated that exogenous ET-1 infusion causes peripheral insulin resistance in healthy human beings by decreasing insulin-dependent whole body glucose uptake [10]. Similarly, Teuscher et al [11] have shown that intravenous infusion of subpressor doses of ET-1 significantly reduced the acute insulin response to glucose and insulin sensitivity in normal nondiabetic men. Other studies observed that ET-1 administered intraperitoneally in conscious Sprague-Dawley rats increased plasma glucose and the plasma glucose-to-insulin ratio [12,13]. These investigators demonstrated by an oral

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glucose tolerance test that glucose levels were significantly higher in rats preinjected with ET-1 than in those preinjected with saline [12,13].

It has also been reported that ET-1 infusion reduces splanchnic glucose production in healthy men [14]. Interestingly, others have shown opposite results in rats [15,16]. It has been demonstrated that ET-1 infusion causes an increase in glucose production in the perfused rat liver owing to the stimulation of hepatic glycogenolysis [15,16]. In agreement with these findings, it has been reported that ET-1 or ET-3 infusion directly into the portal vein increased glucose output in the perfused rat liver and that selective ET_A antagonism attenuated this increase [17].

Taken together, there is evidence to support the idea that increased ET-1 could have detrimental effects on glucose homeostasis, leading possibly to insulin resistance and/or diabetes. We have previously shown that long-term treatment with atrasentan, an ET_A-selective antagonist, positively impacts metabolic responses (glucose and insulin levels) in one of the most commonly used models of insulin resistance, the Zucker fatty rat [18]. We investigated the effects of long-standing ET_A blockade via atrasentan treatment on whole body glucose metabolism and whether there was a direct effect on insulin signaling in the liver and muscle of severely insulin-resistant Zucker fatty rats.

2. Methods

2.1. Animal studies

Six- to seven-week-old Zucker lean and fatty rats (Charles River, Wilmington, Mass) were acclimated to the animal research facilities for 1 week. The following investigations were conducted in accordance with the guidelines of the Abbott Laboratories Institutional Animal Care and Use Committee. Animals were housed and maintained on a Harlan Teklad rodent diet 8640 (Harlan, Madison, Wis) ad libitum.

After acclimation and following a 3-hour fast, rats were weighed and tail-snip glucose levels were determined by the glucose oxidase method (Precision G glucose meter, Abbott Laboratories, Bedford, Mass). Basal insulin levels were measured with a rat insulin ELISA kit (Alpco Diagnostics, Winham, NH) using rat insulin standards. Endothelin-1 levels were also measured using a QuantiGlo human ET-1 chemiluminescent immunoassay (R&D Systems, Minneapolis, Minn). The animals were randomized to the various treatment groups based on plasma glucose levels and body weight. Treatment groups were lean (n = 10), fatty control (n = 20), and fatty atrasentan (n = 20).

Table 1
Effects of atrasentan on body weight, water intake, glucose, and insulin levels in Zucker fatty rats

	Lean	Fatty control	Fatty atrasentan
Body weight (g)	328 ± 3	528 ± 10*	516 ± 8*
Daily water intake (mL)	30 ± 1	46.2 ± 1.3*	44.6 ± 1.7*
Basal glucose levels (mg/dL)			
Before treatment	115.8 ± 3.5	112.5 ± 3.4	110.4 ± 1.8
After 6 wk of treatment	106.8 ± 2.7	96.1 ± 6.7	104.2 ± 2.3
Basal insulin levels			
Before treatment	0.39 ± 0.05	6.7 ± 1.6*	6.8 ± 1.7*
After 6 wk of treatment	1.0 ± 0.2	20.0 ± 1.5*	10.7 ± 1.2*,†

* $P < .01$, compared with lean.
† $P < .01$, fatty atrasentan vs fatty control.

Table 2
Basal ET-1 levels in Zucker lean and fatty rats before and after treatment with atrasentan

	ET-1 level (pg/mL)		
	Lean	Fatty control	Fatty atrasentan
Week 0	3.8 ± 0.3	3.9 ± 0.2	3.6 ± 0.1
Week 2 (after starting treatment)	3.1 ± 0.2	3.9 ± 0.3*	8.9 ± 0.7**,***
Week 4 (after starting treatment)	2.2 ± 0.1	3.7 ± 0.4***	10.6 ± 0.6**,***
Week 6 (after starting treatment)	2.9 ± 0.2	3.6 ± 0.4*	10.7 ± 0.9**,***

* $P < .05$, compared with lean.
** $P < .01$, fatty atrasentan vs fatty control.
*** $P < .001$, compared with lean.

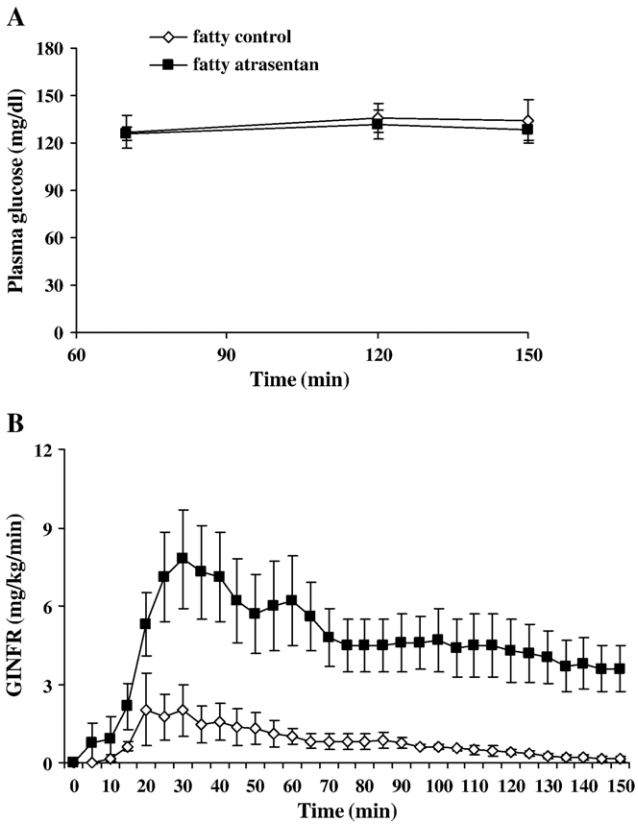

Fig. 1. Glucose levels (A) and GINFR (B) in control and atrasentan-treated Zucker fatty rats during a 150-minute euglycemic-hyperinsulinemic clamp. Each column with a bar represents the mean±SEM of 10 rats per group. GINFR, $P < .05$ (15-20 minutes); $P < .01$ (25-150 minutes), fatty atrasentan vs fatty control.

Fatty rats were treated for 6 weeks with atrasentan in their drinking water (5 mg/kg per day). Lean and fatty control rats were maintained on regular drinking water.

2.2. Drug administration

Atrasentan (5 mg/kg per day, ABT-627, HCl salt, Abbott Laboratories) [19] was dissolved with 2 molar-equivalent 1N NaOH and brought to the desired volume with regular drinking water.

2.3. Euglycemic-hyperinsulinemic clamp

After 5 weeks of treatment with atrasentan, a subset of fatty animals (n = 10/treatment) was anesthetized with ketamine/xylazine (75/2 mg/kg). A midline incision was made on the ventral aspect of the neck. Chronic cannulas were then inserted into the left carotid artery and the right jugular vein (microrenathane, 0.033, Braintree Scientific, Braintree, Mass), brought around the neck subcutaneously, and passed through a small skin incision at the base of the neck. After a 1-week recovery period and following an overnight fast, the animals underwent a euglycemic-hyperinsulinemic clamp. The protocol consisted of a 5-minute priming period with 10 mU/kg per minute of insulin (Humulin-R, Eli Lilly, Indianapolis, Ind), followed by a constant infusion at 4 mU/kg per minute for 140 minutes. Blood samples were taken every 5 minutes and glucose levels were measured using a glucose oxidase method (Precision G glucose meter, Abbott Laboratories). Glucose (50% dextrose; Abbott Laboratories, North Chicago, Ill) was simultaneously infused as necessary to maintain euglycemia (~120-130 mg/dL).

2.4. Blood pressure monitoring

Simultaneously to euglycemic-hyperinsulinemic clamps, blood pressure was monitored for Zucker lean, fatty control, and fatty atrasentan groups. Blood pressure measurement was made from the arterial catheter that was coupled to a fluid-filled strain gauge pressure transducer connected to a digital data acquisition system (Ponemah Physiology Platform, Gould Instrument Systems, Valley View, Ohio).

2.5. Insulin challenge (insulin tolerance test)

In another subset of treated fatty and control lean animals, insulin (0.5 U/kg in 0.1% BSA) or saline control was given intraperitoneally after an overnight fast. Tissue samples from the liver and muscle (soleus) were taken 10 minutes following either saline- or insulin-stimulated conditions (n = 5/treatment). Following the 10-minute challenge, tissues were harvested, immediately frozen in liquid nitrogen, and kept at −80°C for further analysis.

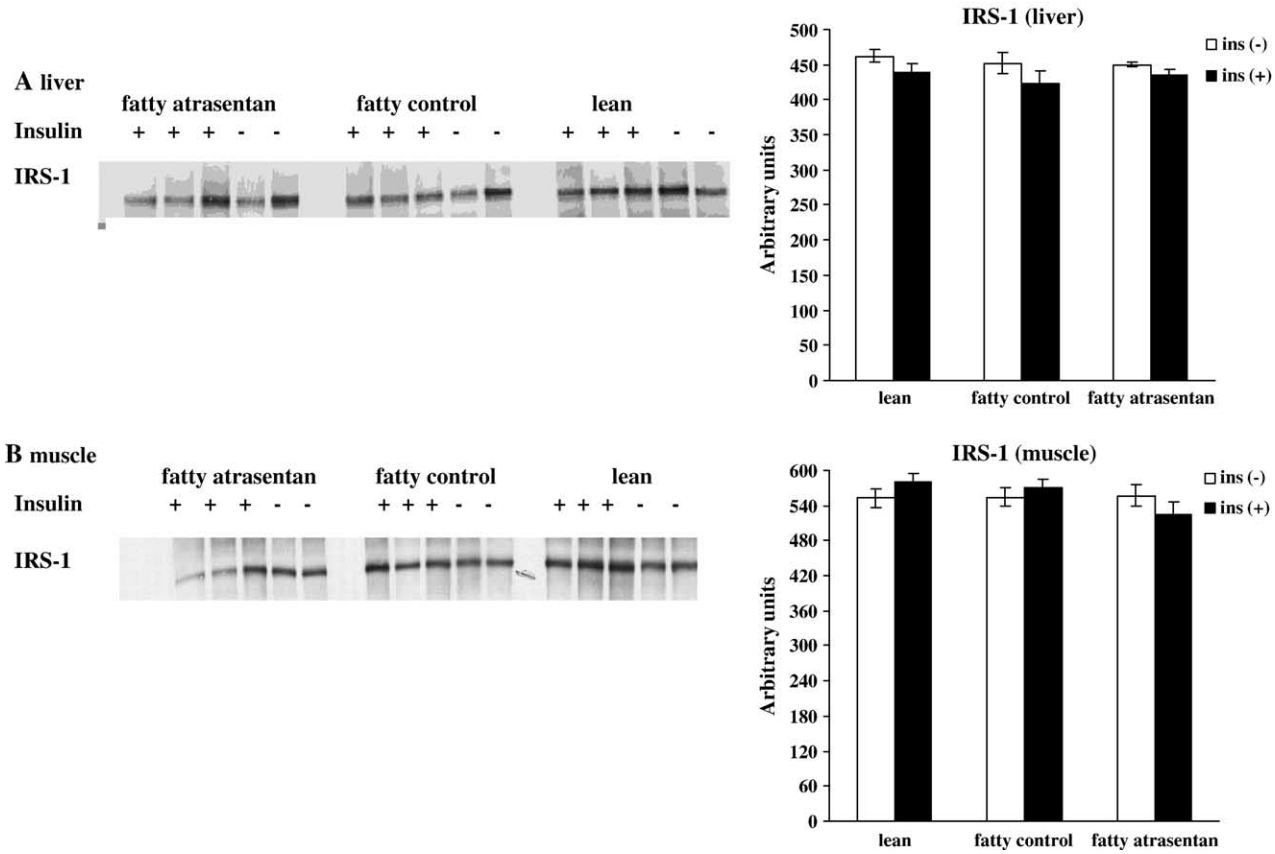

Fig. 2. Western blots of IRS-1 protein levels in the liver (A) and skeletal muscle (B) of Zucker lean and fatty rats with and without atrasentan treatment. Experiments were repeated twice on different sets of extracts. Data are the mean±SEM of 5 rats per group.

2.6. Tissue extracts preparation, immunoprecipitation, and immunoblotting techniques

Tissues were sonicated (using a Branson 450 Sonifier, Danbury, Conn). The lysis buffer for liver tissue contained 20-mmol/L Tris-HCl (pH 7.4), 1% Triton X-100, 10% glycerol, 150-mmol/L NaCl, 2-mmol/L EDTA, 25-mmol/L β-glycerophosphate, 20-mmol/L sodium fluoride, 1-mmol/L sodium orthovanadate, 2-mmol/L sodium pyrophosphate, 10-μg/mL leupeptin, 1-mmol/L benzamidine, 1-mmol/L 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), and 2.5-mg/mL deoxycholic acid. The lysis buffer for skeletal muscle tissue contained 50-mmol/L HEPES, 137-mmol/L NaCl, 1-mmol/L $MgCl_2$, 1-mmol/L $CaCl_2$, 10-mmol/L sodium pyrophosphate, 10-mmol/L sodium fluoride, 2-mmol/L EDTA, 1-mmol/L dithiothreitol, 10% glycerol, 2-mmol/L sodium orthovanadate, 1-mmol/L AEBSF, 10-μg/mL leupeptin, 1-mmol/L benzamidine, and 1% NP40. Tissues were rocked for 30 minutes at 4°C. Detergent-insoluble material was sedimented by centrifugation at 12 000*g* for 15 minutes at 4°C.

For immunoprecipitation, insulin receptor (IR; 4 μg; Transduction Laboratories, San Diego, Calif) or insulin receptor substrate 1 (IRS-1) antibody (4 μg; Upstate Biotechnology, Lake Placid, NY) was added to 500 μg of liver lysate from Zucker lean or fatty rats. Volume was adjusted to 500 μL with lysis buffer and then mixed by inversion for 2 hours at 4°C. Protein A agarose beads (Life Technologies, Rockville, Md) were added (50 μL of a 50% slurry in lysis buffer) to the lysate, and the mixture was inverted at 4°C for 30 minutes. Beads were briefly pelleted in a microfuge (2500*g*, 1 minute) at 4°C and then washed 3 times with 500 μL of lysis buffer followed by addition of 50 μL of Laemmli buffer (Bio-Rad Laboratories, Hercules, Calif). The mixture was boiled for 5 minutes, beads were pelleted, and a 20-μL supernatant was used for Western blotting analysis. Otherwise, cell lysate proteins (20 μg of protein) were directly separated by SDS-PAGE on 7.5% or 10% gels.

Proteins were transferred from the gel to nitrocellulose sheets and blocked in 4% nonfat dry milk in TBST (Sigma, St Louis, Mo). The blots were probed with various primary antibodies—anti-IRβ antibody (Transduction Laboratories), anti–IRS-1, anti-p85α (whole antiserum), antiphosphotyrosine antibodies (Upstate Biotechnology), anti-protein kinase B (PKB), and anti–phospho-PKB (Ser 473; Cell Signaling Technology, Beverly, Mass)—according to the recommendations of the manufacturers. The proteins were detected by enhanced chemiluminescence with horseradish peroxidase–labeled secondary antibodies (Amersham, Pistacaway, NJ). The intensity of the bands was quantified with a laser densitometer (Molecular Dynamics, Sunnyvale, Calif).

2.7. Statistical analysis

Results are given as mean±SEM for the indicated number of rats. A one-way analysis of variance with repeated measures followed by a Tukey-Kramer multiple comparison test was used for the clamp data. Student's *t* test was used for immunoblotting data. *P* values of .05 (2-tailed) and lower were considered significant.

3. Results

3.1. Body weight, water intake, glucose, insulin, and ET-1 levels

Table 1 illustrates body weight, daily water consumption, and basal glucose and insulin levels in Zucker lean and fatty rats with and without atrasentan. As expected, body weight and daily water consumption in fatty animals were significantly higher compared with their lean littermates ($P < .01$). There was no significant effect of atrasentan treatment, however, on these 2 parameters. Baseline and 6 weeks posttreatment results for glucose and insulin are reported in Table 1. There was no effect of atrasentan on glucose levels after treatment. Hyperinsulinemia was significantly reduced (~50%) after 6 weeks of treatment

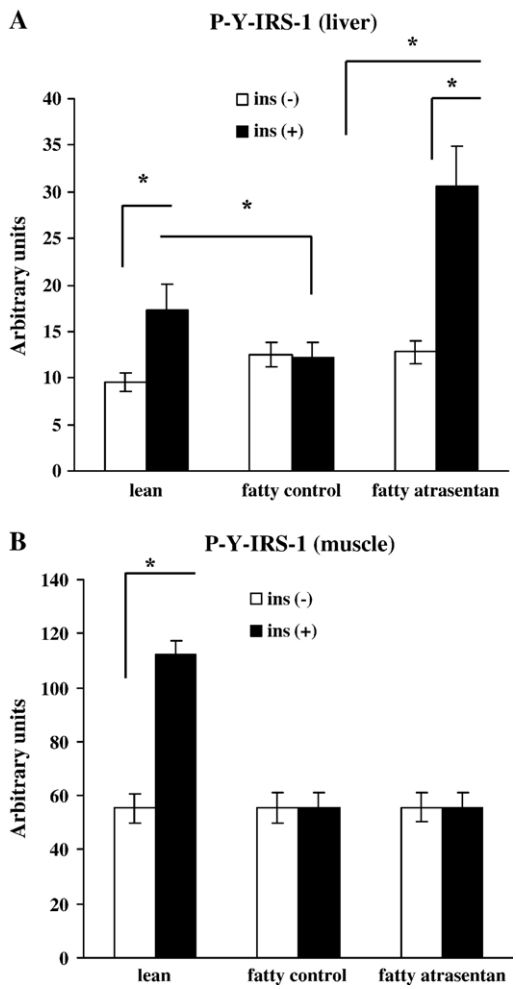

Fig. 3. Basal and insulin-stimulated tyrosine phosphorylation of IRS-1 in the liver (A) and skeletal muscle (B) of Zucker lean and fatty rats with and without atrasentan treatment. Experiments were repeated twice on different sets of extracts. Data are the mean±SEM of 5 rats per group. *$P < .05$.

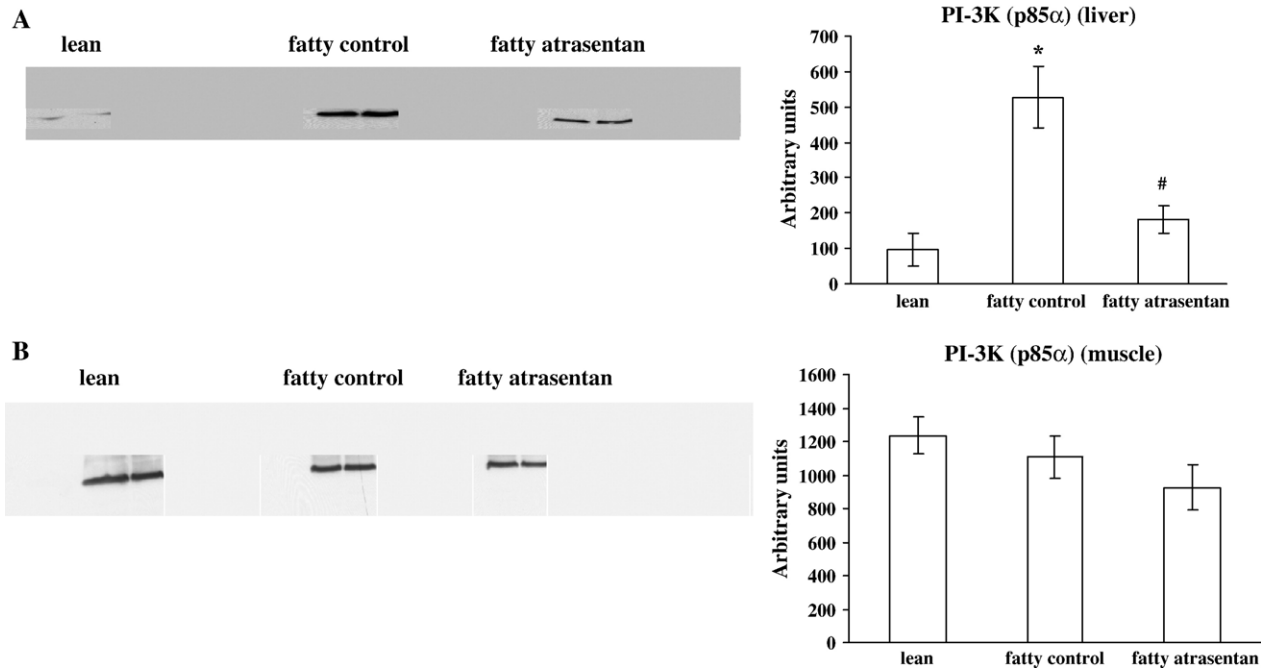

Fig. 4. Western blots of p85α protein levels in the liver (A) and skeletal muscle (B) of Zucker lean and fatty rats with and without atrasentan treatment. Experiments were repeated twice on different sets of extracts. Data are the mean±SEM of 5 rats per group. *$P < .05$, fatty control vs lean; #$P < .05$, fatty atrasentan vs fatty control.

with atrasentan ($P < .01$, fatty atrasentan vs fatty control) (Table 1). Table 2 shows basal ET-1 levels before and after 2, 4, and 6 weeks of treatment with atrasentan. Endothelin-1 levels are significantly elevated in Zucker fatty rats (control group) compared with their lean littermates ($P < .05$, weeks 2 and 6; $P < .001$, week 4) and with atrasentan-treated counterparts ($P < .001$ throughout vs fatty control and lean littermates) (Table 2).

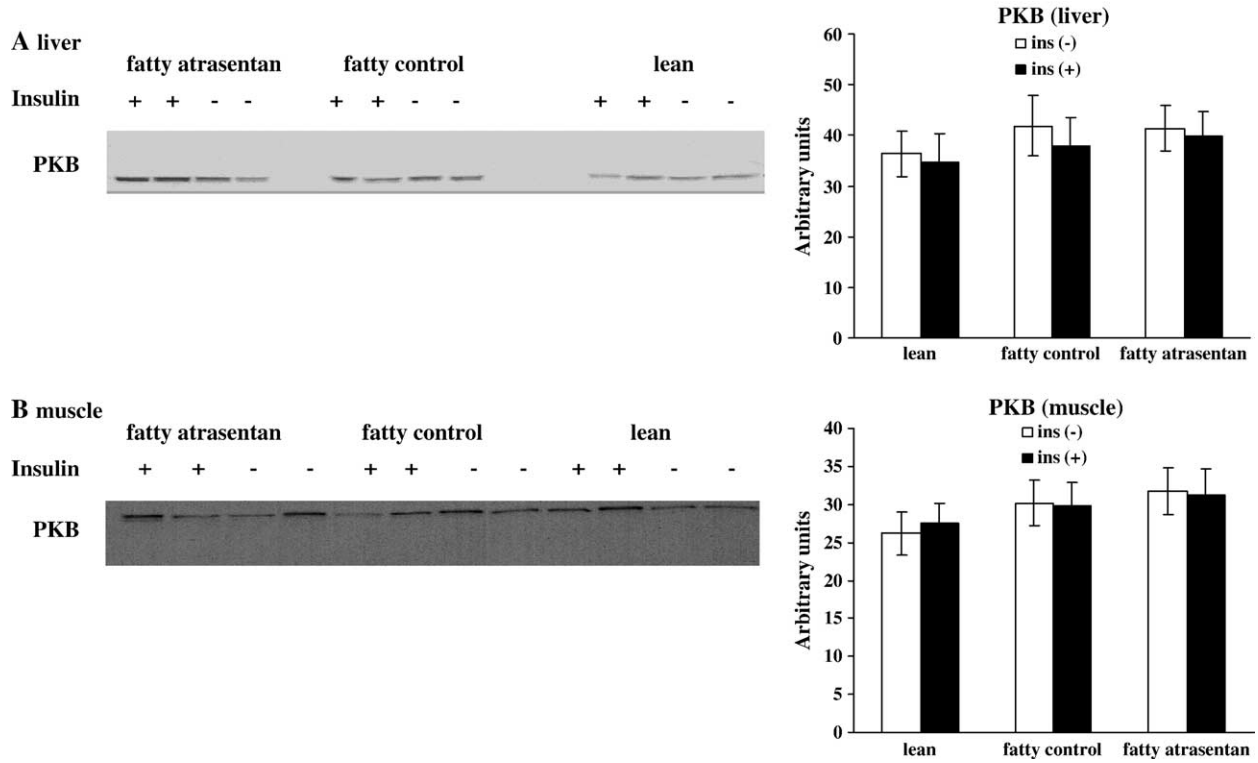

Fig. 5. Western blots of PKB protein levels in the liver (A) and skeletal muscle (B) of Zucker lean and fatty rats with and without atrasentan treatment. Experiments were repeated twice on different sets of extracts. Data are the mean±SEM of 5 rats per group.

3.2. Euglycemic-hyperinsulinemic clamp

Fig. 1 shows plasma glucose levels (A) and GINFR (B) in Zucker fatty rats (control or atrasentan treated) during the euglycemic-hyperinsulinemic clamp (4 mU/kg per minute). There was no significant difference in glucose levels between the fatty control and atrasentan-treated groups after the overnight fast prior to clamp. Glucose levels (~120-130 mg/dL) were maintained throughout the entire experimental period in both groups (Fig. 1A). Illustrated in Fig. 1B is the GINFR for the 150-minute experimental period. The GINFR was significantly higher in the atrasentan-treated rats compared with the fatty control group and reached a steady state by 60 to 70 minutes (Fig. 1B; $P < .05$, 15 and 20 minutes; $P < .01$, 25-150 minutes; fatty atrasentan vs fatty control; n = 10 rats/group).

3.3. Blood pressure

Zucker fatty rats (fatty control) showed a significantly elevated mean arterial pressure (MAP; 122.1 ± 2.4 mm Hg) compared with their lean littermates (108.5 ± 1.2 mm Hg) ($P < .05$). Six weeks of treatment with atrasentan normalized MAP in fatty rats (115.4 ± 3.3 mm Hg, fatty atrasentan).

3.4. Insulin signaling

Despite no significant difference in IRS-1 protein expression in the liver of Zucker fatty rats (fatty control group) compared with their lean littermates (Fig. 2A), insulin-stimulated IRS-1 tyrosine phosphorylation showed a 30% decrease ($P < .05$) in the liver of Zucker fatty rats (fatty control group) compared with their lean littermates (Fig. 3A). This decrease in insulin-stimulated IRS-1 tyrosine phosphorylation in the liver of Zucker fatty rats was improved by 2.2-fold with atrasentan treatment (fatty atrasentan group, Fig. 3A) ($P < .05$). It is noteworthy that basal IRS-1 tyrosine phosphorylation was not significantly different among groups. On the other hand, there was no significant difference in IRS-1 protein expression as well as in basal or insulin-stimulated IRS-1 tyrosine phosphorylation with atrasentan treatment in muscle tissue (Figs. 2B and 3B). Finally, there was no difference in liver or skeletal muscle IR protein expression and in basal or insulin-stimulated IR tyrosine phosphorylation between lean and fatty rats and no effect by atrasentan treatment on IR protein expression or in basal or insulin-stimulated IR tyrosine phosphorylation as compared with the control group (data not shown).

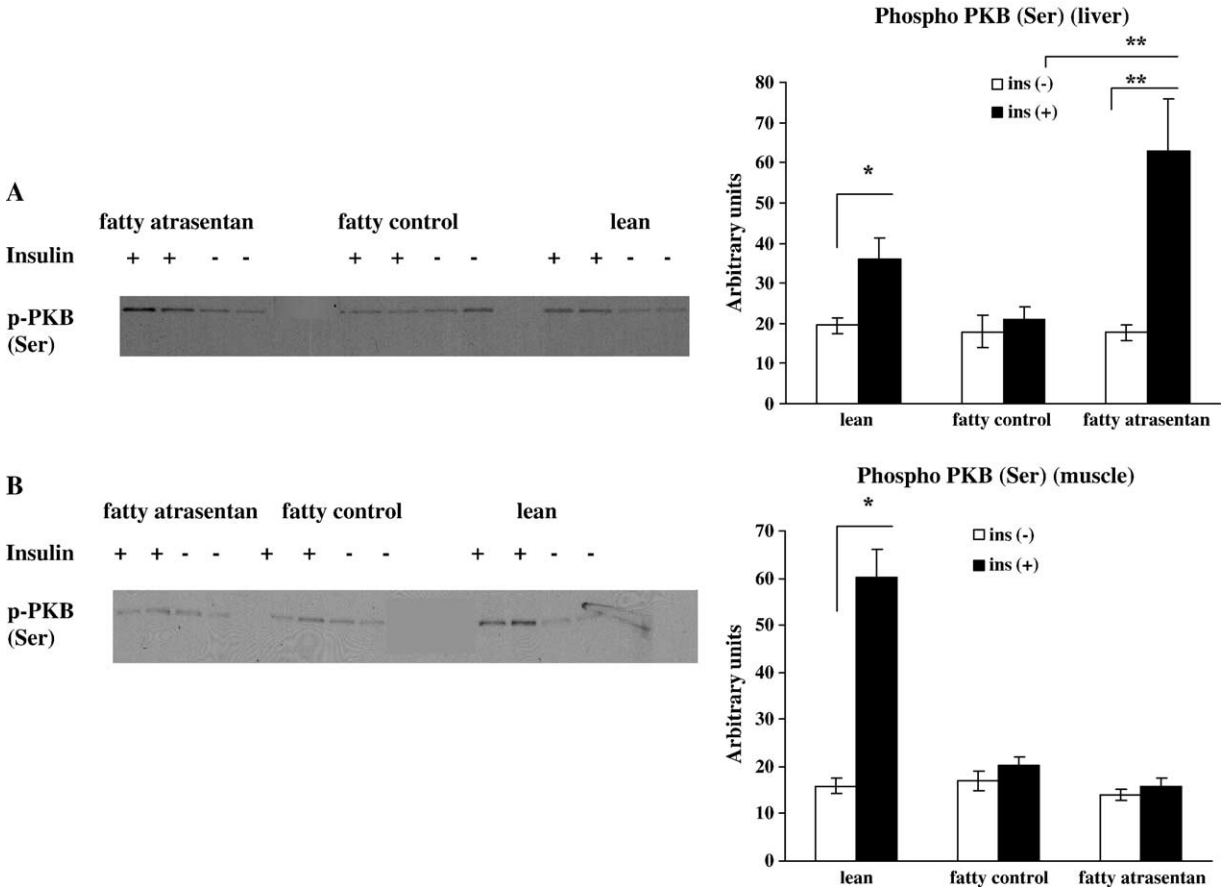

Fig. 6. Western blots of basal and insulin-stimulated PKB serine phosphorylation (Ser 473) in the liver (A) and skeletal muscle (B) of Zucker lean and fatty rats with and without atrasentan treatment. Experiments were repeated twice on different sets of extracts. Data are the mean±SEM of 5 rats per group. *$P < .05$; **$P < .01$.

Fig. 4 illustrates p85α protein levels in the liver and muscle of Zucker lean and fatty rats with and without atrasentan treatment. There was a 5.2-fold increase in p85α protein abundance in the liver of fatty animals compared with their lean counterparts ($P < .05$, lean vs fatty control) (Fig. 4A). This increase was normalized to lean levels with atrasentan treatment ($P < .05$, fatty atrasentan vs fatty control). It is noteworthy that there was no significant difference in p85α protein abundance between lean and fatty animals and no effect with atrasentan treatment in muscle tissue (Fig. 4B).

Protein kinase B protein expression in the liver and muscle was not significantly changed between lean and fatty rats and no effect was observed with atrasentan treatment (Fig. 5A and B). However, insulin-stimulated PKB serine phosphorylation was significantly impaired in fatty animals compared with their lean counterparts ($P < .05$; Fig. 6A and B). On the other hand, insulin-stimulated PKB serine phosphorylation was improved by 3.5-fold in the liver ($P < .01$) (Fig. 6A) but not in the muscle (Fig. 6B) of atrasentan-treated fatty rats compared with the control group. Basal PKB serine phosphorylation was not significantly different among groups (Fig. 6A and B).

4. Discussion

We previously demonstrated that as insulin resistance and glucose intolerance progress in Zucker fatty rats and as they are submitted to meal challenge conditions, plasma ET-1 levels are elevated compared with those in their lean littermates [6]. In addition, basal ET-1 levels appear to be slightly but significantly elevated in Zucker fatty rats compared with their lean littermates (see Table 2). Consistent with other ET receptor antagonists, atrasentan treatment further increases ET-1 levels in Zucker fatty rats (Table 2) [20]. However, this has no detrimental effect on cardiovascular parameters. We observed that atrasentan-treated Zucker fatty rats have significantly reduced MAPs compared with the fatty control group, which were normalized to the level of lean littermate control subjects. This provides evidence that the ET system could have detrimental effects on glucose homeostasis and that ET antagonism might be beneficial in insulin resistance and/or diabetes.

In the present study, we demonstrated that long-term treatment with the ET antagonist atrasentan improves whole body glucose metabolism in the insulin-resistant Zucker fatty rat as determined during the euglycemic-hyperinsulinemic clamp. These results are consistent with a recent publication by da Silva et al [21] using a high fat–fed rat model of obesity and hypertension demonstrating that atrasentan treatment improves the glucose-insulin index (ie, $HOMA_{IR}$) of both the normal and high fat–fed rats.

Atrasentan is an orally active ET_A-selective antagonist with a 1000-fold greater potency on ET_A than on ET_B receptors [19]. We have previously observed that long-term treatment with atrasentan significantly reduces hyperinsulinemia and improves glucose tolerance during a complete nutrient meal challenge in the same model [18]. In the present investigation, basal insulin levels were reduced by 50% after the 6-week treatment period with no change in glucose levels, further demonstrating an increase in insulin sensitivity. Taken together, these results indicate an improvement in glucose tolerance and insulin sensitivity. In the present study, in addition to the euglycemic-hyperinsulinemic clamp results, we investigated how insulin signaling was affected by atrasentan treatment to demonstrate possible cellular mechanisms mediating the beneficial effects observed with this antagonist.

One interesting finding of the present study is that we observed a significant increase in the GINFR in atrasentan-treated Zucker fatty rats compared with their fatty control counterparts. This indicates an improvement in glucose tolerance and insulin sensitivity and is consistent with another study showing that ET-1 treatment leads to skeletal muscle insulin resistance in WKY rats, which was improved by ET antagonism [22]. We did not observe any significant difference in the liver or muscle IR protein levels or in insulin-stimulated IR tyrosine phosphorylation between lean and fatty rats and no effect by atrasentan. The lack of change in IR protein expression between lean and fatty animals is consistent with previous work [23]. On the other hand, we demonstrated a significant decrease in insulin-stimulated IRS-1 tyrosine and PKB serine phosphorylation despite no difference in IRS-1 and PKB protein levels in the liver and muscle of fatty animals compared with their lean littermates, which is consistent with other investigations [23-26]. Interestingly, IRS-1 tyrosine and PKB serine phosphorylation were significantly improved with atrasentan treatment in the liver but not in the skeletal muscle. This improvement in insulin signaling in liver through IRS-1 and PKB suggests an improvement in insulin sensitivity in these insulin-resistant animals.

Previous studies have demonstrated that ET-1 has detrimental effects on insulin signaling in 3T3L1 adipocytes [27-30]. Long-term treatment with ET-1 has been shown to decrease insulin-stimulated IRS-1 tyrosine phosphorylation, PI-3 kinase activity, and GLUT 4 translocation in vitro [28]. It was also determined that these effects are reversed with ET antagonism in vitro [28].

Interestingly, we observed a significant increase in p85α protein levels in the liver but not in the muscle tissue of control fatty rats compared with their lean counterparts, which was normalized with atrasentan treatment. This increase in p85α protein expression in the liver between lean and fatty control rats is not consistent with a previous investigation that reported no significant difference in liver p85α protein abundance in fatty rats compared with their lean littermates [24]. The reason for this discrepancy is not clear. However, the ages of the animals used in this previous study (7-8 weeks) were different compared with those of the animals used in the present study (12-13 weeks) and could account for the difference in results because it is well known

that insulin resistance and glucose intolerance in the Zucker fatty rat model progress over time [6,31,32]. Nevertheless, the normalization in p85α protein levels observed with atrasentan treatment suggests an improvement in insulin sensitivity in the Zucker fatty rat, which is consistent with a recent report showing that reduced expression of the p85α subunit of PI-3 kinase improves insulin signaling and ameliorates diabetes in diabetic mice [33].

Our results suggest that the main target of atrasentan-mediated ET_A blockade on insulin signaling is the liver because no significant difference was shown in the skeletal muscle. It is possible that atrasentan may invoke its beneficial effects by reducing the increased hepatic glucose output in the Zucker fatty rat. To this end, we specifically performed the in vivo clamp experiments at a moderate insulin infusion rate in this severely insulin-resistant rat (4 mU/kg per minute) to permit continued hepatic glucose production to remain a modifiable signal of the whole body glucose fluxes. Our results are supported by the finding that in ET_A-blocked isolated perfused rat liver, the increased hepatic glucose production caused by ET-1 infusion was attenuated [17]. In addition, our results demonstrate that atrasentan mediates its actions downstream of the insulin receptor because no effect has been observed on IR protein levels or on IR tyrosine phosphorylation. Furthermore, significant effects were observed downstream of this effector, including the increased insulin-stimulated IRS-1 and PKB activation.

In conclusion, long-standing ET antagonism improves insulin sensitivity as demonstrated by improved whole body glucose metabolism in the insulin-resistant Zucker fatty rat. We believe that whole body glucose fluxes are improved with ET antagonism and our insulin signaling results suggest that this is hepatically driven. Future experiments using radioactive tracers to determine glucose fluxes and quantify hepatic glucose production are necessary to confirm or refute this hypothesis. Furthermore, we believe that this is not exclusive to the Zucker fatty rat model and that ET antagonism might positively impact whole body glucose metabolism in other models. In fact, a recent investigation [21] using a high fat–fed rat model of obesity and hypertension demonstrated that atrasentan treatment improves the glucose-insulin index (ie, $HOMA_{IR}$) of both normal and high fat–fed rats. In addition, the beneficial effects of ET antagonism may be mediated by increased hepatic insulin signaling through IRS-1, p85α, and/or PKB, possibly leading to a reduction in hepatic glucose production. It can be postulated that ET antagonism might reduce or even reverse ET's detrimental effects on insulin resistance and possibly have beneficial effects in the treatment of diabetes and/or insulin resistance. Further investigation in other insulin resistance and/or diabetes models is necessary to confirm or refute this hypothesis.

Acknowledgments

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