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Early acarbose treatment ameliorates resistance of insulin-regulated GLUT4 trafficking in obese Zucker rats

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Abstract

Genetically (*fa/fa*) obese Zucker rats represent an established model of impaired glucose tolerance, with profound insulin resistance. Acarbose, an inhibitor of α-glucosidases, attenuates postprandial blood glucose peaks, and improves glucose tolerance in these animals. In the present study, we have tested the hypothesis that the effect of acarbose is associated with improved glucose transporter isoform 4 (GLUT4) trafficking in muscle tissue. Acarbose was administered to Zucker rats as a dietary admix (40 mg/100 g diet) for 12 weeks starting at the age of 6 weeks. Serum insulin and leptin were reduced by acarbose from 44 to 19 and 144 to 62 ng/ml, respectively. Glucose tolerance test was performed by i.v. injection of glucose (1 g/kg) and determination of serum glucose up to 60 min. Marked impaired glucose tolerance was observed in obese animals with a profound correction of this defect in acarbose-treated rats. Insulin-regulated translocation of GLUT4 to the plasma membrane in soleus muscle was increased twofold in lean animals, with a totally blunted response in obese rats. Acarbose feeding restored a 1.6-fold effect of insulin on GLUT4 translocation. The exocytotic GLUT4 storage pool in cardiac muscle was completely insulin-insensitive in obese animals, with a largely improved response after acarbose feeding. Activation of Akt, an insulin signaling event upstream of GLUT4, was completely normalized in acarbose-treated rats. In conclusion, we show here that early application of acarbose to obese Zucker rats can prevent the development of impaired glucose tolerance and obesity-associated insulin resistance at the level of the muscle cell, as reflected by an amelioration of defective GLUT4 trafficking in both cardiac and skeletal muscles.

Keywords: Zucker rat; Acarbose; GLUT4 trafficking; Insulin resistance

1. Introduction

The majority of patients with type 2 diabetes are obese, and it is generally accepted that obesity represents one of the major risk factors in the pathogenesis of type 2 diabetes (Spiegelman and Flier, 2001; Kahn and Flier, 2000). One of the most prominent metabolic perturbations associated with the obesity syndrome consists of a pronounced insulin resistance of insulin-stimulated glucose uptake into skeletal (Sherman et al., 1988) and cardiac muscles (Uphues et al., 1995; Kolter et al., 1997). Peripheral insulin resistance causes hyperinsulinaemia and impaired glucose tolerance, and predisposes obese subjects for the development of cardiovascular complications and type 2 diabetes (Reaven, 1988; DeFronzo et al., 1992). Recent evidence supports the notion that a variety of circulating factors secreted by adipocytes play a pivotal role for the induction of insulin resistance (Kahn and Flier, 2000). These factors include free fatty acids, angiotensin, cytokines and many others, and are thought to modulate the insulin signaling pathway in a complex and potentially synergistic fashion (Kahn and Flier, 2000).

Early therapeutic intervention in obesity may help to prevent or delay the progression from impaired glucose tolerance to overt type 2 diabetes. Acarbose is a competitive inhibitor of α-glucosidases in the brush border of the small intestine that delays glucose absorption and decreases the postprandial rise in glucose and insulin (Jenkins et al., 1981). In a recent study, Chiasson et al. (1996) reported that in obese subjects with impaired glucose tolerance, acarbose treatment improves insulin sensitivity. Similar results were also obtained in obese elderly type 2 diabetic patients (Meneilly et al., 2000). However, controversial data were reported in poorly controlled type 2 diabetic patients (Reaven et al.,

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1990; Jenney et al., 1993; Schnack et al., 1989). Furthermore, despite decreasing the weight gain and reducing the rise in glycaemia and insulinaemia, acarbose was not able to improve muscle insulin resistance in obese mice (Le March-and-Brustel et al., 1990). Thus, the potential effects of acarbose on insulin resistance have remained elusive.

In the present investigation, we have reassessed the effect of early acarbose treatment on insulin action and insulin signaling in cardiac and skeletal muscles using genetically obese (*fa/fa*) Zucker rats. These animals represent a well-characterized animal model of insulin resistance associated with extreme obesity, hyperinsulinaemia and impaired glucose tolerance (Uphues et al., 1995), and early treatment with acarbose during the onset of obesity was used to evaluate the potential improvement of insulin sensitivity. This was addressed by (i) measuring glucose transporter isoform 4 (GLUT4) translocation in skeletal muscle, (ii) monitoring different GLUT4 pools and GLUT4 trafficking in cardiac muscle, and (iii) assessing insulin sensitivity and insulin signaling in cardiomyocytes and cardiac tissue. The data show that acarbose treatment improves the metabolic performance of obese Zucker rats and restores near normal insulin action in muscle cells.

2. Materials and methods

2.1. Chemicals

3-*O*-[^{14}C]-Methyl-D-glucose (57 Ci/mmol) and L-[1-^{14}C]-glucose (58 Ci/mmol) was purchased from Amersham (Braunschweig, Germany). Reagents for sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) were supplied by Pharmacia and Sigma (Deisenhofen, Germany). Collagenase was a product of Serva (Heidelberg, Germany). Insulin (Actrapid HM, 100 U/ml) was supplied by Novo (Germany). The polyclonal GLUT4 antiserum was obtained from a rabbit injected with a peptide corresponding to the C-terminal 12 amino acids of GLUT4 (TELEYLGP-DEND) coupled to keyhole limpet hemocyanin (Eurogentec, Belgium). Horseradish peroxidase conjugate (anti-rabbit immunoglobulin G (IgG)) as the secondary antibody for enhanced chemiluminescence (ECL) was purchased from Promega (Mannheim, Germany). Rabbit polyclonal antibodies recognizing phospho-Thr^{308} of Akt and anti-Akt antibodies were obtained from New England Biolabs (Beverly, MA, USA). Monoclonal anti-transferrin receptor antibody was purchased from Zymed (San Francisco, CA, USA).

2.2. Animals

Male lean (*Fa/?*) and obese (*fa/fa*) Zucker rats were provided at the age of 5 weeks by IFFA Credo (L'Arbresle, France). Groups of animals had free access to food and water and were fed either regular laboratory chow (Altromin R. containing 10% sucrose, Lage, Germany), or chow containing 40 mg acarbose per 100 g of chow. Animals were submitted to a 12-h light/12-h dark cycle. Body weight was determined 2–3 times per week. At the end of the treatment period, the animals were killed and heart and soleus muscle was removed. Blood samples were collected for determinations of glucose, insulin and leptin as outlined previously (Eckel et al., 1985; Rizk et al., 1998). For intravenous glucose tolerance test, animals were fasted for 22 h. Rats were anesthetized and tail vein blood was collected for determination of basal glucose and insulin. After i.v. injection of glucose (1 g glucose/kg body wt.), blood was again collected from the tail vein after 5, 10, 30 and 60 min for determination of glucose and insulin. For the insulin-stimulated studies, rats received a tail vein injection of regular insulin (4 U/100 g), and hearts and skeletal muscles were removed 20 min later (Uphues et al., 1995).

2.3. Fractionation of cardiac and skeletal muscles

Crude membrane fractions from cardiac muscle of lean and obese Zucker rats were prepared as previously described by us (Uphues et al., 1995; Kolter et al., 1992). Briefly, ventricular tissue was removed and homogenized in a buffer containing 10 mM Tris–HCl, 0.1 mM phenylmethylsulfonyl fluoride and 2.6 mM dithiothreitol by using an Ultra-Turrax (Ika, Neu-Isenburg, Germany) for 60 s. Homogenization was continued by 10 strokes in a glass-Teflon homogenizer, followed by 3×3 strokes in a tight fitting Potter–Elvehjem homogenizer. After centrifugation at $3000 \times g$ for 10 min, the supernatant was centrifuged at $200{,}000 \times g$ for 90 min to pellet the crude membrane fraction. For fractionation of crude membranes in a 10–35% (w/v) continuous sucrose-density gradient, the crude membranes (10 mg of protein) were suspended in the above buffer, loaded on the gradient and centrifuged for 16 h in a SW-60 rotor at $40{,}000 \times g$. Fractions of 200 μl were collected from the top of the tube to the bottom and analyzed.

For fractionation of skeletal muscle, about 1 g of soleus muscle was homogenized in a buffer containing 20 mM Tris–HCl (pH. 7.4), 250 mM sucrose and protease inhibitors. The homogenate was centrifuged at $12{,}000 \times g$ and the supernatant was collected and kept on ice. The pellet was resuspended in the above-mentioned buffer and centrifuged again. The pooled supernatants were then centrifuged in a Ti 50 rotor at 40,000 rpm for 2 h to yield the crude membrane fraction. Crude membranes were resuspended in Tris buffer (20 mM Tris–HCl, pH 7.2, 50 mM $Na_2P_2O_7$, 300 mM KCl, 250 mM sucrose), homogenized and loaded on top of a discontinuous density gradient consisting of 2 ml 23%, 2 ml 26%, 2 ml 29% and 3 ml 35% (w/v) sucrose. After centrifugation for 16 h at 20,000 rpm in a SW 60 rotor, the plasma membrane fraction was harvested from the 23% layer and the interphase 23–26%. Fractions were diluted with Tris–HCl, pH. 7.4, centrifuged at $150{,}000 \times g$, and stored at -70 °C until further use. Protein was determined

Table 1
Effect of acarbose feeding on body weight and serum concentrations of glucose, insulin and leptin in lean and obese Zucker rats

	Lean (*Fa/?*)		Obese (*fa/fa*)	
	Control	+ Acarbose	Control	+ Acarbose
Body weight (g)	412 ± 8 (20)	361 ± 13 (20)	559 ± 8	452 ± 9[a] (20)
Glucose (mg/dl)	249.3 ± 21.8 (4)	236.3 ± 5.6 (4)	250.7 ± 17.9 (4)	205.7 ± 13.6 (4)
Insulin (ng/ml)	6.9 ± 2.1 (3)	2.9 ± 0.7[b] (3)	44.0 ± 2.2 (4)	19.5 ± 5.3[c] (4)
Leptin (ng/ml)	4.3 ± 2.1 (3)	1.3 ± 0.1[b] (3)	144.2 ± 2.2 (4)	62.8 ± 14.1[c] (4)

Animals were fed with acarbose (40 mg/100 g) for 3 months. At the end of the feeding period, blood samples were collected and analyzed for glucose, insulin and leptin.

[a] Significantly different from acarbose treated lean rats ($P < 0.01$).

[b] Significantly different from lean controls ($P < 0.05$).

[c] Significantly different from lean and obese controls ($P < 0.05$).

by a modification of the Bio-Rad protein assay with bovine serum albumin as a standard.

2.4. Isolation of cardiomyocytes and determination of 3-O-methylglucose transport

Ca^{2+}-tolerant myocytes were isolated by perfusion of the heart with collagenase as previously described by us (Eckel et al., 1983; Russ and Eckel, 1995). The final cell suspension was washed three times with HEPES buffer (composition: NaCl 130 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, HEPES 25 mM, glucose 5 mM, bovine serum albumin 20 g/l, pH 7.4, equilibrated with oxygen) and incubated in silicone-treated Erlenmeyer flasks in a rotating water bath shaker at 37 °C. After 20 min, $CaCl_2$ and $MgSO_4$ (final concentration 1 mM) were added and incubation was continued for at least 60 min until further use. Cell viability was checked by determination of the percentage of rod-shaped cells and averaged 90–95% under all incubation conditions.

Determination of 3-*O*-methylglucose transport was performed at 37 °C in HEPES buffer containing $MgCl_2$ (1 mM) and $CaCl_2$ (1 mM). The reaction was started by pipetting a 50-μl aliquot of the cell suspension to 50 μl of HEPES buffer containing 3-*O*-[^{14}C]methyl-D-glucose (final concentration 100 μM). Carrier-mediated glucose transport was then determined using a 10-s assay period and L-[^{14}C]glucose in order to correct for simple diffusion as described in earlier reports from this laboratory (Kolter et al., 1997; Bähr et al., 1996).

2.5. Immunoblotting

Proteins were separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) using Excel 8–18% gels (Pharmacia, Freiburg, Germany) or 9% and 12% polyacrylamide gels, as described by Laemmli (1970) and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore) in a semidry blotting apparatus. The membrane was blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween 20 for 1 h at room temperature and incubated with different antibodies overnight at 4 °C afterwards. After washing the membrane with PBS containing 0.05% Tween 20, detection was carried out by incubating the membrane with a horseradish peroxidase-conjugated goat anti-rabbit antiserum as a secondary antibody. After several washing steps, the membrane was developed by enhanced chemiluminescence (ECL) using SuperSignal® Substrate (Pierce, Rockford, USA), then visualized and evaluated on a LUMI Imager (Boehringer, Mannheim, Germany). Significance of reported differences was evaluated by using the null hypothesis and *t*-statistics for unpaired data.

3. Results

3.1. Effect of acarbose treatment on glucose tolerance in obese Zucker rats

As reported in earlier investigations (Le Marchand-Brustel et al., 1990; Krause et al., 1982; Vasselli et al., 1987; Michel et al., 1997), administration of acarbose at 40 mg/100 g food to obese Zucker rats resulted in a reduced body weight gain that was detectable in the first month of the feeding period. We did not observe any difference in food intake between obese and acarbose-treated obese Zucker rats. Further, the body weight of acarbose-treated obese Zucker rats at the end of the feeding period was significantly higher when compared to lean controls (452 ± 9 versus 361 ± 12 g, $n = 20$). As presented in Table 1, serum glucose concentrations remained unaffected by acarbose in both lean and obese Zucker rats. Acarbose treatment of obese rats resulted in a marked reduction of both insulin and leptin; however, the serum concentrations of both peptides remained significantly elevated when compared to lean controls (Table 1).

In order to test the potential effect of acarbose on insulin responsiveness, animals were stimulated with insulin by tail vein injection (4 U/100 g) and blood glucose was determined 20 min later. In lean animals, insulin produced a marked decrease of the serum glucose level that remained unaffected by acarbose treatment (Fig. 1). Obese animals are completely resistant to this effect of insulin. Acarbose treatment partially restored the insulin response in obese rats (64% of lean rats) with an incremental decrease of

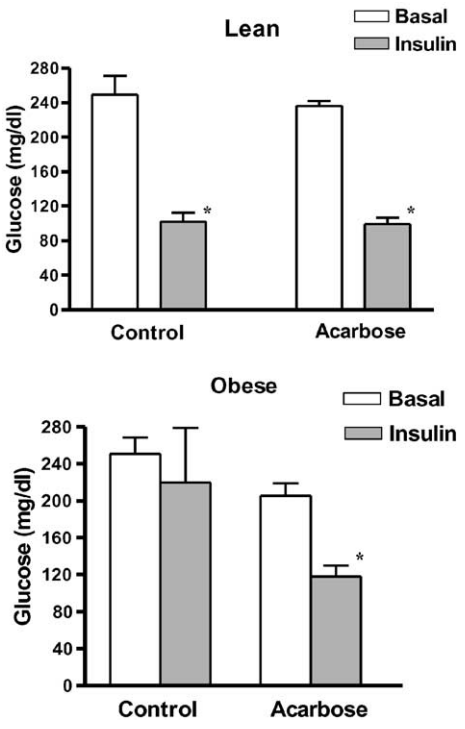

Fig. 1. Effect of insulin on blood glucose levels in lean and obese Zucker rats treated with acarbose. Animals were fed with acarbose for a period of 3 months. Insulin was applied by tail vein injection (4 U/100 g) and blood samples were collected for the determination of glucose, as detailed in Materials and methods. Data are mean values ± S.E.M. of 3–4 individual animals. * Significantly different at $P < 0.05$.

serum glucose by 87.7 ± 7.0 mg/dl compared to 137.3 ± 8.2 mg/dl in lean rats (Fig. 1). Improvement of the metabolic performance of acarbose-treated Zucker rats was then confirmed by intravenous glucose tolerance test. As presented in Fig. 2 (upper panel), the glycaemic response of acarbose-treated Zucker rats was significantly improved at early time points reaching basal glucose levels within 60 min. At that time point, the serum insulin level returned to basal in the acarbose-treated animals but remained elevated in untreated obese control rats (Fig. 2, lower panel).

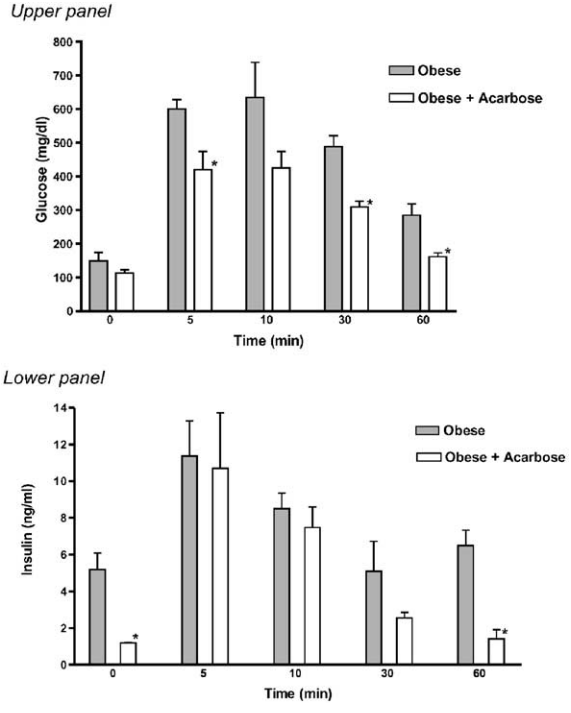

Fig. 2. Intravenous glucose tolerance test in acarbose-treated obese Zucker rats. Animals were fasted for 22 h and after i.v. injection of glucose (1 g glucose/kg body wt.) blood was collected from the tail vein at the indicated times. Glucose (upper panel) and insulin (lower panel) was then determined as detailed in Materials and methods. Data are mean values of 3–4 individual animals. * Significantly different at $P < 0.05$.

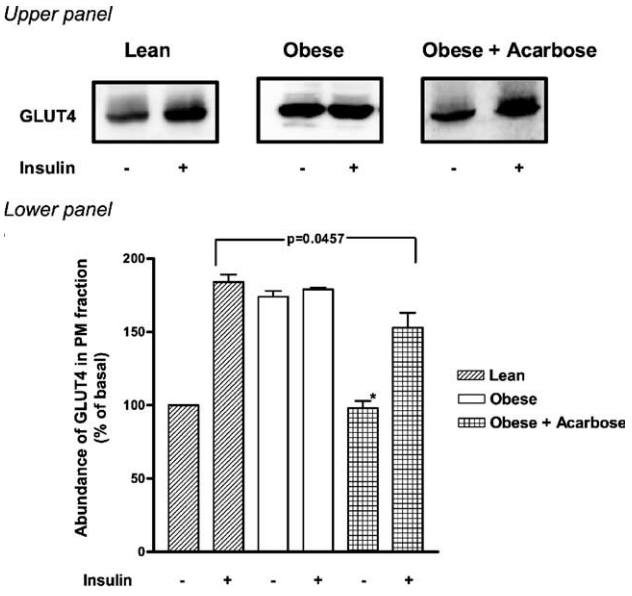

Fig. 3. Effect of acarbose on GLUT4 translocation in soleus muscle of obese Zucker rats. Animals were fed with acarbose as described in Fig. 1 and stimulated with insulin for 20 min. Soleus muscle was then excised and plasma membrane (PM) fractions were obtained as outlined in Materials and methods. (Upper panel) Plasma membranes (20 μg per lane) were subjected to SDS/PAGE and immunoblotted with antiserum against GLUT4, as detailed in Materials and methods. Bands were detected by ECL system and LUMI Imager analyser. (Lower panel) Quantification of Western blots was done by using LUMI Imager software, where the amount of basal was assigned to 100%. Data are mean values ± S.E.M. of four separate experiments. * Significantly different from both basal and insulin-stimulated obese values ($P < 0.05$).

3.2. Effect of acarbose treatment on GLUT4 trafficking in skeletal and cardiac muscles

Improved glucose tolerance and insulin sensitivity in acarbose-treated obese Zucker rats may result from prevention of muscle insulin resistance, increased suppression of hepatic glucose output and/or enhanced β-cell responsiveness. It is well established that muscle insulin resistance in obese Zucker rats reflects both defective insulin signaling and altered GLUT4 trafficking (Uphues et al., 1995; Kolter et al., 1997; Eckel et al., 2000; Kessler et al., 2001). In order to assess if acarbose targets GLUT4 translocation, we

initially determined this process in skeletal muscle of obese and acarbose-treated obese rats. After in vivo stimulation with insulin, the soleus muscle was excised, plasma membrane fractions were prepared and analyzed for the presence of GLUT4. As illustrated in the Western blot in Fig. 3 (upper panel), in lean controls, insulin induced an increased abundance of GLUT4 in the plasma membrane with a completely blunted response in obese rats. In theses animals, GLUT4 abundance in the plasma membrane was significantly elevated in the basal state, most likely reflecting the hyperinsulinaemia of obese rats. Acarbose treatment normalized the GLUT4 distribution (Fig. 3). Quantification of the data showed that insulin increases plasma membrane GLUT4 to $184 \pm 5\%$ of basal in lean animals and $156 \pm 10\%$ of basal in acarbose-treated obese animals (Fig. 3). Thus, insulin regulated GLUT4 translocation in skeletal muscle was partially (67% of lean) restored due to acarbose feeding. It should be noted that GLUT4 expression

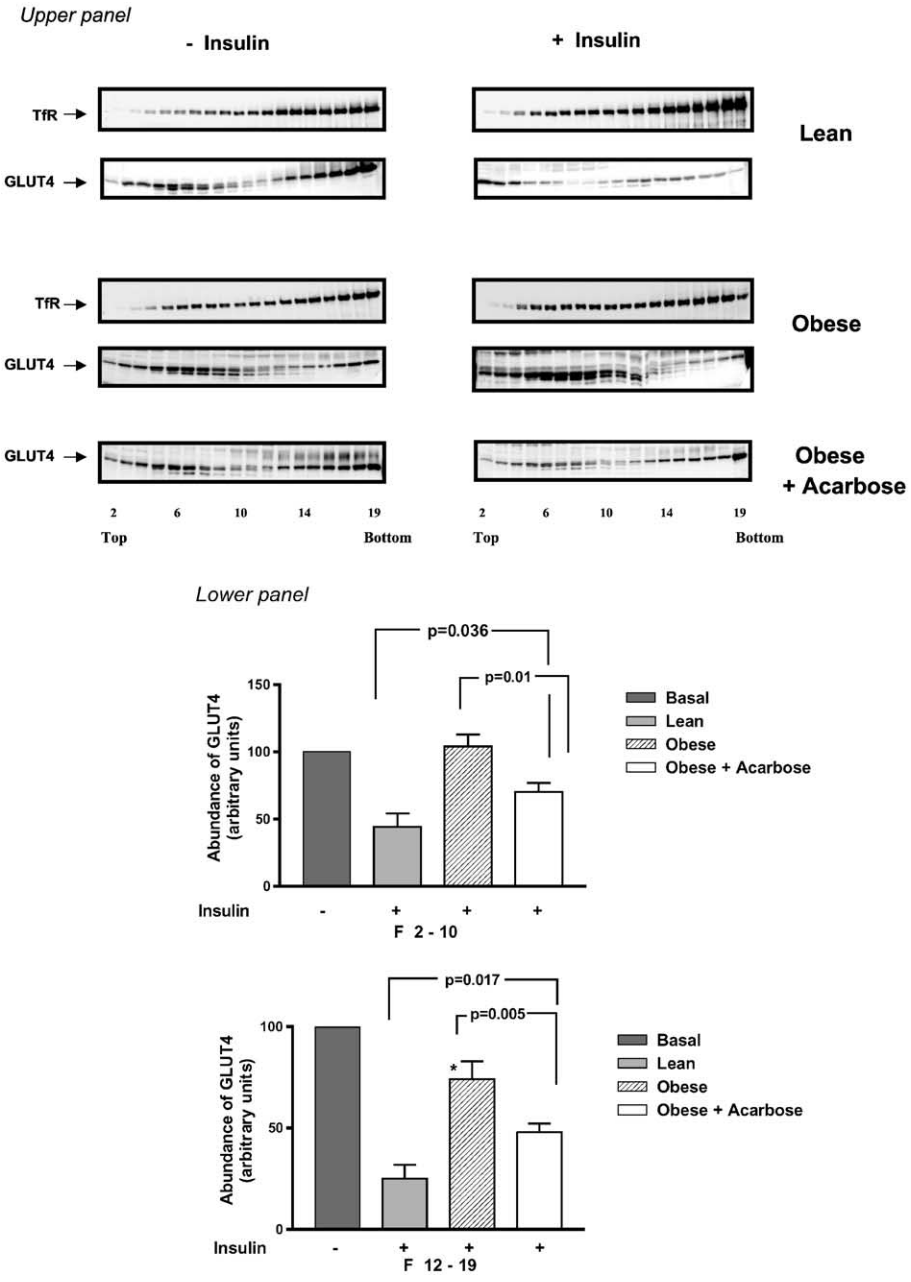

Fig. 4. Fractionation of rat heart crude membranes in a continuous sucrose density gradient. (Upper panel) Crude membranes (10 mg of protein) obtained from lean, obese and acarbose-treated obese Zucker rats were fractionated in a 10–35% continuous sucrose gradient. Fractions (200 μl) were collected and aliquots (30 μl) were subjected to SDS/PAGE, followed by immunoblotting for the transferrin receptor (TfR) and GLUT4. Representative blots out of four separate experiments are shown. (Lower panel) Quantification of Western blots was done by LUMI Imager software, where the amount of the corresponding basal value was assigned to 100%. Signal intensities in fractions 2–10 and 12–19 were individually quantified and subsequently pooled. Data are mean values ± S.E.M. of four separate experiments. * Significantly different from basal ($P < 0.05$).

is not altered in muscle of obese Zucker rats (Uphues et al., 1995) and it was not affected by acarbose (data not shown).

To further characterize the defective recruitment of GLUT4 and the effect of acarbose, we fractionated crude membranes from basal and insulin-stimulated cardiac muscle in a continuous sucrose density gradient. This fractionation procedure results in the separation of two immunoreactive insulin-sensitive GLUT4 pools: (i) a high-density pool co-sedimenting with the transferrin receptor, a generally accepted marker of the endosomal recycling compartment; (ii) a low-density pool, most likely representing the exocytotic storage pool (Kessler et al., 2000). As presented in Fig. 4, both the storage pool (F 2–10) and the endosomal pool were highly sensitive towards insulin. The same distribution of GLUT4 pools was also observed in cardiac tissue of obese rats (Fig. 4, upper panel). However, the storage pool was completely resistant towards insulin, with a minor but significant effect of insulin on the endosomal GLUT4 pool (Fig. 4, lower panel). Acarbose treatment partially restored the insulin sensitivity of both the storage pool and the endosomal GLUT4 recycling pool (Fig. 4, lower panel).

3.3. Effect of acarbose treatment on insulin sensitivity and insulin signaling

We have recently reported a rightward shift of the dose–response curve of insulin-stimulated glucose transport in isolated cardiomyocytes of obese Zucker rats, most likely resulting from a defect of insulin signaling (Kolter et al., 1997). Therefore, we have isolated cardiomyocytes from acarbose-treated Zucker rats and assessed the dose-dependent stimulation of glucose transport by insulin. As presented in Fig. 5, a completely blunted response of glucose transport was observed at physiological insulin concentrations. This defect was completely prevented by administration of acarbose to obese Zucker rats (Fig. 5).

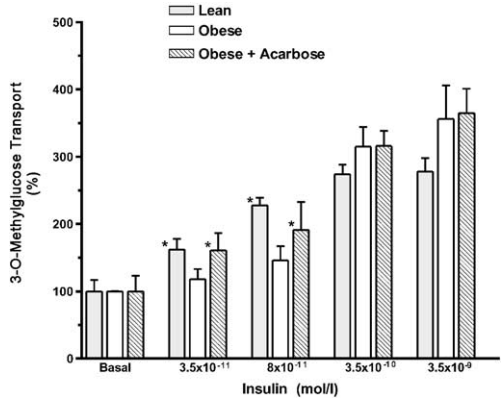

Fig. 5. Dose–response relationship for insulin-stimulated transport of 3-*O*-methylglucose in isolated cardiomyocytes from lean, obese and acarbose-treated obese rats. Animals were fed as described in Fig. 1 and cardiomyocytes were isolated. After preincubation, the cells were incubated with increasing concentrations of insulin (35 pM to 3.5 nM) and sugar transport was monitored as detailed in Materials and methods using a 10-s assay period. Data are mean values ± S.E.M. of three separate experiments. * Significantly different from obese ($P < 0.05$).

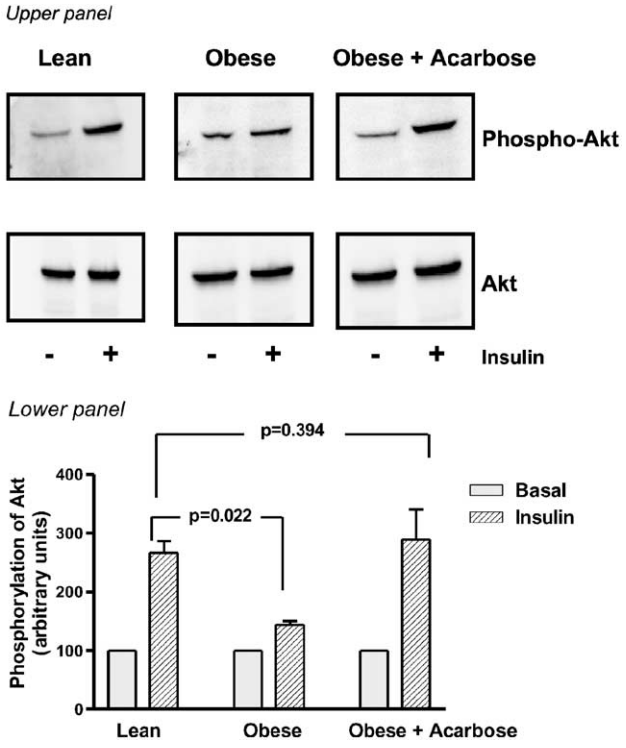

Fig. 6. Insulin-mediated activation of Akt in cardiac muscle of acarbose-treated Zucker rats. After acarbose feeding, the rats were stimulated with insulin for 20 min and the heart was removed. (Upper panel) Lysates of cardiac tissue were resolved by SDS/PAGE and blotted. Phosphorylation of Akt was determined by immunoblotting with phosphospecific antibodies recognizing phospho-Thr^{308} of Akt. Equal loading was confirmed by reprobing the blots with an anti-Akt antiserum. Representative blots out of three separate experiments are shown. (Lower panel) Quantification of Western blots was obtained using LUMI Imager software, where the amount of basal was assigned to 100%. Date are mean values ± S.E.M. of three separate experiments.

To address the question if acarbose may indeed also target the insulin signaling pathway, we studied the activation of Akt/PKB kinase, a Ser/Thr kinase that is thought to play an essential role for the regulation of glucose transport (Wang et al., 1999). For this assay, we used cardiac tissue from control and acarbose-treated animals and a phospho-specific (Thr^{308}) antibody that recognizes the protein only in its phosphorylated (active) state. As presented in Fig. 6, the phosphorylation of Akt was 2–3-fold stimulated in response to insulin in lean rats with a much smaller response in obese animals. Acarbose completely prevented the decreased activation of this kinase by insulin (Fig. 6).

4. Discussion

Obesity is almost invariably associated with insulin resistance and any reduction of excess body fat contributes to an improvement of insulin sensitivity (Hauner, 1999).

Acarbose diminishes postprandial blood glucose excursions (Jenkins et al., 1981) and results of the U.K. Prospective Diabetes Study show improved glycaemic control over 3 years with a reduced weight gain during the first year (Holman et al., 1999). However, in two earlier studies on overweight subjects with impaired glucose tolerance, acarbose was without effect on body weight (Chiasson et al., 1996; Laube et al., 1998) despite an improvement of insulin sensitivity (Chiasson et al., 1996). We show here a reduced weight gain in acarbose-treated genetically obese Zucker rats fed a diet enriched with 10% sucrose, in agreement with earlier data obtained in obese animal models (Krause et al., 1982; Vasselli et al., 1987; Michel et al., 1997; Vasselli et al., 1983). It has been reported that the reduction in body weight gain can be attributed to a 50% reduction of the total lipids in the carcass of obese Zucker rats (Puls et al., 1980). However, this does not affect adipocyte size, lipoprotein lipase activity and the obese body composition of these animals (Vasselli et al., 1983). Consistently, we report here a 50% reduction of serum leptin concentrations in acarbose-treated obese animals; however, leptin remained still 15-fold elevated when compared to lean controls, reflecting the obese phenotype of these animals.

It may be argued that the reduction in body fat mass and the concomitant reduction of fat cell secretory products (fatty acids, cytokines) leads to the improved glucose tolerance of obese Zucker rats observed in the present study and in earlier investigations (Vasselli et al., 1987; Keup et al., 1982). However, acarbose most likely induces a more complex pattern of action since (i) the reduction in body weight gain in obese mice did not ameliorate muscle insulin resistance (Le Marchand-Brustel et al., 1990), and (ii) the increased insulin sensitivity in obese patients with IGT or obese elderly patients with type 2 diabetes was not accompanied by any change in body weight (Chiasson et al., 1996; Meneilly et al., 2000). Thus, the action of acarbose may also involve the decrease in glucose toxicity (Rossetti et al., 1990), potentially modulating the secretory activity of the adipose cell and the insulin sensitivity of muscle tissue. It has also been suggested that acarbose enhances the intestinal release of glucagon-like peptide 1, an incretin hormone that increases insulin sensitivity (Nauck et al., 1997). Most likely, a combination of these pathways, including the reduction in hyperinsulinaemia, contributes to the prevention of insulin resistance in obese animals and humans by acarbose.

Defective recruitment of the glucose transporter GLUT4 from the intracellular storage site plays a pivotal role for the insulin resistance of obesity in both animals and humans (Kahn, 1992). A major finding of the present investigation consists of the observation that early acarbose administration to obese Zucker rats can partially prevent the development of this abnormality in both cardiac and skeletal muscles. For the first time, we show here that the GLUT4 storage pool of cardiac muscle is completely insulin-resistant in obese rats, whereas GLUT4 transporters from the endosomal pool can still be mobilized in response to insulin. This would fit to the notion (Fletcher and Tavare, 1999) that the two GLUT4 pools are activated by different insulin signaling pathways, which could be differentially affected by the obesity syndrome. Most importantly, acarbose treatment largely prevented the insulin resistance of the GLUT4 storage pool despite significant obesity of these animals, supporting the view that multiple pathways contribute to the metabolic effects of acarbose. Decreased expression of GLUT4 was observed in diabetic Zucker fatty rats and this defect was prevented by application of acarbose to prediabetic rats (Friedman et al., 1991). These observations point to an additional mechanism of acarbose that might be operative to improve insulin sensitivity in type 2 diabetic patients.

We recently reported a largely reduced sensitivity of 3-*O*-methylglucose transport in obese rats at physiological doses of insulin (Kolter et al., 1997). This reduced sensitivity correlated to a marked increase in the serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1) and a completely blunted response of IRS-1-associated phosphatidyl inositol (PI) 3-kinase activity (Kolter et al., 1997). This reduced sensitivity of glucose transport activation in isolated cardiomyocytes was found to be completely prevented by early administration of acarbose to obese Zucker rats. Our data further suggest that defects of the insulin signaling pathway may also be completely prevented by acarbose treatment, at least at the level of the Akt kinase. This enzyme lies downstream of PI 3-kinase and is essentially involved in the regulation of glucose transport (Wang et al., 1999). It has been suggested that Akt signals to the GLUT4 storage pool (Fletcher and Tavare, 1999), being consistent with our observation that acarbose significantly restores the insulin sensitivity of the GLUT4 storage pool in the heart.

In summary, we show here that early application of acarbose to genetically obese Zucker rats prevents the development of impaired glucose tolerance and insulin resistance in both skeletal and cardiac muscle involving improved GLUT4 trafficking and insulin signaling. Prevention of glucose toxicity, reduced adipose tissue mass and improved metabolic performance of obese animals may contribute to this effect of acarbose.

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