ELSEVIER

Effects of feeding acarbose on muscle glucose transport and GLUT4 protein in lean and obese diabetic (ZDF/Gmi-*fa*) rats

Patricia L. Dolan,* Edward B. Tapscott,† Richard G. Peterson,‡ and G. Lynis Dohm†

**Department of Biology, Pacific Lutheran University, Tacoma, Washington 98447; †Department of Biochemistry, East Carolina University School of Medicine, Greenville, North Carolina 27858; and ‡Department of Anatomy, Indiana University School of Medicine, Indianapolis, Indiana 46202*

*The Zucker diabetic fatty rat (ZDF/Gmi-*fa*), a model for noninsulin-dependent diabetes mellitus (NIDDM), was used to evaluate the action of dietary acarbose (20 week, 40 mg/100 g diet) in improving hyperglycemia. Muscle glucose uptake and glucose transport were measured during hindlimb perfusion. GLUT4 protein was also measured in the perfused muscle. Insulin resistance was not observed in skeletal muscle from diabetic animals compared with lean controls as concluded from equivalent hemicorpus glucose uptake and muscle glucose transport rates. Three fold increases in both GLUT4 protein and glucose transport were observed in acarbose-fed lean rats compared with lean controls. Acarbose feeding significantly decreased hyperglycemia in diabetic rats. This improvement seems not to be the result of an increase in insulin-stimulated glucose transport in muscle as insulin sensitivity and responsiveness were unchanged.* (J. Nutr. Biochem. 8:322–327, 1997) © *Elsevier Science Inc. 1997*

Keywords: acarbose; skeletal muscle; glucose transport

Introduction

The Zucker diabetic fatty rat strain (ZDF/DRT-*fa*, now being distributed as ZDF/Gmi-*fa*) is a recently established genetic model of noninsulin-dependent diabetes mellitus (NIDDM or type II diabetes) that resulted from inbreeding for a diabetic trait periodically expressed in a colony of Zucker rats.[1,2] This partially inbred strain has been named Zucker diabetic fatty (ZDF) to distinguish it from the euglycemic obese Zucker fatty outbred rat strain from which it arose. Although the obese Zucker fatty rat exhibits some abnormalities characteristic of NIDDM, it is a better model for hyperinsulinemic, euglycemic obesity.[3,4] The male ZDF fatty rat genetically develops both obesity and hyperglycemia with a resulting metabolic profile closer to the altered profile associated with NIDDM.[5] These diabetic fatty rats represent a useful research tool to investigate the mechanism causing hyperglycemia in diabetes because the animals are initially euglycemic, become hyperglycemic at 7 to 12 weeks of age, and remain hyperglycemic for the remainder of their lives.[6] Moreover, the obese male ZDF rat demonstrates a more severe state of hyperglycemia than most other obese models of NIDDM and may therefore be an appropriate model for addressing pathophysiologic questions relating to the type II diabetic patient who is extremely hyperglycemic because of insufficient insulin secretion.

Chronic hyperglycemia is hypothesized to be responsible for the central pathophysiologic features of diabetic complications.[7] An American Diabetes Association position statement concerning the results of the Diabetes Control and Complications Trial furnished the first substantial clinical evidence to support the long-held belief that maintaining blood glucose levels close to normal could prevent or delay diabetic complications.[8] For this reason, attention continues

This work was supported by National Institutes of Health Grant DK38416 and a grant from Miles, Inc. West Haven, CT. P.L.D. was supported by a postdoctoral fellowship (F32-DK08823) from the National Institutes of Health.

Address reprint requests to Dr. G. Lynis Dohm at Department of Biochemistry, ECU School of Medicine, Greenville, NC 27858 USA.

Received August 5, 1996; accepted January 31, 1997.

655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(97)00016-8

to focus on strategies aimed at maintaining better glycemic control.

Efforts to minimize the postprandial rise in blood glucose concentration have led to the use of compounds that delay the absorption of glucose from the gastrointestinal tract. Acarbose (BAY G 5421) is a complex oligosaccharide that reversibly inhibits α-glucosidases present in the brush border of the small intestinal mucosa.[9] Inhibition of these enzymes delays the absorption of glucose and therefore results in a smaller rise in blood glucose concentration after a meal containing carbohydrate.

Friedman et al.[10] observed that feeding diabetic-ZDF animals a diet containing acarbose (40 mg/100 g chow) prevented the development of severe hyperglycemia. Protein levels of the insulin-responsive glucose transporter (GLUT4) were decreased in gastrocnemius muscle of untreated diabetic-ZDF animals compared with nondiabetic lean controls. Acarbose-fed diabetic animals maintained GLUT4 protein at levels observed in untreated lean controls. Because skeletal muscle is the primary tissue responsible for disposal of a glucose load,[11] and because GLUT4 content has been shown to correlate with the maximal rate of insulin-stimulated glucose transport,[12] an increase in skeletal muscle GLUT4 protein could account for the acarbose-induced improvement in glucose tolerance in the diabetic-ZDF rats. An evaluation of skeletal muscle insulin action such as measurement of insulin-stimulated glucose transport rate, was not made in the previous study.[10] A study evaluating insulin resistance in skeletal muscle of the ZDF diabetic rat was deemed additionally relevant in light of findings that acarbose feedings were observed to have only a minimal impact on insulin-stimulated glucose transport rate in isolated adipocytes from diabetic-ZDF rats.[13]

In this study, maximally insulin-stimulated glucose transport was measured in hindlimb skeletal muscles of diabetic-ZDF rats that had been fed acarbose for 20 weeks. Glucose transport rates in acarbose-fed diabetic rats were compared to rates in control-fed diabetic rats. Concurrently, acarbose was fed to a group of lean-ZDF rats to assess the effect of acarbose in a nondiabetic animal of the ZDF strain. In addition, nondiabetic lean rats were compared to diabetic rats to assess the contribution of peripheral insulin resistance to the hyperglycemia observed in this model of NIDDM. GLUT4 glucose transporter protein was measured in gastrocnemius muscle to determine whether acarbose-induced changes in transporter protein correlated with changes in skeletal muscle glucose transport rate.

Methods and materials

Animals

Male Zucker diabetic fatty rats (ZDF) (7 weeks of age) and age-matched lean male heterozygotes (+/*fa*, or +/+) were obtained from the Diabetes Animal Support Facility, Department of Anatomy, Indiana University School of Medicine (ZDF/DRT-*fa*) and from the continuation of this colony at Genetic Models, Inc. Indianapolis, IN USA (ZDF/Gmi-*fa*). Animals were housed in pairs in plastic 10-inch × 18-inch shoe box-type rodent containers, maintained on a 12-hr light/dark cycle and provided with food and water ad libitum. The rats were randomly assigned to two dietary groups of 16 animals each (8 ZDF and 8 lean rats per group). The diet consisted of standard lab chow (Purina 5008) with 10% confectioner's sugar incorporated, with or without acarbose supplementation (40 mg/100 g diet). This dose of acarbose was chosen because it was determined previously to be the most effective for reducing postprandial glucose values without entering the pharmacological range.[2,14] The drug was incorporated into the diet because it was reported that uniform distribution of acarbose through a meal was important for optimal effectiveness.[15] Blood was obtained weekly by tail vein collection and analyzed for glucose concentration using a glucose oxidase assay (Sigma kit No. 510-A, St. Louis, MO USA). Animals were weighed just before blood collection from the fourth week of the study onward.

Muscle perfusion

Perfusion of the hemicorpus was performed after an overnight fast when the rats were 28 to 29 weeks of age. The rats were anesthetized intraperitoneally with ketamine (9 mg/100 g body weight) and xylazine (1 mg/100 g body weight). The perfusion system has been described previously.[16] The perfusion medium (4% BSA, 5.5 mM glucose, 33% washed bovine red blood cells, and Krebs–Henseleit buffer) was gassed with O_2/CO_2 (95:5). Flow rate of perfusate was 15 mL/min and chamber temperature was maintained at 35° to 37°C. The first 50 mL of perfusate coming from the animal was discarded and the remaining 100 mL was recirculated. Glucose was infused at a rate sufficient to maintain its concentration in the perfusion medium at 7.5 ± 0.26 mM. This concentration was chosen to match the mean glucose concentration measured in lean animals fed the control diet (7.2 ± 0.17 mM). After the first 45 min of perfusion, medium samples were taken every 5 min to allow calculation of glucose uptake from the medium. During a 30 min "basal" period, no exogenous insulin was added. At the end of the 30 min basal period, insulin was added to the medium at a submaximal concentration (5×10^{-9} M), and once again, glucose uptake from the medium was measured for 30 min by sampling every 5 min. Finally, the concentration of insulin in the medium was raised to 10^{-7} M for the last 30 min of perfusion to assess the effect of a maximal insulin stimulus on hemicorpus glucose uptake. Glucose transport was measured during the last 10 min of the maximal insulin period. Radioactive label was added to the medium at a final concentration of 20 mM sorbitol containing 0.1 μCi/mL [U^{-14}C]sorbitol and 0.2 μCi/mL [^{3}H]2-deoxyglucose (tracer amount). At the end of the perfusion, the soleus, extensor digitorum longus, gastrocnemius, and quadriceps muscles were quickly removed and prepared for measurement of transport rate.

Measurement of glucose uptake and 2-deoxyglucose transport

Medium samples taken at 5 min intervals throughout the perfusion period were assayed for glucose concentration to assess hemicorpus glucose uptake. Uptake was defined as the amount of glucose required to be infused over the 5 min (to maintain a glucose concentration of 7.5 mM) corrected for the change in medium glucose concentration (as measured by the change in medium glucose concentration over the 5 min period).

To determine glucose transport rate, a weighed portion (50 to 100 mg) of each muscle excised from the perfused hindlimb was minced finely and added to 0.5 mL distilled water in a 7 mL scintillation vial. After dispersion of the muscle by sonication (Heat Systems Sonicator, microtip), 5 mL of scintillation fluid (Ecolite+, ICN, Irvine, CA USA) was added to the vial and samples were counted in a Beckman LS 5000TD liquid scintillation counter preset for simultaneous counting of ^{3}H and ^{14}C.

[^{3}H]2-Deoxyglucose and [^{14}C]sorbitol radioactivity (dpm) were counted in perfusate samples taken at 0, 5, and 10 min into

the transport measurement period. Glucose concentration was determined in these same aliquots (Sigma kit) and the glucose values were used to determine specific activity of [^{3}H]2-deoxyglucose. Accumulation of intramuscular [^{3}H]2-deoxyglucose was determined by subtracting extracellular [^{3}H]2-deoxyglucose (determined from the [^{14}C]sorbital space) from total muscle [^{3}H]2-deoxyglucose.

Quantitation of glucose transporter protein

Muscle was frozen in liquid nitrogen for subsequent GLUT4 protein analysis. Briefly, the frozen muscle was pulverized with a steel mortar and pestle (cooled to −60°C) and then homogenized in ice cold buffer (1 mL/40 mg muscle) for two 15-second bursts with an Ultraturrax homogenizer. Homogenization buffer contained 25 mM HEPES, 4 mM EDTA, 25 mM benzamidine, 0.5 mM PMSF, 1 μM leupeptin, 1 μM pepstatin, and 0.15 μM aprotinin, pH 7.4. Homogenates were transferred to microcentrifuge tubes and spun at 16,000 × g for 30 min at 4°C. The pellet was resuspended in 200 μL of buffer containing 1% Triton X-100 and kept on ice for 1.5 hr. The tubes were spun again at 16,000 × g for 30 min at 4°C and the protein content in the supernatants was determined using the bicinchoninic acid method.[17] To minimize any contribution of interassay variability, an equal number of samples from each treatment group were prepared and analyzed in the same assay. To determine muscle GLUT-4 content, duplicate samples of supernatant containing 50 μg protein were dispersed in Laemmli buffer[18] containing 2.5% dithiothreitol and proteins were separated on an 8% polyacrylamide resolving gel. The proteins were then electrotransferred to an Immobilon membrane (Millipore Corp., Bedford, MA USA), blocked overnight at 4°C with 5% nonfat dry milk in tris-buffered saline (TBS-blotto) followed by incubation for 12 hr at 4°C in a protein A-purified polyclonal antibody (4 ug/mL in TBS-blotto) specific for the carboxy-terminal peptide for GLUT4. The membrane was washed in TBS-Tween followed by TBS and then incubated for 1 hr at 25°C in 50 mL TBS-blotto containing horseradish peroxidase conjugated donkey anti-rabbit 1 gG diluted 1:4000 (Amersham Corp., Arlington Heights, IL USA). Antibody-antigen complexes were detected by enhanced chemiluminescence and an exposure obtained on Hyperfilm-ECL film (Amersham). Films were quantified by densitometric scanning performed on a computer-controlled laser densitometer (Ultrascan XL, LKB Pharmacia). Absorbance units from densitometric scanning of multiple films were normalized by inclusion of a rat heart standard in three lanes of each gel.

Statistical analysis

All data are expressed as means ± SE. Phenotype differences and diet effects were tested by a two-way analysis of variance (ANOVA), followed by comparison of means using Tukey's post hoc tests if statistical significance was obtained. P values < 0.05 were considered statistically significant.

Results

In agreement with results of the previous study,[10] plasma glucose concentration was approximately five-fold higher in diabetic than in control animals. Acarbose feeding decreased significantly hyperglycemia in the diabetic animals at all measured time points (*Figure 1*) with glucose levels remaining similar to those of the lean animals during the early portion of the study. At week 13, however, the mean glucose values in the acarbose-fed diabetic rats increased two fold (increase of 7 mM) and remained elevated for the duration of the study. Whereas these glucose concentrations

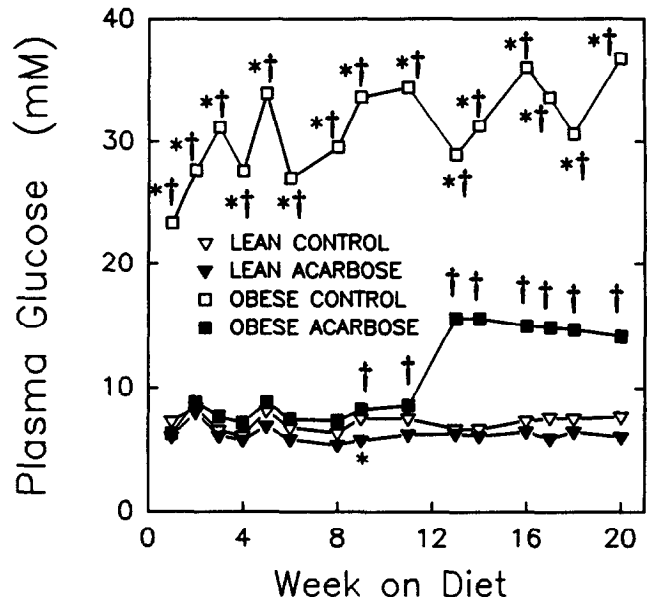

Figure 1 Effect of acarbose feeding (20 weeks) on plasma glucose concentration in nondiabetic (LEAN) and diabetic (OBESE) ZDF rats. Values are means of 8 rats/group. *Significant difference ($P < 0.05$) between acarbose-fed and respective control-fed group. †Significant difference ($P < 0.05$) between LEAN and OBESE of the same dietary regimen.

were still significantly lower than those of untreated diabetics, they were significantly higher than those of lean animals. The plasma glucose concentration in acarbose-fed lean animals was not different from that in control-fed lean rats.

There was a tendency for acarbose feeding to decrease the body weight of nondiabetic lean controls, but this was significant only at weeks 9, 11, and 13 (*Figure 2*). In contrast, from the fifth week of feeding onward, acarbose treatment resulted in a significant increase in body weight of the diabetic animals (*Figure 2*).

Insulin increased ($P < 0.05$) muscle glucose uptake in a dose responsive manner in all four groups (*Figure 3*). There was no significant main effect of phenotype on muscle glucose uptake, but there was a significant interaction of insulin and phenotype such that with maximal insulin stimulation, glucose uptake rate in the control-fed diabetic animals was significantly greater than that of control-fed lean animals. There was also a significant interaction of diet and phenotype at each of the insulin conditions. Acarbose treatment did not alter the glucose uptake response in the diabetic animals, but acarbose-fed lean animals had significantly greater muscle glucose uptake rates than control-fed leans at all three insulin conditions (*Figure 3*).

The interaction of diet and phenotype on maximally insulin-stimulated glucose transport rates in mixed gastrocnemius and red and white gastrocnemius is identical to the diet X phenotype interaction seen with glucose uptake (*Figure 4*). Acarbose treatment significantly increased glucose transport in the lean non-diabetic animals but had no effect on the obese diabetic animals. Similar results were

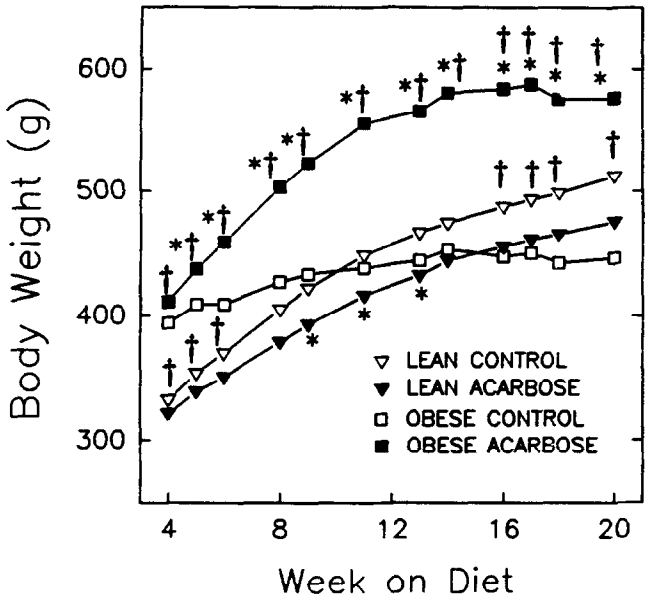

Figure 2 Effect of acarbose feeding (20 weeks) on body weight of nondiabetic (LEAN) and diabetic (OBESE) ZDF rats. Values are means of 8 rats/group. *Significant difference ($P < 0.05$) between acarbose-fed and respective control-fed group. †Significant difference ($P < 0.05$) between LEAN and OBESE of the same dietary regimen.

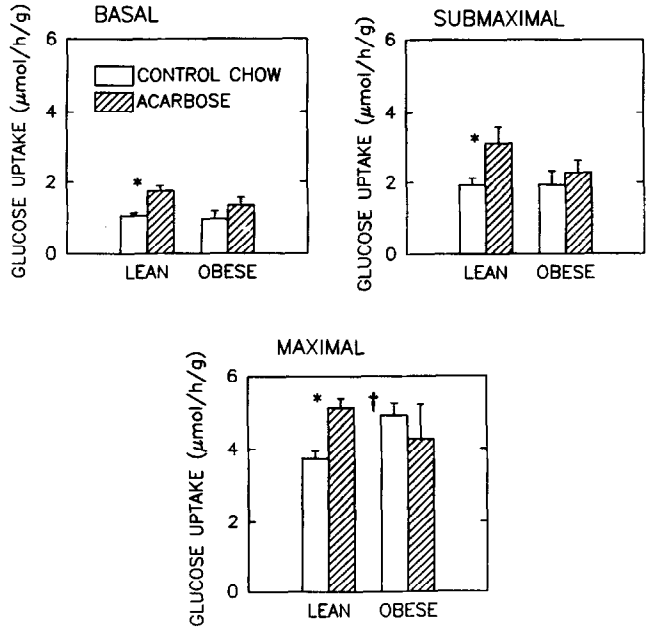

Figure 3 Hemicorpus glucose uptake of nondiabetic (LEAN) and diabetic (OBESE) ZDF rats fed (20 weeks) on control diet or the control diet plus acarbose. Uptake was measured under conditions of basal, submaximal insulin-, and maximal insulin-stimulation. Values are means ± SEM of four to six muscles per group. *Significant difference ($P < 0.05$) between control-fed LEAN and acarbose-fed LEAN groups. †Significant difference ($P < 0.05$) between control-fed LEAN and control-fed OBESE groups.

observed for soleus, EDL, mixed quadriceps, and red and white quadriceps (data not shown).

Acarbose-fed animals of both phenotypes had increased GLUT4 protein in mixed gastrocnemius muscle. The increase in GLUT4 protein in skeletal muscle from diabetic animals, however, was not statistically significant (*Figure 5*). Acarbose treatment restored GLUT4 content in the diabetic animals to the same level observed in the untreated lean animals. Because acarbose feeding also significantly increased the GLUT4 content of the lean animals, however, the glucose transporter content of the acarbose-fed nondiabetic lean rats was still significantly greater than that of the acarbose-treated diabetic animals.

Discussion

Although acarbose feeding dramatically lowered plasma glucose in the diabetic animals as expected, there is no strong evidence in these results that the reduction results from changes in glucose disposal in muscle. This study did, however, produce some interesting observations. Perhaps most striking were the responses of the lean animals to acarbose treatment. Insulin sensitivity in the acarbose treated lean group was markedly improved. This observation is consistent with the large increase in GLUT4 protein and the somewhat lower (though not statistically significant) plasma glucose in the acarbose treated lean animals. Acarbose has been shown to reduce the postprandial rise of blood glucose in healthy men,[9,19] and this reduction in blood glucose concentration was associated with decreased plasma insulin levels.[9] If postprandial plasma glucose and insulin levels are also reduced by acarbose treatment in the rat, insulin sensitivity should be enhanced.

Obesity may be a major effector in these results. Peripheral insulin resistance is observed in most obesity models of glucose intolerance or diabetes and NIDDM patients.[20–22] Reduced insulin sensitivity and responsiveness with obesity is well documented in the rat and in humans.[23,24] The acarbose treated lean animals were slightly smaller than their untreated counterparts and had positive changes in each of the measured parameters, while the treated diabetic animals were significantly heavier than the untreated diabetic animals and exhibited general decreases in glucose transport. The relationship in this study obviously is not simple because insulin responsiveness seems to improve to a greater degree in the leaner animals with a smaller change in body weight.

The mechanism that causes insulin resistance associated with obesity is an area of active investigation. There is a rapidly accumulating body of evidence that serine/threonine phosphorylation of the insulin receptor reduces the activity of the receptor tyrosine kinase.[25,26] Cyclic AMP-activated protein kinase and protein kinase C (PKC) have been implicated in the phosphorylation of the insulin receptor.[27] Recently some PKC isoforms have been observed to be elevated in soleus muscle membrane from the fatty Zucker rat. Additionally muscle membrane diacylglycerols, associated with PKC activation, were observed to be higher in the obese rat.[28] Acarbose treatment of the ZDF rat seems to restore the (fa/fa) Zucker phenotype (except for the hyperinsulinemia), which may include elevated muscle membrane diacylglycerols and associated elevation in membrane PKC.

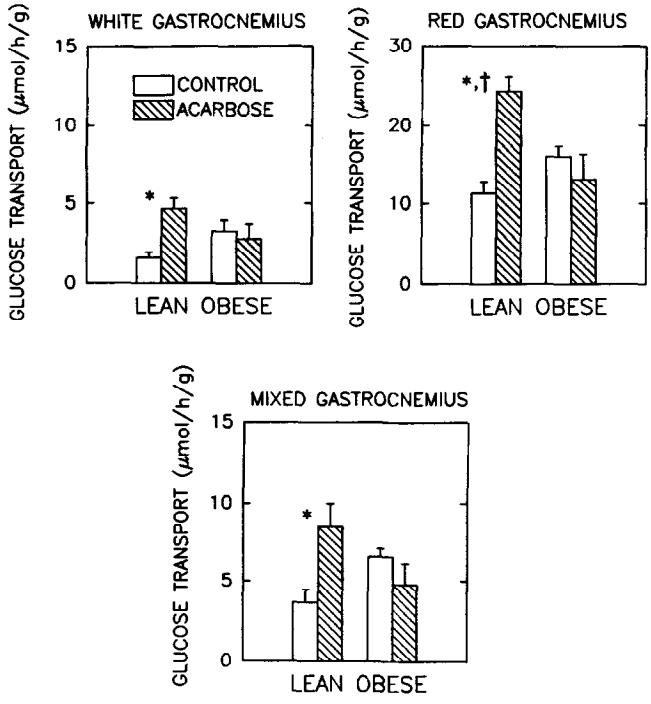

Figure 4 Maximally insulin-stimulated glucose transport rates in white, red, and mixed gastroncnemius muscles. Values are means ± SEM of five muscles per group. *Significant difference ($P < 0.05$) between control-fed LEAN and acarbose-fed LEAN groups. †Significant difference ($P < 0.05$) between acarbose-fed LEAN and acarbose-fed OBESE groups.

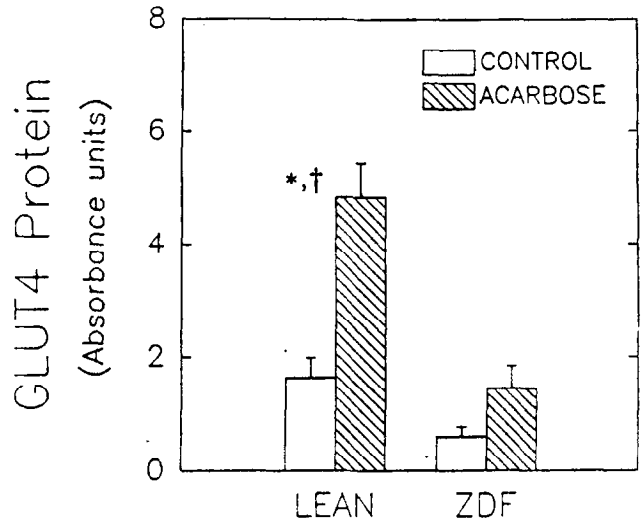

Figure 5 Quantitation (Western blot analysis) of GLUT4 protein in mixed gastrocnemius muscle from nondiabetic (LEAN) and diabetic (OBESE) ZDF rats fed (20 weeks) a control diet or the control diet plus acarbose. Values are means ± SEM of eight muscles per group. Absorbance units from densitometric scanning of multiple films were normalized by inclusion of a rat heart standard in three lanes of each gel. *Significant difference ($P < 0.05$) between control-fed LEAN and acarbose-fed LEAN groups. †Significant difference ($P < 0.05$) between acarbose-fed LEAN and acarbose-fed OBESE groups.

It is still far from clear why acarbose treated diabetic animals gain weight so dramatically. Friedman et al.[10] observed an increase in GLUT4 in gastrocnemius muscle of the acarbose treated animal and we observed a trend in that direction in this study lending support to the argument that the treated animals are better able to use glucose,[29] however, other measurements made in this study indicate that treatment with acarbose does little to change glucose disposal in muscle. Slieker et al.[30] observed that GLUT4 protein was lower by 55% in epididymal adipose from severely diabetic ZDF rats compared fa/fa controls, hinting that there might be major alterations in adipose in the severely diabetic ZDF that might be sensitive to acarbose treatment.

The diabetic animals used in this study did not exhibit the skeletal muscle insulin resistance expected in a model of NIDDM, even though they were severely hyperglycemic. These results are in obvious contrast to other studies where hyperglycemia has been shown to induce insulin resistance[30–34] mainly to decreased glucose transport.[31,32] In another study, where skeletal muscle from animals with chronic hyperglycemia displayed decreased glucose transport and GLUT4 protein content, reduction of hyperglycemia returned glucose transport rates to control values and GLUT4 protein to levels higher than those seen in controls.[35] Acarbose treatment of our diabetic animals greatly reduced plasma glucose, and may have increased muscle GLUT4 levels slightly, yet glucose transport and uptake into perfused muscle were not improved. This suggests that skeletal muscle insulin resistance does not contribute to hyperglycemia in the severely diabetic ZDF rat.

Acknowledgments

We thank Peter J. Dorton for excellent technical assistance.

References

1 Clark, J.B., Palmer, C.J., and Shaw, W.N. (1983). The diabetic Zucker fatty rat. *Proc. Soc. Exp. Biol. Med.* **173,** 68–75

2 Peterson, R.G., Doss, D.J., Neel, M., Little, L.A., Kincaid, J.D., and Eichberg, J. (1993). The effectiveness of acarbose in treating Zucker diabetic fatty rats (ZDF/Drt-*fa*). *Drugs Devel.* **1,** 167–172

3 Zucker, L.M. and Antoniades, H.N. (1972). Insulin and obesity in the Zucker genetically obese rat "fatty". *Endocrinol.* **90,** 1320–1330

4 Kava, R., Greenwood, M.R.C., and Johnson, P.R. (1990). Zucker (fa/fa) rat. *ILAR News.* **32,** 4–8

5 McCaleb, M.L. and Sredy, J. (1992). Metabolic abnormalities of the hyperglycemic obese Zucker rat. *Metabolism* **41,** 522–525

6 Peterson, R.G., Little, L.A., and Neel, M. (1990). Zucker diabetic fatty rat as a model for non-insulin-dependent diabetes. *Ilar News* **32,** 16–19

7 Brownlee, M., Cerami, A., and Vlassara, H. (1988). Advanced glycosylation end products in tissue and the biochemical basic of diabetic complications. *New Eng. J. Med.* **318,** 1315–1321

8 Position Statement (1993) Implications of the diabetes control and complications trial. *Diabetes* **42,** 1555–1558

9 Clissold, S.P. and Edwards, C. (1988). Acarbose. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential. *Drugs* **35,** 214–233

10 Friedman, J.E., De Vente, J.E., Peterson, R.G., and Dohm, G.L. (1991). Altered expression of muscle glucose transporter GLUT-4 in diabetic fatty Zucker rats (ZDF/DRT-*fa*). *Am. J. Physiol.* **261,** E782–E788

11 DeFronzo, R.A., Gunnarsson, R., Bjorkman, O., Olsson, M., and Wahren, J. (1985). Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (Type II) diabetes mellitus. *J. Clin. Invest.* **76,** 149–155

12 Henriksen, E.J., Gourey, R.E., Rodnick, K.J., Korayi, L., Permutt, M.A., and Holloszy, J.O. (1990). Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am. J. Physiol.* **259,** E593–E598

13 Fried, S.K., Maggio, C.A., and Vasselli, J.R. (1993). Effects of acarbose on insulin sensitivity in adipocytes from lean and obese Zucker and Zucker diabetic fatty rats. *Drugs Devel.* **1,** 85–93

14 Koevary, S.B. (1990). Effects of acarbose on the development of diabetes in BB/Wor rats. *Metabolism* **39,** 865–870

15 O'Dea, K. and Turton, J. (1985). Optimum effectiveness of intestinal α-glucosidase inhibitors: importance of uniform distribution through a meal. *Am. J. Clin. Nutr.* **41,** 511–516

16 Dohm, G.L., Kasperek, G.J., Tapscott, E.B., and Beecher, G.B. (1980). Effect of exercise on synthesis and degradation of muscle protein. *Biochem. J.* **188,** 255–262

17 Smith, P.K., Krohn, R.I., Hermanson, G.T., Malli, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150,** 76–85

18 Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of the bateriophage T4. *Nature* **227,** 680–685

19 Andreasson, K., Galuska, D., Thorne, A., Sonnenfeld, T., and Wallberg-Henriksson, H. (1991). Decreased insulin-stimulated 3-0-methylglucose transport in vitro incubated muscle strips from type II diabetic subjects. *Acta. Physiol. Scand.* **142,** 255–260

20 Beck-Nielsen, H., Vaag, A., Damsbo, P., Handberg, A., Nielsen, O.H., Henriksen, J.E., and Thye-Ronn, P. (1992). Insulin resistance in skeletal muscles in patients with NIDDM. *Diabetes Care* **15,** 418–429

21 Dohm, G.L., Tapscott, E.B., Pories, W.J., Dabbs, D.J., Flickinger, E.G., Meelheim, D., Fushiki, T., Atkinson, S.M., Elton, C.W., and Caro, J.F. (1988). An in vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J. Clin. Invest.* **82,** 486–494

22 Taylor, R.H., Jenkins, D.J.A., Baker, H.M., Fielden, H., Goff, D.V., Misiewicz, J.J., Lee, D.A., Allen, H.B., MacDonald, G., and Wallrabe, H. (1982). Effect of acarbose on the 24-hour blood glucose profile and pattern of carbohydrate absorption. *Diabetes Care* **5,** 92–96

23 Dolan, P.L., Tapscott, E.B., Dorton, P.J., and Dohm, G.L. (1993). Contractile activity restores insulin responsiveness in skeletal muscle of obese Zucker rats. *Biochem. J.* **289,** 423–426

24 Elton, C.W., Tapscott, E.B., and Dohm, G.L. (1994). Effect of moderate obesity on glucose transport in human muscle. *Horm. Metab. Res.* **26,** 181–183

25 Takayama, S., White, M.F., and Kahn, C.R. (1988). Phorbol ester-induced serine phosphorylation of the insulin receptor decreases its tyrosine kinase activity. *J. Biol. Chem.* **263,** 3440–3447

26 Stadtmauer, L. and Rosen, O.M. (1986). Increasing the cAMP content of IM-9 cells alters the phosphorylation state and protein kinase activity of the insulin receptor. *Biochem. J.* **250,** 509–519

27 Lee, J. and Pilch, P.F., (1994). The insulin receptor: structure, function, and signaling. *Am. J. Physiol.* **266,** C319–C334

28 Avignon, A., Yamada, K., Zhou, X., Spencer, B., Cardona, O., Saba-Siddique, S., Galloway, L., Standaert, M.L., and Farese, R.V. (1996). Chronic activation of protein kinase C in soleus muscles and other tissues of insulin-resistant type II diabetic Goto-Kakizaki (GK), obese/aged, and obese/Zucker rats. *Diabetes* **45,** 1396–1404

29 Peterson, R.G. (1994). α-Glucosidase inhibitors in diabetes: lessons from animal studies. *Eur. J. Clin. Invest.* **24,** Suppl. 3, 11–18

30 Slieker, L.J., Sundell, K., Heath, W., Osborne, J., Manetta, J., and Sportsman, R. (1992). Glucose transporter levels in tissues of spontaneously diabetic Zucker fa/fa Rat (ZDF/drt) and viable yellow mouse (A^{vy}/a). *Diabetes* **41,** 187–193

31 Hager, S.R., Jochen, A.L., and Kalhoff, R.K. (1991). Insulin resistance in normal rats infused with glucose for 72 h. *Am. J. Physiol.* **260,** E353–E362

32 Hansen, B.F., Hansen, S.A., Ploug, T., Bak, J.F., and Richter, E.A. (1992). Effects of glucose and insulin on development of impaired insulin action in muscle. *Am. J. Physiol.* **262,** E440–E446

33 Richter, E.A., Hansen, B.F., and Hansen, S.A. (1988). Glucose-induced insulin resistance of skeletal muscle glucose transport and uptake. *Biochem. J.* **252,** 733–737

34 Rossetti, L., Smith, D., Shulman, G.I., Papachristou, D., and DeFronzo, R.A. (1987). Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. *J. Clin. Invest.* **79,** 1510–1515

35 Sherman, W.M., Friedman, J.E., Gao, J.P., Reed, M.J., Elton, C.W., and Dohm, G.L. (1993). Glycemia and exercise training alter glucose transport and GLUT4 in the Zucker rat. *Med. Sci. Sports Exerc.* **25,** 341–348