Volume 268, number 1, 13–16 FEBS 08611 July 1990

Exercise training increases glucose transporter protein GLUT-4 in skeletal muscle of obese Zucker (*fa/fa*) rats

Jacob E. Friedman[1], William M. Sherman[2], Michael J. Reed[2], Charles W. Elton[1] and G. Lynis Dohm[1]

[1]*Department of Biochemistry, School of Medicine, East Carolina University, Greenville, NC 27858–4354* and [2]*School of HPER, The Ohio State University, 337 17th Ave, Columbus, OH 43210–1284, USA*

Received 18 May 1990

The present study examined the level of GLUT-4 glucose transporter protein in gastrocnemius muscles of 36 week old genetically obese Zucker (*fa/fa*) rats and their lean (*Fa/−*) littermates, and in obese Zucker rats following 18 or 30 weeks of treadmill exercise training. Despite skeletal muscle insulin resistance, the level of GLUT-4 glucose transporter protein was similar in lean and obese Zucker rats. In contrast, exercise training increased GLUT-4 protein levels by 1.7 and 2.3 fold above sedentary obese rats. These findings suggest endurance training stimulates expression of skeletal muscle GLUT-4 protein which may be responsible for the previously observed increase in insulin sensitivity with training.

Insulin resistance; Skeletal muscle; Obesity; Exercise; Glucose transporter

1. INTRODUCTION

The genetically obese (*fa/fa*) Zucker rat is hyperinsulinemic with impaired glucose tolerance. Previous studies have shown the insulin resistance in the obese Zucker (*fa/fa*) rat is due to impaired insulin-stimulated glucose uptake by muscle, the primary site of glucose disposal [1,2]. Because glucose transport is the rate-limiting step in glucose utilization in muscle [3,4] it suggests the defect in glucose uptake could involve a reduction in the abundance of glucose transporter protein.

Recent studies indicate that skeletal muscle expresses a unique tissue-specific glucose transporter protein GLUT-4, which is responsible for facilitated glucose transport in response to insulin [5–9]. The insulin resistance of streptozotocin diabetic and fasted rats has been shown to correlate closely with decreased GLUT-4 glucose transporter protein [10–13]. In the present study, we measured skeletal muscle GLUT-4 protein levels in genetically obese (*fa/fa*) Zucker rats and their lean littermates (*Fa/−*) in an effort to define the defect in glucose utilization at the biochemical level.

Endurance exercise training has been demonstrated to be an effective means of reducing muscle insulin resistance in the obese Zucker rat [14,15]. However, the cellular mechanism(s) underlying the improvement in insulin sensitivity with chronic exercise training is not known. Glucose transport activity is increased in skeletal muscles of trained rats despite little or no alteration in insulin receptor binding [16–19], or tyrosine kinase activity [17,19] suggesting the mechanism of increased glucose transport activity with training involves sites distal to the insulin receptor. Here we report that chronic endurance training results in a significant increase in GLUT-4 glucose transporter protein levels in muscle of the obese (*fa/fa*) Zucker rat.

Correspondence address: J.E. Friedman, Department of Biochemistry, School of Medicine, East Carolina University, Greenville, NC 27858-4354, USA

2. MATERIALS AND METHODS

2.1. *Reagents*

An affinity purified polyclonal antiserum (ECU4) specific for a COOH-terminal synthetic peptide of the GLUT-4 glucose transporter [6] was raised in rabbits. Reagents for polyacrylamide gel electrophoresis were obtained from Bio-Rad Laboratories, Richmond, CA. ^{125}I-goat anti-rabbit IgG was obtained from ICN Radiochemicals (Irvine, CA). Unless otherwise stated, all other reagents were obtained from Sigma Chemical Co. (St Louis, MO).

2.2. *Animals, exercise training*

Four week old male lean (*Fa/−*) and obese (*fa/fa*) Zucker rats were purchased from Charles River Laboratories (Cambridge, MA), and provided standard laboratory chow and water ad libitum. Obese animals were randomly assigned to an exercise or sedentary group. Exercise training began at either a young (6 weeks of age) or later age (18 weeks of age) and continued until the rats were 36 weeks of age. The exercise protocol consisted of treadmill running at 18 m/min, 15% grade, 5 days/week for gradually increasing durations during the first 2 weeks and thereafter at 20 m/min for 1.5 h/day. This training protocol has been demonstrated to increase cytochrome oxidase and citrate synthase activity 2 fold in plantaris muscle of obese Zucker rats [20,21]. To eliminate any residual effects of the last training session, all animals were sacrificed 48 h after the last exercise session after a 10 h fast. The gastrocnemius muscle was excised and immediately frozen at −70°C until preparation. Fasting blood samples were obtained at the time of sacrifice.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

2.3. *Glucose and insulin determinations*

Frozen aliquots of serum were thawed and insulin analyzed by double antibody radioimmunoassay with a commercially available kit (Immunonuclear) using a rat insulin standard (Eli Lilly & Co.). Glucose was measured by the glucose-oxidase method (Boehringer).

2.4. *Western blot analysis*

Muscle (200 mg) was homogenized using a Polytron homogenizer in buffer containing 25 mM Hepes, 4 mM EDTA, 25 mM benzamidine, and 1 μM each of leupeptin, pepstatin and aprotinin. Samples were incubated for 1.5 h in 1% Triton X-100 at 4°C, centrifuged at 2000 rpm, and supernatant removed. Fifty μg of protein (BCA procedure, Pierce Chemical Co., Rockford, IL) was subjected to SDS-polyacrylamide gel electrophoresis on 8% resolving gel according to Laemmli [22], followed by electrophoretic transfer to an Immobilon membrane. Membranes were blocked using Carnation Low-fat Instant milk, followed by incubation in anti GLUT-4 antiserum (ECU4, 2 μg protein/ml) and ^{125}I-anti-rabbit IgG. Autoradiographs were quantitated using laser scanning densitometry and results expressed relative to an internal standard (30 μg of rat heart membrane). For statistical purposes, repeat blots of muscle samples from 6–8 separate rats per group were measured by dot blot analysis in triplicate. The amount of GLUT-4 protein was scanned and compared with dots obtained with the standard. Comparisons between lean, obese, and obese-trained were analyzed using one-way ANOVA with Neuman-Keuls post-hoc analysis, $P<0.05$ = significance.

3. RESULTS

All observations were made on lean (*Fa/−*) and obese (*fa/fa*) Zucker rats sacrificed at 36 weeks of age. At this age, the *fa/fa* rat is obese (body mass 160 ±4%, mean ±SE, $P<0.01$), hyperinsulinemic (528 ±80%, $P<0.01$), and normoglycemic (105 ±8%, NS) compared with lean (*Fa/−*) littermates (Table I). Obese rats were exercise trained for either 18 or 30 weeks. Trained rats had significantly ($P<0.05$) lower body mass than sedentary obese rats. Fasting insulin levels were lower but not significantly less in both exercise groups compared to obese controls. However, fasting glucose was significantly less ($P<0.05$) in the 30 week trained obese rats compared to obese controls.

The effect of obesity and exercise training on levels of GLUT-4 glucose transporter protein was measured by Western blot analysis using polyclonal antiserum ECU4 (Fig. 1). Quantitation by laser densitometry showed similar levels of GLUT-4 for lean and sedentary obese rats (Fig. 2). However, exercise training of obese rats for 18 or 30 weeks increased GLUT-4 levels by 1.7 and 2.3 fold, respectively, relative to sedentary obese rats. To confirm these finndings, muscles (6–8/group) from lean, obese, and obese 30 week trained rats were analyzed by dot blot, and revealed a significant increase in GLUT-4 protein ($P<0.05$) in the 30 week trained group, and no differences between lean and obese sedentary rats.

Table I

Body weight, fasting plasma glucose and insulin concentrations in 36 week old lean (*Fa/−*), obese (*fa/fa*), and obese-trained Zucker rats

Group	Body mass (g)	Plasma insulin (μU/ml)	Plasma glucose (mm)
Lean (*Fa/−*)	398 ± 4**	92± 13**	8.6 × 0.4
Obese (*fa/fa*)	637 ± 17	486 ± 74	9.0 ± 0.7
Obese-trained 18 week	542 ± 26*	372 ± 58	8.5 ± 0.7
Obese-trained 30 week	570 ± 17*	432 ± 49	7.4 ± 0.3*

Mean ± SE, n = 4–7 animals/group. *Significantly less than obese nontrained (*fa/fa*) group, $P<0.05$ **Significantly less than all obese groups, $P<0.01$

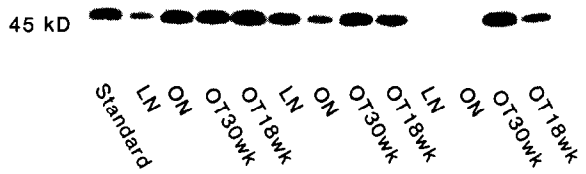

Fig. 1. Muscle glucose transporter GLUT-4 protein in skeletal muscle of lean *Fa/−* (LN), obese *fa/fa* (ON), obese exercise trained 30 week (OT30wk), and obese exercise trained 18 week (OT18wk) Zucker rats. Protein (50 μg) from mixed gastrocnemius muscle was extracted, subjected to SDS-PAGE, transferred to immobilion membrane, and immunoblotted with polyclonal antibody (ECU4) to GLUT-4 protein as described in section 2.

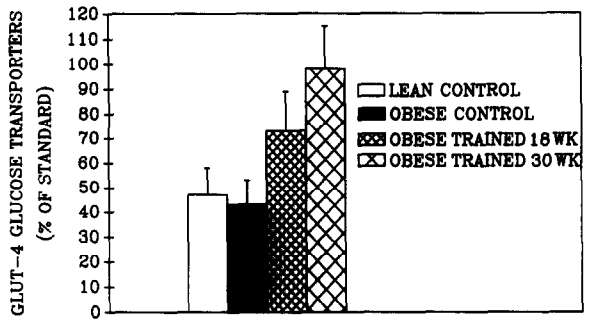

Fig. 2. GLUT-4 protein in gastrocnemius muscle from lean (*Fa/−*), obese (*fa/fa*), obese exercise trained (18 week), and obese exercise trained (30 week) Zucker rats. GLUT-4 protein was measured by quantitative immunoblotting as described in section 2. Results are based on scanning densitometry obtained from Western blot analysis shown in Fig. 1, mean ±SE, $n=3$. Similar results were obtained using dot blot analysis: % of standard = lean 34.6 ±1.51 ($n=6$); obese 32.5 ±1.27 ($n=6$); obese trained 30 week 45.4 ±4.9 ($n=7$), $P<0.05$ vs sedentary obese rats.

4. DISCUSSION

One of the primary biochemical adaptations to endurance exercise training is an increase in mitochondrial protein and oxidative capacity of the muscle [23]. Our results now show that adaptation to chronic endurance training also results in the synthesis of a specific protein which facilitates glucose transport into the muscle cell. As yet, very little is known about the mechanism(s) (neural, humoral, biochemical) responsi-

ble for the increase in GLUT-4 protein with training. However, we recently observed that in addition to increased glucose transport and insulin sensitivity, red slow twitch skeletal muscle has up to 5 times more GLUT-4 protein than white muscle [24]. This observation suggests that increased GLUT-4 protein with exercise training could be mediated by contractile activity per se, since a number of investigators have shown that muscles chronically stimulated to contract take on the biochemical characteristics of slow twitch muscle [25,26].

Several studies have found that the mechanism of increased glucose uptake by skeletal muscle following acute endurance exercise is through translocation of pre-existing glucose transporters to the cell surface [27–29]. The present results demonstrate that exercise training results in the synthesis of new glucose transporters, specifically the GLUT-4 transporter isoform. Increased number of glucose transporters as estimated by D-glucose inhibitable cytochalasin-B binding have been demonstrated previously in adipocytes of endurance trained rats along with increased glucose transport [30,31]. Presumably, these changes are the result of transcriptional regulation of glucose transporter mRNA.

Previous studies have shown that skeletal muscle is the primary tissue responsible for whole body insulin resistance in human obesity [32]. The genetically obese Zucker (*fa/fa*) rat has proven to be a useful model for the study of mechanisms of insulin resistance because of the well characterized defect in insulin-stimulated glucose uptake by skeletal muscle [1,2,15]. Although insulin receptor binding is decreased in soleus muscle from obese Zucker rats compared with lean rats [1,33], the major mechanism underlying the insulin resistance in obese Zucker rats resides in the glucose transport system [2,34]. In the current study we found that the previously observed defect in glucose transport is not accompanied by a depletion of the GLUT-4 glucose transporter protein. These data are similar to a recent report examining GLUT-4 protein in muscles from the young *ob/ob* mouse, another model of obesity and insulin resistance [35]. Thus, the insulin resistance in these genetic forms of obesity is not due to depletion of GLUT-4 glucose transporter protein in skeletal muscle. While very little is known about the signal transduction pathway involved, a major mechanism by which insulin increases glucose uptake is through translocation of glucose transporters from an intracellular compartment to the cell surface [36]. The present results suggest that the defect in glucose transport in obese Zucker rats may be related to changes in signal transduction, translocation, or functional activity of the glucose transporter.

Both insulin and glucose have been suggested as important factors regulating expression of glucose transporters. In insulin deficient states such as diabetes and fasting, there is a 50–60% decrease in GLUT-4 mRNA along with a 50–87% decrease in GLUT-4 protein in adipose cells [10–12] and skeletal muscle [13]. However, when glucose is increased independantly of insulin, glucose transporter protein gene expression and protein are reduced in skeletal muscle [37–38]. Our finding that GLUT-4 protein levels are unchanged in obese Zucker rats, despite severe hyperinsulinemia and normal blood glucose suggests, at least in this model, that GLUT-4 protein levels are not altered by hyperinsulinemia. On the other hand, exercise training which decreased fasting glucose without significantly altering insulin levels, induced a significant increase in GLUT-4 protein. This finding supports, albeit indirectly, the role of glucose in the regulation of GLUT-4 protein levels.

Acknowledgements: The authors would like to thank Peggy Plato, Ann Albright and Terri Marble for assistance in training the animals. This work was supported by NIH Research Grant DK38416 to G.L.D. and a Central Ohio Diabetes Association grant to W.M.S. J.E.F. is supported by NIH National Research Service Award no. DK08477-01.

REFERENCES

[1] Crettaz, M., Prentki, M., Zaninettie, M. and Jeanrenaud, B. (1980) Biochem. J. 186, 525–534.
[2] Sherman, W.M., Katz, A.L., Cutler, C.L., Withers, R.T. and Ivy, J.L. (1988) Am. J. Physiol. 255, E374-E382.
[3] Chiasson, J.L., Germain, L., Srivastava, A.K. and Dupis, P. (1984) Metabolism 33, 617–620.
[4] Ziel, F.H., Venkatesan, N. and Davidson, M.B. (1988) Diabetes 37, 885–890.
[5] James, D.E., Brown, R., Navarro, J. and Pilch, P.F. (1988) Nature 333, 183–185.
[6] James, D.E., Strube, M. and Mueckler, M. (1989) Nature 338, 83–87.
[7] Fukumoto, H., Kayano, T., Buse, J.B., Edwards, Y., Pilch, P.F., Bell, G.I. and Seino, S. (1989) J. Biol. Chem. 264, 7776–7779.
[8] Birnbaum, M.J. (1989) Cell 57, 305–315.
[9] Charron, M.J., Brosius, F.C., Alper, S.L. and Lodish, H.F. (1989) Proc. Nat. Acad. Sci. USA 86, 2535–2539.
[10] Kahn, B.B., Charron, M.J., Lodish, H.F., Cushman, S.W. and Flier, J.S. (1989) J. Clin. Invest. 84, 404–411.
[11] Berger, J., Biswas, C., Vicario, P.P., Strout, H.V., Saperstein, R. and Pilch, P.F. (1989) Nature 340, 70–73.
[12] Sivitz, W.I., DeSautel, S.L., Kayano, T., Bell, G.I. and Pessin, J.E. (1989) Nature 340, 72–74.
[13] Garvey, W.T., Huecksteadt, T.P. and Birnbaum, M.J. (1989) Science 245, 60–63.
[14] Becker-Zimmerman, K., Berger, M., Berchtold, P., Gries, F.A., Jerberg, L. and Schwenen, M. (1982) Diabetologia 22, 468–474.
[15] Ivy, J.L., Sherman, W.M., Cutler, C.L. and Katz, A. (1986) Am. J. Physiol. 251, E299-E305.
[16] Bonen, A., Tan, M.H., Clune, P. Kirby, R.L. (1985) Am. J. Physiol. 248, E403-E408.
[17] Dohm, G.L., Sinha, M.K. and Caro, J.F. (1987) Am. J. Physiol. 252, E170-E175.
[18] Grimditch, G.K., Barnard, R.J., Kaplan, S.A. and Sternlicht, E. (1986) Am. J. Physiol. 250, E57-E575.
[19] Treadway, J.L., James, D.E., Burcel, E. and Ruderman, N.B. (19) Am. J. Physiol. 256, E138-E144.

[20] Crettaz, M., Horton, E.S., Wardzala, L.J., Horton, E.D. and Jeanrenaud, B. (1983) Am. J. Physiol. 244, E374-E382.
[21] Sherman, W.M., Katz, A.L., Cutler, C.L., Van Dyke, J. and Ivy, J.L. (1985) Med. Sci. Sports Exerc. 17, S279.
[22] Laemmli, U.K. (1970) Nature 227, 680-685.
[23] Holloszy, J.O. and Coyle, E.F. (1984). J. Appl. Physiol. 56, 831-838.
[24] Kern, M., Elton, C.W., Stephens, J.M., Pekala, P.H. and Dohm, G.L. (1990) Med. Sci. Sports Exerc., in press.
[25] Williams, R.S., Garcia-Moll, M., Mellor, J., Salmons, S. and Harlan, W. (1987) J. Biol. Chem. 262, 2764-2767.
[26] Jeffery, S., Kelly, C.D., Carter, N., Kaufmann, M. Termin, A. and Pette, D. (1990) FEBS Lett. 262, 225-227.
[27] Douen, A.G., Ramlal, T., Cartee, G.D. and Klip, A. (1990) FEBS Lett. 261, 256-260.
[28] Fushiki, T., Wells, J.A., Tapscott, E.B. and Dohm, G.L. (1989) Am. J. Physiol. 256, E580-E587.
[29] Hirshman, M.F., Walberg-Henriksson, H., Wardzala, L.J., Horton, E.D. and Horton, E.S. (1988) FEBS Lett. 238, 235-239.
[30] Vinten, J., Peterson, L.N., Sonne, B. and Galbo, H. (1985) Biochim. Biophys. Acta. 842, 223-227.
[31] Hirshman, M.F., Wardzala, L.J., Goodyear, L.J., Fuller, J.P., Horton, E.D. and Horton, E.S. (1989) Am. J. Physiol. E520-E530.
[32] DeFronzo, R.A. (1988) Diabetes 37, 667-687.
[33] Czech, M.P., Richardson, D.K., Becjer, S.G., Walters, C.G., Gitomer, W. and Heinrich, J. (1978) Metabolism 27, Suppl. 2, 1967-1981.
[34] Zaninetti, D., Greco-Perotio, R., Assimacopoulos-Jeannet, F. and Jeanrenaud, B. (1989) Diabetologia 32, 56-60.
[35] Koranyi, L., James, D., Mueckler, M. and Permutt, M.A. (1990) J. Clin. Invest. 85, 962-967.
[36] Simpson, I.A. and Cushman, S.W. (1986) Annu. Rev. Biochem. 55, 1059-1089.
[37] Wang, P.H., Moller, D., Flier, J.S., Nayak, R.C. and Smith, R.J. (1989) J. Clin. Invest. 84, 62-67.
[38] Wertheimer, E., Benneriah, Y., Sasson, S. and Cerasi, E. (1989) Diabetes 38, 40A.