Contractile activity restores insulin responsiveness in skeletal muscle of obese Zucker rats

Patricia L. DOLAN,* Edward B. TAPSCOTT, Peter J. DORTON and G. Lynis DOHM
Department of Biochemistry, School of Medicine, East Carolina University, Greenville, NC 27858, U.S.A.

Both insulin and contraction stimulate glucose transport in skeletal muscle. Insulin-stimulated glucose transport is decreased in obese humans and rats. The aims of this study were (1) to determine if contraction-stimulated glucose transport was also compromised in skeletal muscle of genetically obese insulin-resistant Zucker rats, and (2) to determine whether the additive effects of insulin and contraction previously observed in muscle from lean subjects were evident in muscle from the obese animals. To measure glucose transport, hindlimbs from lean and obese Zucker rats were perfused under basal, insulin-stimulated (0.1 μM), contraction-stimulated (electrical stimulation of the sciatic nerve) and combined insulin- + contraction-stimulated conditions. One hindlimb was stimulated to contract while the contralateral leg served as an unstimulated control. 2-Deoxyglucose transport rates were measured in the white gastrocnemius, red gastrocnemius and extensor digitorum longus muscles. As expected, the insulin-stimulated glucose transport rate in each of the three muscles was significantly slower ($P < 0.05$) in obese rats when compared with lean animals. When expressed as fold stimulation over basal, there was no significant difference in contraction-induced muscle glucose transport rates between lean and obese animals. Insulin- + contraction-stimulation was additive in skeletal muscle of lean animals, but synergistic in skeletal muscle of obese animals. Prior contraction increased insulin responsiveness of glucose transport 2–5-fold in the obese rats, but had no effect on insulin responsiveness in the lean controls. This contraction-induced improvement in insulin responsiveness could be of clinical importance to obese subjects as a way to improve insulin-stimulated glucose uptake in resistant skeletal muscle.

INTRODUCTION

The cause of the insulin resistance in obesity and non-insulin-dependent diabetes mellitus (NIDDM) has been the topic of continuing research since it was realized that a primary source of the hyperglycaemia in these individuals was a failure of their peripheral tissues (skeletal muscle and fat) to take up glucose in response to normal insulin levels. Since skeletal muscle is responsible for nearly 90% of the glucose disposal following a glucose load (DeFronzo et al., 1985), a defect in skeletal-muscle glucose uptake would have a large impact on total-body glucose homoeostasis. Our laboratory and others have demonstrated that the decreased glucose uptake seen in skeletal muscle of obese/and or NIDDM individuals is due to a decrease in glucose transport (Dohm et al., 1988) rather than a defect in utilization (Shulman et al., 1991). Alterations in insulin-receptor number and/or glucose-transporter protein do not account entirely for the insulin resistance seen with obesity and/or NIDDM (Caro et al., 1987; Horton et al., 1990; Pedersen et al., 1990; Handberg et al., 1990; Dohm et al., 1991; Betts et al, 1991; Friedman et al., 1992; Eriksson et al., 1992). The decreased insulin-stimulated glucose transport must therefore be due in part to a defect in insulin signalling. Impairments in both insulin-stimulated tyrosine kinase activity of the insulin receptor (Caro et al., 1987; Arner et al., 1987; Obermaier-Kusser et al., 1989) and insulin-induced suppression of soluble protein phosphatase activity (McGuire et al., 1991) have been documented for insulin-resistant humans. The obese Zucker rat was chosen as the insulin-resistant model for the present experiments, because it mimics human obesity and NIDDM in several regards. First, decreased total glucose-transporter protein (plasma and microsomal membranes) cannot account for the insulin resistance observed in obese animals compared with lean littermates (Friedman et al., 1990; Zarjevski et al., 1992). Second, receptor tyrosine kinase activity is decreased in the muscle of the obese Zucker rat (Slieker et al., 1990); and third, insulin binding to soleus muscle is decreased by 25–35% in the obese Zucker rat (Crettaz et al., 1980), which is similar to the decreased insulin binding to muscle insulin receptors in obese humans (Caro et al., 1987).

Previous research has suggested that glucose transport can be stimulated via two separate pathways in mammalian skeletal muscle. One pathway is activated by insulin, and the other by contractile activity (Nesher et al., 1985; Zorzano et al., 1986; Constable et al., 1988; Wallberg-Henriksson et al., 1988). It is not known if the contraction-stimulated pathway is normal in skeletal muscle of obese individuals or if, like the insulin-stimulated pathway, it is also depressed. Therefore, the first aim of the present study was to determine if contraction-stimulated glucose transport in skeletal muscle of obese individuals was comparable with that seen in lean subjects.

The combined effects of insulin and contraction on glucose uptake are additive in insulin-sensitive skeletal muscle (Nesher et al., 1985; Constable et al., 1988; Wallberg-Henriksson et al., 1988; Henriksen et al., 1990). This additivity has not been examined in a model of insulin resistance in obesity. Hence, the second aim of the present study was to determine whether the additive effects of insulin and contraction would be observed in skeletal muscle of the obese Zucker rat.

In order to address the above two aims, perfused skeletal muscle was examined under the following four conditions: (1) basal, (2) insulin stimulation, (3) contraction stimulation, and (4) combined insulin + contraction stimulation. Results indicate that the contraction-stimulated pathway, unlike the insulin pathway for glucose transport, is not impaired in skeletal muscle of obese

Abbreviation used: NIDDM, non-insulin-dependent diabetes mellitus.
* To whom correspondence should be addressed.

rats. In addition, insulin-+contraction-stimulation produces a synergistic glucose transport response in obese skeletal muscle, rather than an additive one.

METHODS

Animals

Male obese Zucker rats (*fa/fa*) (12 weeks of age, 412±16.2 g) and their lean littermates (*Fa/?*) (279±10.0 g) were obtained from Charles River Laboratories (Raleigh, NC, U.S.A.). Animals were housed individually, maintained on a 12 h-light/12 h-dark cycle (light from 06.00 to 18.00 h) and provided with food (Purina rodent chow) and water *ad libitum*. Perfusion experiments were performed after an overnight fast.

Hindquarter perfusion

Rates of glucose transport were determined in the perfused rat hindlimb (Ruderman et al., 1971) by using 2-deoxy[^{3}H]glucose, a glucose analog that is transported and phosphorylated, but not metabolized further. Half of the perfusions contained a maximal concentration of insulin in the perfusate and the other half had no insulin added. One limb of each hindquarter was electrically stimulated via the sciatic nerve, while the contralateral leg served as an unstimulated control. This protocol resulted in four different treatments: (1) basal, (2) contraction, (3) insulin, and (4) insulin+contraction.

Perfusion of the hemicorpus was performed when the rats were approx. 12 weeks of age. The rats were anaesthetized intraperitoneally with ketamine (9 mg/100 mg body wt.) and xylazine (1 mg/100 g body wt.) after an overnight fast. The perfusion system has been described previously (Dohm et al., 1980). The perfusion media (4% BSA, 5.5 mM glucose, 33% washed bovine red blood cells and Krebs–Henseleit buffer) was gassed with O_2/CO_2 (19:1). Flow rate was 18 ml/min and chamber temperature was maintained at 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 glucose concentration in perfusion media at 5.5±0.14 mM. Insulin, when appropriate, was present at a maximal concentration of 0.1 μM. At the end of a 30 min pre-perfusion period, radioactive label was added to the media at a final concentration of 20 mM sorbitol containing 0.1 μCi of [U-^{14}C]sorbitol and 0.2 μCi of 2-deoxy[^{3}H]glucose (tracer amount). After an additional 30 min perfusion, the soleus, extensor digitorum longus and red and white gastrocnemius muscles were quickly removed and prepared for measurement of transport rate.

Electrical stimulation

During the surgical procedures, a piece of 3-O silk suture was tied to the achilles tendon of the leg to be stimulated, and the sciatic nerve was isolated. The other end of the suture was attached to a force-displacement transducer (Grass) which was connected to a polygraph (Grass model 7D). During the initial contractions, muscle length was adjusted to achieve the maximal increase of tension during the contractions. An electrode was attached to the sciatic nerve, and electrical stimulation was delivered by a Grass 88 stimulator. The muscles were stimulated during the last 12 min of the pre-perfusion period for two 5 min periods separated by a 1 min rest period. Stimulation consisted of 2 ms trains delivered at a rate of 1 per s. Each train consisted of repeated 10 V pulses of 0.1 ms duration delivered at 100 Hz.

Measurement of 2-deoxyglucose transport and glucose uptake

A weighed portion (50–100 mg) of each muscle excised from the perfused hindlimb was finely minced and added to 0.5 ml of 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) was added to the vial and samples were counted for radioactivity in a Beckman LS 5000TD liquid-scintillation counter preset for simultaneous counting of ^{3}H/^{14}C.

2-Deoxy[^{3}H]glucose and [^{14}C]sorbitol radioactivity (d.p.m.) were counted in perfusate samples taken at 5 min intervals. Glucose concentration was determined in the same samples (Sigma kit, glucose oxidase determination) and these glucose values were used to determine specific radioactivity of 2-deoxy[^{3}H]glucose, since glucose was the carrier for the 2-deoxy[^{3}H]glucose. Accumulation of intramuscular 2-deoxy[^{3}H]glucose was determined by subtracting extracellular 2-deoxy[^{3}H]glucose (determined from the [^{14}C]sorbitol space) from total muscle 2-deoxy[^{3}H]glucose.

Statistical analysis

All data are expressed as means±S.E.M. Differences between treatment groups were evaluated by using a three-way ANOVA with repeated measures of the contraction factor. For statistically significant interaction effects, Tukey *post hoc* tests were used to determine the site of significance. P values < 0.05 were considered statistically significant.

RESULTS

Table 1 shows the glucose transport (μmol/h per g) data. The four treatment conditions (basal, insulin, contraction and insulin+contraction) are displayed for white gastrocnemius, red gastrocnemius and extensor digitorum longus muscles. These muscles were chosen because they represent muscle of dissimilar fibre-type composition (Ariano et al., 1973), which has been

Table 1 Effect of electrical stimulation and insulin on rates of glucose transport in selected skeletal muscle

Values are means±S.E.M. of 9 muscles/group: *$P < 0.05$, significantly less than lean. 'Predicted I+C' are values expected if strict additivity was observed.

	Glucose transport (μmol/h per g)		
Treatment	White gastrocnemius	Red gastrocnemius	Extensor digitorum longus
Basal			
Lean	1.23±0.30	1.00±0.15	1.52±0.27
Obese	0.82±0.06	0.96±0.04	1.05±0.07
Insulin			
Lean	5.14±1.05	19.93±1.42	21.14±2.56
Obese	1.50±0.24*	4.80±0.70*	3.06±0.54*
Contraction			
Lean	7.52±1.82	1.71±0.50	7.02±1.28
Obese	2.59±0.71*	1.32±0.34	4.82±1.16
Insulin+contraction (I+C)			
Lean	12.04±2.04	19.82±0.96	33.62±3.22
Obese	5.79±1.51*	9.28±2.66*	10.97±2.24*
Predicted I+C			
Lean	11.43	20.64	26.64
Obese	3.27	5.16	6.83

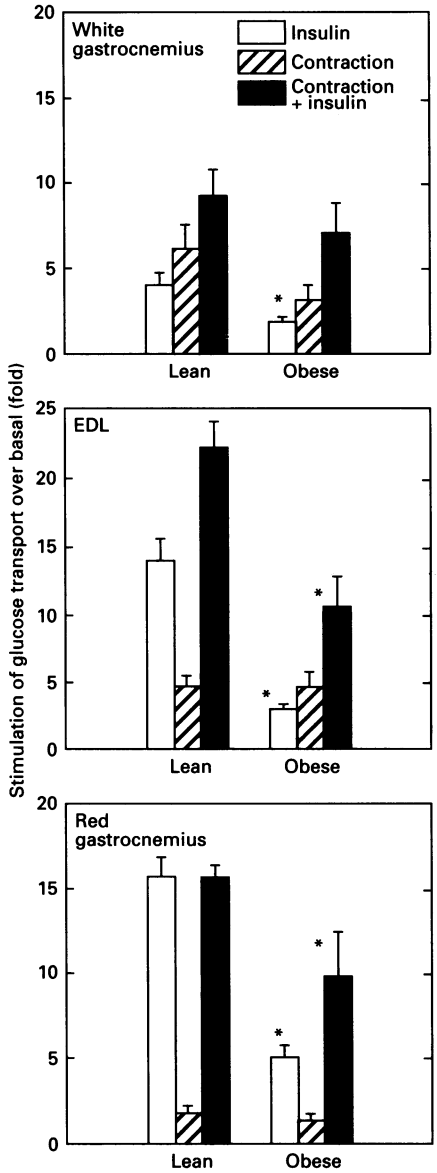

Figure 1 Stimulation (fold) of glucose transport above basal induced by insulin, contractile activity, and the combination of the two

There were 9 muscles per group: *significantly different from comparable lean group ($P < 0.05$). Abbreviation: EDL, extensor digitorum longus.

shown previously to differ in insulin binding (Bonen et al., 1986), glucose transporters (Kern et al., 1990) and glucose transport capacities (James et al., 1985; Kern et al., 1990). The stimulation protocol used in the present study did not elicit an effect of muscle contraction on glucose transport in the soleus, and therefore the soleus will not be included in the current discussion.

Figure 1 summarizes the data from Table 1 as fold stimulation above basal. Dividing the stimulated rate by basal rate decreases variability due to minor differences in basal rates between lean and obese muscle and between the different muscle fibre types. As expected, maximal insulin stimulation was significantly less in the insulin-resistant obese animals compared with lean controls for each of the three muscles (Figure 1). However, the contraction-stimulated fold increase over basal was not significantly different between obese and lean animals. Whereas the insulin- + contraction-stimulated fold increase in the obese animals was significantly lower than for lean controls in the extensor digitorum longus and red gastrocnemius, it was closer to the control level than were the insulin-stimulated values in the same muscles. Insulin- + contraction-stimulation in the obese-rat white gastrocnemius muscle did not differ from the lean-rat muscle.

The combined effect of insulin + contraction on glucose transport was additive in the skeletal muscle of lean animals (Table 1). The glucose transport rate for insulin + contraction in skeletal muscle of the obese animals was 2-fold greater than expected if straight additivity had been observed.

DISCUSSION

The present findings demonstrate that, although the insulin-stimulated pathway for glucose uptake into the cell is abnormal in obesity, the contraction-stimulated pathway functions normally. This conclusion is further strengthened by the finding that the translocation defect observed with maximal insulin stimulation in skeletal muscle of the obese Zucker rat (Horton et al., 1990) is not present after high-intensity treadmill exercise (Betts et al., 1991).

Insulin (Wardzala and Jeanrenaud, 1981; Klip et al., 1987) and muscle contraction (Hirshman et al., 1988; Douen et al., 1989; Fushiki et al., 1989) both increase glucose transport via the recruitment/activation of the GLUT4 glucose-transporter isoform (Douen et al., 1990; Henriksen et al., 1990; Horton et al., 1990; Betts et al., 1991) to/at the cell membrane. Skeletal-muscle expression of GLUT4 protein (Friedman et al., 1990; Zarjevski et al., 1992) or mRNA (Zarjevski et al., 1992) does not differ between lean and obese Zucker rats. The fact that contraction (known to recruit the same transporter isoform as insulin) stimulates glucose transport normally in the obese rats indicates that these GLUT4 transporters are functional if properly signalled. These findings suggest that the insulin resistance observed in skeletal muscle of obese and NIDDM patients cannot be attributed to defects intrinsic to the GLUT4 glucose-transporter protein. Rather, the decreased insulin-stimulated glucose uptake in obese-rat skeletal muscle may be due to a defect in the insulin signalling pathway which prevents optimal recruitment and/or activation of the glucose-transporter protein.

Prior investigations have shown that the maximal effects of insulin and contractions on glucose transport are additive in mammalian skeletal muscle (Nesher et al., 1985; Constable et al., 1988; Wallberg-Henriksson et al., 1988; Henriksen et al., 1990). In the present investigation, we also demonstrate an additive relationship of insulin + contraction in lean-rat skeletal muscle. However, in obese-rat skeletal muscle, the combined effect of insulin + contraction had synergistic, rather than additive, effects. We believe that this 'extra' transport rate (above additivity) represents an improvement in insulin responsiveness. If the contraction-stimulated transport rate is subtracted from the insulin- + contraction-stimulated rate, the remaining transport rate, that attributed to insulin, can be compared with the insulin-stimulated transport rate measured in the absence of prior contraction. If insulin responsiveness is unaltered by prior contraction, then the two transport rate values should be similar and produce a ratio close to 1. Skeletal muscle of lean rats had a ratio close to 1 (white gastrocnemius 1.16, red gastrocnemius 0.96, extensor digitorum longus 1.35), but the insulin-stimulated glucose transport rate after contraction was increased 2–5-fold in the skeletal muscle of obese animals (white gastrocnemius 4.7, red gastrocnemius 2.08, extensor digitorum longus 3.06). Our data suggest that contraction may modify a defect present in the insulin pathway of the resistant skeletal muscle, thereby allowing

the insulin responsiveness of these muscles to approach more closely the non-defective response of lean controls.

Our laboratory and others have shown that the insulin-stimulated tyrosine kinase activity of the insulin receptor is depressed in skeletal muscle of obese patients with and without NIDDM (Caro et al., 1987; Arner et al., 1987; Obermaier-Kusser et al., 1989). Additionally, it has been shown that insulin-induced suppression of soluble protein phosphatase activity is severely impaired in insulin-resistant humans (McGuire et al., 1991). Both decreased tyrosine kinase activity and increased phosphatase activity would be expected to decrease subsequent tyrosine phosphorylation. In the context of our data, we suggest that contractile activity may inhibit a protein phosphatase leading to increased phosphorylation of a signalling peptide, which in the phosphorylated form is responsible for stimulating translocation and/or activation of GLUT4. If this phosphorylated signalling peptide is a common intermediate to both the exercise and insulin-stimulated pathways, then contractile activity (exercise) may result in an attenuated inhibition of the associated protein phosphatase in insulin-resistant muscle in such a way that subsequent insulin signalling is amplified.

Support for the occurrence of a common step in the insulin- and contraction- or hypoxia-stimulated pathways is provided by studies showing that polymyxin B inhibits both insulin-stimulated and contraction/hypoxia-stimulated glucose transport (Henriksen et al., 1989; Young et al., 1991). In addition, Cartee and Holloszy (1990) reported that submaximal hypoxia-stimulated glucose transport was greatly amplified in muscles studied 3 h after exercise. This increased stimulation by hypoxia is similar to the increased insulin sensitivity seen after exercise and suggests that exercise mediates its effect on a late step that is common to both the insulin- and the hypoxia-stimulated pathway. Additionally, a study in our laboratory found that the absolute rate of submaximal contraction-stimulated glucose transport (electrical stimulation of sciatic nerve in perfused hindlimb) was significantly increased in the white gastrocnemius muscle of previously exercised obese Zucker rats compared with non-exercised controls (P. L. Dolan, unpublished work). It appears that exercise was able to amplify previously submaximal contraction-stimulated glucose transport, just as it did with the submaximal hypoxia-stimulated glucose transport in the study by Cartee and Holloszy (1990).

In summary, contractile activity stimulates glucose transport normally in skeletal muscle of obese rats. In fact, contractile activity partially restores insulin-responsiveness in skeletal muscle of obese animals and may therefore improve glucose homoeostasis in skeletal muscle of obese insulin-resistant subjects.

This work was supported by National Institutes of Health Grant DK38416. P.L.D. was supported by a Postdoctoral Fellowship (F32-DK08823) from the National Institutes of Health.

REFERENCES

Ariano, M. A., Armstrong, R. B. and Edgerton, V. R. (1973) J. Histochem. Cytochem. **21**, 51–55

Arner, P., Pollare, T., Lithell, H. and Livingston, J. N. (1987) Diabetologia **30**, 437–440

Betts, J. J., King, P. A., Horton, E. D. and Horton, E. S. (1991) Med. Sci. Sports Exercise **23**, S86

Bonen, A., Clune, P. and Tan, M. H. (1986) Am. J. Physiol. **251**, E196–E203

Caro, J. F., Sinha, M. K., Raju, S. M., Ittoop, O., Pories, W. J., Flickinger, E. G., Meelheim, D. and Dohm, G. L. (1987) J. Clin. Invest. **79**, 1330–1337

Cartee, G. D. and Holloszy, J. O. (1990) Am. J. Physiol. **258**, E390–E393

Constable, S. H., Favier, R. J., Cartee, G. D., Young, D. A. and Holloszy, J. O. (1988) J. Appl. Physiol. **64**, 2329–2332

Crettaz, M., Prentki, M., Zaninetti, D. and Jeanrenaud, B. (1980) Biochem. J. **186**, 525–534

DeFronzo, R. A., Gunnarsson, R., Bjorkman, O., Olsson, M. and Wahren, J. (1985) J. Clin. Invest. **76**, 149–155

Dohm, G. L., Kasperek, G. J., Tapscott, E. B. and Beecher, G. B. (1980) Biochem. J. **188**, 255–262

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) J. Clin. Invest. **82**, 486–494

Dohm, G. L., Elton, C. W., Friedman, J. E., Pilch, P. F., Pories, W. J., Atkinson, S. M., Jr. and Caro, J. F. (1991) Am. J. Physiol. **260**, E459–E463

Douen, A. G., Ramlal, T., Klip, A., Young, D. A., Cartee, G. D. and Holloszy, J. O. (1989) Endocrinology (Baltimore) **124**, 449–454

Douen, A. G., Ramlal, T., Rastogi, S., Bilan, P. J., Cartee, G. D., Vranic, M., Holloszy, J. O. and Klip, A. (1990) J. Biol. Chem. **265**, 13427–13430

Eriksson, J., Koranyi, L., Bourey, R., Schalin-Jantti, C., Widen, E., Mueckler, M., Permutt, A. M. and Groop, L. C. (1992) Diabetologia **35**, 143–147

Friedman, J. E., Sherman, W. M., Reed, M. J., Elton, C. W. and Dohm, G. L. (1990) FEBS Lett. **268**, 13–16

Friedman, J. E., Dohm, G. L., Keggett-Frazier, N., Elton, C. W., Tapscott, E. B., Pories, W. P. and Caro, J. F. (1992) J. Clin. Invest. **89**, 701–705

Fushiki, T., Wells, J. A., Tapscott, E. B. and Dohm, G. L. (1989) Am. J. Physiol. **256**, E580–E587

Handberg, A., Vaag, A., Damsbo, P., Beck-Nielsen, H. and Vinten, J. (1990) Diabetologia **33**, 625–627

Henriksen, E. A., Sleeper, M. D., Zierath, J. R. and Holloszy, J. O. (1989) Am. J. Physiol. **256**, E662–E667

Henriksen, E. J., Bourey, R. E., Rodnick, K. J., Koranyi, L., Permutt, M. A. and Holloszy, J. O. (1990) Am. J. Physiol. **259**, E593–E598

Hirshman, M. F., Wallberg-Henriksson, H., Wardzala, L. J., Horton, E. D. and Horton, E. S. (1988) FEBS Lett. **238**, 235–239

Horton, E. D., King, P. A., Hirshman, M. F. and Horton, E. S. (1990) Diabetes **39**, 83A

James, D. E., Kraegen, E. W. and Chisholm, D. J. (1985) Am. J. Physiol. **248**, E575–E580

Kern, M., Wells, J. A., Stephens, J. M., Elton, C. W., Friedman, J. E., Tapscott, E. B., Pekala, P. H. and Dohm, G. L. (1990) Biochem. J. **270**, 397–400

Klip, A., Ramlal, T., Young, D. A. and Holloszy, J. O. (1987) FEBS Lett. **224**, 224–230

McGuire, M. C., Fields, R. M., Nymoba, B. L., Raz, I., Bogardus, C., Tonks, N. K. and Sommercorn, J. (1991) Diabetes **40**, 939–942

Nesher, R., Karl, I. E. and Kipnis, D. M. (1985) Am. J. Physiol. **249**, C226–C232

Obermaier-Kusser, B., White, M. F., Pongratz, D. E., Su, Z., Ermel, B., Muhlbacher, C. and Haring, H. U. (1989) J. Biol. Chem. **264**, 9497–9504

Pederson, O., Bak, J. F., Andersen, P. H., Lund, S., Moller, D. E., Flier, J. S. and Kahn, B. B. (1990) Diabetes **39**, 865–870

Ruderman, N. B., Houghton, C. R. S. and Hems, R. (1971) Biochem. J. **124**, 639–651

Shulman, R. G., Rothman, D. L. and Shulman, G. I. (1991) Diabetes **40**, 22A

Slieker, L. J., Roberts, E. F., Shaw, W. N. and Johnson, W. T. (1990) Diabetes **39**, 619–625

Wallberg-Henriksson, H., Constable, S. H., Young, D. A. and Holloszy, J. O. (1988) J. Appl. Physiol. **65**, 909–913

Wardzala, L. J. and Jeanrenaud, B. (1981) J. Biol. Chem. **256**, 7090–7093

Young, J. C., Kurowski, T. G., Maurice, A. M., Nesher, R. and Ruderman, N. B. (1991) J. Appl. Physiol. **70**, 1650–1654

Zarjevski, N., Doyle, P. and Jeanrenaud, B. (1992) Endocrinology (Baltimore) **130**, 1564–1570

Zorzano, A., Balon, T. W., Goodman, M. N. and Ruderman, N. B. (1986) Am. J. Physiol. **251**, E21–E26

Received 26 May 1992/19 August 1992; accepted 25 August 1992