British Journal of Pharmacology (1995) 115, 1182-1187

Effects of metformin treatment on glucose transporter proteins in subcellular fractions of skeletal muscle in (*fa/fa*) Zucker rats

[1]J. Rouru, M. Koulu, *J. Peltonen, E. Santti, V. Hänninen, U. Pesonen & †R. Huupponen

Department of Pharmacology, University of Turku, Kiinamyllynkatu 10 FIN-20520 Turku, Finland; *Department of Medical Biochemistry, University of Turku, Finland and †Department of Clinical Pharmacology, Turku University Central Hospital, Turku, Finland

1 The present study was designed to clarify the cellular mechanism through which the antihyperglycaemic drug, metformin, exerts its effects. For this purpose the contents of glucose transporter protein isoforms GLUT1 and GLUT4 were measured in plasma membrane and intracellular membrane fractions of skeletal muscle obtained from genetically obese, insulin-resistant Zucker rats.

2 Hindlimb muscles were dissected from metformin-treated (300 mg kg^{-1} day^{-1}, p.o., for 12 days) and control rats in basal treatment state, and after acute stimulation with insulin (22 u kg^{-1}, i.p.). Since metformin treatment reduces food intake, we also used a pair-fed control group to investigate the effects of altered insulinaemia *per se*. Glucose transporter levels were analysed by Western blot and slot blot-techniques. In addition, 2-deoxy-[^{14}C]-glucose uptake in isolated muscle strips was evaluated.

3 No changes were noted in the contents of GLUT1 proteins in any of the subcellular fractions after metformin treatment. The contents of GLUT4 in subcellular fractions were not altered in the basal treatment state. After acute insulin exposure the content of GLUT4 in the intracellular membrane fraction declined significantly in the metformin-treated group, while no significant effect was seen in the plasma membrane fraction. In agreement with these results, metformin treatment did not alter 2-deoxyglucose uptake into isolated muscle strips.

4 In conclusion, the present study does not support the concept that metformin would enhance translocation of glucose transporter proteins from the intracellular compartment to the plasma membrane in skeletal muscle *in vivo*.

Keywords: Metformin; GLUT4; GLUT1; glucose transporter; skeletal muscle; Zucker rat; genetic obesity

Introduction

Skeletal muscle is the primary site for insulin-dependent glucose utilization (Yki-Järvinen, 1993). Glucose transport into this tissue appears to be a rate limiting step in glucose utilization under most physiological conditions (Yki-Järvinen, 1993). Glucose is transported inside the myocyte by a process of facilitated diffusion mediated by two isoforms of glucose transporter proteins, GLUT1 and GLUT4 (Mueckler, 1994). The primary vehicle for insulin-stimulated glucose uptake is GLUT4, which is translocated from the intracellular pool to the plasma membrane in response to insulin, whereas GLUT1 acts mainly as a basal transporter (Shepherd & Kahn, 1993). Insulin resistance at the level of skeletal muscle is a major feature of obesity and non-insulin-dependent diabetes mellitus (NIDDM) (Yki-Järvinen, 1994). The biochemical background for the development of insulin resistance is as yet unknown, but defects have been proposed to exist at the postreceptor level (Seely & Olefsky, 1993), such as in inappropriate regulation of glucose transporter proteins (Shepherd & Kahn, 1993).

Metformin is an antihyperglycaemic agent used widely for the treatment of NIDDM (Bailey, 1993). Therapeutic effects of metformin have been attributed to a combination of improved peripheral glucose utilization, decreased hepatic glucose output and decreased rate of intestinal absorption of carbohydrates (Bailey, 1993), but its cellular mode of action is still incompletely understood. Metformin treatment potentiates the effects of insulin on glucose uptake in streptozotocin diabetic mouse skeletal muscle (Bailey & Puah, 1986). In cultured L6 muscle cells, metformin translocates GLUT1 proteins from the intracellular pool to the plasma membrane (Hundal *et al.*, 1992) and in adipocytes isolated from obese Zucker rats *in vivo*, metformin treatment has been shown to potentiate insulin-stimulated translocation of both GLUT1 and GLUT4 proteins from the intracellular compartment to the plasma membrane (Matthaei *et al.*, 1993). In recent studies, metformin treatment failed to alter GLUT1 or GLUT4 protein content in skeletal muscle crude membranes obtained from obese Zucker rats (Handberg *et al.*, 1993; 1994). However, it remains to be established whether the effect of metformin on glucose transporter protein translocation applies to skeletal muscle cells *in vivo* (Bailey, 1993).

The purpose of the present study was to characterize the mechanism of action of metformin in skeletal muscle by studying the subcellular distribution of GLUT1 and GLUT4 proteins, and the uptake of 2-deoxyglucose in isolated muscle strips after 12 days metformin-treatment. As an animal model we used genetically obese, hyperinsulinaemic and insulin-resistant Zucker rats (Bray *et al.*, 1989). Many features in these rats resemble human NIDDM; however, the obese Zucker rats are not hyperglycaemic at the postabsorptive state.

Methods

Animals

Genetically obese 12-week-old (*fa/fa*) male Zucker rats were purchased from IFFA CREDO, (L'Arbresle, France). All rats were individually housed, maintained on a regular light-dark cycle, lights on from 06 h 00 min to 20 h 00 min and provided with normal laboratory rat chow.

Experiment 1

Thirty obese animals were divided into three experimental groups (metformin-treated, pair-fed and control) matched with

[1] Author for correspondence.

body weight and 24 h food intake recordings ($n=10$ in each group). The animals were the same as those used for the determination of brain neuropeptide Y mRNA expression in the experiments described by Rouru *et al.* (1995). The metformin-treated group received 300 mg kg^{-1} day^{-1} metformin hydrochloride dissolved in the drinking water. The dose was based on our previous experiments where the treatment was found effective in decreasing plasma insulin levels and resulted in plasma metformin concentrations comparable to those found in NIDDM patients during maintenance metformin therapy (Rouru *et al.*, 1993). The 24 h fluid intake was monitored and the concentration of metformin in the drinking water adjusted every second day to maintain its correct daily dose. The control group and pair-fed group received drinking water without metformin *ad libitum*. Because rats in the metformin group ate less than those in the control group, the pair-fed group received a restricted amount of food (at 16 h 00 min) to ensure similar caloric intake with the metformin-treated rats. Food intake and body weights were measured every day.

After 12 days treatment food and water were withdrawn from all animals at 07 h 00 min and they were decapitated between 09 h 30 min and 13 h 30 min. Blood was collected into prechilled EDTA tubes, and plasma was separated and stored at −70°C until analysed for insulin and glucose. Hindlimbs were fixed and cleaned free of fat and connective tissue. Four strips from soleus muscles were prepared from each animal as described earlier (Crettaz *et al.*, 1980) and kept in 0.9% NaCl solution until used for the determination of 2-deoxyglucose uptake. Tibialis muscles were removed and quickly frozen in isopenthane on liquid nitrogen for the immunohistochemical staining procedures. The rest of the hindlimb muscles were dissected out and frozen in liquid nitrogen for the subcellular fractionation procedure. The frozen muscle samples were stored at −70°C until processed further.

Experiment 2

Twenty obese animals were divided into two experimental groups (metformin-treated and control) matched with body weight ($n=10$ in each group). Metformin-treated group received 300 mg kg^{-1} day^{-1} metformin hydrochloride dissolved in the drinking water for 12 days as in experiment 1. The control group received drinking water without metformin *ad libitum*.

Food was withdrawn from all animals on the day before decapitation at 17 h 00 min and restored 1 h before decapitation. In order to obtain a maximal insulin stimulus all rats were given 22 u kg^{-1} insulin i.p. 30 min before decapitation, which was carried out between 09 h 30 min and 14 h 15 min. Blood was collected into prechilled EDTA tubes, and plasma was separated and stored at −70°C until analysed for insulin and glucose. Hindlimbs were fixed and cleaned free of fat and connective tissue and muscles were dissected out and frozen in liquid nitrogen for the subcellular fractionation procedure. The frozen muscle samples were stored at −70°C until processed further.

2-Deoxyglucose transport

The determination of 2-deoxyglucose transport was carried out essentially as described earlier (Crettaz *et al.*, 1980). In brief, once prepared, muscle strips were weighed and stretched on a stainless-steel holder. After preincubation two strips from each animal were incubated with 1 mu ml^{-1} insulin and two strips without insulin in the presence of 2-deoxy-[^{14}C]-glucose and [^{3}H]-sucrose as extracellular marker. At the end of the incubations, muscles were washed in cold 0.9% NaCl, hydrolysed in 1 M NaOH and counted with the Opti Phase 'High Safe' II scintillation cocktail (FSA Laboratories Supplies, Loughborough, U.K.) in a liquid scintillation counter (LKB Wallac 1219, Turku, Finland), enabling the samples to be counted for both ^{14}C and ^{3}H radioactivity.

Membrane isolation and characterization

Plasma membranes and internal membranes from 8–16 g hindlimb muscles were isolated by the method described earlier by Klip *et al* (Klip *et al.*, 1987; Marette *et al.*, 1992). After centrifugation in sucrose gradient the membrane fractions floating on 25 and 35% sucrose were collected and stored at −70°C until used for Western blot, slot blot and protein assays and determination of 5'-nucleotidase activity. Protein content of membrane suspensions were assayed according to the method of Peterson (1977). 5'-Nucleotidase activity was measured as described by Aronson & Touster (1974).

The 25% sucrose fraction (plasma membrane fraction) was enriched in the plasma membrane marker 5'-nucleotidase activity 4.2–5.1 fold when compared with 35% sucrose fraction representing intracellular membranes (data not shown). This is in the same range as plasma membrane marker enrichment reported earlier by this method (Klip *et al.*, 1987).

Western blot and slot blot analyses

The specificity of GLUT1 and GLUT4 antibody binding on proteins of subcellular membrane fractions was determined by Western blot analysis. Membranes (10 μg of protein) were supplemented with 2.5% sodium dodecyl sulphate (SDS), electrophoresed on 12% polyacrylamide gels (PAGE) with Bio-Rad Mini-PROTEAN II system (Bio-Rad Laboratories Inc., Hercules, CA, U.S.A., buffer: 25 mM Tris, 192 mM glycine, 0.1% SDS), electrophoretically transferred to nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany, buffer: 48 mM Tris, 39 mM glycine, 20% methanol, 30 min) and air dried. Before primary antibody incubation, the filters were blocked for 1 h with 5% nonfat dry milk in buffer containing 50 mM Tris, 2 mM $CaCl_2$, 0.2% Nonidet P-40 and 0.02% NaN_3, pH 8 and washed in five changes of TBS-T (20 mM Tris, 137 mM NaCl, 0.2% Tween-20). The filters were incubated with GLUT1 or GLUT4 antibody (1:2000 dilution in TBS-T, East Acres Biologicals, Southbridge, MA, U.S.A.) for 1 h and washed again in five changes of TBS-T. The filters were then incubated with Horseradish peroxidase labelled second antibody (1:20000 dilution in TBS-T, Amersham, Buckinghamshire, U.K.) for 1 h and washed in seven changes of TBS-T. The immunolabelled bands were visualized by placing the membranes against Hyperfilm ECL (Amersham) after addition of a chemiluminescence reagent (ECL, Amersham).

The determination of GLUT1 and GLUT4 protein levels were carried out by slot blot hybridization manifold (Schleicher & Schuell): 1 μg of total membrane protein was loaded into each well in duplicate or triplicate, transferred to nitrocellulose filter membrane and air dried. The immunolabelling was carried out as described for Western blots. Slot blots were quantitated with a OS/2 based image analysis system (MCID, Imaging Research Inc., Ontario, Canada), and the results are expressed as relative optical density values per μg protein. Because the exposure times were very short, the values are not comparable between separate experiments.

Immunohistochemistry of GLUT1 and GLUT4 and plasma insulin and glucose

Tibialis muscles were cut on 8 μm sections at −23°C. Sections were thaw-mounted on gelatine-coated microscopic slides and fixed in −18°C acetone for 10 min. The immunostaining and detection was performed as described by Muona *et al.* (1992). The dilutions for antibodies were 1:250 for GLUT1 and 1:50 for GLUT4. In control reactions, the primary antibody was omitted.

Plasma insulin and glucose were analysed as in Rouru *et al.* (1995).

Drugs

Meftormin hydrochloride was a generous gift from Leiras OY, Turku, Finland. Insulin (Velosulin Human) was purchased from Nordisk Gentofte A/S, Gentofte Denmark.

Statistical analysis

Statistical analysis of the data was carried out by one-way analysis of variance (ANOVA) followed by Newman-Keuls procedure for multiple comparisons or by Student's t test for unpaired data. The 2-deoxyglucose uptake data were analysed by analysis of covariance (ANCOVA) the weight of the muscle strip being the covariate. If necessary, logarithmic transformation of the data was performed prior to analysis. The calculations were performed using BMDP software (BMDP Statistical Software Inc., Los Angles, CA, U.S.A.). A P-value less than 0.05 was considered statistically significant. The values presented are means ± s.e.mean.

Results

Food intake, body weights, plasma insulin and glucose

During the 12 day treatment the rats in the metformin group (both in the experiments 1 and 2, $P<0.05$) and the rats in the pair-fed group (in experiment 1, $P<0.05$) ate about 85% of the food consumed by the control group as described earlier in more detail (Rouru *et al.*, 1995). In experiment 1, body weight gains were reduced in the metformin-treated ($P<0.05$) and pair-fed ($P<0.05$) groups when compared with the control group (Rouru *et al.*, 1995). In experiment 2, the body weight gain was 1 ± 3 g in the metformin-treated group and 22 ± 2 g in the control group ($P<0.05$, values are means ± s.e.mean).

In experiment 1, fasting plasma insulin concentrations were 40% lower both in the metformin-treated ($P<0.05$) and pair-fed ($P<0.05$) rats when compared with the control rats (Rouru *et al.*, 1995). There were no significant differences in plasma glucose levels between the treatment groups (Rouru *et al.*, 1995).

In experiment 2, plasma insulin concentrations rose 10 fold after insulin injections when compared with the basal values in experiment 1, (1153 ± 79 and 1269 ± 223 ng ml^{-1}, in the metformin-treated and control rats, respectively), but there were no significant differences between the metformin-treated and control groups in this respect. Plasma glucose levels fell after insulin injection about 2 mmol l^{-1} when compared with experiment 1 but again there were no significant differences between the groups (5.7 ± 0.3 and 6.1 ± 0.5 mmol l^{-1} in the metformin-treated and control rats, respectively).

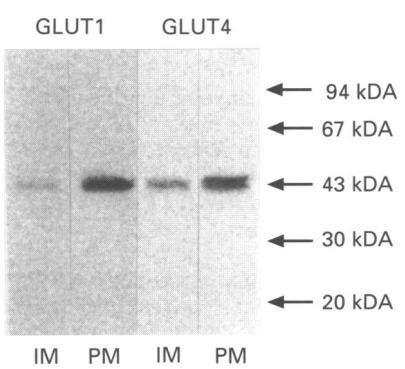

Figure 1 Representative Western blots of GLUT1 and GLUT4 from internal membrane (IM) and plasma membrane (PM) fractions of skeletal muscle: 10 μg membrane proteins were separated on 12% polyacrylamide gels, blotted onto nitrocellulose membrane and probed with GLUT1 or GLUT4 antibodies.

2-Deoxyglucose uptake

Insulin significantly increased 2-deoxyglucose uptake in muscle strips in all treatment groups (insulin effect: $P<0.0001$, adjusted cell means from ANCOVA were 239, 233 and 241 d.p.m. mg^{-1} wet weight when no insulin was added and 290, 304 and 285 d.p.m. mg^{-1} wet weight in insulin-stimulated conditions in the control, pair-fed and metformin-treated groups, respectively, $n=20$ in each group). However, there was no significant difference in 2-deoxyglucose uptake between the control, pair-fed and metformin-treated groups.

GLUT4 and GLUT1 protein contents in subcellular fractions

Representative Western blots from both subcellular fractions of hindlimb muscle preparations are shown in Figure 1. Because Western blotting resulted in a single band of approximately 43 kDa size with very little background we were able to perform the determination of GLUT1 and GLUT4 protein levels by slot blot method, which resulted in much less variation than Western blotting between duplicate samples and separate membranes.

In the basal state (experiment 1) and after insulin stimulation (experiment 2) there were no significant differences in the content of GLUT1 protein either in the plasma membrane or in internal membrane fractions in the separate treatment groups (Figure 2). Similarly, basal GLUT4 protein content

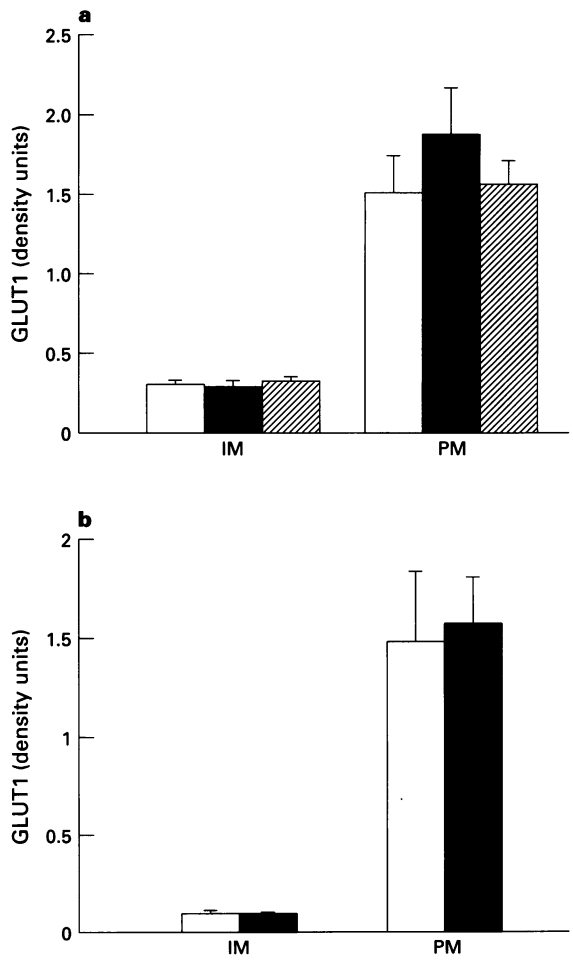

Figure 2 Relative GLUT1 protein levels in internal membrane (IM) and plasma membrane (PM) fractions of skeletal muscle in control (open columns), metformin-treated (solid columns) and pair-fed (hatched columns) obese, Zucker rats in the basal state (a) and after insulin-stimulation (b). Values are means ± s.e.mean, $n=10$ in each group.

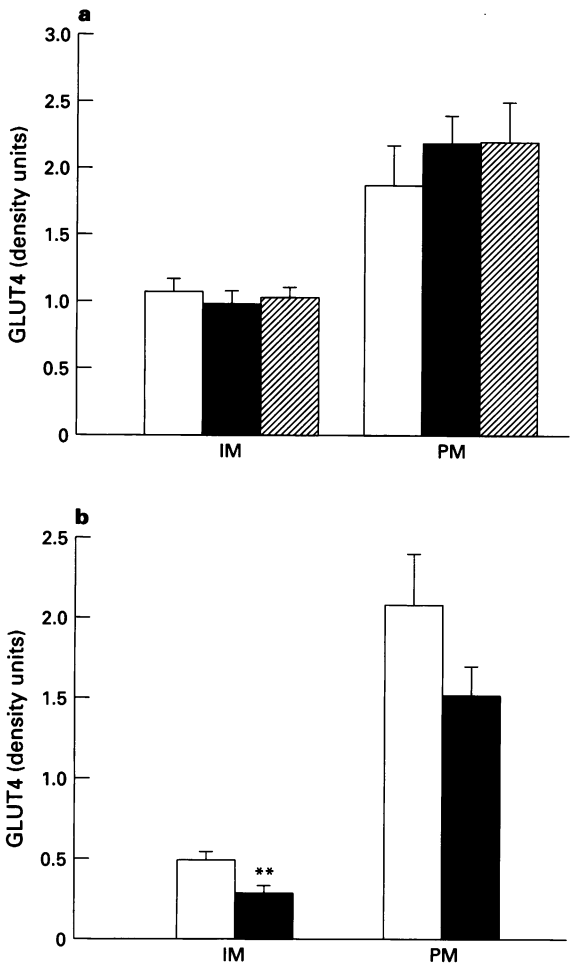

Figure 3 Relative GLUT4 protein levels in internal membrane (IM) and plasma membrane (PM) fractions of skeletal muscle in control (open columns), metformin-treated (solid columns) and pair-fed (hatched columns) obese, Zucker rats in the basal state (a) and after insulin stimulation (b). $^{**}P<0.01$, Student's t test for unpaired data. Values are means ± s.e.mean, $n=10$ in each group.

was altered neither in plasma nor in internal membrane fractions after metformin treatment or pair-feeding (Figure 3). After insulin stimulation, GLUT4 protein content was significantly lowered in internal membrane fraction of metformin-treated animals ($P<0.01$). However, in the plasma membrane fraction the change in GLUT4 content was not statistically significant ($P=0.15$, Figure 3).

Immunohistochemistry

Microscopic pictures of GLUT1- and GLUT4-labelled tibialis muscle sections from metformin-treated, control and pair-fed animals are presented in Figure 4. With both antibodies the plasma membranes appear rich in immunoreactive epitopes while they are less concentrated inside the muscle cells. However, individual variations in the staining of muscle sections were so great that no quantitative evaluation between treatment groups was considered appropriate. Importantly, it is evident that at least two distinct types of fibre coexist in the muscle sections. Irrespective of treatment, approximately half of the fibres show an intense staining whereas the other half were stained less.

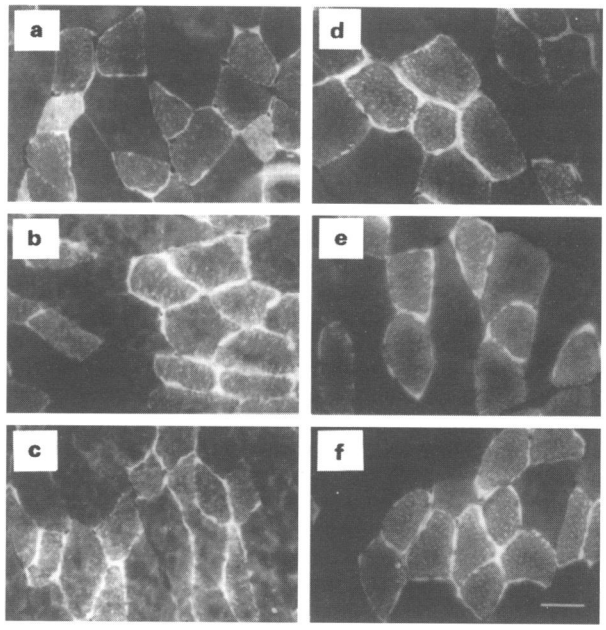

Figure 4 Immunohistochemical localization of GLUT4 (a-c) and GLUT1 (d-f) in transverse cryosections of tibialis muscles in control (a, d), metformin-treated (b, e) and pair-fed (c, f) obese, Zucker rat in the basal state. Bar = 20 μm.

Discussion

The main finding of the present study was that the contents of GLUT1 and GLUT4 proteins were not increased in the plasma membranes of skeletal muscle samples obtained either in the basal state or after insulin stimulation in obese, insulin-resistant, Zucker rats treated with metformin for 12 days. In line with these results we did not find differences in the uptake of 2-deoxyglucose in isolated muscle strips after metformin-treatment.

In an earlier *in vitro* study, in which cultured L6 muscle cells were used, metformin increased GLUT1 (but not GLUT4) protein content in plasma membrane fraction and decreased its content in the intracellular pool both in basal and in insulin-stimulated conditions (Hundal *et al.*, 1992). In our study, there was a clear reduction of GLUT4 protein concentration in the internal membrane fraction after insulin stimulus, and also a nonsignificant decline in the plasma membrane fraction. The design of the present study does not provide an explanation for this reduction. The possibility that differences in circulating insulin concentrations could mask effects of metformin on glucose transport molecules was controlled in our study by the presence of the pair-fed control group. Indeed, plasma insulin concentrations were very similar in the metformin-treated and pair-fed control groups.

Our study is the first to address the effect of metformin on translocation of glucose transporter proteins in skeletal muscle during *in vivo* treatment. In a study where white adipose tissue was used, *in vivo* metformin treatment increased insulin stimulated translocation of both GLUT1 and GLUT4 from the intracellular pool to the plasma membrane in obese Zucker rats (Matthaei *et al.*, 1993). This effect was not evident in obese rats in the basal state or in lean Zucker rats either in the basal or insulin-stimulated state (Matthaei *et al.*, 1993). Thus, glucose transporters seem to be regulated differentially after metformin-treatment in L6 muscle cells, in white fat and in skeletal muscles obtained from Zucker rats. This does not necessarily conflict with the data in our study, because glucose uptake in muscle and adipose tissue is known to be regulated in a different manner during the development of insulin resistance in Zucker rats (Pénicaud *et al.*, 1987). However, due to its major role in whole body glucose handling, skeletal muscle is of special importance in this respect.

The same GLUT1 and GLUT4 antibodies were used in the determination of muscle glucose transporter levels and in the immunohistochemical labelling of the muscle cryosections. The results from the immunohistochemistry suggest that structures with a physiologically relevant subcellular distribution were recognized with the antibodies. Because the immunohistochemical labelling of muscle sections with glucose transporter antibodies demonstrate that muscle fibres appear to stain differentially, it is supposed that well-stained muscle fibres represent oxidative muscle fibres and weakly stained fibres represent glycolytic fibres as demonstrated earlier (Marette *et al.*, 1992).

The ability of metformin to improve insulin-mediated glucose utilization in insulin-resistant patients and rats is well established by glucose clamp studies (Rossetti *et al.*, 1990; Bailey, 1993). In line with this we found reduced fasting plasma insulin concentrations in metformin-treated rats when compared to control animals without alterations in the glycaemic status of the rats, which indirectly suggests improved insulin sensitivity in these insulin-resistant animals. However, we did not find differences in the uptake of 2-deoxyglucose in isolated muscle strips obtained from metformin-treated, pair-fed or control animals either in basal state or after insulin stimulation, suggesting that metformin-treatment did not influence the ability of the muscle to take up glucose. Because no metformin was added to the incubation medium, the results primary reflect the effects of chronic exposure of the tissue to metformin; direct addition of metformin into the incubation medium could have resulted in a different effect. Nevertheless, it seems clear that metformin does not have any sustaining effect on glucose transport into skeletal muscle cells obtained from obese Zucker rats.

These obese Zucker rats are hyperinsulinaemic and insulin-resistant. However, they are not markedly hyperglycaemic in the basal state. The insulin-sensitizing action of metformin is demonstrable both in hyperglycaemic NIDDM patients (Bailey, 1993) and in normoglycaemic insulin-resistant, hypertensive patients (Landin *et al.*, 1991), but not in healthy individuals with normal insulin sensitivity (Landin *et al.*, 1994). Thus, the effects of metformin on insulin-sensitivity are specific merely to insulin-resistant states rather than hyperglycaemic states. The rationale for studying the effects of metformin in normoglycaemic insulin-resistant animals instead of hyperglycaemic animals is that the expected changes in glycaemia caused by metformin-treatment could *per se* alter transporter content in muscle membranes (Dimitrakoudis *et al.*, 1992). However, it remains possible that some of the results presented here would be different if hyperglycaemic animals were used.

In conclusion, the present study does not support the concept that the mechanism of action of metformin *in vivo* is based on increased glucose uptake and translocation of glucose transporter proteins in skeletal muscle *per se*.

This study was financially supported by the Medical Research Council of the Academy of Finland, the Finnish Medical Society Duodecim, Alfred Kordelin Foundation, Research and Science Foundation of Farmos, Pharmacal Research Foundation, Leiras Research Foundation and Turku University Foundation which are gratefully acknowledged. We thank Dr Anne Marjamäki for valuable advice concerning Western blot techniques and Dr Kaija Pyykkö and Dr Markku Ahotupa for the 5'-nucleotidase assays. We are also grateful to Ms Helena Hakala, Ms Ulla Heikonen, Ms Raija Kaartosalmi, Ms Elina Kahra and Ms Taina Lehti for their skilful technical assistance.

References

ARONSON, N.N.J. & TOUSTER, O. (1974). Isolation of rat liver plasma membrane fragments in isotonic sucrose. In *Methods in Enzymology*. ed. Fleisher, S. & Packer, L. pp. 90–102. New York: Academic Press.

BAILEY, C.J. (1993). Metformin–an update. *Gen. Pharmacol.*, **24,** 1299–1309.

BAILEY, C.J. & PUAH, J.A. (1986). Effect of metformin on glucose metabolism in mouse soleus muscle. *Diabetes Metab.*, **12,** 212–218.

BRAY, G.A., YORK, D.A. & FISLER, J.S. (1989). Experimental obesity: a homoeostatic failure due to defective nutrient stimulation of the sympathetic nervous system. *Vitam. Horm.*, **45,** 1–125.

CRETTAZ, M., PRENTKI, M., ZANINETTI, D. & JEANRENAUD, B. (1980). Insulin resistance in soleus muscle from obese Zucker rats. *Biochem. J.*, **186,** 525–534.

DIMITRAKOUDIS, D., VRANIC, M. & KLIP, A. (1992). Effects of hyperglycaemia on glucose transporters of the muscle: use of the renal glucose reabsorption inhibitor phlorizin to control glycaemia. *J. Am. Soc. Nephrol.*, **3,** 1078–1091.

HANDBERG, A., KAYSER, L., HOYER, P.E., MICHEELSEN, J. & VINTEN, J. (1994). Elevated GLUT 1 level in crude muscle membranes from diabetic Zucker rats despite a normal GLUT 1 level in perineural sheaths. *Diabetologia*, **37,** 443–448.

HANDBERG, A., KAYSER, L., HOYER, P.E., VOLDSTEDLUND, M., HANSEN, H.P. & VINTEN, J. (1993). Metformin ameliorates diabetes but does not normalize the decreased GLUT 4 content in skeletal muscle of obese (fa/fa) Zucker rats. *Diabetologia*, **36,** 481–486.

HUNDAL, H.S., RAMLAL, T., REYES, R., LEITER, L.A. & KLIP, A. (1992). Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. *Endocrinology*, **131,** 1165–1173.

KLIP, A., RAMLAL, T., YOUNG, D.A. & HOLLOZY, J.O. (1987). Insulin-induced translocation of glucose transporters in rat hindlimb muscles. *FEBS Lett.*, **224,** 224–230.

LANDIN, K., TENGBORN, L. & SMITH, U. (1991). Treating insulin resistance in hypertension with metformin reduces both blood pressure and metabolic risk factors. *J. Int. Med.*, **229,** 181–187.

LANDIN, K., TENGBORN, L. & SMITH, U. (1994). Metformin and metoprolol CR treatment in non-obese men. *J. Int. Med.*, **235,** 335–341.

MARETTE, A., RICHARDSON, J.M., RAMLAL, T., BALON, T.W., VRANIC, M., PRESSIN, J.E. & KLIP, A. (1992). Abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. *Am. J. Physiol.*, **263,** C443–C452.

MATTHAEI, S., REIBOLD, J.P., HAMANN, A., BENECKE, H., HÄRING, H.U., GRETEN, H. & KLEIN, H.H. (1993). In vivo metformin treatment ameliorates insulin resistance: Evidence for potentiation of insulin-induced translocation and increased functional activity of glucose transporters in obese (fa/fa) Zucker rat adipocytes. *Endocrinology*, **133,** 304–311.

MUECKLER, M. (1994). Facilitative glucose transporters. *Eur. J. Biochem.*, **219,** 713–725.

MUONA, P., SOLLBERG, S., PELTONEN, J. & UITTO, J. (1992). Glucose transporters of rat peripheral nerve. *Diabetes*, **41,** 1587–1596.

PÉNICAUD, L., FERRÉ, P., TERRETAZ, J., KINEBANYAN, M.F., LETURQUE, A., DORÉ, E., GIRARD, J., JEANRENAUD, B. & PICON, L. (1987). Development of obesity in Zucker rats: Early insulin resistance in muscles but normal sensitivity in white adipose tissue. *Diabetes*, **36,** 626–631.

PETERSON, G.L. (1977). A simplification of the protein assay method of Lowry *et al.* which is generally more applicable. *Anal. Biochem.*, **83,** 346–356.

ROSSETTI, L., DEFRONZO, R.A., GHERZI, R., STEIN, P., ANDRAGHETTI, G., FALZETTI, G., SHULMAN, G.I., KLEIN-ROBBENHAAR, E. & CORDERA, R. (1990). Effect of metformin treatment on insulin action in diabetic rats: In vivo and in vitro correlations. *Metabolism*, **39,** 425–435.

ROURU, J., HUUPPONEN, R., SANTTI, E. & KOULU, M. (1993). Effect of subchronic metformin treatment on macronutrient selection in genetically obese Zucker rats. *Pharmacol. Toxicol.*, **72,** 300–303.

ROURU, J., PESONEN, U., KOULU, M., HUUPPONEN, R., SANTTI, E., ROUVARI, T. & JHANWAR-UNIYAL, M. (1995). Anorectic effect of metformin in obese Zucker rats: lack of evidence for the involvement of neuropeptide Y. *Eur. J. Pharmacol.*, **273,** 99–106.

SEELY, B.L. & OLEFSKY, J.M. (1993). Potential cellular and genetic mechanisms for insulin resistance in the common disorders of diabetes and obesity. In *Insulin Resistance*. ed. Moller, D.E. pp. 187–252. Chichester: John Wiley & Sons.

SHEPHERD, P.R. & KAHN, B.B. (1993). Cellular defects in glucose transport: Implications for human insulin resistance. In *Insulin Resistance*. ed. Moller, D.E. pp. 253–300. Chichester, John Wiley and Sons.

YKI-JÄRVINEN, H. (1993). Action of insulin on glucose metabolism in vivo. *Baillieres Clin. Endocrinol. Metab.*, **7**, 903–927.

YKI-JÄRVINEN, H. (1994). Pathogenesis of non-insulin-dependent diabetes mellitus. *Lancet*, **343**, 91–95.

(Received January 27, 1995
Revised April 7, 1995
Accepted April 21, 1995)