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Effect of diabetes and fasting on GLUT-4 (muscle/fat) glucose-transporter expression in insulin-sensitive tissues

Heterogeneous response in heart, red and white muscle

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1. GLUT-4 glucose-transporter protein and mRNA levels were assessed in heart, red muscle and white muscle, as well as in brown and white adipose tissue from 7-day streptozotocin-induced diabetic and 48 h-fasted rats. 2. In agreement with previous data, white adipose tissue showed a substantial decrease in GLUT-4 mRNA and protein levels in response to both diabetes and fasting. Similarly, GLUT-4 mRNA and protein markedly decreased in brown adipose tissue in both insulinopenic conditions. 3. Under control conditions, the level of expression of GLUT-4 protein content differed substantially in heart, red and white skeletal muscle. Thus GLUT-4 protein was maximal in heart, and red muscle had a greater GLUT-4 content compared with white muscle. In spite of the large differences in GLUT-4 protein content, GLUT-4 mRNA levels were equivalent in heart and red skeletal muscle. 4. In heart, GLUT-4 mRNA decreased to a greater extent than GLUT-4 protein in response to diabetes and fasting. In contrast, red muscle showed a greater decrease in GLUT-4 protein than in mRNA in response to diabetes or fasting, and in fact no decrease in GLUT-4 mRNA content was detectable in fasting. On the other hand, preparations of white skeletal muscle showed a substantial increase in GLUT-4 mRNA under both insulinopenic conditions, and that was concomitant to either a modest decrease in GLUT-4 protein in diabetes or to no change in fasting. 5. These results indicate that (*a*) the effects of diabetes and fasting are almost identical and lead to changes in GLUT-4 expression that are tissue-specific, (*b*) white adipose tissue, brown adipose tissue and heart respond similarly to insulin deficiency by decreasing GLUT-4 mRNA to a larger extent than GLUT-4 protein, and (*c*) red and white skeletal muscle respond to insulinopenic conditions in a heterogeneous manner which is characterized by enhanced GLUT-4 mRNA/protein ratios.

INTRODUCTION

Experimental diabetes and fasting are characterized by marked modifications in the effect of insulin to stimulate glucose uptake in peripheral tissues. Thus, in streptozotocin-induced diabetes a decrease in insulin-stimulated glucose disposal has been reported in isolated adipocytes [1–3], perfused heart [4], isolated cardiac myocytes [5] and skeletal muscle [6–8]. In fasting, there is also an impaired stimulation of glucose transport in response to insulin in adipocytes [9–11] and heart [4]. However, an increased sensitivity and responsiveness to insulin has been reported in perfused and incubated preparations of skeletal muscle from fasted rodents [12–15].

Tissues which acutely respond to insulin by increasing glucose uptake express at least two glucose transporter isoforms, i.e., type GLUT-1 (erythrocyte/Hep G2) and type GLUT-4 (muscle/fat) [16–21]. On the basis of studies performed in isolated rat adipocytes, GLUT-1 transporters play a major role in glucose uptake under basal conditions. GLUT-4 transporters, which represent the vast majority of glucose carriers expressed in rat adipocytes, are responsible for the stimulation of glucose transport observed in response to insulin [20].

Recently published data have suggested that the level of GLUT-4 expression determines the maximal effect of insulin on glucose transport. Thus, diabetes leads to a marked decrease in GLUT-4 expression in skeletal muscle and adipose tissue by suppression at a pretranslational step [22–28], paralleling insulin action in consequence. In addition, the greater content of GLUT-4 glucose transporters in red muscle compared with white muscle has been reported [29,30], also in parallel to the maximal effect of insulin on glucose transport [31].

At present, no information is available about the effect of insulinopenic conditions on GLUT-4 protein content in insulin-sensitive tissues such as heart or brown adipose tissue. Furthermore, it is also unknown whether red and white muscle respond in a similar fashion to diabetes or fasting. In order to determine whether there is a similar regulation of GLUT-4 expression in response to insulinopenic states in all insulin-sensitive tissues, we have investigated the alterations in GLUT-4 mRNA and protein from heart, red muscle, white muscle, brown and white adipose tissue, in response to diabetes and fasting.

METHODS

Materials

^{125}I-labelled goat anti-mouse IgG and Hybond N were purchased from Amersham. Random-priming DNA-labelling kit was from Boehringer. Immobilon was obtained from Millipore. All electrophoresis reagents, molecular-mass markers and reagents for protein assay were obtained from Bio-Rad. γ-Globulin and most commonly used chemicals were from Sigma.

Animals and tissue sampling

Male Wistar rats (150–200 g) obtained from our own colony were used. The rats were fed with Purina Laboratory chow *ad*

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libitum and housed in animal quarters maintained at 22 °C with a 12 h-light/12 h-dark cycle. Diabetes was produced by an intraperitoneal injection of streptozotocin (70 mg/kg body wt.) 1 week before the study. Some animals were fasted for 48 h before the experimental day. All animals were anaesthesized with sodium pentobarbital (5–7 mg/100 g body wt.), and epididymal fat-pads, heart, interscapular brown adipose tissue and red and white skeletal muscle were rapidly collected. Red muscle consisted of pooled soleus, red portions of the gastrocnemius and quadriceps muscles, and similarly white portions of the gastrocnemius and quadriceps were pooled as the source of white muscle. On the basis of previous studies, documenting fibre composition in different muscles of the rat [32,33], the red muscle fraction consists primarily of fast-twitch oxidative fibres and the white muscle largely of fast-twitch glycolytic fibres. After collection, tissues were rapidly frozen and kept in liquid nitrogen until analysis.

Preparation of membrane fractions from tissues

Tissues were homogenized in 10 vol. of ice-cold buffer containing 25 mM-Hepes, 250 mM-sucrose, 4 mM-EDTA, 1 trypsin-inhibitory unit of aprotinin/ml, 25 mM-benzamidine, 0.2 mM-phenylmethanesulphonyl fluoride, 1 μM-leupeptin and 1 μM-pepstatin, pH 7.4. Homogenates from white adipose tissue, brown adipose tissue and heart were centrifuged at 5000 *g* for 5 min at 4 °C. The supernatant was then centrifuged at 150000 *g* for 2 h at 4 °C to obtain the membrane fractions. The homogenates from red and white muscle were centrifuged at 15000 *g* for 20 min at 4 °C. The supernatants were adjusted to 0.8 M-KCl, incubated at 4 °C for 30 min, and then centrifuged for 90 min at 200000 *g* at 4 °C to obtain the membranes. The membrane pellets were resuspended in homogenization buffer and repeatedly passed through a 25-gauge needle before storage at −20 °C. Proteins were measured by the method of Bradford [34], with γ-globulin as a standard.

Electrophoresis and immunoblotting of membranes

SDS/PAGE was performed on membrane protein by the method of Laemmli [35]. Proteins were transferred to Immobilon as previously reported [20] in buffer consisting of 20% (v/v) methanol, 200 mM-glycine and 25 mM-Tris, pH 8.3. After transfer, the filters were blocked with 5% (w/v) non-fat dry milk/0.02% NaN_3 in phosphate-buffered saline (134 mM-NaCl/2.6 mM-KCl/6.4 mM-Na_2HPO_4/1.46 mM-KH_2PO_4, pH 7.4) for 1 h at 37 °C and were incubated with antibody 1F8 [17] for the same time at the same temperature. Transfer was confirmed by Coomassie Blue staining of the gel after the electroblot. Antibody 1F8 purified by Protein A chromatography (kindly given by Dr. Paul F. Pilch) was used at 5–10 μg/ml in 1% non-fat dry milk/0.02% NaN_3 in phosphate-buffered saline for immunoblotting. Detection of antibody–antigen complexes was effected with goat anti-mouse ^{125}I-labelled antibody and autoradiography. The autoradiograms were quantified by using scanning densitometry (Ultrascan × L enhancer laser densitometer; LKB). Immunoblots were performed under conditions in which autoradiographic detection was in the linear-response range, and data were expressed as percentages of control values.

RNA isolation and Northern-blot analysis

Total RNA from heart, adipose tissues, red and white muscle was extracted by the acid guanidinium isothiocyanate/phenol/chloroform method as described by Chomczynski & Sacchi [36]. All samples had an A_{260}/A_{280} ratio above 1.7.

After quantification, total RNA (15–30 μg) was denatured at 65 °C in the presence of formamide, formaldehyde and ethidium bromide [37] to allow observation of RNA. RNA was separated on a 1.2%-agarose/formaldehyde gel and blotted on Hybond N filters. The RNA in gels and filters was detected with ethidium bromide and photographed by u.v. trans-illumination to ensure the integrity of RNA, to check the loading of equivalent amounts of total RNA and to confirm proper transfer. RNA was transferred in 10 × standard saline citrate (SSC; 0.15 M-NaCl/0.015 M-sodium citrate, pH 7.0).

Blots were initially prehybridized for 4 h at 45 °C in 50% (v/v) formamide/5 × Denhardt's (1 × Denhardt's solution is 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% BSA)/0.5% SDS/5 × SSPE (1 × SSPE is 0.15 M-NaCl, 1 mM-EDTA and 10 mM-NaH_2PO_4, pH 7.4) and 100 μg of denatured salmon sperm DNA/ml. The blots were then hybridized to the corresponding probes for 12 h at 42 °C in 50% formamide/5 × Denhardt's/0.5% SDS/5 × SSPE/10% dextran sulphate and

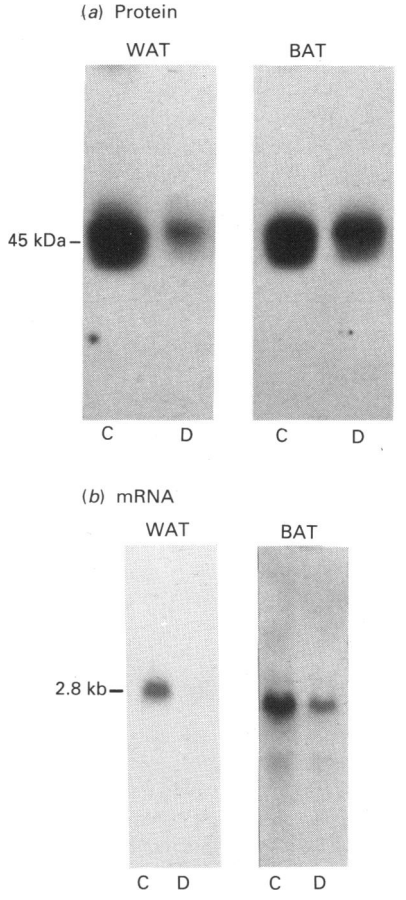

Fig. 1. Effect of diabetes on the expression of GLUT-4 protein and mRNA in white and brown adipose tissue

Total RNA and membrane proteins were purified from epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) obtained from control (C) and 7-day streptozotocin-induced diabetic rats (D); 200 μg of membrane proteins or 15 μg of total RNA from control or diabetic groups was applied on gels. After blotting, GLUT-4 protein was detected by incubation with monoclonal antibody 1F8 (*a*). GLUT-4 mRNA was detected after hybridization with a 2007-base-pair *Sal*I fragment as a cDNA probe (*b*) (see the Methods section). Representative autoradiograms, obtained after various times of exposure, from three to five separate experiments are shown.

100 μg of denatured salmon sperm DNA/ml. The cDNA probe for GLUT-1 is a 1346-base-pair *Eco*RI fragment, and the cDNA probe for GLUT-4 is a 2007-base-pair *Sal*I fragment. Both cDNA probes were obtained from Dr. Graeme I. Bell (University of Chicago). The cDNA probe for β-actin is a 4500-base-pair *Hin*dIII and *Eco*RI fragment [38]. The cDNA probes were labelled with [^{32}P]CTP by random oligonucleotide-priming. The probes were included at 1.5×10^6 c.p.m./ml. Filters from GLUT-1 and GLUT-4 assays were washed for 15 min in $2 \times$ SSC at room temperature, and then twice in $0.4 \times$ SSC/0.1 % SDS (first wash for 20 min and second wash for 30 min) at 55 °C. Filters from β-actin assay were washed once for 30 min in $0.1 \times$ SSC/0.1 % SDS at 55 °C. The abundance of specific glucose transporter or β-actin mRNA was quantified by scanning densitometry of autoradiograms as described above, and data were expressed as percentages of control values.

RESULTS

Effect of diabetes and fasting on GLUT-4 expression in white and brown adipose tissue

It has been previously reported that diabetes and fasting cause a profound decrease in the expression of GLUT-4 mRNA and protein in adipose tissue and isolated rat adipocytes [22–25]; this is a selective effect, since no changes were reported in GLUT-1 expression [22–25]. Therefore, we initially investigated the comparative alterations caused by these insulinopenic conditions in white and brown adipose tissue. In keeping with previous data, 7-day streptozotocin-induced diabetes led to a marked decrease in GLUT-4 protein, as assessed by Western blotting using the specific monoclonal antibody 1F8 [17], both in white and brown adipose tissue (Fig. 1). Nevertheless, the extent of the decrease was greater in white adipose tissue than in brown adipose tissue (values in diabetes, expressed per μg of protein, were 27 ± 8 % and 59 ± 5 % of control levels in white and brown adipose tissue respectively; $n = 4$). The presence of GLUT-4 mRNA was determined by Northern-blot methodology using a human cDNA probe [39] at high stringency conditions. Thus Northern-blot analysis of brain tissue, which does not express GLUT-4, did not reveal any labelling (results not shown). Under these conditions, GLUT-4 mRNA markedly decreased in white and brown adipose tissue in response to diabetes (Fig. 1) and changes were greater in white adipose tissue (values in diabetes, expressed per μg of total RNA, were 11 ± 2 % of control values; $n = 3$) than in brown adipose tissue (values expressed per μg of RNA were 28 ± 9 % of control values; $n = 3$). mRNA levels for β-actin markedly decreased in white adipose tissue from diabetic rats (60 ± 16 % and 36 ± 10 % of control values in white adipose tissue and brown adipose tissue respectively, when data were expressed per μg of RNA; $n = 3$), so its use as a housekeeping mRNA was avoided for these tissues.

We also investigated the changes associated with fasting, a situation which is characterized by low circulating insulin and glucose concentrations. GLUT-4 protein diminished in white and brown adipose tissue (Fig. 2) (values, expressed per μg of protein, were 9 ± 5 % and 53 ± 5 % of control levels in white and brown adipose tissue respectively; $n = 5$). Under these conditions, GLUT-4 mRNA levels also diminished in white and brown adipose tissue (Fig. 2), and changes were greater in white adipose tissue (values expressed per μg of RNA, from two

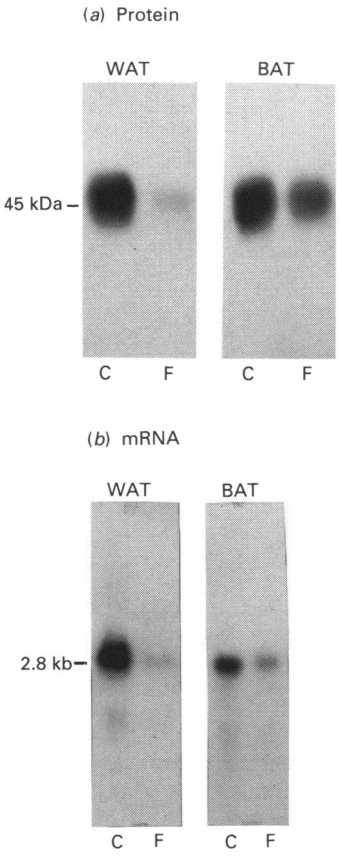

Fig. 2. Effect of fasting on the expression of GLUT-4 protein and mRNA in white and brown adipose tissue

Total RNA and membrane proteins were purified from epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) obtained from control (C) and 48 h-fasted rats (F), and GLUT-4 protein (*a*) and mRNA (*b*) were determined as described in the Methods section and in the legend to Fig. 1. Representative autoradiograms, obtained after various times of exposure, from two to five separate experiments are shown.

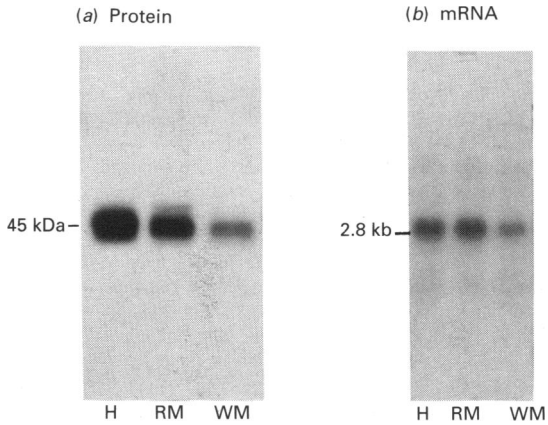

Fig. 3. Comparative expression of protein and mRNA for GLUT-4 in heart, red and white muscle from control rats

Total RNA and membrane proteins were purified from heart (H), red (RM) and white (WM) skeletal muscle obtained from control rats; 50 μg of heart membrane proteins or 200 μg of proteins from red and white muscle was applied on gels for Western blots, and 15 μg of total RNA from heart, red and white muscle was applied on gels for Northern blots. GLUT-4 protein (*a*) and mRNA (*b*) were determined as described in the Methods section and the legend to Figure 1. Representative autoradiograms, obtained after various times of exposure, from three to five separate experiments are shown.

Table 1. GLUT-4 protein and mRNA and GLUT-1 mRNA levels in muscles under control conditions

Total RNA and membrane proteins were purified from heart, red and white skeletal muscle obtained from control rats. GLUT-4 protein, GLUT-4 mRNA and GLUT-1 mRNA were determined as described in the Methods section and legend to Fig. 1. Autoradiographs were subjected to scanning densitometry. The results of three to seven separate experiments (means ± S.E.M.) are shown, and expressed as percentages of values detected in heart: * indicates a significant difference from heart tissue, at $P < 0.05$; † indicates a significant difference between red and white muscle, at $P < 0.05$.

	Red muscle	White muscle	Heart
GLUT-4 protein (units/μg of protein)	9.2±2.7*	4.0±1.4*†	100
GLUT-4 mRNA (units/μg of RNA)	101.7±2.7	76.7±1.8†	100
GLUT-4 mRNA/ protein ratio	11.1±0.3*	19.2±0.5*†	1
GLUT-1 mRNA (units/μg of RNA)	55±5*	45±6*	100

Table 2. Effect of diabetes and fasting on tissue contents of RNA and membrane protein in heart, red and white muscle

Data on total RNA and membrane protein extractable from heart, red and white muscle from control, 7-day streptozotocin-induced diabetic and 48 h fasted rats are expressed as mg per g of tissue; for details see the Methods section. Results are means ± S.E.M. of 6–12 observations per group: * indicates significant differences between control and experimental groups, at $P < 0.05$.

	Control	Diabetes	Fasting
Heart			
Protein	11.8±1.35	11.4±2.4	10.5±1.8
RNA	1.19±0.10	0.91±0.11	0.99±0.11
Red muscle			
Protein	7.1±0.6	6.6±0.9	6.4±1.9
RNA	1.04±0.05	0.85±0.05*	0.87±0.03*
White muscle			
Protein	8.0±0.7	6.5±1.1	10.3±1.3
RNA	0.89±0.03	0.71±0.06*	0.57±0.03*

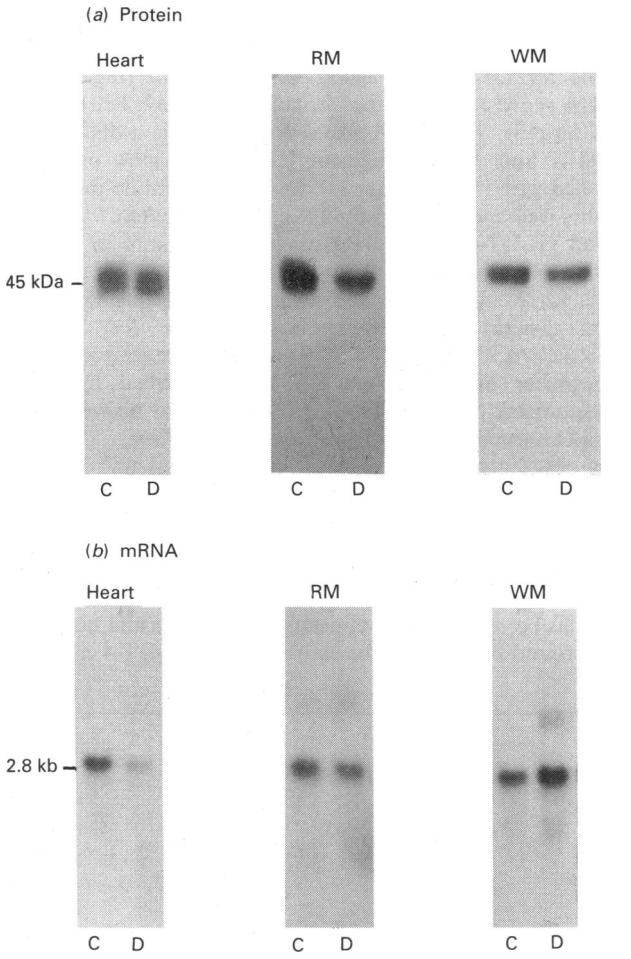

Fig. 4. Effect of diabetes on GLUT-4 mRNA and protein levels in heart, red and white muscle

Total RNA and membrane proteins were purified from heart, red (RM) and white (WM) portions of skeletal muscle obtained from control (C) and 7-day streptozotocin-induced diabetic rats (D); 50 μg of heart membrane proteins or 200 μg of proteins from red and white muscle was applied on gels for Western blots, and 15 μg of total RNA from heart, red and white muscle was applied on gels for Northern blots. GLUT-4 protein (*a*) and mRNA (*b*) levels were determined as described in the Methods section and the legend to Fig. 1. Representative autoradiograms, obtained after various times of exposure, from five to eight separate experiments are shown.

independent observations, were 1 and 2% of control values) than in brown adipose tissue (values expressed per μg of RNA, from two independent observations, were 20 and 30% of control values). β-Actin mRNA levels also decreased in response to fasting (values in fasting, expressed per μg of RNA, were 19% and 47% of control values in white and brown adipose tissue respectively).

GLUT-4 expression in heart, red and white muscle

Next, we investigated the expression pattern of the GLUT-4 glucose transporter in muscle tissues as well as its alterations in diabetes and fasting. Under basal conditions, GLUT-4 protein was expressed to a greater level in heart muscle than in skeletal-muscle tissue, and there were also wide differences in GLUT-4 expression between red and white skeletal muscle (Fig. 3 and Table 1). These differences were not due to a differential yield of GLUT-4-containing membranes in muscles and, in fact, identical results were obtained when GLUT-4 protein was determined from crude solubilized extracts (results not shown).

Differences in the amount of GLUT-4 protein between heart and red muscle occurred in the absence of differences in GLUT-4 mRNA (Fig. 3 and Table 1), whereas GLUT-4 mRNA content was lower in white muscle (Fig. 3). As a corollary of all the above, broad differences in GLUT-4 mRNA/protein ratio were detected among muscle tissues, so the heart showed much lower mRNA/protein ratios compared with red and white skeletal muscle (Table 1).

mRNA levels for GLUT-1 were very low in heart, red and white muscle (indistinguishable under conditions that allow optimal measurement of GLUT-1 mRNA levels from rat brain). Analysis of GLUT-1 mRNA values, obtained by long exposure of autoradiographs, revealed a greater concentration in heart

Table 3. Effect of diabetes and fasting on GLUT-4 mRNA and protein in heart, red and white muscle

Total RNA and membrane proteins were purified from heart, red and white skeletal muscle obtained from control, streptozotocin-induced diabetic and 48 h-fasted rats. GLUT-4 protein, GLUT-4 mRNA, GLUT-1 mRNA and β-actin mRNA were determined as described in the Methods section and the legend to Fig. 1. Autoradiographs were subjected to scanning densitometry. The results of four to seven separate experiments (means ± S.E.M.) are shown, except for β-actin mRNA in fasted rats, which represents mean of two values (individual values in parentheses). Data are expressed as percentages of control values: * indicates a significant difference between control and experimental groups, at $P < 0.05$.

	Control	Diabetic	Fasted
Red skeletal muscle			
GLUT-4 mRNA	100	74±9*	114±23
GLUT-4 protein	100	52±11*	66±12*
β-Actin mRNA	100	119±7	111±23
White skeletal muscle			
GLUT-4 mRNA	100	194±36*	341±94*
GLUT-4 protein	100	65±11*	110±16
β-Actin mRNA	100	95±14	67 (64, 69)
Heart			
GLUT-4 mRNA	100	40±13*	31±2*
GLUT-4 protein	100	79±10*	76±10*
β-Actin mRNA	100	34±16*	49±27*
GLUT-1 mRNA	100	44±8*	58±9*

than in red or white muscle (Table 1). The very low expression of GLUT-1 mRNA in skeletal muscles precluded its study in further experiments.

Effect of diabetes on GLUT-4 expression in skeletal muscle and heart

In view of the marked differences among muscle tissues, we studied the effect of 7 days of streptozotocin-induced diabetes separately in heart, red and white skeletal muscle. No differences were detected in the yield of membrane protein obtained per g of tissue in heart, red or white muscle from control and diabetic rats (Table 2). However, a decrease of approx. 20% in the yield of total RNA was obtained in red and white muscle in response to diabetes (Table 2).

Streptozotocin-induced diabetes caused a marked fall in GLUT-4 protein levels in red and white muscle (Fig. 4 and Table 3). In contrast, red and white muscle differed in the response of their GLUT-4 mRNA levels to diabetes. Thus diabetes caused a decrease in GLUT-4 mRNA content in red muscle (Fig. 4 and Table 3), in the presence of unaltered levels of β-actin mRNA (Table 3). However, levels of GLUT-4 mRNA were greater in white muscle from diabetic rats compared with controls (Fig. 4 and Table 3), and this again occurred in the absence of changes in β-actin mRNA levels (Table 3). This overall pattern of changes in GLUT-4 mRNA was evident when data were expressed either per μg of total RNA or per g of tissue (results not shown).

Heart responded to diabetes in a differential manner compared with that in skeletal muscles. Levels of mRNA for GLUT-4, GLUT-1 and β-actin, expressed per μg of RNA, were markedly decreased in streptozotocin-induced diabetes (Fig. 4 and Table 3). This occurred in the absence of changes in the yield of RNA extractable per g of tissue (Table 2). In spite of the large decrease in GLUT-4 mRNA levels, tissue content of GLUT-4 protein in diabetes was decreased by only 21% compared with controls (Fig. 4 and Table 3). This heart-specific GLUT-4 protection observed in response to large changes in GLUT-4 mRNA during experimental diabetes is not just due to the fact that the heart is a working muscle. Thus we found that in the diaphragm, another constantly working muscle, diabetes caused a marked decrease in GLUT-4 protein content (results not shown).

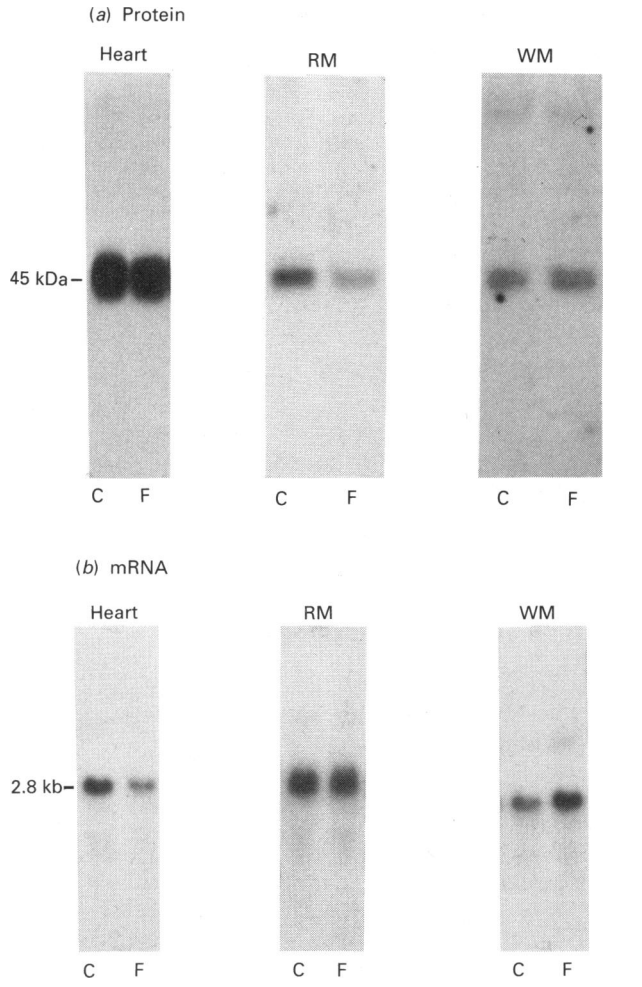

Fig. 5. Effect of fasting on GLUT-4 mRNA and GLUT-4 protein in heart, red and white muscle

Total RNA and membrane proteins were purified from heart, red (RM) and white (WM) skeletal muscle obtained from control (C) and 48 h-fasted rats (F); 50 μg of heart membrane protein or 200 μg of proteins from red and white muscle was applied on gels for Western blots, and 15 μg of total RNA from heart, red and white muscle was applied on gels for Northern blots. GLUT-4 protein (*a*) and mRNA (*b*) were determined as described in the Methods section and the legend to Fig. 1. Representative autoradiograms, obtained after various times of exposure, from five to six separate experiments are shown.

Effect of fasting on GLUT-4 expression in skeletal muscle and heart

Fasting for 48 h did not alter GLUT-4 or β-actin mRNA content in red muscle, but caused a marked decrease in GLUT-4 protein (Fig. 5 and Table 3). The pattern obtained in response to fasting was the same whether data were expressed per μg of

Table 4. Effect of diabetes and fasting on GLUT-4 mRNA/GLUT-4 protein ratio in insulin-sensitive tissues

GLUT-4 mRNA content expressed as units/μg of RNA and GLUT-4 protein expressed as units/μg of protein (data from Figs. 1 and 2 and Table 3) were recalculated as a ratio between GLUT-4 mRNA and GLUT-4 protein (mean ± S.E.M.). Ratios in diabetic and fasted groups were expressed as percentages of control values: * indicates a significant difference between control and experimental groups, at $P < 0.05$.

	Control	Diabetic	Fasted
White adipose tissue	1.0	0.40±0.07*	0.23±0.10*
Brown adipose tissue	1.0	0.47±0.15*	0.47±0.12*
Heart	1.0	0.50±0.16*	0.41±0.03*
Red muscle	1.0	1.43±0.17*	1.72±0.35*
White muscle	1.0	2.97±0.55*	3.09±0.85*

protein or per g of tissue, for both GLUT-4 protein and GLUT-4 mRNA (results not shown).

GLUT-4 protein was not altered in white skeletal muscle in response to 48 h of fasting (Fig. 5 and Table 3). However, mRNA levels in white muscle were increased by fasting (Fig. 5 and Table 3) when expressed either per μg of RNA or per g of tissue, and this occurred in spite of a 36% decrease in the yield of total RNA (Table 2). In contrast with the increase in GLUT-4 mRNA, fasting was associated with a decrease in β-actin mRNA (Table 3).

mRNA levels for GLUT-4, GLUT-1 and β-actin were markedly decreased in heart tissue from 48 h-fasted rats (Fig. 5 and Table 3). In spite of the large alterations in GLUT-4 mRNA, GLUT-4 protein content in heart was only moderately decreased by 48 h of fasting (Fig. 5 and Table 3).

Effect of diabetes and fasting on GLUT-4 mRNA/GLUT-4 protein ratio in insulin-sensitive tissues

Based on the observation that different tissues respond to insulinopenic conditions in a heterogeneous manner, we calculated the GLUT-4 mRNA/GLUT-4 protein ratio. Data, expressed as percentages of control values, are shown in Table 4. The GLUT-4 mRNA/protein ratio markedly decreased in white adipose tissue, brown adipose tissue and heart with diabetes and fasting (Table 4). In contrast, both red and white muscle displayed the opposite pattern in response to diabetes and fasting, so mRNA/protein ratios increased significantly in insulinopenic conditions (Table 4). This latter suggests the existence of either a translational impairment or an enhanced rate of protein degradation.

DISCUSSION

The results of the present study indicate that diabetes and fasting lead to tissue-specific changes in the expression of GLUT-4 protein. Although each particular tissue responded to insulinopenia in a very unique manner regarding GLUT-4 expression, a closer inspection allowed us to detect two different general patterns of response. On the one hand, white adipose tissue, brown adipose tissue and heart responded to insulinopenia by decreasing GLUT-4 mRNA and protein levels, in keeping with observations previously obtained in white fat [22–25] and which indicated the existence of suppression of GLUT-4 expression at a pretranslational step, owing to changes in the rate of transcription, maturation or stability of mRNA. A characteristic of these tissues was that GLUT-4 mRNA decreased to a larger extent than GLUT-4 protein during insulinopenia. That suggests the triggering of adaptive mechanisms at translational or post-translational steps, presumably oriented to the maintenance of tissue GLUT-4 protein content. In fact, changes at translational or post-translational levels have also been detected during rat perinatal development for GLUT-4 in brown adipose tissue and heart [40].

On the other hand, red and white muscle displayed a differential pattern of response to insulinopenia compared with white adipose tissue, brown adipose tissue and heart. Red and white muscle showed a heterogeneous response to diabetes and fasting concerning GLUT-4 expression; however, these tissues shared in common the enhancement of GLUT-4 mRNA/protein ratios. In fact, a decrease in GLUT-4 protein substantially larger than the decrease in GLUT-4 mRNA was also found by Strout *et al.* [27] in preparations of skeletal muscle from streptozotocin-diabetic rats. Overall, that suggests that GLUT-4 gene expression is relatively independent of circulating insulin in skeletal muscle, in contrast with what occurs in adipose tissues or in heart, where GLUT-4 mRNA levels fall drastically under insulinopenic conditions. Furthermore, it is likely that most of the decrease in GLUT-4 protein content detected in skeletal muscle under diabetes or fasting is due to alterations either at a translational level (that is, decreased translational efficiency) or at a post-translational step (that is, increased rate of protein degradation). In support of this view, a decreased rate of protein synthesis and polysomal aggregation has been reported in skeletal muscle from diabetic rats [41,42].

Our data also indicate that 7 days of streptozotocin-induced diabetes or 2 days of fasting induced almost identical changes in the expression of GLUT-4 glucose carriers in insulin-sensitive tissues. A common feature of streptozotocin-induced diabetes and fasting is the low plasma concentration of insulin. Therefore, most of the complex alterations in GLUT-4 expression detected in all insulin-sensitive tissues must be due either directly or indirectly to the low circulating insulin levels, rather than to alterations in blood glucose concentrations. This conclusion is also supported by data from Cusin *et al.* [43], which indicate that 4 days of chronic hyperinsulinaemia markedly alters GLUT-4 expression in white fat and skeletal muscle, and the pattern of changes is similar when hyperinsulinaemia occurs in the presence of euglycaemia or hypoglycaemia.

The cellular mediators responsible for the alterations in GLUT-4 expression in fasting or diabetes are unknown. It has been proposed that cyclic AMP could play such a role, and in this regard an increase in cellular cyclic AMP has been reported to decrease GLUT-4 expression in 3T3 adipocytes [44], thus mimicking the effect of the diabetic state in adipose tissue [22–25].

The existence of wide differences in insulin action between red and white muscle has been previously reported. Thus the effects of insulin in stimulating tyrosine kinase activity of receptors for insulin or insulin-like growth factor I, glucose uptake or incorporation of glucose into glycogen are greater in red muscle than in white muscle [31,45–48]. In connection with glucose-transporter expression, we report here the greater expression of GLUT-4 in red than in white skeletal muscle, which is also in agreement with recently published data [29,30]. Furthermore, we substantiate the existence of a differential diabetes- and fasting-induced regulation of GLUT-4 expression between red and white muscle. Thus in streptozotocin-induced diabetes, GLUT-4 protein content decreases in red and white portions of skeletal muscle, concomitantly with either decreased (red muscle) or augmented (white muscle) GLUT-4 mRNA levels. In contrast, in fasting, GLUT-4 protein is decreased in red muscle in the presence of unaltered mRNA levels, whereas GLUT-4 protein

does not change in white muscle, and this is concomitant with a marked increase in GLUT-4 mRNA levels. The mechanism by which various muscle fibres respond in such a different manner to diabetes or fasting modifying GLUT-4 gene expression remains to be investigated. Our data, revealing a differential regulation of GLUT-4 expression in red and white muscle, could explain some discrepancies previously reported about the effect of fasting [28,49] or diabetes [24,27,50] in skeletal muscle.

In the present study we have demonstrated that heart muscle is highly efficient in its GLUT-4 expression. This is based on two different observations. First, under control conditions, GLUT-4 protein content is much greater in heart than in skeletal muscle (red or white muscle), whereas GLUT-4 mRNA levels are comparable in heart and red muscle. Secondly, in response to diabetes and fasting, GLUT-4 mRNA markedly decreases in heart muscle, whereas GLUT-4 content is only marginally decreased. Whether this pattern found in heart is due to a greater translational efficiency or to a low rate of protein degradation remains to be investigated.

On the basis of recently published data on the diabetes-induced decrease in GLUT-4 in peripheral tissues [22–28] and on the differential content of GLUT-4 in red and white muscle [29,30], it has been proposed that the level of GLUT-4 expression influences the insulin effect on glucose transport. Supporting this view, we have observed that, in heart and skeletal muscle from diabetic rats, a fall in GLUT-4 protein content coincides with a decreased effect of insulin stimulating glucose transport [4,6–8]. However, our study also reveals that this is not a general finding, and, whereas the effect of insulin on glucose utilization is increased in skeletal muscle during fasting [12–15], this is concomitant with either unaltered or even decreased GLUT-4 protein levels. In this regard, it should be kept in mind that insulin action relies not only on the content of cellular GLUT-4 but also on the biological activities of the insulin receptor, as well as on several post-receptor events distal to the glucose carrier itself. In this regard, it should be mentioned that streptozotocin-induced diabetes leads to a marked impairment of tyrosine receptor kinase activity in skeletal muscle [51], and that fasting enhances insulin binding as assessed in the incubated muscle preparation [13,14].

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