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Insulin receptor and glucose transporter mRNA expression in skeletal muscle of genetically obese Zucker rats

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Insulin resistance and defective glucose transport are associated with muscle tissue in the genetically obese Zucker rat and are accompanied by changes in the number of insulin receptors and the availability of glucose transporters. The present study was carried out to assess whether, in male Zucker rats at 10 weeks of age, these defects were reflected by changes in the levels of mRNAs for the insulin receptor and for the insulin responsive glucose transporter (GLUT-4). Total RNA was extracted from plantaris and soleus muscles and the levels of insulin receptor and GLUT-4 mRNAs and 18 S rRNA were determined by Northern hybridization and quantified by image analysis of the autoradiographs. A 50% increase in the level of insulin receptor mRNA was detected in both the plantaris and the soleus muscle from the obese rats compared to the lean rats. No change in the level of GLUT-4 mRNA was detected in the plantaris muscle although increases were observed in the soleus muscle from the obese rats.

Zucker rats; Insulin receptor; Glucose transporter; mRNA

1. INTRODUCTION

Skeletal muscle, by virtue of its mass, is a major target organ for insulin in the body and is the primary tissue responsible for insulin-dependent whole-body glucose uptake [1]. Consequently, if glucose utilization and/or insulin responsiveness of the muscle are altered this may significantly affect the efficiency of whole-body glucose metabolism.

An insulin-resistant state has been well documented in the genetically obese Zucker rat [2] and this is reflected in defects at the receptor and the post-receptor level [3] in skeletal muscle and liver [4]. A reduction in insulin binding and the phosphorylation of the insulin receptor [2,5] may contribute to the insulin-resistant state, although this cannot fully explain the condition. Decreased glucose uptake, oxidation and incorporation into glycogen have been characteristically associated with skeletal muscle of genetic obesities [2]. The concomitant increase in basal and insulin-stimulated glucose oxidation in adipose cells [6] are also important features associated with the obese syndrome. In addition, metabolic defects have been detected in the glucose transport system in cultured adipose cells isolated from young (30 day old) Zucker rats and these are associated with a marked increase in the glucose transporters in the intracellular pool [7].

Evaluation of glucose transporter or insulin receptor mRNA levels allows assessment of which mechanisms (i.e. translational or pre-translational) are defective in tissues from obese animals. The mRNA for the insulin-responsive glucose transporter, GLUT-4, has been shown to be elevated in cardiac myocytes [8] and in adipocytes from young obese Zucker rats [9]. However, there is no direct relationship between insulin receptor number and the insulin receptor mRNA in the liver of genetically obese mice (*ob/ob*) since the number of insulin receptors in the liver of such mice [10] is reduced, whereas the level of insulin receptor mRNA is increased [11].

Despite the above studies, the mechanisms which lead to the insulin resistance of muscle in genetically obese animals are not clearly understood. The aim of the present study was to investigate if the obese phenotype is associated with changes in the level of mRNA for the insulin receptor and the insulin-responsive glucose transporter, GLUT-4, in skeletal muscle of Zucker rats. Muscle samples were taken when the animals were 10-weeks old, an age at which a state of insulin resistance is known to exist [4,12]. Both soleus muscle, consisting predominantly of slow oxidative fibres, and the plantaris muscles, of mixed fibre type, were examined, as insulin sensitivity depends upon muscle fibre type and inherent oxidative capability [13,14].

2. MATERIALS AND METHODS

2.1. *Experimental animals: tissue sampling and plasma insulin determination*

Male lean (*FaFa* or *Fafa*) and obese Zucker rats (*fafa*) were group housed and fed ad libitum. When the animals were 10-weeks old they

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were killed by decapitation and blood samples were collected from severed blood vessels. The plantaris and the soleus muscles were removed and quickly frozen in liquid nitrogen and subsequently stored at −70°C until required. The insulin level in the plasma of lean and obese Zucker rats was determined by radioimmunoassay using [^{125}I]-insulin as a tracer and rat insulin (Novo Biolaboratories, UK) as a standard.

2.2. *RNA extraction, purification and electrophoresis*

RNA was extracted from the muscle tissue using the method described by Chomczynski and Sacchi [15]. Briefly, the tissues were homogenized in a denaturing solution containing 4 M guanidinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl and 0.1 M 2-mercaptoethanol. The RNA was extracted using phenol-chloroform-isoamyl alcohol and the aqueous layer was removed. The RNA was precipitated with isopropanol and further concentrated by a second precipitation with isopropanol. The RNA pellet was then washed with 70% ethanol and finally resuspended in 0.5% sodium dodecyl sulphate (SDS). The concentration of the RNA was determined by its absorbance at 260 nm.

The RNA was then resolved by electrophoresis in a 1.2% agarose gel containing formaldehyde [16]. Each sample was analysed in duplicate or in triplicate using 20 μg of RNA per lane. The gel was run at 55 V for 3 h and the RNA was subsequently visualized by staining with ethidium bromide. The RNA was transferred to a nylon membrane (Genescreen) by overnight capillary blotting and immobilized by exposure to short-wave ultraviolet light.

2.3. *Northern hybridizations*

Northern hybridizations were carried out on total RNA extracted from skeletal muscle in order to determine the level of mRNA both for the insulin receptor and GLUT-4 and for the 18 S rRNA: the cDNA probe for the human insulin receptor was a 1.5 kb *Eco*RI/*Pst*I fragment corresponding to the region 1,101–2,602 base pairs which codes for a region in the extracellular domain [17]. The human GLUT-4 probe was a 2.1 kb, *Sal*I fragment from a cDNA [18] and the 18 S rRNA probe was a 1.4 kb *Bam*HI fragment [19].

All of the hybridizations were carried out under similar conditions at 42°C. The nylon membranes were pre-hybridized for 16 h in buffer consisting of 50% formamide, 10% dextran sulphate, 0.2% polyvinylpyrrolidone (PVP), 0.2% bovine serum albumin, 0.2% Ficoll, 0.05 M Tris-HCl (pH 7.5), 1.0 M NaCl, 0.1% sodium pyrophosphate, 1.0% SDS and denatured salmon sperm DNA (>100 μ/ml). The appropriate probe (25–50 ng) was labelled by random priming with [α-^{32}P]dCTP using the Multiprime DNA labelling system (Amersham International, UK) and separated from labelled nucleotides using a Sephadex G-50 column. The labelled probe was made up in the hybridization buffer (excluding dextran sulphate) and added to the existing buffer. Hybridization was carried out for 16 h at 42°C. Non-specific hybridization was removed from the filters by the following washing procedures: 2 × SSC (0.3 M NaCl/30 mM sodium citrate, pH 7.0) at room temperature for 5 min; 0.5 × SSC/1% SDS at 65°C for 30 min; 0.2 × SSC at room temperature for 30 min. Each procedure was carried out in duplicate and the filters were then laid down in autoradiography cassettes with Hyperfilm-MP and stored at −70°C. The length of exposure was assessed using a Geiger counter after the membranes had been washed, and exposure time varied from 3 h (18 S rRNA) to 7 days (insulin receptor, GLUT-4).

The level of mRNA that was detected using these procedures was determined by quantifying the intensity of the signal on the autoradiograph using a QUIPS image processing work-station, operating with VCS image processing software. The signals for the mRNAs under scrutiny were calculated relative to the level of 18 S rRNA (optical density ratio) in each sample lane. This enabled correction for any differences in signal intensity that may be due to variation in the initial loading of the RNA onto the gel. The optical density ratio of the respective mRNAs in skeletal muscle of obese Zucker rats were then expressed relative to values for their lean counterparts (100%). Quantitation was carried out on duplicate samples from 5 animals in each test group as specified in the text. Data are presented throughout as means ± S.E.M. Statistical analysis of the data was carried out using a Student's *t*-test.

3. RESULTS

3.1. *Plasma insulin*

Plasma insulin levels were determined in 6 animals of both lean and obese Zucker rats. The obese rats were clearly hyperinsulinaemic with an insulin level of 4.70 ng/ml (± 0.24) compared to 1.76 ng/ml (± 0.16) for the lean Zucker rats.

3.2. *Northern hybridization*

Using Northern hybridization of total RNA from soleus and plantaris muscle of lean and obese Zucker rats, mRNA species for the insulin receptor (7.2 and 8.5 kb) and for GLUT-4 (2.8 kb) were detected (Fig. 1). As observed in normal rodent species, two major bands were observed for the insulin receptor mRNA. Insulin receptor mRNA was quantified by combining the optical density of both bands. No significant changes in the level of GLUT-4 mRNA were observed between the lean and the obese rats in the plantaris muscle, although the insulin receptor mRNA increased significantly (Fig. 2) to 153%. In the soleus muscle significant increases were observed in the level of insulin receptor (189%) and GLUT-4 (141%) mRNA species in the obese rats compared with their lean counterparts (Fig. 2).

4. DISCUSSION

The results demonstrate an increased level of insulin receptor mRNA in the soleus and the plantaris muscles of obese Zucker rats when compared to lean rats. In the soleus muscle the level of GLUT-4 mRNA was also increased. The alterations in the level of insulin receptor mRNA in the plantaris muscle appear to be relatively specific as the concentrations of GLUT-4 mRNA were unchanged.

In the light of previous observations on the low number of insulin receptors in muscle tissue of genetically obese Zucker rats [3,20], it appears that the decrease in receptors may be due to alterations of translation of the mRNA and/or to post-translational processes. Although post-receptor defects can also occur, the decreased number of insulin receptors is likely to contribute to the insulin resistance of the skeletal muscle tissue.

An increase in the level of insulin receptor mRNA was also observed in the soleus muscle, suggesting that a similar defective translation process may be operating. However, unlike the situation in the plantaris muscle, this was accompanied by an increase in the concentration of GLUT-4 mRNA. An increase of similar magnitude has been observed in isolated cardiac myocytes from Zucker rats [8], with a decrease in basal and insulin-stimulated transport. The increase in the level of

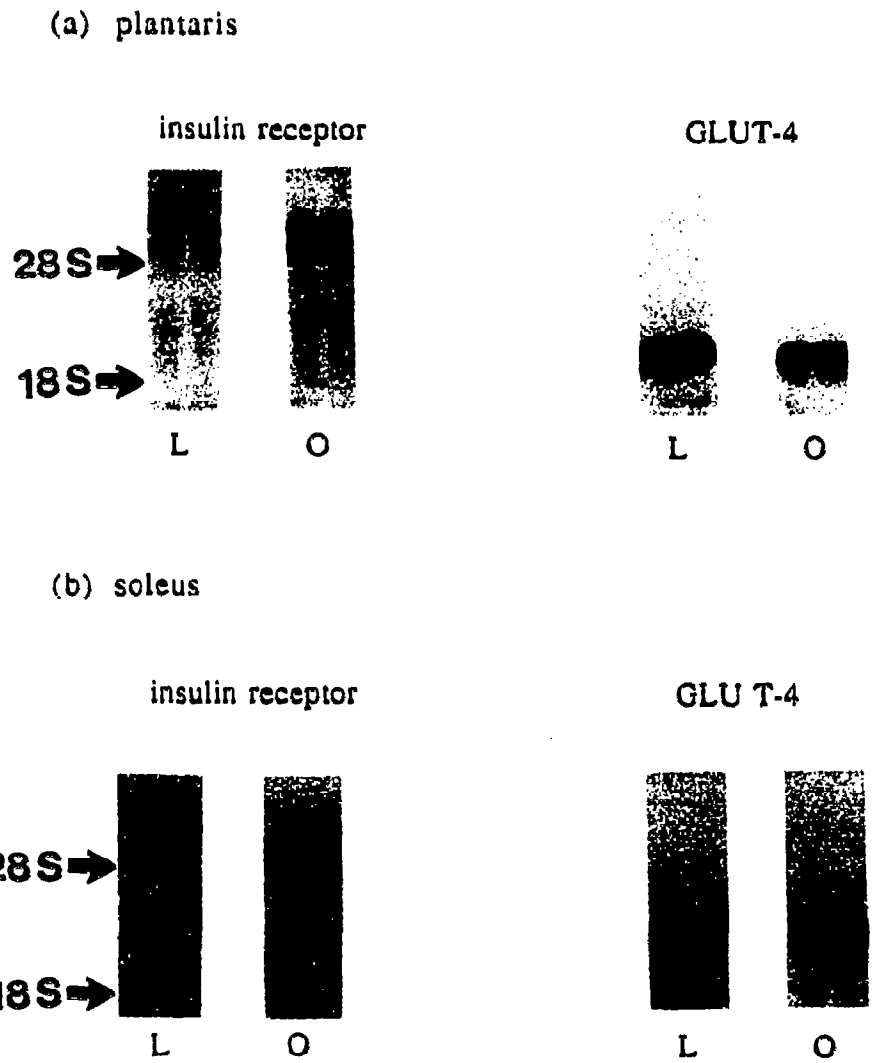

Fig. 1. Northern hybridization of total RNA (20 μg) extracted from (a) plantaris and (b) soleus muscle using cDNA probes for the insulin receptor and GLUT-4. Results indicated the specific detection of the mRNA species in both muscle types and the relative differences between the lean and the obese rats. For further technical details, see Materials and Methods.

GLUT-4 mRNA may be in response to changes in circulating insulin concentration, as GLUT-4 has been identified in insulin responsive tissues [21]. The absence of any increase in the plantaris muscle implies the existence of differential regulatory mechanisms that are associated with the varying fibre type of the two muscles [13]. This observation is consistent with previous observations [9] which demonstrated that in a mixed fibre-type muscle (gastrocnemius), no change in the level of GLUT-4 mRNA was detected in the obese phenotype while changes were observed in adipocytes from the obese animals. The requirement for an increased number of glucose transporters may not be as critical in the plantaris as in the soleus because the plantaris has a higher proportion of oxidative fibres. There still remains a reduction in the overall glucose utilization of muscle tissue from genetically obese Zucker rats [2] which may be mediated through defects in other pathways.

Insulin regulates the levels of insulin receptors [22] and insulin receptor mRNA [23], in vitro. It has also been shown that acute nutritional changes [24–26] significantly increases the level of insulin receptor mRNA in skeletal muscle, possibly through increased transcription of the insulin receptor gene or increase in the stability of the mRNA itself. Since the obese Zucker rats are characteristically hyper-insulinaemic, the changes in insulin receptor mRNA in their muscle may be a response to increased levels of circulating insulin.

In summary, an increased level of insulin receptor mRNA has been detected in the plantaris and the soleus muscle of genetically obese Zucker rats. Therefore, the

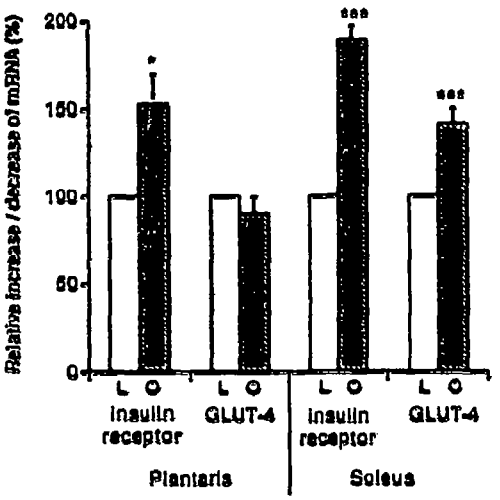

Fig. 2. Quantitation of insulin receptor and GLUT-4 mRNA levels in the plantaris and the soleus muscle of lean (L) and obese (O) Zucker rats. A significant increase ($^{*}P < 0.05$) in the level of insulin receptor mRNA was detected in the plantaris muscle of obese Zucker rats when compared to lean counterparts. No significant changes were observed in the GLUT-4 mRNA in this muscle. Significant increases were observed in the soleus muscle for the insulin receptor mRNA ($^{***}P<0.001$) and GLUT-4 mRNA ($^{**}P< 0.01$). The level of mRNA in the muscle samples from the lean rats is shown as 100% and the changes that are observed are indicated relative to this.

decreased number of insulin receptors in the muscle tissue of obese Zucker rats cannot be simply explained by reduced amounts of available mRNA. On the contrary this suggests both that there is additional control at translational and/or post-translational levels and that the increased mRNA levels reflect a compensatory mechanism which is attempting to overcome the tissue's resistance to insulin. Increases in the level of GLUT-4 mRNA were also evident in the soleus muscle but not in the plantaris muscle. This may indicate specific differences that are associated with the fibre types of the muscles or a generalized enrichment of the mRNAs in the soleus muscle.

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