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Development of insulin sensitivity in rat skeletal muscle

Studies of glucose transporter and insulin receptor mRNA levels

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Expression of GLUT-4 and insulin receptor mRNAs was investigated in rat skeletal muscle by Northern hybridization. GLUT-4 mRNA was barely detectable in foetal muscle, was expressed at low levels by 1–8 days and at 2–3-fold higher levels during and after weaning (18–40 days). In contrast there was little change in insulin receptor mRNA levels prior to weaning and a reduction in mRNA abundance between 18 and 40 days. Weaning rats on to a diet rich in fat prevented the increase in GLUT-4 abundance seen between 15 and 29 days in animals weaned on a high-carbohydrate diet.

Insulin; Glucose transporter; Messenger RNA; Weaning; Diet; Fat

1. INTRODUCTION

There are dramatic differences between new-born and adult animals in the ability of their skeletal muscle carbohydrate metabolism to respond to circulating insulin [1]. In the adult rat skeletal muscle is an important organ in the regulation of whole body glucose homeostasis [2], whereas in the muscle of new-born rats glucose transport does not respond to insulin and the tissue is apparently insulin-resistant [1,3]. Studies in young rats suggest that insulin sensitivity appears after 15 days of age (15d) during the transition period (weaning) from suckling to eating a solid diet [1]. Furthermore weaning on to a high-fat diet produces a lower sensitivity to insulin than weaning on to a high carbohydrate diet [4], suggesting that dietary factors are partly responsible for the development of insulin sensitivity.

The biochemical basis for the change in insulin sensitivity of muscle during post-natal development remains unknown. One possibility is that such changes may be related to the differential expression of different glucose transporter molecules. Numerous studies have demonstrated the expression of two distinct facilitative glucose transporter isoforms in muscle; GLUT-1, the erythrocyte-type glucose transporter and GLUT-4, the insulin-sensitive glucose transporter [5,6]. In addition recent data have suggested that a third transporter isoform (GLUT-3) is present at low levels in adult skeletal muscle but at high levels in foetal muscle [7]. In order to investigate the development of insulin sensitivity in muscle we have analysed the abundance of GLUT-4 and insulin receptor mRNAs by Northern hybridization.

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2. MATERIALS AND METHODS

The human insulin receptor cDNA probe [8], a gift from Dr. Leland Ellis, Howard Hughes Medical Institute, University of Texas, Dallas, USA, was a 1.5 kb *Pst*I/*Eco*RI fragment corresponding to a region of the gene (1,101–2,602 bp) which codes for a part of the extracellular domain of the receptor. The human GLUT-4 cDNA probe was a 2.1 kb *Sal*I fragment [9] kindly donated by Dr. G. Bell, Howard Hughes Medical Institute, Chicago, USA. The 1.4 kb probe for the 18 S rRNA [10] was kindly donated by Dr. R. Fulton, Beatson Institute, Glasgow. Multiprime labelling kits, Hyperfilm-MP and [^{32}P]dCTP were purchased from Amersham International Ltd., Amersham, UK. Laboratory chemicals were of molecular biology grade.

Hooded Lister rats of the Rowett strain were used in two studies. In the development study rats were obtained as 19d foetuses or at 1, 8, 18, 21 and 40d post partum. Animals were allowed access to solid laboratory chow (CRM nuts, Labsure; K. & K. Greff, Croydon, UK) throughout but normally did not consume this until 15d; animals were weaned at 19d and fed on the chow diet ad libitum. The animals were killed by cervical dislocation and either whole-limb muscles (foetal, 1d and 8d animals) or gastrocnemius muscles removed immediately, weighed and rapidly frozen in liquid nitrogen. Muscles were stored at −70°C until analysed. In the diet study three groups of rats were weaned at 19d on to one of three diets; normal laboratory chow or a casein/corn-oil/lard/sucrose diet which was either high in carbohydrate content and low in fat (high carbohydrate diet) or high in fat and relatively low in carbohydrate (high fat diet); the composition of the two semi-synthetic diets are shown in Table 1.

Muscle was ground whilst frozen, and homogenized in 4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarkosyl and 0.1 M 2-mercaptoethanol using an Ultra-Turrax. RNA was extracted

using the procedure of Chomczynski and Sacchi [11], subjected to electrophoresis [12] and transferred to nylon membranes (Genescreen, NEN Dupont) by capillary blotting. DNA probes (50–100 ng) were restricted and separated by electrophoresis in 0.6% low-melting-point agarose and labelled with [^{32}P]dCTP by random priming. Hybridization was carried out as described previously [13,14] and the membranes washed to remove non-specifically bound DNA; twice for 5 min in 2 × SSC (1× SSC is saline sodium citrate, 0.3 M NaCl, 0.03 M sodium citrate) at room temperature, followed by two washes for 30 min at 65°C, in 0.5× SSC, 1% SDS for both the GLUT-4 and insulin receptor probes and 0.2× SSC, 1% SDS for the 18 S rRNA probe. Specific hybridization was detected by autoradiography using Hyperfilm-MP at −70°C and the absorbance of the bands on the film quantified by image analysis [13]. After autoradiography membranes were washed in 0.1% SDS for 5–7 min at 95°C before re-hybridization to other probes.

Data from the diet experiment was analysed by comparing the values obtained for the intensity of the GLUT-4 mRNA band/intensity of the 18 S rRNA using the technique of residual maximum likelihood [15]. Filters contained samples from rats fed either the chow, high-carbohydrate or high-fat diets together with, in each case, samples from the pre-experimental group (15d animals) and aliquots of a control RNA sample. Thus the blocking factor in the analysis was the filters and the comparison between diets involved combining within-filter and between-filter information.

Table 1

Composition of diets

Nutrient	Content in g/kg diet	
	High-carbohydrate diet	High-fat diet
Casein	188	254
Corn-oil	41	169.5
Lard	0	169.5
Glycerol	100	0
Sucrose	119	85
Corn starch	397.7	132
Cellulose	38	51
Methionine	2.3	5
Gelatin	14	19

59% of the total energy content of the high-fat diet was provided by fat, 20% by carbohydrate and 21% by protein. This compared with the 10% provided by fat, 69% by carbohydrate and 21% by protein in the high-carbohydrate diet and the 3% provided by fat, 72% by carbohydrate and 24% by protein in the stock laboratory chow. All diets contained mineral and vitamin supplements in accordance with recommended daily intakes.

3. RESULTS

As shown in Fig. 1, Northern hybridization of RNA samples with the GLUT-4 cDNA probe detected one mRNA species which migrated to a position corresponding to a size of 2.8 kb. In contrast to the GLUT-4 mRNA, hybridization of muscle RNA with the insulin receptor probe detected two mRNA species of approximate size 8.2 and 7.5 kb; this is consistent with earlier studies which have shown multiple insulin receptor mRNA species of comparable size in rat tissue [13,16].

Visual inspection of autoradiographs obtained for Northern hybridizations with the GLUT-4 probe showed considerable differences in the GLUT-4 mRNA levels from skeletal muscle of rats of different ages (Fig. 1a). Such differences were confirmed (Fig. 2a) by quantification of absorbance levels by image analysis and expression of the GLUT-4 mRNA level per unit of hybridization to the 18 S rRNA probe in order to elim-

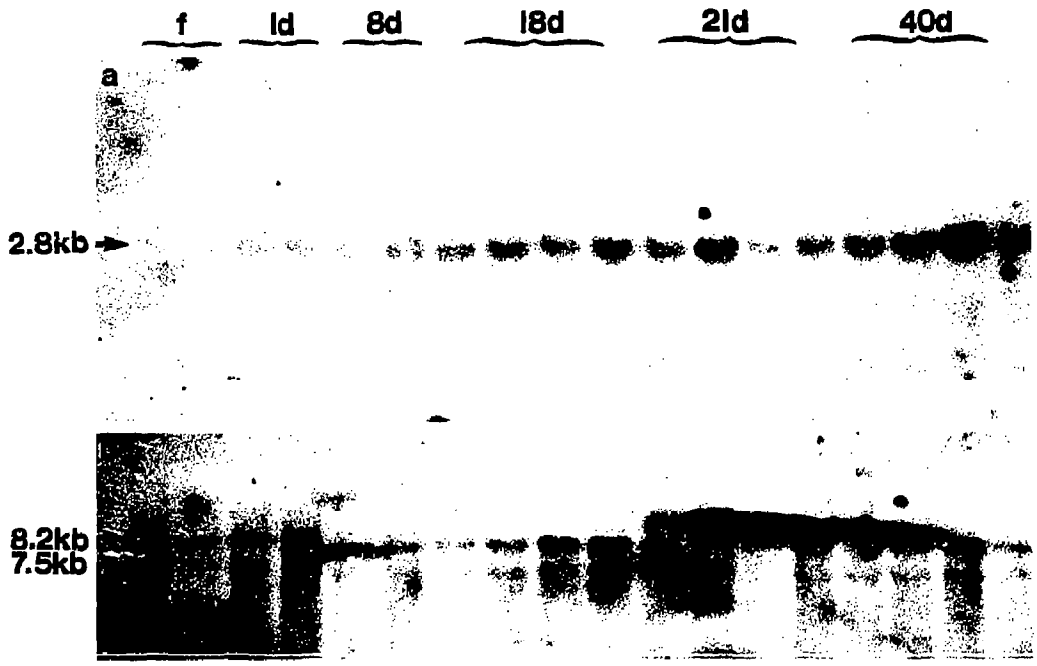

Fig. 1. Northern hybridization showing the abundance of GLUT-4 (a) and insulin receptor (b) mRNAs in skeletal muscle from rats of different ages. All lanes were loaded with 14 μg of total RNA and filters hybridized successively with GLUT-4, insulin receptor and 18 S rRNA probes. Results show the abundance of GLUT-4 and insulin receptor mRNAs detected by autoradiography.

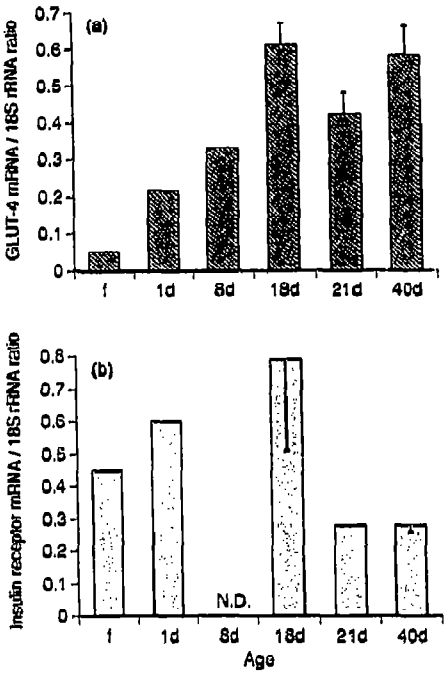

Fig. 2. Quantification of changes in GLUT-4 mRNA/18 S rRNA (a) and insulin receptor mRNA/18 S rRNA (b) ratios in skeletal muscle during rat development. Samples of total RNA were subjected to electrophoresis and capillary blotting. The filters were then analysed by successive hybridization with GLUT-4, insulin receptor and 18 S rRNA probes. The degree of hybridization was detected by autoradiography and quantified by image analysis. Ratios of hybridization to a particular probe to that achieved with the 18 S rRNA probe were calculated and means ± S.E.M. calculated from groups of 3 or 4 animals. N.D., not determined; f, foetal.

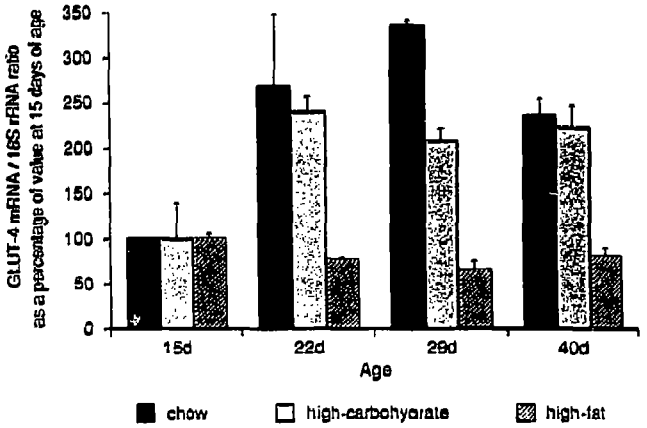

Fig. 3. Quantification of changes in the GLUT-4 mRNA/18 S rRNA ratio in rat skeletal muscle during weaning on to diets rich in either fat or carbohydrate. Samples of total RNA were analysed by Northern hybridization successively probed to GLUT-4 mRNA and 18 S rRNA. Hybridization was detected by autoradiography and quantified by image analysis and the extent of hybridization achieved with the GLUT-4 probe expressed as a ratio of that achieved with the 18 S rRNA probe. The ratio values were analysed by the technique of residual maximum likelihood (see section 2), $^{*}P < 0.05$. Mean values of the data expressed as percentages of the pre-experimental (15d) values were calculated for each group.

inate any errors due to variation in loading of the gels. As shown in both figures GLUT-4 mRNA was barely detectable in foetal muscle but was present at low abundance in muscle from 1d animals; the abundance of GLUT-4 mRNA was further increased in muscle from 8d animals and considerably higher in rats immediately prior to weaning (18d). The expression of GLUT-4 mRNA was maintained at a high level of expression at 21–40d. In comparison the insulin receptor mRNA was expressed in foetal and neonatal muscle and increased only marginally during the pre-weaning and weaning period (Figs. 1b and 2b); after weaning the abundance appeared to decrease over the period from 21–40d in contrast to the level of GLUT-4.

The influence of diet was investigated in rats which were weaned on to diets in which fat and carbohydrate made different contributions to the energy content of the diets (Table I). In rats weaned on to either the normal laboratory chow or the high-carbohydrate semi-synthetic diet the abundance of GLUT-4 mRNA increased between 15d (pre-weaning) and 22d (post-weaning) and then remained high at 29 and 40d (Fig. 3). In contrast GLUT-4 mRNA showed no increase in rats weaned on to the high-fat diet (Fig. 3). Analysis of the data from all three treatment groups showed that in 21 and 29d rats the abundance of GLUT-4 mRNA was significantly lower ($P < 0.05$) in those animals fed the high-fat diet compared with those fed the high-carbohydrate diet.

4. DISCUSSION

The present results show that during the first three weeks of post-natal development there is a large increase in the abundance of GLUT-4 mRNA in rat muscle. This increase commences by 1–8d but occurs largely between 8 and 18d, just prior to weaning. Furthermore, data from the diet experiment show that in animals offered standard laboratory chow there is a marked increase in GLUT-4 mRNA abundance between 15 and 21d. Recently published data have shown that in the heart GLUT-4 mRNA is present at 7d but does not appear in significant amounts in skeletal muscle until after 11d [17]. Thus it appears that although at birth the GLUT-4 gene is switched on in muscle, albeit at a low level of expression, the major increase in muscle GLUT-4 mRNA occurs after 8d during the immediate pre-weaning and weaning period. The present data do not allow assessment of whether such changes in mRNA abundance are reflected in concentrations of the GLUT-4 protein. However the changes in mRNA abundance coincide with the low sensitivity of muscle to insulin at birth, the responsiveness of skeletal muscle carbohydrate metabolism to circulating insulin concentrations at 30–40 d and the appearance of insulin sensi-

tivity predominantly at the time of weaning from a milk diet with a high-fat content to a laboratory chow diet high in carbohydrate [1–4]. It is likely therefore that the changes in GLUT-4 mRNA abundance are reflected both in levels of the protein and the sensitivity of carbohydrate metabolism to insulin.

In contrast to GLUT-4, the abundance of mRNA for the insulin receptor is comparatively high in neonatal and foetal muscle, increases only slightly before weaning and decreases from 21–40d. These changes do not parallel changes in insulin sensitivity, supporting the view that changes in receptor number are probably relatively unimportant in regulating tissue sensitivity to insulin during development [18,19].

As shown in Fig. 3 the feeding of diets of markedly different fat and carbohydrate composition during the weaning period affects the GLUT-4 mRNA abundance in muscle. Presentation of a chow or high-carbohydrate, semi-synthetic diet to 15d rats leads to increased GLUT- 4 mRNA levels by 21d. However this increase is prevented in animals offered a high-fat diet at this period, as recently also observed by others [20]. This is consistent with the observations that high-fat diets cause a reduced insulin sensitivity or partial insulin resistance at weaning [4] and decreased insulin sensitivity in adult rats [21]; thus the biochemical basis for the change in insulin sensitivity at this period may involve modulation of the expression of GLUT-4 mRNA, either through altered gene transcription or changes in mRNA stability.

In conclusion, during rat muscle development GLUT-4 isoform expression is switched on at birth such that there is low expression during the first week of life. Subsequently the expression of GLUT-4 mRNA increases markedly between 8d and weaning, particularly during the immediate pre-weaning/weaning period when suckling rats start to nibble food, and when sensitivity of muscle to insulin normally develops [1–4]. During this period GLUT-4 mRNA expression can be modulated by dietary factors, such that the normal increase which occurs during weaning on to a carbohydrate-rich diet is inhibited by feeding a diet high in fat.

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