Nutritional Regulation of Insulin-Sensitive Glucose Transporter Gene Expression in Rat Cardiac Muscle (43588)

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Abstract. **The effects of fasting and refeeding on the levels of mRNA encoding the insulin-sensitive glucose transporter (GLUT4) in rat cardiac and skeletal muscle were investigated using solution hybridization/RNase protection assays with a rat GLUT4 antisense RNA probe. In addition, the effects of these nutritional states on GLUT1 mRNA levels in several non-insulin-sensitive tissues were examined using a GLUT1 antisense RNA probe. Fasting for 48 hr significantly decreased GLUT4 mRNA levels in heart, with levels significantly increased over control levels by 24 hr after refeeding. In contrast, GLUT4 mRNA levels in skeletal muscle increased with fasting and returned to control levels with refeeding. No significant changes in GLUT1 mRNA were seen after fasting and refeeding in several non-insulin-sensitive tissues studied. These results suggest that altered GLUT4 gene expression is observed in different nutritional (insulin) states in insulin-sensitive tissues, and suggests a potential role for insulin in mediating these changes in gene expression.** [P.S.E.B.M. 1993, Vol 203]

The facilitated glucose transporters are structurally related proteins with multiple transmembrane domains, and are encoded by members of a glucose transporter gene family, of which at least five members have been identified to date. The protein encoded by the GLUT1 gene has been found in all cell types studied, but it is particularly abundant in red blood cells and certain brain cells (1). The GLUT4 gene product, on the other hand, is found exclusively in adipose and muscle tissue and is thought to be the primary insulin-sensitive glucose transporter (2). Previous studies have shown that the rat GLUT4 gene is nutritionally regulated in adipose tissue, with fasting causing a decrease in GLUT4 gene expression and refeeding resulting in an overshoot in GLUT4 gene expression (3). A proposed mechanism of this gene regulation has been speculated to involve the direct effects of diminished and increasing levels of circulating insulin which accompany these different nutritional states (4). To further examine the effects of such insulin states on gene expression, we have studied the effects of fasting and refeeding on GLUT4 gene expression in other insulin-sensitive tissues, cardiac muscle and skeletal muscle, as well as their effects on GLUT1 gene expression in several non-insulin-sensitive tissues.

Materials and Methods

Adult male Sprague-Dawley rats were fed *ad libitum* for 48 hr (three rats), fasted for 48 hr (three rats), or fasted for 48 hr and then refed for 24 hr (two rats). RNA was prepared from heart, skeletal muscle, brain, kidney, testes, stomach, lung, and liver from individual animals by homogenization of tissue in a guanidine thiocyanate buffer followed by phenol/chloroform extraction and precipitation with lithium chloride, as described previously (5). RNA was quantitated by determining absorbance at 260 nm. mRNA content was determined by slot blot analysis of varying amounts of RNA, followed by hybridization with ^{32}P-labeled oligo-dT, and scanning densitometry of the autoradiograph. Based upon these determinations, aliquots of total RNA containing equal amounts of poly(A)$^+$ mRNA were analyzed in solution hybridization/RNase protection assays, as described previously (5). A 364-base pair *Bg*III-*Xba*I fragment of a rat GLUT4 cDNA (clone SMI-1-2; provided by M. Birnbaum, Harvard Univer-

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sity Medical School, Boston, MA), including sequences corresponding to 95 bases of the C-terminal coding region and 269 bases of the 3′-untranslated region, was subcloned into the *Bam*H1 and *Xho*I sites of pGem-4Z (Promega Biotech). After linearization of this construct with *Eco*R1 and gel purification, T7 RNA polymerase and [^{32}P]UTP were-used to transcribe a ^{32}P-labeled antisense RNA probe, 363 bases of which were complementary to rat GLUT4 mRNA. The construction of the GLUT1 antisense RNA probe has been described previously (6). RNA samples containing equal amounts of mRNA were hybridized with probe for 15 to 20 hr at 45°C, followed by RNase treatment, ethanol precipitation, and electrophoresis of the protected hybrids, as described previously (5). Quantitation of protected bands was performed by scanning densitometry of the autoradiographs. Statistical analysis was performed using unpaired *t* tests.

Results

Figure 1 shows the results of experiments for two different treatment groups in which RNA preparations from individual hearts of fed, fasted, or refed rats were hybridized with the GLUT4 antisense RNA probe. The results of six sets of mRNA determinations using three independent treatment groups are represented graphically in Figure 2. Clearly, fasting resulted in a statistically significant decrease in GLUT4 mRNA levels in cardiac muscle, to levels 60% lower than in the fed control state. Conversely, refeeding resulted in a statistically significant overshoot in GLUT4 mRNA levels in cardiac muscle, to levels 1.7-fold greater than in the fed control state. (Data were analyzed using unpaired *t* tests.) Skeletal muscle RNA from the same rats was similarly examined in five sets of determinations (Fig. 3). As illustrated, fasting resulted in an increase in GLUT4 mRNA levels in skeletal muscle, to nearly twice the levels seen in the fed control state. Conversely, refeeding resulted in a decrease in GLUT4 mRNA levels relative to the fasted state, with a return to approximately those levels found in the fed control state. These changes in skeletal muscle are in agreement with the findings of other groups (7). In addition to these experiments, several non-insulin-sensitive tissues (including brain, kidney, testes, stomach, lung, and liver) were assayed using a GLUT1-specific antisense RNA probe. No significant changes in GLUT1 mRNA levels were seen after fasting and refeeding in any of these tissues studied (data not shown).

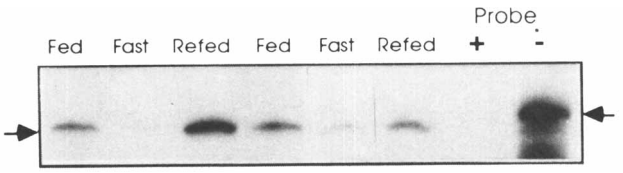

Figure 1. Representative solution hybridization/RNase protection assay using the GLUT4 antisense RNA probe with 20-μg aliquots of total RNA from hearts of individual fed, fasted, and refed rats. The arrow on the left shows the position of the protected probe band corresponding to GLUT4 mRNA. The arrow on the right shows the position of the full-length probe alone with (+) and without (−) treatment with RNases. Autoradiographic exposure was for 3 days at −70 C with two intensifying screens.

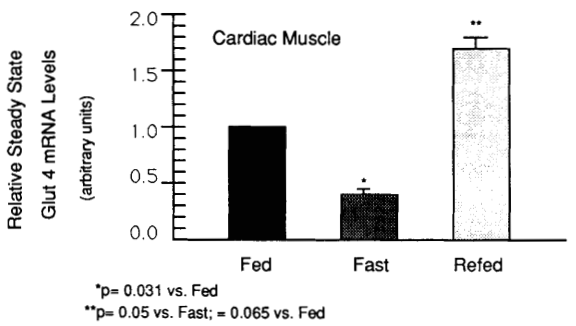

Figure 2. Quantitation of data from RNase protection assays using rat cardiac muscle RNA and the rat GLUT4 antisense RNA probe.

Discussion

The expression of the rat GLUT4 gene in insulin-sensitive tissues and the rat GLUT1 gene in non-insulin-sensitive tissues in fasted, fed, and refed states was studied using solution hybridization/RNase protection assays. We report that GLUT4 gene expression in rat cardiac muscle is nutritionally regulated, with significantly decreased expression (to 40% of control) in the fasted state and significantly increased expression (to 170% of control) in the refed state. Previous studies in the rat have demonstrated the same pattern of nutritional regulation of the GLUT4 gene in adipose tissue. These data suggest that for these two tissues, conditions of low insulin (the fasted state) significantly decrease GLUT4 gene expression and that conditions of higher insulin (fed and refed states) significantly increase glucose transporter gene expression. The magnitude of the changes in GLUT4 gene expression that we have observed in cardiac muscle is comparable to that described

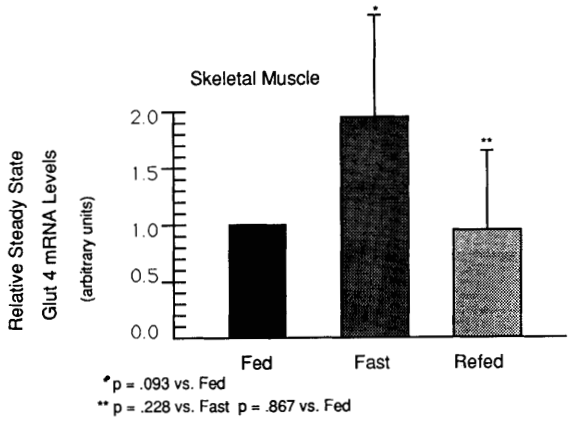

Figure 3. Quantitation of data from RNase protection assays using rat skeletal muscle RNA and the rat GLUT4 antisense RNA probe.

for GLUT4 mRNA levels in adipose tissue. Although significant, the magnitude of these changes is modest relative to that for the regulation of other genes, and may reflect the rigid control inherent to glucose homeostasis. Furthermore, the fact that GLUT1 gene expression in non-insulin-sensitive tissues did not change in these nutritional states also underscores the probable role of insulin in regulating gene expression in tissues that are sensitive to it.

On the other hand, in skeletal muscle, we found that fasting increased GLUT4 gene expression, which returned to control levels upon refeeding. This finding suggests that diminished levels of insulin in the fasted state alone are not responsible for controlling the level of GLUT4 gene expression in insulin-sensitive tissues, and that a complex interplay of several possible mediators (including glucosamine, glucose 6-phosphate, and/or glucokinase) are involved in determining the direction and extent of changes in GLUT4 gene expression.

Our work presents evidence that altered glucose transporter gene expression is observed in different nutritional states in insulin-sensitive tissues, and suggests that altered levels of circulating insulin may have a role in mediating these changes in gene expression. Further studies are required to elucidate the molecular basis of this complex mode of gene regulation. In addition, the possible utility of GLUT4 gene expression as a potential marker of nutritional status should be assessed.

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