Different role of insulin in GLUT-1 and -4 regulation in heart and skeletal muscle during perinatal hypothyroidism

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Ramos, S., L. Goya, C. Alvarez, M. A. Martín, M. Agote, F. Escrivá, and A. M. Pascual-Leone. Different role of insulin in GLUT-1 and -4 regulation in heart and skeletal muscle during perinatal hypothyroidism. Am J Physiol Endocrinol Metab 281: E1073–E1081, 2001.—Two groups of hypothyroid rats were used; one group was given 2-mercaptop-1-methylimidazole (MMI) treatment in the drinking water of the mothers and was killed at 2 and 4 days of life, and the other group was given similar MMI treatment and then was thyroidectomized at 5 days of life and killed at 8 or 20 days. Serum insulin, growth hormone (GH), and insulin-like growth factor I (IGF-I) were decreased in MMI-treated rats but increased in MMI-treated plus thyroidectomized rats. No significant reduction of thyroid hormones was observed in 2-day-old MMI rats. Protein and mRNA expression of GLUT-1 increased, and those of GLUT-4 decreased, in the heart in all populations independent of changes in insulin, GH, and IGF-I levels. However, GLUT-4 protein and mRNA expression in quadriceps and gastrocnemius skeletal muscles decreased at 4 days and increased at 8 and 20 days of life in parallel with insulin, GH, and IGF-I levels. GLUT-1 in the skeletal muscles seemed regulated posttranscriptionally and presented a decrease of mRNA expression in all stages studied. A differential sensitivity to insulin regulation of GLUT-1 and GLUT-4 glucose transporters seems to be one of the causes for the tissue-specific regulation of these glucose transporters in heart and skeletal muscles during the perinatal period.

perinatal period; thyroid hormones; GLUT-1 and GLUT-4 regulation; insulin

The facilitated diffusion of glucose into cells from interstitial space is mediated by a family of related glucose transport proteins, which differ in their distribution and in functional characteristics (3, 14). GLUT-4 (muscle/fat) is the main glucose transporter expressed in skeletal muscle from adult rats, whereas GLUT-1 (erythroid/Hep G2) accounts for only 5–10% of total glucose carriers (25). Cardiac muscle expresses both GLUT-4 and GLUT-1 glucose transporters, and in rat cardiomyocytes these account for ~70 and 30%, respectively, of total glucose carriers (13). In addition to the different tissue expression of GLUT-1 and GLUT-4, these transporters show differential localization in the muscle fiber. GLUT-1 is located mainly in the plasma membrane, whereas GLUT-4 is more abundant in intracellular membranes (42). Moreover, the relative abundance of both transporters in skeletal and cardiac muscle depends on the developmental stage (31). GLUT-1 is the predominant glucose carrier during fetal life (5, 31). GLUT-4 levels are low in the fetal rat, and a continuous induction of GLUT-4 mRNA and protein takes place in the perinatal phase (5, 31, 36). In fetal growth retardation, glucose transport is decreased in the lung, an organ whose growth is restricted, and treatment with insulin or insulin-like growth factor I (IGF-I) has been shown to restore body growth and to increase glucose uptake and levels of GLUT-1 protein and mRNA in a dose-dependent manner (32). Insulin and IGF-I (9) are known also to regulate growth in the fetus, and an important mode of action may be at a cellular level through their effects on glucose entry into the cell. In most fetal tissues, glucose uptake is controlled by glucose transporters.

The perinatal period is decisive in the development of mammals, and myelination in the rat takes place during days 7–20 of life, when a growth spurt occurs in the brain. To cope with this great energy requirement, the maintenance of glucose homeostasis is crucial during the perinatal period. Both thyroxine, a critical regulator of nervous system development, and insulin, an overall fetal growth regulator, have important metabolic and endocrine effects on the maintenance of glucose homeostasis during development. However, the role of both hormones in the regulation of glucose transporters GLUT-1 and GLUT-4 has been considered separately (4, 5); that is, insulin has not been determined in hypothyroid conditions, and thyroid hormones have not been measured in a diabetic situation.

Changes in glucose transport modulation seem to contribute to the regulation of tissue growth (32) and, although it has been reported that thyroid hormones increase basal and insulin-stimulated glucose transport in skeletal muscle (39), little is known about the role of insulin in the regulation of GLUT-1 and -4 in
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muscle under conditions of hypothyroidism during development. In addition, increased expression of IGF-I during postnatal stages (the first 2 wk of life) seems to be regulated by thyroid hormones (15), as occurs with the ontogenic rise of GLUT-4 around day 4 of life (39, 40), but the role of IGF-I in the regulation of glucose carriers in postnatal stages of development remains unknown. In rat cardiomyocytes, the role of insulin in GLUT-4 regulation seems to be more relevant than that in GLUT-1 (13, 43), because GLUT-1 does not seem to show insulin-dependent recruitment (44). Thyroid hormones increase GLUT-4 (muscle/fat transporter) gene expression in rat skeletal muscle, but they do not affect GLUT-1 (erythroid glucose transporter) protein and mRNA (40), which are weakly expressed in skeletal muscle. However, at present, the function of thyroid hormones on GLUT-1 and -4 during perinatal development is still confusing, because it is not known whether it results from a direct effect of the hormones or through other systems that might be involved.

The aims of this work were 1) to observe whether the different levels of insulin and IGF-I in the two populations of hypothyroid rats affect the regulation of GLUT-1 and -4 in heart and skeletal muscle, and 2) to study whether the ontogenic increase of GLUT-4 at 4 days or that of IGF-I at 2 wk influences the glucose carrier regulation in hypothyroid rats.

MATERIALS AND METHODS

Animals. Wistar rats bred in our laboratory with controlled temperature and an artificial light-dark cycle (light 0600–1800) were used throughout the study. After birth, the number of pups in each litter was evened out to eight, and males and females were used in equal numbers. Animals were fed a standard laboratory diet ad libitum. Thyroidectomy (Tx) was performed under ether anaesthesia, and control rats were sham operated. To prevent a possible hypocalcaemia from loss of the parathyroid glands after Tx, 1% calcium lactate was added to the drinking water of experimental and control rats. Blood was harvested from the trunk after decapitation, and plasma or serum was stored at −80°C until assayed. Livers and pituitaries were frozen in liquid N2.

European Community regulations for the use of experimental models and other scientific purposes were followed. All experiments were conducted in accordance with the principles and procedures outlined in the National Research Council Guide for the Care and Use of Laboratory Animals.

Experimental models. Three populations of neonatal rats were used (Fig. 1). A first group of rats received 2-mercapto-1-methylimidazole (MMI; through the mother) from day 14 of gestation and were killed on day 2 or day 4 of life (T rats). A second group of rats received MMI from day 14 of gestation and then they were thyroidectomized on day 5 of life and killed on day 8 or day 20. Control (C) rats were sham operated and killed on day 2, 4, 8, or 20 of life.

Fig. 1. Populations of neonatal rats (N) and manipulations: MMI rats received 0.02% wt/vol 2-mercapto-1-methylimidazole (MMI, through the mother) from day 14 of gestation and were killed on day 2 or day 4 of life. T rats also received MMI and then were thyroidectomized on day 5 of life and killed on day 8 or day 20. Control (C) rats were sham operated and killed on days 2, 4, 8, or 20 of life. They were still rather immature. MMI was obtained from Sigma Chemical (St. Louis, MO).

Serum glucose, T₃ and T₄, plasma insulin and growth hormone, and pituitary growth hormone determinations. Serum glucose was determined with a Reflotron IIM (Boehringer Mannheim, Leverkusen, Germany) glucose analyzer (12). Serum T₃ and T₄ were determined at Centro de Investigaciones Biomedicas by highly specific RIAs previously described by Weeke and Orskov (38) and modified for rat plasma by Obregón et al. (26). The minimal detectable doses were 2.5 pg for T₃ and 0.7 pg for T₄ per assay tube. Plasma immunoreactive insulin was estimated with purified rat insulin as standard (NOVO, Bagsvaerd, Denmark), an antibody to porcine insulin, which cross-reacted similarly with pork and rat insulin standards, and moniodinated ¹²⁵I-labeled human insulin. The minimal detectable dose was 0.04 ng/ml, with a coefficient of variation within and between assays of 10%.

Because of the great number of parameters measured in blood, samples from 3–4 different rat neonates at any age were pooled. Therefore, each individual datum in sera determinations resulted from a pool of 3–4 individual sera.

Iodination, purification, and determination of serum IGF-I. Recombinant human IGF-I was labeled by a modified chloramine T method (20). The specific activity achieved was ~90–175 μCi/μg. Before IGF-I determination, serum IGF-binding proteins were removed by standard acid gel filtration. This method has proved to be the most reliable one for use with rat serum (30).

The radioimmunoassay for IGF-I was carried out as previously described (30). The coefficients of variation within and between assays were 8.0 and 12.4%, respectively. Recombinant human IGF-I (Boehringer Mannheim, Leverkusen, Germany) was used for iodination.

Riboprobes and solution hybridization/RNase protection assay. Rat IGF-I cDNAs were kindly provided by Drs. C. T. Roberts Jr. and D. LeRoith (NIH). Rat IGF-I cDNA ligated...
into a pGEM-3 plasmid (Promega Biotech, Madison, WI) was linearized with HindIII, and an antisense riboprobe was produced by T7 RNA polymerase. pT7 RNA 18S antisense control template (Ambion, Austin, TX) was used for lane-loading control. The riboprobe was incubated with T7 RNA polymerase to produce a 109-nucleotide runoff transcript. [32P]UTP was purchased from ICN (Nuclear Ibérica, Madrid, Spain). The Riboprobe Gemini II Core System (Promega, Madison, WI) was used for the generation of RNA probes. Solution hybridization/RNase protection assays were performed as previously described (18, 29). Autoradiography was performed at −70°C against a Hyperfilm MP film between intensifying screens. Bands representing protected probe fragments were quantified using a Molecular Dynamics scanning densitometer and accompanying software. RNase A and RNase T1 were purchased from Boehringer Mannheim. A. Zorzano (Depar-tamento de Bioquimica y Biologia Molecular, Universidad de Barcelona, Barcelona, Spain) were kindly provided by Dr. A. Zorzano (Depar-tamento de Bioquimica y Biologia Molecular, Universidad de Barcelona, Barcelona, Spain) and were used as probes. Mem-branes were autographed, and relative densities of signals were determined by densitometric scanning of the autoradiograms in a laser densitometer.

**Statistical analysis.** All data in Figs. 1–7 are presented as means ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by the protected least significant difference test.

**RESULTS**

Table 1 shows that rats treated with MMI from day 14 of gestation present a decrease of plasma and pituitary GH at 2 days of life, although serum T₃ and T₄ remained unchanged vs. control, whereas body weight, serum T₃ and T₄ and plasma and pituitary GH were found reduced vs. controls at 4 days, indicating a situation of hypothyroidism. Glycemia remained unchanged in both stages of MMI rats. Rats treated with MMI from day 14 of gestation and thyroidectomized at day 5 of life (T rats) show at 8 and 20 days a greater reduction of body weight vs. controls than that of MMI rats, as well as a greater decrease of serum T₃ and T₄. However, an increase of plasma and pituitary GH vs. controls at 8 and 20 days was found in T rats. T rats show a decreased glycemia vs. controls at both periods. Figure 2 shows another interesting difference between the hypothyroid populations; plasma insulin was decreased in MMI rats vs. controls at 4 days, but it was elevated in T rats vs. controls at 8 and 20 days. Figure 2 also shows that serum IGF-I was decreased in MMI rats at 4 days and increased in T rats at 8 and 20 days vs. controls. No changes in serum IGF-I were found in MMI rats at 2 days. Liver mRNA expression of IGF-I was decreased at 2 and 4 days in MMI rats, whereas it...
was elevated vs. controls in T rats at 8 and 20 days, suggesting a transcriptional regulation for liver IGF-I gene expression by thyroid deprivation (Fig. 2B). Plasma insulin changed in parallel to serum IGF-I in all populations and periods studied (Fig. 2A).

Protein levels of GLUT-1 in heart tissue were found increased in MMI rats at 4 days and in T rats at 8 days vs. controls, whereas a decrease in GLUT-4 was observed in MMI rats at 4 days and in T rats at 8 and 20 days (Fig. 3). Neither GLUT-1 nor GLUT-4 changed in 2-day-old MMI rats. Variations in mRNA expression of GLUT-1 and GLUT-4 in heart run parallel to those of protein levels, in most of the conditions, suggesting a local transcriptional regulation of the gene expression (Fig. 4).

In quadriceps, an increase in the protein levels of GLUT-1 in T rats at 8 and 20 days and in MMI rats at 2 days was observed (Fig. 5A). An increase in GLUT-4 was also observed in T rats vs. controls at 8 and 20 days, whereas this transporter was decreased in MMI rats at 4 days and remained unchanged at 2 days. As shown in Fig. 6, mRNA expression of GLUT-1 in quadriceps decreased at 4 days in MMI and 20 days in T and increased at 2 days in MMI and at 8 days in T vs. controls, whereas mRNA expression of GLUT-4 in the same tissue decreased in MMI rats at 2 and 4 days and increased in T rats at 8 and 20 days. These results suggest a transcriptional regulation of GLUT-4 gene expression and a posttranscriptional regulation of GLUT-1 by thyroid deprivation in quadriceps.

In gastrocnemius (Fig. 5B), protein levels of GLUT-1 decreased in MMI rats at 4 days and increased, as in quadriceps, in T rats vs. controls at 8 and 20 days. GLUT-4 decreased in MMI rats vs. controls at 4 days and increased in T rats at 8 and 20 days, with changes in protein levels similar to those observed for the same proteins in quadriceps. Variations in mRNA expression for GLUT-1 and GLUT-4 in gastrocnemius can be observed in Fig. 7. Again, changes in GLUT-4 mRNA expression paralleled those of protein levels, suggesting a transcriptional regulation of the gene in this skeletal muscle. However, no resemblance between the changes in protein levels and mRNA abundance was observed in GLUT-1 in hypothyroid rats, suggesting a posttranscriptional regulation of the GLUT-1 gene expression by thyroid deprivation in gastrocnemius.

Therefore, a different regulation for GLUT-1 and -4 in heart and skeletal muscle can be inferred from these results. In heart, changes in GLUT-1 and -4 induced by thyroid hormone deprivation were similar for both populations and at the two periods studied, whereas in skeletal muscle, significant differences can be observed between MMI rats, which present decreased plasma insulin, and T rats with elevated plasma insulin (Fig. 2). Moreover, GLUT-1 does not seem to be transcriptionally regulated in either quadriceps or gastrocnemius skeletal muscle.

DISCUSSION

Thyroid hormones play a pivotal role in controlling the transition of glucose transporters GLUT-1 to GLUT-4 from fetal to neonatal levels in heart and brown adipose tissue (5). Besides, the mechanisms of IGF-I secretion and glucose transporter regulation by insulin and thyroid hormones are intimately connected. Synthesis of IGF-I, GLUT-1, and GLUT-4 in cardiac muscle and adipose tissue is regulated by thyroid hormones (5, 29), and insulin mediates the thyroid hormone effects on IGF-I secretion in immature stages of development (29, 30). Overall, the aim of this work was to study the influence of two experimental models of hypothyroidism that present different levels of insulin and IGF-I on the regulation of GLUT-1 and GLUT-4.

Rats treated with MMI and killed at 4 days show low insulin and IGF-I as well as body weight, whereas the same parameters did not change in rats killed at 2 days. It is worth mentioning that MMI rats show a decrease of plasma and pituitary GH at 2 and 4 days of life.

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<th>Table 1. Body weight, serum T3 and T4, plasma and pituitary GH, and glycemia of 5 groups of neonatal rats</th>
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Values are means ± SD of 8–10 animals. H, hypothyroid neonatal rats; C, control rats; MMI, treated with mercapto-1-methylimidazole (MMI), 0.02 wt/vol in drinking water of mothers from 14 days of pregnancy; T, receiving MMI in drinking water from 14 days of pregnancy and thyroidectomized at 5 days of life; T3, 3,5,3’-triiodothyronine; T4, thyroxine; GH, growth hormone. MMI group rats were killed at 2 or 4 days of life; T group rats were killed at 8 or 20 days of life. *P < 0.05 vs. C rats.
life, indicating a high sensitivity of the somatotroph cell to thyroid deprivation in immature stages, because in day 2 of life, although differences in the biologically active thyroid hormone (T₃) did not reach statistical significance, they show a tendency to decrease in MMI rats compared with controls and almost within the range of statistical significance. However, rats treated with MMI and thyroidectomized at 5 days show a significant decrease of thyroid hormones and, consequently, a reduced body weight and glycemia; however, surprisingly, they present an increase of plasma and pituitary GH as well as plasma insulin and serum IGF-I. These interesting differences between the two neonatal hypothyroid populations (our MMI and T groups) have been previously reported at 10, 15, and 20 days of life (29), whereas in the same work, decreased GH, insulin, and IGF-I were found to be decreased in thyroidectomized adult rats (28).

The mechanism by which thyroid hormones contribute to the regulation of glucose and insulin homeostasis is a complex subject (23); i.e., normal, increased, and decreased plasma insulin (23) and increased insulin secretion from the isolated pancreas (24) have been
reported in hypothyroidism. Changes of insulin parallel those of GH in both hypothyroid populations (MMI and T), in agreement with what has been reported in diabetic rats, characterized by decreased plasma levels and pituitary GH secretion that are restored by insulin treatment (17). However, the increase of plasma GH and insulin found in T neonates, contrary to the decrease observed in adults, does not seem to depend on the maturity of the animal, because this increase was not found in MMI rats at 2 and 4 days. Likely, this result suggests that, in immature stages of the hypothalamic system, the imbalance provoked by thyroid withdrawal increases plasma insulin and GH by β-adrenergic stimulation (33) or glucocorticoid secretion (1, 21), although a more precise explanation awaits further investigation. Moreover, the effects per se of goitrogen drugs such as MMI on insulin (19, 20) could also play a role in the decrease of insulin in the MMI rats. We have recently found (data not shown) a reduced stomach milk content in MMI neonatal rats that was not found in T neonates (28), and such a difference could also play a role in the decreased insulin and, therefore, GH and IGF-I, observed in these rats.

In this work, we have presented two groups of thyroid hormone-deprived rats, one with low insulin, GH, and IGF-I at 4 days of life and another with elevated insulin, GH, and IGF-I at 8 and 20 days of life; these
different groups permit us to study the role of the two endocrine factors together in the regulation of GLUT-1 and GLUT-4. It is worth mentioning that, in a previous study, the decreased serum insulin, GH, and IGF-I found in nonthyroidectomized MMI-treated neonatal rats at 2 and 4 days remained reduced at 10, 15, and 20 days of life (29). However, an earlier stage of neonatal development (2–4 days) was selected for MMI-treated rats in the present study to test whether the ontogenic increase of GLUT-4 influences the changes of protein levels and mRNA expression of GLUT-1 and -4 in the hypothyroid condition. In the same line, stages of 8 and 20 days were selected for T neonatal rats to test whether the development shift from IGF-II to IGF-I (during the first 2 wk of life) affects protein levels and mRNA expression of GLUT-1 and GLUT-4.

The results of this work demonstrate that GLUT-1 increases and GLUT-4 decreases in the heart of all hypothyroid rats regardless of the levels of insulin, GH, and IGF-I, changes that parallel those of cardiac mRNA expression of both genes; these results agree with previously released data obtained in a similar experimental model of hypothyroidism in which insulin was not determined (5). The results from this work also show, for the first time to our knowledge, that the changes of GLUT-1 and -4 in the heart seem essentially to depend on the plasma thyroid hormone status and not on the insulin levels. In addition, as previously described (5), the parallel changes in protein levels and mRNA expression observed for GLUT-1 and -4 in heart suggest a pretranslational regulation of both genes. Our results obtained in heart tissue show the gradual decrease of GLUT-1 and increase of GLUT-4 protein and mRNA expression, in agreement with previous observations regarding the higher levels of GLUT-1 in immature stages and the progressive induction of GLUT-4 from day 4 onward (31). Thus regulation of GLUT-1 and GLUT-4 in heart tissue in hypothyroid rats is not mediated by insulin, GH, and IGF-I during the perinatal period of the rat. The insulin-independent increase of GLUT-1 in cardiac muscle in conditions of hypothyroidism agrees with the fact that GLUT-1, which represents 40% of total glucose transporters, is localized in plasma membrane of the cardiomyocytes and does not need to be translocated from inner vesicles as shown with GLUT-4 (27). Probably because of the high rate of glucose uptake (7) in immature heart tissue, the animal must upregulate GLUT-1 to cope with actual needs in a situation of thyroid hormone deprivation.

Skeletal muscle accounts for nearly 40% of body mass (41) at any stage of life, and it is the main tissue involved in the insulin-induced stimulation of glucose intake (41). Euglycemic-hyperinsulinemic clamps have shown that, at circulating levels of insulin in the upper physiological range, most of the infused glucose is taken up by skeletal muscle and converted mainly into glycogen (8). Besides, alterations in insulin sensitivity of the skeletal muscle have a profound impact on whole body glucose disposal in adults. In this regard, patients with type 2 (non-insulin-dependent) diabetes show a deficient insulin-induced glucose transport in skeletal muscle (2, 10). The most important glucose carrier in skeletal muscle is GLUT-4, because GLUT-1 accounts for only 5–10% of total glucose carriers (25). All of these studies point out the crucial role of insulin in the regulation of GLUT-4 in skeletal muscle and in the control of glucose homeostasis in adulthood.

On the other hand, previous studies have shown that thyroid hormones increase basal and insulin-stimulated glucose transport (39) and GLUT-4 in adult skeletal muscle (40), but the level of insulin was not measured in those studies, which were carried out only during the adult period. In the present study, with two populations of hypothyroid neonatal rats (MMI and T), we found that the decreased mRNA expression of GLUT-1, contrary to that observed in heart showing the tissue-specific regulation of these genes, is independent of the levels of insulin, GH, and IGF-I both in quadriceps and gastrocnemius. Although GLUT-1 protein was found increased in skeletal muscle of T rats at 8 and 20 days, when insulin, GH, and IGF-I were elevated, no parallel changes were observed in the tissue mRNA expression. These results indicate that, during the perinatal period of the rat, GLUT-1 regulation in skeletal muscle takes place at a posttranscriptional level and, as observed in heart in the same populations, seems to be independent of plasma insulin variations. These data agree with previous observations showing a weak effect of insulin on GLUT-1 (13, 43).

Contrary to the observations with GLUT-1, the results of this study obtained in MMI rats at 4 days, with low insulin, show a decrease of protein levels and mRNA expression of GLUT-4 in quadriceps and gastrocnemius. But at 8 and 20 days, when insulin, GH, and IGF-I were elevated, T rats showed an increase of GLUT-4 protein and mRNA expression in the same tissues. This increase of GLUT-4 has been previously observed in adipose tissue of hypothyroid rats similar to those used in this work, MMI plus T, but insulin was not determined in that study (5). The regulation of GLUT-4 in skeletal muscles by insulin shown in our results agrees with the previously reported decrease of protein levels and mRNA expression of GLUT-4 in insulin-sensitive tissues found in diabetes and fasting (4), conditions also with reduced serum thyroid hormones (37). In addition, the results agree with the diabetes-induced decrease in GLUT-4 mRNA expression in skeletal muscle and adipose tissue (4) at a pretranslational step (16, 34). In summary, these results not only support but explain the previously described tissue-specific regulation of GLUT-1 and -4 during the neonatal period (5); changes of GLUT-1 and -4 in neonatal heart in conditions of thyroid hormone deprivation seem to be independent of insulin, GH, and IGF-I, whereas regulation of GLUT-4 in skeletal muscles, which takes place at a pretranslational step, strongly depends on circulating insulin. Besides, in such a hypothyroid condition, the regulation of GLUT-1 and GLUT-4 in both muscles does not seem influenced by the change from GLUT-1 to -4 at 4 days
(31), nor by the ontogenic increase of IGF-I at 8 and 20 days of life, although both changes are regulated by thyroid hormones (11, 15). The results also suggest that, in perinatal rat hypothyroidism, diffusion of glucose into cardiac cells from interstitial space seems to be mainly carried out by GLUT-1, whereas in skeletal muscle it is carried out by GLUT-4. Changes in IGF-I parallel those of insulin, but further research is needed to unravel the specific role of IGF-I, independent of that of insulin, in the glucose transporter regulation, because parallel changes in GLUT-4 and IGF-I are also observed in skeletal muscle.

Finally, it is necessary to point out that MMI rats at 2 days, with no significant decrease of thyroid hormones, show a reduced mRNA expression of hepatic IGF-I and heart and quadriceps GLUT-4. Perhaps the increase of protein levels of GLUT-1 in quadriceps and the increase of mRNA expression of GLUT-1 in both skeletal muscles of MMI rats at 2 days could indicate a compensatory process to maintain glucose homeostasis in conditions of thyroid hormone and insulin deprivation during the immature period, when GLUT-1 is the most abundant glucose transporter. We believe that the present results should also be taken into account when one considers the regulation of GLUT-4 in skeletal muscles by insulin during immature stages of development, when a decrease of circulating insulin does not always follow thyroid deprivation, contrary to what has been observed in adult animals (29).

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