Thyroid hormones differentially modulate enolase isozymes during rat skeletal and cardiac muscle development

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MUSCLE PHYSIOLOGY is greatly influenced by thyroid status. Thyroid deficiency leads to muscular weakness and excessive fatigability, characterized by a fall in production of energetic compounds. In developing muscle, thyroid hormones control not only myosin isoform transitions but also the expression of several genes, such as those encoding the Ca2+-ATPase of the sarcoplasmic reticulum (41, 42, 45), the Na+-K+-ATPase pump (2, 12), and enzymes of the oxidative and glycolytic pathways (1, 22, 36, 43). Thus they modulate energy-consuming reactions (contraction and relaxation) as well as reactions of the energy-producing pathways. In a study of the metabolic specialization of individual fibers, in fast- and slow-twitch rat muscles, it has been suggested that thyroid hormones could be useful to optimally coordinate the expressions of contractile and metabolic proteins during development (36). Thus patterns of energy metabolism could adjust to specific energy requirements. Many studies dealing with the control of energy metabolism by thyroid hormones have been devoted to enzymes of the oxidative pathway; fewer studies have been devoted to glycolytic enzymes.

Enolase (2-phospho-α-glycerate hydrolyase, EC 4.2.1.11) is a glycolytic enzyme that is active as a dimer. In higher vertebrates it exhibits cell type-specific isoforms constituted from the three subunits, α, β, and γ, encoded by different genes (7, 13, 38). The α-α-homodimer is predominant in all embryonic tissues and remains expressed at various levels in most adult tissues. During development, accumulation of specific isoforms accompanies the differentiation of two tissues with high energy demands: αγ and γγ in brain and αα and ββ in striated muscles (14, 24, 30, 31, 49). At the adult stage, β-enolase transcript and subunit accumulate preferentially in fast-twitch fibers. In skeletal muscle, total enolase activity is highest in fast-twitch muscle, and this activity is almost entirely due to the muscle-specific homodimer ββ. In rat cardiac muscle, which exhibits a lower enolase activity, the three isoforms αα, αβ, and ββ are almost equally abundant (18, 23–25). In cardiac muscle the decrease of total enolase activity observed during postnatal development results from a drop of αα-enolase activity (23, 25).

Quantitative analyses of β-enolase gene expression during development of mouse hindlimb muscles have shown a biphasic accumulation of β-enolase transcripts (30). A prenatal increase accompanies the establishment of innervation, and a postnatal rise is temporally correlated with the increase in thyroid hormone levels in serum (9, 32) and with the accumulation of the transcripts of the rapid adult form of myosin, myosin heavy chain (MHC) IIb, known to be dependent on these hormones (8, 9, 40).

The influence of thyroid hormones on enolase gene expressions has not been studied. Here, we present the effects of rat thyroid status on the αα- and ββ-isoform gene expressions during development in two types of muscle:
hindlimb muscles, which are mostly glycolytic and fast twitch, and heart, which is oxidative.

**EXPERIMENTAL PROCEDURES**

Animals. Wistar rats (IFFA-CRÉDO, L’Arbresle, France) were kept under controlled housing conditions. Animals in three different thyroid statuses were used. Control rats were euthyroid. Hypothyroidism was induced in pregnant rats by feeding a diet containing 0.1% methylthiouracil (MTU) from 16 days of gestation and suppressing iodine throughout the feeding a diet containing 0.1% methylthiouracil (MTU) from 16 days of gestation and suppressing iodine throughout the experiment. In the third group, young MTU-treated rats were injected daily, from birth, with 3,5,3'-triiodothyronine (Sigma Chemical, Lisle d'Abeau Chesnes, France, T₃, 30 ng/10 g body wt ip). Litters were maintained at seven pups per dam.

The rats were used at birth and at 4, 8, 13, 15, 21, and 28 days after birth. Muscles were dissected and immediately frozen in liquid nitrogen for protein and mRNA extraction. Northern blot analysis. Total RNA was prepared according to the RNA-Plus manufacturer’s protocol (Bioprobe, Montreuil, France).

Northern blot and hybridization procedures were carried out as previously described (30).

Probes. The probe for β-β-enolase mRNA corresponds to the 3'-untranslated region of the murine β-enolase mRNA (European Molecular Biology Laboratories/GenBank Data Library ACX57747). The specific α-enolase probe corresponds to the 3'-untranslated region of the mouse α-enolase mRNA (European Molecular Biology Laboratories/GenBank Data Library ACX52379). Probe for MHC IIB mRNA was provided by Shin’ichi Takeda and corresponds to the 5'-noncoding region of the mouse mRNA (44). A 30mer oligonucleotide probe corresponding to the 18S ribosomal rat RNA (6) was used to normalize the results to the same amount of total RNA (30).

Enolase enzyme activity. Enolase activity was measured at 30°C. Activity was measured spectrophotometrically at 240 nm as the conversion of sodium 2-phospho-D-glycerate to phosphoenolpyruvate and expressed in international units, as previously described (26).

Protein concentration was determined by using the Bio-Rad (Bradford) protein assay and BSA as a standard.

Western blot analysis. Protein extraction and Western blot analysis, and the two-dimensional gel electrophoresis were performed according to a standardized protocol (33). Briefly, the blots were kept dry until immunologic treatments were applied, according to the protocol indicated by Amersham (Les Ulis, France) for the enhanced chemiluminescence Western blotting detection system. The anti-α- and anti-β-enolase rabbit sera were diluted 1:5,000 and 1:50,000, respectively. The peroxidase-coupled secondary antibody was anti-rabbit IgG (Biosys, Compiègne, France).

Immunocytochemistry. After rapid removal, the muscles were dissected and immersed in 2% paraformaldehyde in PBS for 3 h at 4°C. They were then treated as previously described (25). After overnight incubations at 4°C with the primary antibodies, cryostat sections were washed and incubated for 1 h at room temperature with the appropriate secondary antibodies. Muscle sections were then mounted in Immunofluor (New England Nuclear) and observed by confocal microscopy. Control preparations were obtained using the second antibody alone or rabbit preimmune serum. No fluorescence staining was visible in these conditions (data unshown). Production of specific antibodies directed against the rabbit ββ-enolase in sheep (30) and against mouse αα- and ββ-enolase in rabbits (26, 33) has been previously described. The anti-enolase sera produced in rabbits were diluted 1:400 for anti-α-enolase and 1:1,000 for anti-β-enolase. For double-labeling experiments, together with the anti-α-enolase rabbit serum, the anti-β-enolase serum produced in sheep was affinity purified and used at 1:30 dilution.

For fiber type identification, the following monoclonal antibodies directed against MHC were used: MY32, diluted 1:2,000 (Sigma Chemical), reveals fibers expressing fast and neonatal MHCs (47); NCL, diluted 1:5 (Novoceastra, Newcastle Upon Tyne, UK) directed against slow-type MHC, reveals type I fibers.

For subcellular localization of αα- and β-β-enolase, the Z line was visualized by a monoclonal anti-α-actinin serum diluted 1:400 (Sigma Chemical) and the M band was visualized by a specific goat antiserum diluted 1:200 (a kind gift of T. Wallimann, Zurich, Switzerland) directed against chicken M band proteins (myomesin and protein M) (46). The following secondary antibodies produced in donkey were used (Jackson Laboratory, diluted 1:100): fluorescein (FITC) anti-mouse IgG, fluorescein anti-goat IgG, and Texas red anti-rabbit IgG.

In some experiments we also used goat FITC anti-rabbit IgG diluted 1:200 (Amersham, Little Chalfont, UK) and rabbit Texas red anti-goat IgG diluted 1:100 (Immunootech, Marseille, France). The choice of Texas red as second fluorescent conjugate is important, since its excitation spectrum is far from the emission spectrum of fluorescein. The excitation intensity at the two wavelengths was carefully adjusted to avoid the artifact of microscopic observation.

Confocal laser scanning microscopy was performed using a confocal imaging system (model TCS-4D, Leica instrument, Heidelberg, Germany) equipped with a ×63 objective.

Statistical analysis. Data were statistically analyzed using the STATVIEW program, by ANOVA or Student’s t-test. P<0.05 was considered significant.

**RESULTS**

Thyroid status. Three series of neonatal rat pups were analyzed: controls (C), which were euthyroid; rats treated with MTU, an antithyroid drug that induces hypothyroidism; and MTU-treated rats that received a daily injection of T₃ from birth (MTU + T; see EXPERIMENTAL PROCEDURES). The body weight of control rats increased more rapidly than that of hypothyroid rats (Fig. 1A). At 21 days, body weights and heart weights of MTU-treated rats were only 40% of those of the controls. T₃ treatment (30 ng/10 g) of hypothyroid rats did not significantly modify the developmental profile of body weight (Fig. 1A) or heart weight (Fig. 1B). Thus the dose of T₃ used in this protocol did not induce the cardiac hypertrophy known to be produced by hyperthyroidism. It is known that induction of the rapid adult form of MHC, MHC IIB, is dependent on thyroid hormones. We have measured the levels of MHC IIB mRNA in extracts from hindlimb muscles (Fig. 1C). MHC IIB mRNA level started to increase in muscle extracts of control rats after 8 postnatal days. In MTU-treated rats, MHC IIB mRNA level remained undetectable by Northern blot analysis throughout the experiment. In MTU + T rats, the developmental profile of MHC IIB mRNA was comparable to that of controls, indicating the effectiveness of T₃ treatment.

Differential regulation of αα- and ββ-enolase gene expressions by thyroid hormones in rat hindlimb muscles. We have compared the developmental profiles of αα- and ββ-enolase transcripts in hindlimb muscles from control and hypothyroid rats (Fig. 2, A and C). During normal
muscle development, the level of α-enolase transcripts did not vary significantly (Fig. 2C). At all the examined times, except at birth, this level was significantly reduced in hypothyroid animals. At 21 and 28 days the α-enolase level in muscles of hypothyroid rats corresponded to 35% of the control levels.

The β-enolase transcript level increased about ninefold from birth to 28 days in hindlimb muscles of control rats. In MTU-treated rats this level increased only by a factor of 5 (Fig. 2A). In contrast to α-enolase transcripts, no modification was induced by hypothyroidism in β-enolase transcript level until 13 days. This time corresponds to the expression of MHC IIB transcripts in control muscles (Fig. 1C), reflecting the diversification of fiber contractile properties. Thereafter, β-enolase transcript level was reduced in hypothyroid animals, representing 60% of control levels at 28 days.

The effects of T₃ injections into hypothyroid rats on the levels of α- and β-enolase transcripts in hindlimb muscles during development are presented in Fig. 2, B and D, respectively. T₃ injections stimulated α-enolase gene expression at all the examined times and β-enolase gene expression starting at 13 days. Transcript levels almost reached values obtained in controls.

Total enolase activity is decreased by hypothyroidism in hindlimb muscles. Total enolase activity was measured in hindlimb muscle extracts of control and hypothyroid rats. This activity increased linearly during the postnatal muscle maturation in both groups of animals, but the slope of the line was significantly lower in hypothyroid rats than in controls (Fig. 3A). During the same period, an increase in the muscle-specific enolase transcript level, β, was observed (Fig. 2A). Statistical analysis indicated a positive linear relationship between total enolase activity and β-enolase transcript level, independently of the animal group (Fig. 3B).

Microheterogeneity of α- but not β-enolase subunit is influenced by the thyroid status in developing hindlimb muscle. Biochemical analyses of α- and β-enolase subunits have shown a microheterogeneity of these proteins that is modulated during mouse hindlimb muscle maturation (33). We have now examined whether similar developmental changes occur in rats. Hindlimb muscle extracts were analyzed by two-dimensional gel electrophoresis and then by Western blot from embryonic day 18 (E18) to postnatal day 21 (P21). To visualize both subunits, the blots were sequentially reacted with the anti-β-enolase and with the anti-α-enolase serum. In the less mature muscles (E18), a large amount of the ubiquitous α-subunit and a small amount of β-enolase subunit were each expressed as one major basic spot (Fig. 4, control). A large increase in the concentration of β-enolase subunit occurred between E18 and postnatal day 15 (P15). Between postnatal day 8 (P8) and P15 there was a transition in the expression of α-enolase variants, with the acidic spot becoming predominant. At P15 and later, α- and β-enolase subunits exhibited two electrophoretic variants differing in their isoelectric points.

Comparison of muscle extracts from control, MTU, and MTU + T animals uncovered differences in α-subunit microheterogeneity (Fig. 4). Strikingly, at P15 and P21, the acidic variant of the α-subunit could not be detected in hindlimb muscle extracts of MTU animals.

The injection of thyroid hormone in these hypothyroid
animals partially restored the normal pattern of α-enolase microheterogeneity.

Localization of α- and β-enolase in hindlimb muscle fibers by indirect immunofluorescence labeling and confocal microscopy. Taking advantage of antibodies produced in different species, we have compared the fiber localization of α- and β-enolase subunits in double-labeling experiments. As shown on gastrocnemius muscle sections of 15-day-old rats (Fig. 5, a and b), some fibers expressed both subunits (1), some expressed α- but not β-subunits (2), and some expressed β- and not α-subunits (3). Other double-labeling experiments were performed using monoclonal antibodies specific for slow- or fast-type MHCs and the polyclonal antibodies specific for each enolase isoform. The results shown here on gastrocnemius sections of 28-day-old rats demonstrated that the muscle-specific enolase subunit was expressed in all fast-twitch and not in slow-twitch fibers (Fig. 5, c and d). Similar results were obtained when the immunocytochemical analysis was conducted with muscles of hypothyroid animals: the β-subunit was never detected in slow-twitch fibers (Fig. 5, g and h), in contrast to the α-subunit (Fig. 5, e and f).

The subcellular localization of the enolase isoforms was investigated in double-labeling experiments with a monoclonal antibody directed against a specific compound of the Z disk, α-actinin, or an antiserum specific for the M band (Fig. 6). Immunolabeling with the anti-β-enolase serum yielded a diffuse appearance (Fig. 6, b), although striations were visible and could be localized to the M band (data not shown). In contrast, a striated fluorescent pattern was observed with the anti-α-enolase serum (Fig. 6, a). This immunoreactivity was dissociated from the Z line (Fig. 6, c) but colocalized with the M band (Fig. 6, d). Similar analyses performed with MTU rats indicated that the subcellular localization of enolase isoforms was independent of the thyroid status, with a localization of the α-subunit at the M band (Fig. 6, e) and a partial localization of the β-subunit at the M band (Fig. 6, f).

Note the immunolabeling of nonmuscle cells by anti-α-enolase (Fig. 6, e) but not anti-β-enolase serum (Fig. 6, f).

Hypothyroidism increases β-enolase gene expression in the heart. It is assessed that the adult heart relies on oxidative metabolism. Hypothyroidism did not modify significantly the α-enolase transcript level in developing cardiac muscle (Fig. 7A) but increased the transcript level of the muscle-specific isoform after 8 days (Fig. 7B). At 13–15 days, the β-enolase transcript levels are significantly higher in hypothyroid than in control rats, but values obtained at 21–28 days are more...
dispersed, and in this case the differences are not statistically significant. Interestingly, an increase in the β-subunit level compared with control level was visible in hypothyroid rats at 21 and 28 days (Fig. 8).

**DISCUSSION**

Among glycolytic enzymes, enolase is unique for the strict cell type specificity of its muscle isoforms, αβ and ββ. Our previous results suggested a role of thyroid hormones in the postnatal rise of β-enolase gene expression (30). Thus the enolase isozymic system has allowed us to study the effect of these hormones simultaneously on the ubiquitous (α) and the muscle-specific (β) genes, both expressed throughout development.

Differential regulation of α- and β-enolase genes by thyroid hormones during hindlimb muscle development. We demonstrate here, for the first time, an important role of thyroid hormones on α- and β-enolase gene expressions during rat muscle maturation. Data obtained from hypothyroid rats show that α- and β-enolase gene expressions are responsive to thyroid hormones, but in different ways. Starting at birth, the ubiquitous enolase transcript level is diminished by hypothyroidism. Thus, throughout the period studied, α-enolase gene expression appears sensitive to thyroid hormones, even to the low levels of these hormones already present in the circulation of neonates. On the contrary, the muscle-specific enolase gene expression is insensitive to the thyroid status of the rats before the establishment of the functional diversification of fibers, indicated in our experiments by the MHC IIB transcript accumulation. The fraction of β-enolase expression responding to thyroid hormones thereafter is less important than that of α-enolase. However, because of the major abundance of the ββ-isof orm in postnatal skeletal muscles (23, 25, 30), the decreased expression of the β-enolase gene should be the determinant for the modulation of total enolase activity. Indeed, we have established that there is a linear relationship between β-enolase transcript level and total enolase activity during normal or hypothyroid postnatal development.

![Fig. 3](http://ajpendo.physiology.org/)

**Fig. 3.** Relationship between total enolase activity and β-enolase transcript levels in developing hindlimb muscles from control and MTU-treated rats. A: enolase activity expressed in international units (IU) per mg protein. There is a linear relationship as a function of time: R = 0.96 for controls, R = 0.91 for MTU-treated animals. Differences between 2 groups of values are significant: P < 0.05 by ANOVA. Each point corresponds to a different animal. B: linear relationship between β-enolase transcript level (arbitrary units) and total enolase activity (IU/mg): R = 0.88. See Fig. 1 legend for explanation of symbols.

In heart, no developmental change in the microheterogeneity of α- and β-enolase subunits, comparable to that of skeletal muscles, was observed. The predominant spot of each subunit remained the basic variant at all the examined times. When heart extracts of control and hypothyroid animals were compared, no change in the microheterogeneity of the α-subunit was observed (Fig. 8).

![Fig. 4](http://ajpendo.physiology.org/)

**Fig. 4.** Changes in microheterogeneity of enolase subunits during hindlimb development: influence of thyroid status. Muscle extracts were analyzed by 2-dimensional gel electrophoresis, then by Western blot. In all cases, 5 µg of protein extracts were analyzed, except at embryonic day 18 (E18), where more material was used (20 µg). Sequential incubations of membranes with specific antibodies directed against α- and β-enolase allowed visualization of both subunits. Analyses of samples corresponding to rats of same age but in different thyroid status were always treated simultaneously and in identical conditions. Exposure time was 3 min, except at postnatal day 21 (P21), where it was 10 min. Control, euthyroid; MTU, hypothyroid; MTU + T, hypothyroid rats injected with T3. Each protein analysis corresponds to 1 rat extract. ▽, α-Enolase; oblique arrows, β-enolase; P8 and P15, postnatal days 8 and 15.
Daily injections of hypothyroid rats with \( T_3 \) from birth restore the control developmental profile of MHC IIB and almost restore that of \( \alpha \)- and \( \beta \)-enolase transcripts, confirming the important role of the hormones in the expression of these genes during development. It is worth noting that hyperthyroidism, induced in mouse from birth, did not modify \( \alpha \)- or \( \beta \)-enolase developmental transcript levels in hindlimb muscles, indicating that circulating levels of thyroid hormones are sufficient to maximally stimulate enolase gene expressions in skeletal muscles of euthyroid animals (unpublished observations).

Our results support the hypothesis of Nemeth et al. (36) suggesting a role of these hormones during skeletal muscle development to coordinate the expressions of contractile proteins and metabolic enzymes and, thus, contribute to the adjustment of energy supply and energy needs. Furthermore, our analyses of enolase conducted during muscle development (present study), together with previous results obtained at the adult stage, underline the possibility that a time window of thyroid hormone responsiveness in hindlimb muscles might exist. Indeed, after thyroidectomy of adult rats, there is an irreversibility of the mature myosin phenotype in the rat extensor digitorum longus (20). We have also established that thyroidectomy of adult mice does not modify MHC IIB or \( \alpha \)- and \( \beta \)-enolase transcript levels in hindlimb muscles (unpublished observations).

Fig. 5. Fiber type localization of \( \alpha \)- and \( \beta \)-enolase subunits in normal and hypothyroid rats. a–d: Muscle sections from control animals that were immunostained in double-labeling experiments and analyzed by confocal microscopy. a and b: Section of 15-day-old rat gastrocnemius, reacted with anti-\( \alpha \)- and anti-\( \beta \)-enolase serum produced in rabbit and sheep, respectively. c and d: Section of 28-day-old rat gastrocnemius reacted with rabbit anti-\( \beta \)-enolase serum and monoclonal antibody MY32 directed against fast MHC (MHCf). e–h: Muscle sections from 28-day-old hypothyroid animals immunostained with anti-\( \alpha \) (e) or anti-\( \beta \)-enolase serum (g) and with monoclonal antibody NCL (f and h) directed against slow-type MHC (MHCs). eno, Enolase immunoreactivity.
Cellular and subcellular localization of muscle enolase isoforms is independent of thyroid status. The immunocytochemical analyses have shown that, after the establishment of functional fiber diversity in control rats, the muscle-specific enolase subunit, β, is specifically expressed in fast-twitch fibers. Although neonatal MHC persists in many myofibers of hypothyroid rats (5, 16), β-enolase immunoreactivity still presents a nonuniform myofiber distribution and is absent from slow-twitch fibers, demonstrating that this heterogeneous distribution is not under thyroid hormone control. In this study we have observed, for the first time, that the ubiquitous subunit of enolase, α, is expressed not only in nonmuscle cells (see labeled vessel cells in Figs. 5 and 6) but also in skeletal muscle fibers. No fiber type-related distribution of this isoform is evident. Hypothyroidism induces an important decrease of α-enolase gene expression (at P21, α-enolase transcript level is 40% of control), and at least part of this drop probably takes place within myofibers.

Observations by confocal microscopy provided insights into the subcellular localization of the α- and β-enolase subunits in hindlimb muscles. The β-enolase subunit, expressed at high levels, mostly exhibits a diffuse immunoreactivity, although some striations are visible at the level of the M band. By contrast, the α-enolase subunit, which is much less abundant, exhibits a striking striated pattern of immunoreactivity, corresponding to the M band. Thus only the β-subunit is localized at the I band, as previously described for muscle glycolytic enzymes (11). The subcellular localizations of α- and β-enolase subunits are not changed in hypothyroid rats. Thus the acidic variant of the α-subunit, which appears, as demonstrated here, under thyroid hormone control and, thus, is undetectable in hypothyroid rats, does not seem important for this localization.

Mechanisms of action of thyroid hormones on enolase gene expressions. The effects of thyroid hormones on α- and β-enolase expressions can be direct or indirect. Direct effects are mediated via T₃ receptors bound to a thyroid hormone response element on the gene. Sequence analysis of the β-enolase gene did not reveal the canonical motif composed of two half-sites, which are usually separated by four nucleotides, and we were able to characterize half-sites only. Such a sequence analysis cannot be done for the rat α-enolase gene, which has not yet been cloned.
Many factors may mediate indirect effects of thyroid hormones on enolase gene expressions. We will discuss some of them. It has been suggested that the concentration of $\beta$-enolase might be related to the functional state of the muscles and, thus, to the energy demands (23, 25, 34). Thyroid hormones have pleiotropic effects, and many of them can influence the energy balance. They control the isomyosin switch during development and, thus, the energetic cost of contraction (8, 40). The relaxing properties of fibers are also modified, since the expressions of $\mathrm{Ca}^{2+}$-ATPases of the sarcoplasmic reticulum vary depending on the thyroid status (41, 42, 45).

Indirect effects of thyroid hormones may also be mediated via its transcriptional regulation of the pituitary growth hormone gene and the subsequent effects on the expression of the insulin-like growth factor (IGF) (4, 10, 35). The important role of IGFs in vitro myogenic differentiation and in innervation is well established (for review see Ref. 48). The expression of IGF-II has been correlated with myogenic differentiation in vivo and in vitro (15, 27, 39). IGF-II mRNA is present in large amounts in fetal skeletal muscles, and its level decreases dramatically after birth (3) at the time of elimination of superfluous synapses (19). The high level of IGF-II gene expression in developing muscles may reflect a state receptive to innervation (17, 29). It is well known that superfluous synapse elimination is delayed in muscles from hypothyroid animals (28).

In the course of this study, we have observed that IGF-II transcript levels decreased postnatally in control hindlimb muscles and remained high in muscles of hypothyroid rats (unpublished observations). Therefore, it is possible that an increased release of IGF-II from the muscles might sustain a persistent multiaxonal innervation in these animals and, as a consequence, modify $\beta$-enolase gene expression.

Thyroid hormone sensitivity of enolase genes is muscle type specific. In heart, in contrast to hindlimb muscles, enolase gene expressions are not depressed by hypothyroidism. On the contrary, thyroid hormone withdrawal results in an increase in the muscle-specific form of enolase gene expression.

It had been proposed that hypothyroidism induces mitochondrial metabolism impairment, and this could lead to an abnormal recruitment of several metabolic pathways, such as glycolysis (21). The effects of thyroid hormones on the enzymes of the respiratory chain are also developmentally modulated and are specific to the tissue examined. These differences may be a consequence of the different energy requirements of tissues such as heart and skeletal muscle during development (37, 43). The interdependency of these two energy-producing pathways, oxidative and glycolytic, should be considered to interpret the resulting effect of thyroid hormones on energy production during skeletal and cardiac muscle development.

From our results, we can establish some correlation between the expression of the $\beta$-enolase gene during development and its response to different thyroid statuses in different muscles. When seric levels of thyroid hormones increase, $\beta$-enolase gene products increase in hindlimb muscles, whereas the $\beta$-subunit decreases in heart (25, 30). Hypothyroidism results in a diminution of $\beta$-enolase gene expression in hindlimb muscles and an increase of this expression in heart.

In conclusion, the differences in the developmentally and hormonally controlled expressions of the isoforms of the glycolytic enzyme enolase, between hindlimb
muscles and heart, most probably reflect a physiological adjustment to the differences in energy requirements of these two types of muscles.

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