Thyroid hormones and the mRNA of the GH receptor and IGFs in skeletal muscle of fetal sheep

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Forhead, A. J., J. Li, R. S. Gilmour, M. J. Dauncey, and A. L. Fowden. Thyroid hormones and the mRNA of the GH receptor and IGFs in skeletal muscle of fetal sheep. Am J Physiol Endocrinol Metab 282: E80–E86, 2002—Thyroid hormones are required for the normal development of skeletal muscle in utero, although their mechanism of action is poorly understood. The present study examined the effects of the thyroid hormones on the gene expression of the growth hormone receptor (GHR) and the insulin-like growth factors (IGFs) IGF-I and IGF-II, in skeletal muscle of fetal sheep during late gestation (term: 145 ± 2 days) and after manipulation of plasma thyroid hormone concentration. Thyroidectomy at 105–110 days of gestation suppressed muscle GHR and IGF-I gene expression in fetuses studied at 127–130 and 142–145 days. Muscle GHR mRNA abundance remained unchanged with increasing gestational age in intact and thyroidectomized fetuses. In the intact fetuses, a decrease in muscle IGF-I gene expression was observed between 127–130 and 142–145 days, which coincided with the normal prepartum surges in plasma cortisol and triiodothyronine (T3). At 127–130 days, down-regulation of muscle IGF-I mRNA abundance was induced prematurely in intact fetuses by an infusion of cortisol for 5 days (2–3 mg·kg−1·day−1·iv), which increased plasma cortisol and T3 concentrations to values seen near term. However, increasing plasma T3 alone by an infusion of T3 for 5 days (8–12 μg·kg−1·day−1·iv) in intact fetuses at this age had no effect on GHR or IGF-I gene expression in skeletal muscle. In the thyroidectomized fetuses, no additional change in the low level of muscle IGF-I mRNA abundance was seen with increasing gestational age, but at 127–130 days, IGF-I gene expression was reduced further when plasma cortisol and T3 concentrations were increased by exogenous cortisol infusion. Muscle IGF-II mRNA abundance was not affected by thyroidectomy, gestational age, or exogenous hormone infusion. These findings show, in the sheep fetus, that thyroid hormones may influence the growth and development of skeletal muscle via changes in the local activity of the somatotrophic axis.

growth hormone receptor; cortisol; fetus; insulin-like growth factors; thyroxine; triiodothyronine

THYROID HORMONES ARE ESSENTIAL for normal fetal growth and development (15). In fetal sheep, hypothyroidism causes growth retardation and is associated with ab-

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have been shown to depend on the rise in fetal plasma cortisol concentration that normally occurs over the last few days of gestation (29, 30). Because the prepartum cortisol surge is also responsible for stimulating the deiodination of thyroxine (T\textsubscript{4}) to T\textsubscript{3}, there is a rise in plasma T\textsubscript{3} concentration in the fetus toward term that coincides with the ontogenic changes in tissue IGF expression (13, 14, 17, 18). However, the role of the increase in plasma T\textsubscript{3} concentration near term in mediating the cortisol-induced decline in IGF-I gene expression in skeletal muscle is unknown.

Therefore, the aims of the present study were 1) to investigate the role of the thyroid hormones in the control of GHR, IGF-I, and IGF-II gene expression in the skeletal muscle of fetal sheep during late gestation and 2) to determine whether the prepartum rise in plasma T\textsubscript{3} mediates the maturational effects of cortisol on IGF-I mRNA abundance close to term. The effects of the thyroid hormones on GHR and IGF gene expression were examined in skeletal muscle taken from sheep fetuses after manipulation of plasma thyroid hormone concentration by thyroidectomy and exogenous hormone infusion.

**MATERIALS AND METHODS**

**Animals.** Thirty-one Welsh Mountain sheep fetuses of known gestational age were used in this study and were randomly assigned to the treatment groups. All but two of the fetuses were twins. The ewes were housed within the laboratory animal house in individual pens and were maintained on 200 g/day concentrates (Ewe Ration 18; 10.5 MJ/kg digestible energy, 18% protein; H & C Beart, Stowbridge, UK) with free access to hay, water, and a salt lick block. Food, but not water, was withheld for 18–24 h before surgery. Table 1 shows the number, gestational ages, and body weights at postmortem of the fetuses used in each experimental group of the study. All surgical and experimental procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act of 1986.

**Surgical procedures.** All surgical operations were performed under halothane anesthesia (1.5% in O\textsubscript{2}-N\textsubscript{2}O) with positive pressure ventilation. Between 105 and 110 days of gestation (term 145 ± 2 days), 12 fetuses were thyroidectomized (TX) in utero using surgical techniques described previously (23). Four of these TX fetuses were catheterized in a second operation at 115–120 days, along with 12 of the intact fetuses. Intravascular catheters were inserted into the femoral artery and a branch of the femoral vein in the fetuses and into the maternal femoral artery as described previously (6). All catheters were exteriorized through the flank of the ewe and secured in a plastic pouch sutured to the skin. The catheters were flushed daily with heparinized saline solution (100 IU heparin/ml in 0.9% wt/vol saline) from the day after surgery. At surgery, all fetuses were administered intravenously 100 mg ampicillin (Penbritin; Beecham Animal Health, Brentford, UK) and 2 mg gentamicin (Frangene-100; Biotvet, Mullingar, Ireland). The ewes received antibiotic intramuscularly [900 mg procaine penicillin (Depocillin); Mycofarm, Cambridge, UK] on the day of surgery and for 3 days thereafter. Normal feeding patterns were restored within 24 h of surgery.

**Experimental procedures.** Blood samples of 2 ml were taken daily from the catheterized fetuses and ewes to monitor fetal well-being and blood gas status and to determine plasma hormone concentrations.

Four intact and four TX fetuses were infused with cortisol (2–3 mg·kg\textsuperscript{-1}·day\textsuperscript{-1} iv Efocortilan; Glaxo, Ware, UK) for 5 days before delivery for tissue collection at 127–130 days. In addition, eight intact fetuses were infused with either T\textsubscript{3} (8–12 μg·kg\textsuperscript{-1}·day\textsuperscript{-1} iv, n = 5; Sigma, Poole, UK) or saline (n = 3) for 5 days before delivery at 127–130 days. The doses of exogenous cortisol and T\textsubscript{3} infused were calculated to mimic the plasma concentrations normally observed in the immediate prepartum period (13). Of the remaining untreated fetuses, three intact and four TX fetuses were delivered at 127–130 days, and four intact and four TX fetuses were delivered at 142–145 days of gestation.

All fetuses were delivered by Caesarean section under general anesthesia (20 mg/kg iv pentobarbital sodium). On the day of delivery, normal feeding patterns were observed in all ewes, and none of the animals showed any signs of labor. At delivery, blood samples were taken by venipuncture of the umbilical artery. All blood samples obtained during the study were placed into EDTA-containing tubes and centrifuged for 5 min at 1,000 g and 4°C; the plasma aliquots were stored at −20°C until analysis. A number of tissues were collected from the fetuses immediately after the administration of a lethal dose of pentobarbital sodium (200 mg/kg). Samples of skeletal muscle from a hindlimb were snap frozen in liquid nitrogen and stored at −80°C until analysis. At delivery, there was no evidence of thyroidal remnants in any of the TX fetuses.

**Biochemical analyses.** Plasma cortisol concentration was measured by radioimmunoassay validated for use with ovine plasma, as described previously (38). The lower limit of detection was 2–5 nmol/l, and the interassay coefficient of variation was 11%. Plasma T\textsubscript{3} and T\textsubscript{4} concentrations were also measured by radioimmunoassay using a commercial kit validated for ovine plasma (ICN Biochemicals, Thame, UK) (16). The lower limits of detection were 0.21 nmol/l for T\textsubscript{3} and 8.8 nmol/l for T\textsubscript{4}. The interassay coefficient of variation was 10% for both assays.

Table 1. Numbers and gestational ages of the fetuses used in the different experimental groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Fetuses</th>
<th>Gestational Age, days</th>
<th>Body Weight at Postmortem, kg</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>At thyroidectomy</td>
<td>At catheterization</td>
</tr>
<tr>
<td>Intact and untreated</td>
<td>6</td>
<td>115–120</td>
<td>127–130</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>142–145</td>
</tr>
<tr>
<td>Intact and cortisol infusion</td>
<td>4</td>
<td>115–120</td>
<td>127–130</td>
</tr>
<tr>
<td>Intact and T\textsubscript{3} infusion</td>
<td>5</td>
<td>115–120</td>
<td>127–130</td>
</tr>
<tr>
<td>TX and untreated</td>
<td>4</td>
<td>105–110</td>
<td>127–130</td>
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<tr>
<td></td>
<td>4</td>
<td>105–110</td>
<td>142–145</td>
</tr>
<tr>
<td>TX and cortisol infusion</td>
<td>4</td>
<td>105–110</td>
<td>127–130</td>
</tr>
</tbody>
</table>

Gestational ages are ranges; body weights are means ± SE. T\textsubscript{3}, triiodothyronine; TX, thyroidectomized.
Tissue RNA isolation and RNase protection assay. Total RNA was extracted from 1 g of frozen tissue by use of the guanidinium thiocyanate method of Chomczynski and Sacchi (4) and was quantified by absorbance at 260 nm (1 OD + 35 μg/ml). To check the equivalence of the RNA samples, total poly(A)+ was also measured as described previously (39). When RNA samples from all tissues were considered, there was a constant relationship between absorbance and total poly(A)+ content. The RNase protection assay was carried out on 50-μg samples of total RNA with the use of the ovine GHR, IGF-I, and IGF-II riboprobes as described previously (30, 32). The relative intensities of the protected band on the X-ray film were quantified by densitometry and normalized using a number of control samples run on all gels.

Statistical analyses. All data are presented as means ± SE. Previous studies in this laboratory have shown that there are no differences in plasma cortisol, T3, or T4 concentrations in intact fetuses that have and have not been catheterized (16); therefore, the values from the untreated and catheterized intact fetuses were pooled. Significant differences in the measurements made between the different groups of fetuses were assessed by unpaired t-test. Within each group of fetuses infused with either cortisol or T3, paired t-tests were used to compare the values observed before the infusion began and 5 days later. All the actual measured values of plasma T3 and T4 were used in the statistical analysis, even if they fell below the lower limit of assay detection. Differences where P < 0.05 were regarded as significant.

RESULTS

Ontogeny and effect of thyroidectomy. Between 127–130 and 142–145 days of gestation, a significant increase in plasma cortisol concentration was observed in both the intact and TX fetuses (P < 0.05 in both cases, Table 2). No significant difference in plasma cortisol concentration was seen between the two groups of fetuses at each gestational age (Table 2). In the intact, but not TX fetuses, the prepartum cortisol surge was accompanied by a significant increase in plasma T3 concentration from an undetectable level (P < 0.05, Table 2). At 142–145 days, plasma T3 in the intact fetuses was significantly greater than that observed in the TX fetuses (P < 0.05, Table 2). At both gestational ages studied, fetal thyroidectomy reduced the plasma concentration of T4 to a value below the limit of assay detection and significantly less than that seen in the intact fetuses (P < 0.05 at both ages, Table 2). Plasma T4 remained unchanged between 127–130 and 142–145 days in both groups of fetuses (Table 2).

Fetal thyroidectomy caused a significant decrease in GHR gene expression in skeletal muscle (Fig. 1A). At both 127–130 and 142–145 days, muscle GHR mRNA abundance was significantly lower in the TX fetuses than in the intact fetuses (P < 0.05 at both ages, Fig. 1A). No ontogenic change in muscle GHR mRNA level was observed in either group of fetuses (Fig. 1A).

At 127–130 days, IGF-I gene expression in skeletal muscle was also suppressed by fetal thyroidectomy (Fig. 1B). Muscle IGF-I mRNA abundance in the TX fetuses was significantly lower than that observed in the intact fetuses at the same gestational age (P < 0.05, Fig. 1B). In the intact fetuses, a significant decrease in IGF-I mRNA abundance in skeletal muscle was observed between 127–130 and 142–145 days (P < 0.05, Fig. 1B). This ontogenic decrease in muscle IGF-I gene expression was not seen in the TX fetuses: the mean IGF-I mRNA level in the skeletal muscle of these animals was already low at 127–130 days of gestation (Fig. 1B). No significant difference in muscle IGF-I mRNA level was seen between the intact and TX fetuses at 142–145 days (Fig. 1B).

No significant differences in muscle IGF-II gene expression were observed between the intact and TX fetuses at either gestational age studied (Fig. 1C). Between 127–130 and 142–145 days, a decrease in mean IGF-II mRNA abundance was seen in the skeletal muscle of both groups of fetuses, but this was only significant for the TX fetuses (P < 0.05, Fig. 1C).

Effect of cortisol infusion. An infusion of cortisol for 5 days significantly increased plasma cortisol concentration in the intact and TX fetuses by a similar extent and to a value normally seen near term (P < 0.05 in both groups, Table 2). At delivery at 127–130 days, values of concentrations are means ± SE in nmol/l. T3, thyroxine; ND, not detectable (below the lower limit of assay detection). *Significantly different from the control intact fetuses at 127–130 days, P < 0.05; †significantly different from the control TX fetuses at 127–130 days, P < 0.05; ‡significantly different from the intact fetuses at 142–145 days, P < 0.05; §significantly different from the preinfusion value, P < 0.05.

Table 2. Plasma concentrations of cortisol, T3, and T4 in intact and TX fetuses at 127–130 and 142–145 days, and intact and TX fetuses infused with cortisol for 5 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gestational Age at Delivery, days</th>
<th>Cortisol Preinfusion</th>
<th>Cortisol Delivery</th>
<th>T3 Preinfusion</th>
<th>T3 Delivery</th>
<th>T4 Preinfusion</th>
<th>T4 Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>127–130</td>
<td>32.6 ± 2.5</td>
<td>ND</td>
<td>160.0 ± 26.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX</td>
<td>127–130</td>
<td>22.3 ± 2.5</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>142–145</td>
<td>161.4 ± 38.6*</td>
<td>1.00 ± 0.24*</td>
<td>125.4 ± 8.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX</td>
<td>142–145</td>
<td>172.1 ± 42.2†</td>
<td>ND§</td>
<td>ND</td>
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</table>

Intact and TX fetuses infused with cortisol for 5 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cortisol Preinfusion</th>
<th>Cortisol Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact + cortisol</td>
<td>28.7 ± 3.9</td>
<td>175.2 ± 32.6§</td>
</tr>
<tr>
<td>TX + cortisol</td>
<td>22.3 ± 2.5</td>
<td>189.2 ± 14.1‡§</td>
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plasma cortisol concentration in the cortisol-infused intact and TX fetuses was significantly greater than that observed in their respective groups of control fetuses ($P < 0.05$ in both groups, Table 2) and was similar to the value seen in the intact and TX fetuses studied at 142–145 days (Table 2). Over the period of cortisol infusion, plasma $T_3$ concentration increased significantly in both the intact and TX fetuses ($P < 0.05$ in both groups, Table 2). At delivery at 127–130 days, plasma $T_3$ concentration in the cortisol-infused intact and TX fetuses was significantly higher than that observed in their respective groups of control fetuses ($P < 0.05$ in both groups, Table 2) and resembled that seen in the intact fetuses studied at 142–145 days (Table 2). Plasma $T_4$ concentration was unaffected by cortisol infusion in both groups of fetuses (Table 2).

The rise in plasma cortisol induced by exogenous cortisol infusion had no effect on muscle GHR gene expression in either the intact or TX fetuses (Fig. 2A). At 127–130 days, muscle GHR mRNA abundance in the cortisol-infused intact and TX fetuses was significantly higher than that observed in their respective groups of control fetuses ($P < 0.05$ in both groups, Table 2) and resembled that seen in the intact fetuses studied at 142–145 days (Table 2).
both groups of cortisol-infused fetuses remained similar to that seen in their respective groups of control fetuses (Fig. 2A).

Muscle IGF-I gene expression was suppressed in both the intact and TX fetuses when plasma cortisol was increased to a value normally seen at term (Fig. 2B). At 127–130 days, the level of IGF-I mRNA observed in the cortisol-infused intact and TX fetuses was significantly lower than in their respective groups of control fetuses ($P < 0.05$ in both groups, Fig. 2B) and resembled that seen in the intact and TX fetuses at 142–145 days (Figs. 1B and 2B).

Because of the wide interanimal variation, no significant difference in muscle IGF-II gene expression was observed between the cortisol-infused intact and TX fetuses and their respective groups of control fetuses (Fig. 2C). However, all four of the cortisol-infused intact fetuses had a lower muscle IGF-II mRNA level than their respective control twins. Furthermore, muscle IGF-II mRNA abundance in three of the four TX fetuses infused with cortisol was lower than the mean value seen in the control TX fetuses (Fig. 2C).

**Effect of T3 infusion.** In the intact fetuses, a 5-day period of exogenous T3 infusion significantly increased plasma T3 concentration to a value normally seen close to term ($P < 0.05$, Table 3). At delivery at 127–130 days, plasma T3 concentration in the T3-infused fetuses was significantly greater than that observed in the control intact fetuses ($P < 0.05$, Table 3) and resembled the concentration seen in the intact fetuses studied at 142–145 days (Table 2). Plasma concentrations of cortisol and T4 in the T3-infused fetuses at 127–130 days remained similar to those seen in the control intact fetuses at the same gestational age (Table 3).

The gene expression of GHR, IGF-I, and IGF-II in skeletal muscle did not significantly change when plasma T3 concentration was raised by T3 infusion (Table 3). At 127–130 days, muscle GHR, IGF-I, and IGF-II mRNA levels in the T3-infused fetuses remained similar to those observed in the control intact fetuses at the same gestational age (Table 3).

**DISCUSSION**

**Role of the thyroid hormones in the control of GHR and IGF gene expression.** The results of the present study show, in the sheep fetus, that an intact thyroid gland is essential for the normal expression of the GHR and IGF-I genes in skeletal muscle. Thyroidectomy in utero suppressed muscle GHR and IGF-I mRNA levels but had no effect on the gene expression of IGF-II. The thyroid hormones, therefore, appear capable of influencing skeletal muscle development before birth, partly via changes in tissue GHR and IGF-I mRNA abundance. More specifically, it appears to be the deficit in circulating T4 rather than T3 that is responsible for the changes observed after fetal thyroidectomy. Indeed, increasing plasma T3 alone by exogenous infusion did not have any effect on GHR or IGF-I mRNA abundance. These findings are in agreement with those in fetal pigs, where T4 replacement restores the low levels of muscle IGF-I content induced by hypophysectomy (27).

In the present study, the decrease in muscle IGF-I gene expression induced by hypothyroidism was likely to be due, in part, to the reduction in GHR mRNA abundance. Thyroid hormones in utero may act to maintain normal IGF-I mRNA abundance in skeletal muscle primarily by regulating the expression of the GHR. Indeed, in many species, the presence of the GHR in fetal skeletal muscle during late gestation suggests that GH may have an important role in the development and metabolism of muscle in utero, either directly or via changes in IGF-I gene expression (19, 25, 40). In addition, the overall drive for GH-dependent IGF-I production appears to be suppressed by hypothyroidism because in the sheep fetus, thyroidectomy also downregulates pituitary GH mRNA abundance and circulating GH concentration (37). By contrast to the present findings, fetal hypothyroidism induced by maternal glucosinolate treatment in pigs has been shown to increase GHR gene expression in skeletal muscle (9). However, in that earlier study, fetal plasma concentrations of total T4 and T3 were only reduced by up to 50% of values in the control animals, and a compensatory increase in thyroid hormone receptor density has been observed in the skeletal muscle of the fetal pig in response to this form of hypothyroidism (8, 9). Compared with IGF-I, muscle IGF-II gene expression in the sheep fetus appeared unaffected by thyroidectomy or by the subsequent changes in GHR mRNA abundance. Similarly, skeletal muscle IGF-II content was unchanged in pigs hypophysectomized in utero (27). Therefore, the gene expression of IGF-II in skeletal muscle is likely to be regulated by factors other than GH, such as local changes in nutrient availability and other hormones.

**Do thyroid hormones mediate the cortisol-induced changes in GHR and IGF gene expression?** In fetal sheep, T3 has been shown to be involved in the ontogenic changes in hepatic GHR and IGF gene expression.

Table 3. Plasma concentrations of cortisol, $T_3$, and $T_4$ and mRNA abundance of GHR, IGF-I, and IGF-II in skeletal muscle of intact fetuses infused with $T_3$ for 5 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cortisol, nmol/l</th>
<th>$T_3$, nmol/l</th>
<th>$T_4$, nmol/l</th>
<th>GHR mRNA, arbitrary units</th>
<th>IGF-I mRNA, arbitrary units</th>
<th>IGF-II mRNA, arbitrary units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preinfusion</td>
<td>Delivery</td>
<td>Preinfusion</td>
<td>Delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>32.6 ± 2.5</td>
<td>ND</td>
<td>ND</td>
<td>160.0 ± 26.5</td>
<td>15.8 ± 1.2</td>
<td>13.4 ± 1.9</td>
</tr>
<tr>
<td>Intact + $T_3$</td>
<td>29.0 ± 4.1</td>
<td>33.7 ± 4.7</td>
<td>0.85 ± 0.04†</td>
<td>130.6 ± 12.6</td>
<td>107.6 ± 10.6</td>
<td>16.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. GHR, growth hormone receptor; IGF, insulin-like growth factor. *Significantly different from control intact fetuses, $P < 0.05$; †Significantly different from preinfusion value, $P < 0.05$. 

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caused by endogenous and exogenous increments in cortisol (13, 14). However, there was little evidence from the present study to suggest that the maturational effect of cortisol on muscle GHR and IGF gene expression was mediated exclusively by the cortisol-induced rise in plasma T3. In immature fetuses, increasing plasma T3, but not cortisol, concentration to the level normally seen close to term had no effect on GHR or IGF mRNA abundance. Furthermore, the ontogenetic decrease in IGF-II mRNA abundance was still evident in the TX fetuses in which the prepartum T3, but not cortisol, surge was abolished. However, the observation that the low level of muscle IGF-I mRNA could be suppressed further when plasma cortisol and T3 were both raised in the immature TX fetuses infused with cortisol, but not in the mature TX fetuses when cortisol but not T3 concentrations were high, suggests that downregulation of muscle IGF-I gene expression near term may depend on the prepartum surge in both cortisol and T3. In the sheep fetus, the ontogeny and effects of hormones on GHR and IGF-I gene expression in skeletal muscle contrast with those seen in the liver, where exogenous and endogenous increments in cortisol and T3 upregulate both GHR and IGF-I mRNA abundance (14). Therefore, the effects of these hormones are tissue specific.

The inability of T3 alone to suppress IGF-I gene expression in skeletal muscle in utero may be a consequence of low exposure of the tissue to T3. Compared with other tissues, a high rate of inner ring monodeiodinase enzyme activity (which both metabolizes T4 to biologically inactive reverse-T3, and inactivates T3 to diiodothyronine) has been reported in the skeletal muscle of fetal rats (24). Alternatively, the lack of effect of exogenous T3 on muscle GHR or IGF-I gene expression at 127–130 days may have been due to the relatively low number of thyroid hormone receptors present in fetal tissues at this gestational age (35). Although not quantified in skeletal muscle, thyroid hormone binding in the liver of fetal sheep increases progressively from 80 days of gestation to peak at birth (35). In fetal pigs, there are increases in thyroid hormone receptor density in both the liver and skeletal muscle toward term (8). The effects of increasing plasma cortisol alone without a concomitant rise in plasma T3 could not be investigated in the present study, as an increment in circulating T3 was observed in the immature TX fetuses infused with cortisol. This rise may have resulted from the desulfation of T3 sulfate, which is known to persist in the circulation of the sheep fetus for 2 wk after thyroidectomy (13, 42).

Mechanisms of hormone action. The mechanisms by which cortisol and the thyroid hormones alter GHR and IGF gene expression in fetal skeletal muscle remain unclear. There may be changes in gene transcription and/or mRNA stability and nucleocytoplasmic transport in response to these hormones. Previous studies have shown that the ovine IGF-II gene has no thyroid hormone response element (TRE) but is downregulated transcriptionally by cortisol in the liver via a specific promoter in leader exon 7 (31). The thyroid hormones and cortisol are unlikely to have direct effects on the ovine IGF-I gene because the published genomic sequence, which contains extensive regions 5’ to the start site for transcription, does not contain either TRE or glucocorticoid response elements (GRE; Ref. 7). In sheep, two untranslated leader exons (1A and 1B) of the GHR gene, which are alternately spliced to the coding exons, have been identified (1). Although the two transcripts could not be distinguished with the common coding exon riboprobe used in the present study, GHR mRNA in fetal ovine skeletal muscle has been shown to be derived primarily from exon 1B (1). Sequence analysis of the 5’ flanking region of this leader exon has revealed a putative TRE but not GRE (1). Taken together, the current findings suggest that T4 may affect muscle GHR gene expression directly, but that the effects of cortisol and thyroid hormones on muscle IGF-I mRNA abundance may occur via changes in GHR abundance and/or GH activation by GH. Alternatively, the reductions in GHR and IGF-I mRNA abundance may have occurred secondarily to more generalized changes in the morphology of skeletal muscle after fetal thyroidectomy. In fetal sheep, thyroidectomy at 90–110 days of gestation has been shown to induce muscle hypoplasia (10) and, at 70–75 days, to reduce myofiber size and alter the ratio of fast-type to slow-type fibers seen at term (11). However, the extent to which the endocrine-induced changes in muscle GHR and IGF-I gene expression seen in the present study are the cause or the consequence of the changes in the structural and functional properties of skeletal muscle remains to be established.

Perspectives. The findings of the present study have provided one specific mechanism by which the thyroid hormones may influence the development of skeletal muscle in utero. Changes in the bioavailability of thyroid hormones before birth may alter muscle growth and differentiation via modulation of the local somatotrophic axis. Because skeletal muscle accounts for a large proportion of body mass at birth, the effects of the thyroid hormones on the expression of the GHR and IGF-I genes are likely to have an important role in the determination of body size in the newborn animal. Furthermore, in species like sheep, in which the skeletal muscle cell number is determined primordially in fetal life (2), the effects of the thyroid hormones on muscle development may have long-term consequences for the structural and functional properties of skeletal muscle after birth.

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REFERENCES


E86 TH AND mRNA OF MUSCLE GHR AND IGFS IN UTERO


