Control of ovine hepatic growth hormone receptor and insulin-like growth factor I by thyroid hormones in utero

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Forhead, A. J., J. Li, J. C. Saunders, M. J. Dauncey, R. S. Gilmour, and A. L. Fowden. Control of ovine hepatic growth hormone receptor and insulin-like growth factor I by thyroid hormones in utero. Am J Physiol Endocrinol Metab 278: E1166–E1174, 2000.—By use of RNase protection assays, hepatic growth hormone receptor (GHR) and insulin-like growth factor I (IGF-I) mRNA abundances were measured in sheep fetuses after experimental manipulation of fetal plasma thyroid hormone concentrations by fetal thyrodectomy (TX) and exogenous infusion of triiodothyronine (T3) and cortisol. TX abolished the normal prepartum rise in hepatic GHR abundance but had little effect on hepatic GHR gene expression at 127–130 days (term 145 ± 2 days). By contrast, it upregulated basal IGF-I expression in immature fetal liver by increasing both Class 1 and Class 2 transcript abundance but had no further effects on IGF-I gene mRNA levels at 142–145 days. Raising plasma T3 to prepartum values by exogenous infusion of either T3 or cortisol into immature intact fetuses prematurely raised hepatic GHR and IGF-I mRNA abundances to values similar to those seen in intact fetuses at 142–145 days. In TX fetuses, cortisol infusion increased hepatic GHR mRNA but not total IGF-I mRNA abundance at 127–130 days. These findings show that thyroid hormones have an important role in the regulation of hepatic GHR and IGF-I gene expression in fetal sheep during late gestation and suggest that T3 mediates the maturational effects of cortisol on the hepatic somatotrophic axis close to term.

fetal tissues, but their abundance in the liver is low until around birth (1, 8, 15, 16, 18, 33). In fetal sheep and pigs, upregulation of hepatic GH receptor gene expression begins before delivery and is accompanied by an increase in hepatic IGF-I gene expression (7, 21, 33). Therefore, during the perinatal period, there is a developmental shift from GH-independent, local synthesis of IGF-I in utero to GH receptor-stimulated production of hepatic IGF-I characteristic of the postnatal animal.

In sheep, induction of the hepatic GH receptor and the maturational switch in hepatic IGF-I synthesis are initiated by the prepartum rise in fetal plasma cortisol (21). This cortisol increment begins 10–15 days before delivery and escalates rapidly in the last 3–5 days before birth (11). It is also responsible for many of the maturational changes that prepare the fetus for extrauterine life (11, 23). For example, cortisol suppresses IGF-II gene expression and enhances gluconeogenic enzyme activities in the fetal liver (11, 22, 26). It also stimulates deiodination of thyroxine (T4) to triiodothyronine (T3) and, thereby, leads to a prepartum rise in plasma T3 that coincides with the increases in hepatic GH receptor and IGF-I gene expression toward term (21, 23). In several tissues, the prepartum rise in plasma T3 is believed to mediate the maturational effects of cortisol (10, 11, 23).

Thyroid hormones are known to be essential for normal fetal growth and development (10). Hypothyroidism causes intrauterine growth retardation and is associated with low IGF-I concentrations in the fetal circulation (12, 24). It also alters hepatic GH receptor and IGF-I gene expression in neonatal and adult animals (2, 3, 7, 26, 35). Furthermore, recent studies have shown that thyroid hormones are involved in the ontogenic changes in hepatic IGF-II gene expression induced by cortisol in sheep fetuses close to term (9). However, little is known about the role of the thyroid hormones in the regulation of hepatic GH receptor and IGF-I mRNA abundance in utero, especially during late gestation when fetal plasma cortisol levels rise and the major changes in expression of these genes occur. The present study, therefore, investigated 1) the role of thyroid hormones in the control of hepatic GH receptor and IGF-I gene expression during late gestation and 2) whether the prepartum rise in plasma T3 is responsible for the maturational action of cortisol on these genes. The effects of thyroid hormones on hepatic GH receptor

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and IGF-I gene expression were examined in sheep fetuses after experimental manipulation of plasma thyroid hormone concentrations by fetal thyroidectomy and exogenous hormone infusion.

**METHODS**

**Animals**

A total of 33 fetuses from 22 Welsh Mountain ewes of known gestational age were used in this investigation. All but two of the fetuses studied were twins. The ewes were 3–4 yr old and weighed between 40 and 50 kg at the time of study. They were housed in individual pens and fed concentrates (200 g/day; H & G Beart, Stowbridge, Kings Lynn, UK) and hay ad libitum. Food but not water was withheld for 16–24 h before surgery. Normal feeding patterns were restored within 24 h of operation. All procedures were approved and licensed by the Home Office of the UK government under the Animals (Scientific Procedures) Act 1986.

**Surgical Procedures**

Under halothane anesthesia (1.5% in O₂-N₂O), one of the three following procedures was carried out using surgical methods already published (13): 1) thyroidectomy of the fetus, 2) intravascular catheterization of fetuses thyroidectomized (TX) at a previous operation, and 3) intravascular catheterization of intact fetuses. The numbers and gestational ages of the fetuses at operation are shown in Table 1 (term 145 ± 2 days).

**Experimental Procedures**

Blood samples of 2 ml were taken daily throughout the experimental period from all the catheterized fetuses to monitor fetal well-being and to determine plasma cortisol, T₄, and T₃ concentrations. At least 6 days after catheterization, 13 intact fetuses were infused intravenously with cortisol (2–3 mg·kg⁻¹·day⁻¹) in 3.0 ml 0.9% saline; EF-Cortelan, Glaxo, Greenford, Middlesex, UK, n = 4) or saline (3.0 ml/day 0.9% wt/vol saline; Sigma, Poole, Dorset, UK, n = 5) for 5 days beginning between 123 and 125 days of gestation (Table 1). Similarly, 5 TX fetuses were infused intravenously with cortisol (2–3 mg·kg⁻¹·day⁻¹) in 3.0 ml 0.9% saline; Sigma, Poole, Dorset, UK, n = 5) for 5 days beginning between 123 and 125 days of gestation (Table 1). The fetuses to be saline or hormone infused were chosen randomly. The doses of cortisol and T₃ were chosen to mimic the plasma concentrations normally observed in the immediate preparum period (23).

**Sample Collection**

All operated fetuses, irrespective of previous treatment, and seven additional untreated intact fetuses were delivered by cesarean section under sodium pentobarbital anesthesia (20 mg/kg iv); details of the numbers, body weight, and ages of the animals at delivery are given in Table 1. Blood samples were taken from all of the fetuses at the time of delivery either through the indwelling catheters or by venipuncture from the umbilical artery after anesthesia had been induced. After administration of a lethal dose of anesthesia (200 mg/kg sodium pentobarbital), 3- to 5-g samples of the right lobe of the liver, adjacent to the gall bladder, were collected and snap frozen in liquid nitrogen before storage at −80°C. All blood samples were centrifuged immediately at 4°C, and the plasma was stored at −20°C until analysis. At delivery, no obvious thyroidal remnants were found in any of the TX fetuses.

**Biochemical Analyses**

Hormone determinations. Plasma concentrations of cortisol, T₄, and T₃ were measured by radioimmunoassay validated for use with ovine plasma as described previously (12). The limits of detection were 1.5 ng/ml for cortisol and 7.0 and 0.1 ng/ml for T₄ and T₃, respectively. The interassay coefficients of variation for cortisol, T₄, and T₃ were 10%.

RNAase protection assays. Total RNA was isolated from 1-g portions of frozen liver with a guanidine thiocyanate method and quantified by absorbance at 260 nm (1 A = 40 µg/ml). To check the equivalence of RNA samples, total poly (A)⁺ content was also measured as published previously (32). A constant relation between absorbance and poly (A)⁺ content was found for ovine fetal tissues (20). RNAase protection assays were performed on 50 µg of total RNA. The ovine growth hormone receptor (GHR) and IGF-I riboprobes were derived from published sequences, as described previously (21). The GHR riboprobe protected a 138 nucleotide fragment of GHR mRNA from the intracellular domain of the receptor (21). Ovine IGF-I mRNA has multiple forms, which have been classified as Class 1 or Class 2 transcripts depending on which of two possible 5’ leader exons (exon 1 or exon 2) is spliced to the IGF-I first coding exon 3 (32). The IGF-I riboprobe used in this study spanned exons 2–3 and protected two fragments of 132 and 147 nucleotides derived from hybridization to the Class 1 and Class 2 IGF-I mRNA transcripts, respectively (21). Protected fragments were separated on 6% polyacrylamide denaturing gels and exposed to X-ray film (Kodak, Cambridge, UK). Protected bands were quantified by measuring the integrated optical density of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>At thyroidectomy</th>
<th>At catheterization</th>
<th>At tissue collection</th>
<th>Body Weight at Delivery, kg</th>
<th>No. of Fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline infused</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol infused</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T₃ infused</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol infused</td>
<td></td>
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</tr>
</tbody>
</table>

Values are ranges (gestational age), means ± SE (body weight at delivery), and single nos. (fetus nos.). T₃, triiodothyronine.
Table 2. Concentrations of plasma cortisol, T₄, and T₃ at delivery in control intact and TX sheep fetuses at 127–130 days and 142–145 days and in intact and TX fetuses at 127–130 days after 5 days of cortisol or T₃ infusion

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>n</th>
<th>Cortisol</th>
<th>T₄</th>
<th>T₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>Control</td>
<td>7</td>
<td>11.1 ± 1.0</td>
<td>123 ± 19†</td>
<td>ND</td>
</tr>
<tr>
<td>127–130 days</td>
<td>Cortisol infused</td>
<td>4</td>
<td>63.5 ± 11.8*</td>
<td>106 ± 6†</td>
<td>1.08 ± 0.27†</td>
</tr>
<tr>
<td>142–145 days</td>
<td>T₃ infused</td>
<td>5</td>
<td>12.2 ± 1.7</td>
<td>86 ± 9</td>
<td>0.57 ± 0.03*</td>
</tr>
<tr>
<td>TX</td>
<td>Control</td>
<td>4</td>
<td>58.5 ± 13.9*</td>
<td>100 ± 10†</td>
<td>0.67 ± 0.16†</td>
</tr>
<tr>
<td>127–130 days</td>
<td>Cortisol infused</td>
<td>4</td>
<td>8.1 ± 0.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>142–145 days</td>
<td>Cortisol infused</td>
<td>5</td>
<td>64.6 ± 8.2*</td>
<td>ND</td>
<td>0.41 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in ng/ml; n, no. of sheep fetuses in group. T₄, thyroxine; TX, thyroidectomized; ND, not detectable. *Significantly different from values at 127–130 days in intact and TX control fetuses (P < 0.05); †significantly different from values in TX fetuses with same treatment (P < 0.05).

Each band with a computerized image analyzer (Seescan, Cambridge, UK). A constant rectangular area of the X-ray film that included the complete mRNA signal plus background was defined. This background area was used to set zero intensity. Exposure times were adjusted so that band intensities were within the linear range of detection of the analyzer. To ensure valid comparison of data from different gel runs, each analysis contained an identical sample of liver total RNA. These quality controls varied by ±5%, and hence only minor adjustments, if any, were required to compare the different gels. Total IGF-I mRNA abundance was calculated as the sum of the individual measurements of the two protected fragments.

Statistical Analyses

Mean and SE values have been given throughout, and statistical analyses were made according to the methods of Snedecor and Cochran (36). Statistical significance was assessed by ANOVA with Fisher’s test and paired and unpaired t-tests as appropriate. Correlation and partial correlation coefficients were calculated by linear regression and assessed for significance using Fisher’s test. Multiple and stepwise linear regression analyses were also used on all of the data (Statview, Abacus Concepts, Berkeley, CA). Probabilities of <5% were considered significant. No significant differences were observed between the untreated and saline-infused fetuses at 127–130 days; hence, all of the values were combined in the subsequent analyses.

RESULTS

Effects of Thyroidectomy on the Ontogenic Changes in Hepatic GHR and IGF Gene Expression

Thyroidectomy of the fetus abolished the prepartum rise in fetal plasma T₃ but not cortisol (Table 2). It also prevented the upregulation of GHR gene expression that normally occurs in fetal liver close to term (Fig. 1A). In common with previous findings (24, 25), hepatic GHR mRNA levels were significantly greater at 142–145 days than at 127–130 days in intact fetuses in the present study (Fig. 1B). In contrast, there was no significant change in hepatic GHR mRNA abundance with increasing gestational age in the TX fetuses (Fig. 1A). Mean hepatic GHR mRNA abundance was similar in intact and TX animals at 127–130 days but was significantly less in TX than in intact fetuses at 142–145 days (Fig. 1B).

Thyroidectomy also altered the ontogeny of IGF-I gene expression in fetal liver (Fig. 2A). In intact fetuses, hepatic total IGF-I mRNA levels were significantly higher at 142–145 days than at 127–130 days (Fig. 2B) because of increases in the abundance of both the Class 1 and Class 2 transcripts (Table 3). The hepatic Class 2 transcript was not quantifiable in intact fetuses at 127–130 days but had risen to detectable levels at 142–145 days (Fig. 2A, Table 3). No significant gestational trends were observed in total IGF-I mRNA (Fig. 2B) or in the abundance of Class 1 or Class 2 transcripts in the TX fetuses (Table 3). At 127–130 days, mean total IGF-I mRNA abundance in the TX fetuses was significantly greater than that in the intact fetuses and was similar to the values seen in intact and TX fetuses at 142–145 days (Fig. 2B). The raised level of total IGF-I mRNA in liver from TX fetuses at 127–130 days was due to increased abundance of both the Class 1 and Class 2 transcripts (Table 3).

![Fig. 1. Autoradiograms (A) and mean (±SE) relative values (B) of hepatic growth hormone receptor (GHR) mRNA abundance in liver from intact (open bars) and thyroidectomized (TX, hatched bars) fetuses at 127–130 days and 142–145 days. Autoradiograms were exposed for 2 days. †Significantly different from fetuses at 127–130 days (P < 0.01). *Significantly different from intact fetuses at 142–145 days (P < 0.05).](http://ajpendo.physiology.org/Downloadedfrom)
Effects of Cortisol Infusion in Intact and TX Fetuses

Cortisol infusion increased the plasma concentrations of cortisol and \( T_3 \) in both the intact and TX groups of fetuses (Table 2). At 127–130 days, mean cortisol and \( T_3 \) levels in the cortisol-infused animals were significantly higher than those in the corresponding groups of control fetuses and were similar to the values observed in intact fetuses at 142–145 days (Table 2). Plasma \( T_4 \) concentrations were unaffected by cortisol infusion in both groups of fetuses (Table 2).

Cortisol infusion increased hepatic GHR gene expression to a similar extent in intact and TX fetuses (Fig. 3). Mean hepatic GHR mRNA abundance in the cortisol-infused intact and TX fetuses was significantly higher than in their respective groups of controls and was similar to the values observed in intact fetuses at 142–145 days (Figs. 1B and 3B). In TX fetuses, cortisol infusion increased the mean hepatic GHR mRNA level at 127–130 days (41.4 ± 1.7 arbitrary units, \( n = 4 \), Fig. 3B) to values significantly higher than the values seen at 142–145 days (28.9 ± 3.9, \( n = 4 \), \( P < 0.01 \); Fig. 1B).

In common with previous findings (21), cortisol infusion also increased total IGF-I mRNA and the abundances of Class 1 and Class 2 transcripts in the liver of intact fetuses (Fig. 4A, Table 3). At 127–130 days, mean hepatic abundances of total IGF-I mRNA and of the Class 1 and Class 2 transcripts in cortisol-infused intact fetuses were significantly higher than those in their age-matched controls and were similar to the values observed in intact fetuses at 142–145 days (Figs. 2B and 4B, Table 3). In contrast, there was no further elevation in the total IGF-I mRNA abundance in liver from TX fetuses infused with cortisol (Fig. 4A). Thus mean hepatic abundances of total IGF-I mRNA and of the Class 1 transcript in the cortisol-infused TX fetuses at 127–130 days were not significantly different from those found in control TX fetuses at either 127–130 days or 142–145 days (Fig. 4B and Table 3). Cortisol infusion did, however, affect Class 2 transcript abundance in the liver of TX fetuses at 127–130 days relative to Class 1 IGF mRNA expression (Fig. 4A). The Class 2 transcript accounted for a significantly greater proportion of the total hepatic IGF-I mRNA in cortisol-infused TX fetuses at 127–130 days than in untreated TX or cortisol-infused intact fetuses at the same gestational age (Table 3).

Effect of \( T_3 \) Infusion in Intact Fetuses

At 127–130 days, infusion of \( T_3 \) into intact fetuses increased plasma \( T_3 \) concentrations from the undetectable values observed in their age-matched controls to levels similar to those observed in intact fetuses at 142–145 days (Table 2). Neither fetal cortisol nor \( T_4 \) concentrations were affected by \( T_3 \) administration (Table 2). The GHR mRNA abundance in intact (open bars) and TX (hatched bars) sheep fetuses at 127–130 days and 142–145 days. Autoradiograms were exposed for 3 days. *Significantly different from intact fetuses at 127–130 days (\( P < 0.01 \)). ‡Significantly different from intact fetuses at 127–130 days (\( P < 0.01 \)).

Table 3. Abundance of Class 1 (exon 1) and Class 2 (exon 2) IGF-I mRNA transcripts in liver from control intact and TX fetuses at 127–130 days and 142–145 days and in intact and TX fetuses treated with cortisol or \( T_3 \) for 5 days before delivery at 127–130 days

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>n</th>
<th>Class 1 Abundance (%Total)</th>
<th>Class 2 Abundance (%Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>Controls</td>
<td>7</td>
<td>7.0 ± 1.0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Cortisol</td>
<td>4</td>
<td>14.0 ± 1.3</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Infused</td>
<td></td>
<td>85.5 ± 0.6</td>
<td>14.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>T3 Infused</td>
<td>5</td>
<td>15.8 ± 1.4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>TX</td>
<td>Controls</td>
<td>4</td>
<td>16.6 ± 1.1</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Infused</td>
<td></td>
<td>83.2 ± 2.7</td>
<td>16.8 ± 2.7</td>
</tr>
<tr>
<td>127–130 days</td>
<td>Cortisol</td>
<td>5</td>
<td>20.0 ± 4.5</td>
<td>3.7 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>Infused</td>
<td></td>
<td>86.7 ± 3.1</td>
<td>13.3 ± 3.1</td>
</tr>
<tr>
<td>142–145 days</td>
<td>Cortisol</td>
<td>4</td>
<td>16.7 ± 2.7</td>
<td>6.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Infused</td>
<td></td>
<td>72.4 ± 3.9</td>
<td>27.6 ± 3.9†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>11.0 ± 3.3</td>
<td>1.8 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in arbitrary units (abundance) or % n, nos. of animals. *IGF-I, insulin-like growth factor I. †Significantly different from values in corresponding group of intact or TX control animals at 127–130 days (\( P < 0.05 \)); ‡significantly different from values in intact fetuses with same treatment (\( P < 0.05 \)); ND, not detectable.
2. Infusion of T₃ increased hepatic GHR and total IGF-I mRNA abundances (Fig. 5A). Mean levels of GHR and total IGF-I mRNA in the T₃-infused intact fetuses at 127–130 days (35.6 ± 3.5 and 15.5 ± 1.4 arbitrary units, n = 5, respectively) were significantly higher than in their age-matched controls (Fig. 5B) and were similar to the values observed both in intact fetuses later in gestation (Figs. 1B and 2B) and in cortisol-infused intact fetuses at 127–130 days (Figs. 3B and 4B). The increment in total IGF-I mRNA in response to fetal T₃ treatment was due primarily to a rise in the abundance of the Class 1 transcript (Fig. 5A, Table 3). The Class 2 IGF-I mRNA transcript was detectable in some of the T₃-infused fetuses (Fig. 5A) but was present at too low an abundance to be quantifiable by densitometry (Table 3).

Relation Between Hepatic GHR and IGF-I Gene Expression and Plasma Concentrations of Cortisol and Thyroid Hormones

When the data from all of the fetuses were combined irrespective of gestational age or treatment, hepatic GHR mRNA abundance was positively correlated to the log plasma concentrations of cortisol ($r = 0.588$, $n = 33$, $P < 0.01$) and plasma T₃ ($r = 0.656$, $n = 33$, $P < 0.01$) but not to log plasma T₄ ($r = -0.164$, $n = 30$, $P > 0.05$) in the individual fetuses. Partial correlation analysis of the four variables showed that both cortisol and T₃ had significant effects on hepatic GHR mRNA abundance but that T₃ was the predominant influence ($T_3 P < 0.0001$, cortisol $P < 0.007$), with no independent effect of T₄ ($P > 0.05$). Multiple and stepwise linear regression analyses also showed that log plasma T₃ was the major influence on hepatic GHR mRNA abundance.

Hepatic IGF-I mRNA abundance was positively correlated to hepatic GHR mRNA levels both in the intact control fetuses alone ($r = 0.921$, $n = 11$, $P < 0.01$) and when the data from all of the fetuses were combined ($r = 0.554$, $n = 33$, $P < 0.01$). Overall, hepatic IGF-I mRNA abundance was also positively correlated to log plasma T₃ ($r = 0.418$, $n = 33$, $P < 0.02$) and inversely correlated to log plasma T₄ ($r = -0.396$, $n = 33$, $P < 0.05$) but was unrelated to log plasma cortisol ($r = 0.127$, $n = 33$, $P > 0.05$) in the individual fetuses. Partial correlation, and multiple and stepwise linear regression analyses of these variables all showed that plasma T₃ and T₄ were equally important in influenc-
deficiency of not only T₄ but also its sulfated metabolites.

Hepatic GHR Expression

In the present study, upregulation of GHR expression in fetal liver was invariably associated with an increment in fetal plasma T₃ level. Abolition of the prepartum rise in T₃ by fetal thyroidectomy prevented the rise in hepatic GHR mRNA abundance that normally occurs close to term. Conversely, raising T₃ levels to prepartum values in intact immature fetuses prematurely raised hepatic GHR mRNA abundance. Increased hepatic GHR gene expression was also observed in immature intact and TX sheep fetuses when T₃ levels were elevated by cortisol infusion. T₃ therefore appears to play an important role in upregulating GHR gene expression in fetal liver close to term. The positive correlation observed between individual values of fetal plasma T₃ and hepatic GHR mRNA abundance in the present study also indicates that T₃ is a physiological regulator of hepatic GHR gene expression over the normal range of T₃ concentrations observed in utero.

Taken together, these findings suggest that the prepartum increase in T₄ deiodination and the concomitant rise in plasma T₃ are responsible, in part, for the normal ontogenic increase in hepatic GHR mRNA abundance observed close to term. Certainly, the current observations are consistent with previous findings that T₃ is essential for the normal rise in hepatic GHR gene expression in fetal pigs near term (7) and enhances GHR gene expression in adult hepatocytes (3, 13, 35).

In the sheep fetus, the prepartum rises in fetal plasma T₃ and hepatic GHR gene expression are both cortisol dependent (20, 23). They can be prevented by fetal adrenalectomy and stimulated prematurely by cortisol infusion early in gestation (21, 33). In several fetal tissues, the prepartum maturational effects of cortisol are dependent on the cortisol-induced increase in T₃ production from T₄ (10, 23). Recent studies have shown that the normal downregulation of IGF-II gene expression seen in fetal ovine liver before birth is dependent on the increase in both fetal plasma cortisol and T₃ close to term (9). In the present study, plasma T₃ appeared to be the dominant factor in regulating GHR
expression in the fetal liver. Increases in hepatic GHR mRNA abundance occurred only in association with elevated fetal plasma T₃ and could be induced by raising fetal T₃ concentrations alone without a simultaneous increment in fetal plasma cortisol or any change in plasma T₄ levels. Consequently, T₃ rather than cortisol may be the direct stimulus to hepatic GHR gene upregulation near term. These observations indicate that T₃ may mediate the maturational effects of cortisol on the hepatic GHR gene in the sheep fetus.

The precise mechanisms by which T₃ acts on the GHR gene are unclear. Nuclear thyroid hormone receptors are present in fetal ovine liver during late gestation and have a 10-fold greater affinity for T₃ than T₄ in utero (28). Two transcripts of the GHR gene, derived from alternate untranslated leader exons 1A and 1B, have also been detected in fetal ovine liver during late gestation (1). Both are upregulated during the prepartum period by the fetal cortisol surge, although the exon 1B transcripts predominate and account for most of the increases in hepatic GHR mRNA abundance observed during late gestation and in response to exogenous cortisol infusion (1, 20). Sequence analyses of the 5′ flanking region of the two leader exons have revealed a putative half-site for binding activated thyroid hormone receptors in the upstream region of exon 1B, but not of exon 1A (1). By contrast, the upstream region of exon 1A, but not exon 1B, has a glucocorticoid response element (1). Although leader exon usage could not be assessed with the GHR coding exon probe used in the present study, the current findings are consistent with the known changes in GHR transcript composition toward term and during manipulation of the fetal cortisol level (20, 21). Overall, these observations indicate that T₃ may upregulate hepatic GHR gene expression through the exon 1B promoter, whereas cortisol acts both indirectly via T₃ and directly on the GHR gene through the exon 1A promoter. However, although T₃ and cortisol generally act via gene transcription (11, 20), part of their observed action on GHR mRNA expression may have been via alteration of mRNA stability and extranuclear transport of specific mRNA to the cytoplasm (40).

Hepatic IGF-I Gene Expression

The marked increase in hepatic IGF-I gene expression in the immature TX fetuses contrasted with previous findings in hypophysectomized fetal sheep and pigs. In these studies, hypothyroidism in utero led to reduced plasma and hepatic IGF-I levels that were ameliorated or prevented by T₄ treatment (19, 24). Thyroid hormone deficiency also attenuates the normal ontogenic increases in plasma IGF-I and hepatic IGF-I mRNA that occur in newborn rats during the early postnatal period (13, 26). In addition, plasma T₄ levels are positively correlated with plasma and hepatic IGF-I levels in adult animals of several species (3, 4, 39). However, inconsistent actions of the thyroid hormone on IGF-I status have been reported previously for young, growing animals (19, 25, 37). In neonatal rats, plasma IGF-I levels and hepatic IGF-I mRNA abundance increased after thyroidectomy but decreased when thyroid hormone deficiency was induced by drug treatment (26, 38). Similarly, in fetal pigs, plasma IGF-I levels are decreased after hypophysectomy but not after thyroidectomy (19, 37). Furthermore, when nutrient availability is closely regulated either in vivo or in cultured hepatocytes from young pigs, T₄ is inversely correlated with both plasma IGF-I and hepatic IGF-I mRNA levels (2, 25). The specific effects that thyroid hormones have on IGF status may, therefore, depend on the prevailing endocrine and nutritional conditions (25, 39). Fetal circulating levels and hepatic expression of IGF-I are known to be influenced by the availability of insulin and glucose in utero (17, 29, 31). However, neither of these factors is altered by thyroidectomy of the sheep fetus (10). Changes in GH status are also unlikely to account for the enhanced hepatic IGF-I mRNA levels in immature TX fetuses, as hepatic GHR mRNA levels were unaltered in these fetuses and fetal GH levels are reduced, not enhanced, by thyroidectomy (30). The effects and molecular actions of the thyroid hormones also depend, in part, on the cellular bioavailability of T₃. This changes as levels of Type I deiodinase and thyroid hormone receptors rise in the fetal liver toward term in parallel with the prepartum cortisol surge (23, 27, 28). Thyroid hormones might, therefore, suppress hepatic IGF-I gene expression when levels of T₃ and thyroid hormone receptors are low, before the fetal cortisol surge, but increase IGF-I mRNA abundance when T₃ bioavailability is enhanced either by exogenous T₃ infusion or by the increases in deiodinase activity and thyroid hormone receptors that occur close to term. However, it is more likely that the high IGF-I mRNA levels observed in the immature TX fetuses were an indirect consequence of thyroid hormone deficiency in utero and reflect, for instance, a reduction in the posttranscriptional processing of mRNA caused by the low metabolic rate of the TX sheep fetus (12).

Thyroidectomy increased expression of both Class 1 and Class 2 IGF-I transcripts in the fetal liver. In intact sheep fetuses, the Class 2 transcript is readily quantifiable only in liver close to term when its expression is upregulated by the fetal cortisol surge (21). Cortisol infusion increased the proportion of Class 2 transcripts in the total IGF-I mRNA in both the intact and TX fetuses in the present study. In contrast, raising T₃ levels to prepartum values by exogenous infusion in intact immature fetuses had no effect on Class 2 transcript expression, although Class 1 and total IGF-I mRNA abundances were increased in these circumstances. Upregulation of the Class 2 transcript in fetal ovine liver close to term may, therefore, depend on either the fetal cortisol surge alone or simultaneous increments in fetal plasma cortisol and T₃. Because the plasma concentrations of cortisol and T₃ were more closely correlated with hepatic GHR mRNA than IGF-I mRNA in the present study, their effects in stimulating the hepatic IGF-I gene may be largely indirect and mediated via upregulation of the hepatic GHR gene. Indeed, neither promoter region of the ovine IGF-I gene
has glucocorticoid or thyroid hormone response elements (6).

Growth and Development

The 5- to 10-fold increments in plasma cortisol and T₃ observed in the last 10 days of gestation are the major endocrine events in fetal sheep preceding delivery and are responsible for many of the prepartum maturational changes that occur in fetal tissues in preparation for extrauterine life (10, 23). The actions of thyroid hormones and cortisol on hepatic GHR and IGF-I gene expression, demonstrated in this and previous studies (10, 21, 24–26), concur with the known maturational effects of these hormones. Around birth, there is a switch from paracrine synthesis of the IGFs to hepatic production of endocrine IGF-I, which is essential for normal postnatal growth (9, 14). In sheep, this switch is cortisol dependent and accompanied by upregulation of GHR abundance, increased IGF-I mRNA production from the GH-sensitive promoter, and downregulation of IGF-I gene expression in the fetal liver (24–26). It also coincides with the onset of GH-dependent growth (14). Together with our previous findings on IGF-II (9), the results of the current study demonstrate that thyroid hormones modify this developmental shift in hepatic IGF synthesis and mediate, in part, the maturational actions of cortisol on the hepatic somatotropic axis. Hence, developmental abnormalities in GH responsiveness and IGF synthesis are likely to occur in adverse conditions, such as intrauterine growth retardation, placental insufficiency, and undernutrition, which lead to hypothyroidism and/or a precocious increase in plasma cortisol in utero (14, 17, 29, 31). Moreover, activation of the switch in hepatic somatotropic gene expression may be impaired in infants delivered prematurely, before the normal prepartum increments in fetal cortisol and T₃ have occurred (10). The current findings therefore have important implications for the control of tissue growth and development both before and after birth and suggest a mechanism for the intrauterine programming of postnatal GH responsiveness and IGF-I synthesis (14).

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