Control of hepatic insulin-like growth factor II gene expression by thyroid hormones in fetal sheep near term

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Forhead, Alison J., Juan Li, R. Stewart Gilmour, and Abigail L. Fowden. Control of hepatic insulin-like growth factor II gene expression by thyroid hormones in fetal sheep near term. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E149–E156, 1998.—The effects of thyroid hormones on hepatic insulin-like growth factor (IGF) II gene expression and their interaction with cortisol in the ontogenic control of this gene were investigated in fetal sheep during late gestation (term 145 ± 2 days) and after experimental manipulation of fetal plasma hormone concentrations. In intact fetuses, a significant decrease in hepatic IGF-II mRNA abundance was observed between 127–130 and 142–145 days of gestation, which coincided with the normal prepartum rise in plasma cortisol and triodothyronine (T3) concentrations. This ontogenic decline in hepatic IGF-II gene expression was abolished in fetuses in which the prepartum rise in plasma T3, but not cortisol, was prevented by fetal thyroidectomy. At 127–130 days, downregulation of hepatic IGF-II mRNA abundance was induced prematurely in intact fetuses by an infusion of cortisol for 5 days (2–3 mg·kg-1·day-1 iv). Plasma concentrations of cortisol and T3 in the cortisol-infused intact fetuses were increased to values seen close to term. Similar findings were observed in thyroidectomized fetuses, in which, despite thyroidectomy, cortisol infusion significantly increased plasma T3 concentrations and caused a premature decrease in hepatic IGF-II mRNA levels. However, in intact fetuses at 127–130 days, the increasing of T3 concentrations alone by exogenous T3 infusion (8–12 µg·kg·day-1 iv for 5 days) had no effect on hepatic IGF-II mRNA levels. Overall, a decrease in hepatic IGF-II mRNA abundance was only observed in fetuses in which there were concurrent increases in plasma cortisol and T3 concentrations. When observations from all fetuses were considered, irrespective of gestational age or treatment, hepatic IGF-II mRNA levels were negatively correlated with plasma cortisol and T3 but not thyroxine concentrations. Partial correlation analysis of hepatic IGF-II, cortisol, and T3 values showed that the plasma concentration of cortisol in the fetus had the predominant effect on hepatic IGF-II mRNA abundance. These findings show that T3 may mediate, in part, the maturational effects of cortisol on hepatic IGF-II gene expression but that it is ineffective without a concomitant rise in fetal plasma cortisol. Hence, increased concentrations of both cortisol and T3 appear necessary to induce downregulation of hepatic IGF-II mRNA abundance in fetal sheep close to term.

The insulin-like growth factors (IGFs) are important regulators of growth in both fetal and neonatal animals, although the mechanisms controlling IGF synthesis differ before and after birth. In many species, IGF-II is the predominant IGF present in utero and, compared with expression in the adult animal, is highly abundant in the fetal circulation and tissues (18, 19, 23). The importance of IGF-II in the normal development of the fetus has been demonstrated in transgenic mice in which the IGF-II gene has been disrupted. Both fetal and placental size are severely impaired in these animals, although normal rates of growth are observed after birth (1, 8). In addition, body weight has been positively correlated with plasma IGF-II concentrations in sheep fetuses during late gestation (32).

In utero, plasma IGF-II concentrations and IGF-II gene expression in hepatic and extrahepatic tissues appear to be independent of pituitary control (21, 30). In fetal sheep, circulating concentrations of growth hormone (GH) are high, but hepatic expression of GH receptor and IGF-I genes remains low until shortly before birth (3, 27). The ontogenic increase in GH-mediated IGF-I production coincides with a decrease in plasma IGF-II concentrations and downregulation of IGF-II gene expression in the liver and other fetal tissues (9, 18, 28). Hence, the transition from fetal to neonatal life is characterized by a developmental shift in hepatic IG synthesis.

In sheep, the maturational switch in hepatic IGF production from IGF-II to IGF-I and the induction of hepatic GH receptors depend on the increase in fetal plasma cortisol concentrations that normally occurs toward term (27, 28). This prepartum cortisol surge is also known to be responsible for a number of other maturational events that prepare the sheep fetus for extrauterine life (29). For example, cortisol stimulates the deiodination of thyroxine (T4) to triodothyronine (T3) and thereby causes a prepartum increase in circulating T3 concentrations that coincides with the decline in hepatic IGF-II gene expression toward term (14, 15, 28).

Thyroid hormones are known to be essential for normal growth and development in utero. Hypothyroidism in fetal sheep causes growth retardation and abnormal maturation of a number of fetal tissues (11, 13, 20). It is also associated with changes in circulating IGF concentrations in the fetus (21, 30). However, little is known about the role of thyroid hormones in the regulation of tissue IGF gene expression in utero, especially during late gestation when major changes in IGF mRNA abundance occur as fetal plasma cortisol concentrations rise.

Therefore, the present study investigated 1) the role of thyroid hormones in the control of hepatic IGF-II gene expression during late gestation and 2) whether the prepartum rise in plasma T3 concentrations may mediate the maturational action of cortisol on hepatic IGF-II mRNA abundance. The effects of thyroid hormones on hepatic IGF-II gene expression were examined in sheep fetuses after experimental manipulation of plasma thyroid hormone concentrations by fetal thyroidectomy and exogenous hormone infusion.
MATERIALS AND METHODS

Animals. A total of 31 Welsh Mountain sheep fetuses of known gestational age were used in this study. 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, 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 and gestational ages of the fetuses used in each experimental group of the study.

Surgical procedures. All surgical operations were performed under halothane anesthesia (1.5% in O₂:N₂O) with positive pressure ventilation. Between 105 and 110 days of gestation (term 145 ± 2 days), 12 fetuses were thyroidectomized (TX) in utero with the use of surgical techniques described previously (20). 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 (7). 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 of ampicillin (Penbritin, Beecham Animal Health, Brentford, UK) and 2 mg of gentamycin (Frangen-100, Biovet, Mullingar, UK). The ewes received antibiotic intramuscularly (900 mg of 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 the operation.

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

Four intact and four TX fetuses were infused with cortisol (2–3 mg·kg⁻¹·day⁻¹ iv; Ecottelan, Glaxo, Ware, UK) for 5 days before delivery for tissue collection at 127–130 days. In addition, eight intact fetuses were infused with either T₃ (8–12 µg·kg⁻¹·day⁻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 T₃ infused were calculated to mimic the plasma concentrations normally observed in the immediate prepartum period. 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 cesarean section under general anesthesia (20 mg/kg iv pentobarbital sodium). 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 liver 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 analysis. Plasma cortisol concentrations were measured by radioimmunoassay validated for use with ovine plasma as described previously (37). The lower limit of detection was 40–80 pmo/l, and the interassay coefficient of variation was 11%. Plasma T₃ and T₄ concentrations were also measured by radioimmunoassay, using a commercial kit validated for ovine plasma (13) (ICN Biomedicals, Thame, UK). The lower limits of detection were 0.21 nmol/l for T₃ and 8.8 nmol/l for T₄. The interassay coefficient of variation was 10% for both assays.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gestational Age, days</th>
<th>Number of Fetuses</th>
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<tbody>
<tr>
<td></td>
<td>At thyrodiectomy</td>
<td>At catheterization</td>
</tr>
<tr>
<td>Intact and untreated</td>
<td>115–120 (n = 3)</td>
<td>127–130</td>
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<tr>
<td>Intact and cortisol infusion</td>
<td>115–120</td>
<td>127–130</td>
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<tr>
<td>Intact and T₃ infusion</td>
<td>115–120</td>
<td>127–130</td>
</tr>
<tr>
<td>Thyroidectomy and untreated</td>
<td>105–110</td>
<td>127–130</td>
</tr>
<tr>
<td>Thyroidectomy and cortisol infusion</td>
<td>105–110</td>
<td>127–130</td>
</tr>
</tbody>
</table>

Statistical analysis. All data are presented as mean values ± SE. 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. Relationships between the variables measured were determined by linear regression and partial correlation analyses (41); plasma hormone concentrations that were below the limit of assay detection were assigned a value of 1 in regression analyses. Differences for which P < 0.05 were regarded as significant.

RESULTS

Ontogeny of hepatic IGF-II gene expression in intact and TX fetuses. The normal ontogenic decline in hepatic IGF-II mRNA abundance was abolished when the immediate prepartum rise in plasma T₃ but not cortisol concentrations was prevented by fetal TX (Fig. 1).

At 127–130 days of gestation, there was no significant difference in hepatic IGF-II mRNA levels between TX and intact fetuses (Fig. 1 and Table 2A). Plasma cortisol and T₃ concentrations were also similar between the two groups of fetuses at this gestational age.
Plasma T4 concentrations were undetectable in the TX fetuses and were significantly lower than those observed in the intact fetuses at 127–130 days (P < 0.01, Table 2A).

Between 127–130 and 142–145 days, a significant decrease in IGF-II mRNA abundance was seen in the intact (P < 0.001, Fig. 1) but not TX fetuses. At 142–145 days, hepatic IGF-II mRNA levels in the TX fetuses were similar to those seen in TX fetuses at 127–130 days and were significantly greater than those observed in the intact fetuses (Fig. 1 and Table 2A).

Between 127–130 and 142–145 days, plasma cortisol concentrations significantly increased in both groups of fetuses (P < 0.05 in both cases, Table 2A), but a significant concomitant increase in plasma T3 concentrations was only observed in the intact fetuses (P < 0.01, Table 2A).

### Table 2. Plasma concentrations of cortisol, T3, and T4 and hepatic IGF-II mRNA levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gestational Age at Delivery, days</th>
<th>Cortisol, nmol/l</th>
<th>T3, nmol/l</th>
<th>T4, nmol/l</th>
<th>IGF-II mRNA at Delivery, arbitrary units</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Preinfusion</td>
<td>Delivery</td>
<td>Preinfusion</td>
<td>Delivery</td>
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<tr>
<td>A</td>
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<tr>
<td>Intact</td>
<td>127–130</td>
<td>32.6 ± 2.5</td>
<td>ND</td>
<td>160.0 ± 26.5</td>
<td>46.5 ± 3.1</td>
</tr>
<tr>
<td>TX</td>
<td>127–130</td>
<td>22.3 ± 2.5</td>
<td>ND</td>
<td>ND</td>
<td>39.2 ± 3.0</td>
</tr>
<tr>
<td>Intact</td>
<td>142–145</td>
<td>161.4 ± 36.6</td>
<td>1.00 ± 0.24</td>
<td>125.4 ± 8.6</td>
<td>8.0 ± 3.1</td>
</tr>
<tr>
<td>TX</td>
<td>142–145</td>
<td>172.1 ± 42.2</td>
<td>ND</td>
<td>ND</td>
<td>37.2 ± 3.9</td>
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<td></td>
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</tr>
<tr>
<td>Intact</td>
<td>127–130</td>
<td>32.6 ± 2.5</td>
<td>ND</td>
<td>160.0 ± 26.5</td>
<td>46.5 ± 3.1</td>
</tr>
<tr>
<td>TX</td>
<td>127–130</td>
<td>22.3 ± 2.5</td>
<td>ND</td>
<td>ND</td>
<td>39.2 ± 3.0</td>
</tr>
<tr>
<td>Intact + cortisol</td>
<td>127–130</td>
<td>175.2 ± 32.6ae</td>
<td>ND</td>
<td>159.0 ± 20.8</td>
<td>19.3 ± 5.4ae</td>
</tr>
<tr>
<td>TX + cortisol</td>
<td>127–130</td>
<td>189.2 ± 14.1ae</td>
<td>ND</td>
<td>ND</td>
<td>39.2 ± 3.0</td>
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<td>C</td>
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</tr>
<tr>
<td>Intact + T3</td>
<td>127–130</td>
<td>32.6 ± 2.5</td>
<td>ND</td>
<td>160.0 ± 26.5</td>
<td>46.5 ± 3.1</td>
</tr>
<tr>
<td>Intact + T3</td>
<td>127–130</td>
<td>33.7 ± 4.7</td>
<td>ND</td>
<td>130.6 ± 12.6</td>
<td>49.5 ± 8.0</td>
</tr>
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Values are mean ± SE plasma concentrations of cortisol, T3, and thyroxine (T4) and hepatic insulin-like growth factor (IGFII) mRNA levels in intact and thyroidectomized (TX) fetuses at 127–130 and 142–145 days (A), intact and TX fetuses infused with cortisol for 5 days (B), and intact fetuses infused with T3 for 5 days (C). ND, not detectable (below lower limit of assay detection). aP < 0.05 and bP < 0.005, significantly different from intact fetuses at 127–130 days, unpaired t-test. cP < 0.05 and dP < 0.005, significantly different from TX fetuses at 127–130 days, unpaired t-test. eP < 0.05 and fP < 0.005, significantly different from preinfusion values, paired t-test.
No gestational change in plasma T₄ concentrations was identified in either group of fetuses; plasma T₄ remained undetectable in the TX fetuses at 142–145 days (Table 2A).

Effect of cortisol infusion on hepatic IGF-II gene expression in intact and TX fetuses. Hepatic IGF-II mRNA abundance was reduced in both intact and TX fetuses at 127–130 days when fetal plasma cortisol concentrations were increased to values seen near term by an exogenous infusion of cortisol for 5 days (Fig. 2).

At 127–130 days, hepatic IGF-II mRNA abundance in the cortisol-infused intact fetuses was significantly lower than that observed in the control intact fetuses (P < 0.005, Fig. 2 and Table 2B). Likewise, hepatic IGF-II mRNA abundance in the TX fetuses infused with cortisol was significantly lower than that seen in the untreated TX fetuses (P < 0.001, Fig. 2 and Table 2B). There was no significant difference in hepatic IGF-II mRNA levels between intact and TX fetuses infused with cortisol (Fig. 2).

Exogenous cortisol infusion significantly increased plasma cortisol concentrations in intact and TX fetuses to a similar extent (P < 0.05 in both cases, Table 2B). At delivery, plasma cortisol concentrations in the cortisol-infused intact and TX fetuses were significantly greater than those observed in their respective groups of control fetuses at 127–130 days (P < 0.005 in both cases, Table 2B) and resembled values seen in intact and TX fetuses at 142–145 days of gestation (Table 2, A and B).

Over the period of cortisol infusion, plasma T₃ concentrations significantly increased in both intact (P < 0.05) and TX fetuses (P < 0.005, Table 2B). At delivery at 127–130 days, concentrations of T₃ in the cortisol-infused intact and TX fetuses were significantly higher than those measured in their respective groups of control fetuses (P < 0.005 in both cases, Table 2B) and were similar to those observed in intact fetuses at 142–145 days (Table 2, A and B). Plasma T₄ concentrations were unaffected by cortisol infusion in both groups of fetuses (Table 2B).

Effect of T₃ infusion on hepatic IGF-II gene expression in intact fetuses. Hepatic IGF-II mRNA levels at 127–130 days were unaffected when fetal plasma T₃, but not cortisol, concentrations were raised to values seen near term by an exogenous infusion of T₃ for 5 days before delivery (Fig. 3).

No significant difference in hepatic IGF-II mRNA abundance was observed between T₃-infused and control intact fetuses at 127–130 days (Fig. 3 and Table 2C). Infusion of T₃ significantly increased plasma T₃ concentrations (P < 0.0001, Table 2C). At delivery at 127–130 days, plasma T₃ concentrations in the T₃-infused fetuses were significantly greater than those observed in control intact fetuses (P < 0.0001, Table 2C) and were similar to the concentrations seen in intact fetuses at 142–145 days of gestation (Table 2, A and C). Plasma cortisol and T₄ concentrations in the T₃-infused fetuses at 127–130 days were similar to...
those seen in control intact fetuses of the same gestational age (Table 2).

Relationship between hepatic IGF-II gene expression and plasma cortisol and thyroid hormone concentrations. When the data from all of the fetuses were combined, irrespective of treatment or gestational age, a significant inverse relationship was identified between hepatic IGF-II mRNA abundance and plasma cortisol concentrations ($r = -0.680$, $n = 30$, $P < 0.0001$). In addition, hepatic IGF-II mRNA levels were inversely correlated with plasma T3 ($r = -0.367$, $n = 31$, $P < 0.05$) but not T4 concentrations ($r = -0.174$, $n = 29$, $P > 0.05$). Partial correlation analysis showed that the plasma concentration of cortisol in the fetus was a more important determinant of hepatic IGF-II mRNA abundance ($r = -0.610$, $n = 30$, $P < 0.05$) than the plasma T3 concentration ($r = -0.019$, $n = 30$, $P > 0.05$). A significant positive relationship was also observed between plasma concentrations of cortisol and T3 in the fetuses ($r = 0.539$, $n = 30$, $P < 0.005$).

**DISCUSSION**

The present study demonstrates that thyroid hormones have an important role in the control of hepatic IGF-II gene expression in fetal sheep close to term. Fetal thyroidec-tomy abolished the decline in hepatic IGF-II mRNA abundance that normally occurs toward term in this species. It also prevented the normal prepartum rise in fetal plasma T3, but not cortisol, concentrations. Downregulation of the hepatic IGF-II gene toward term has been shown previously to be dependent on the prepartum cortisol surge (28). Hepatic IGF-II mRNA abundance was maintained when the cortisol surge was prevented by fetal adrenalectomy and was reduced prematurely when cortisol concentrations were raised to values seen at term (28). However, adrenalectomy also prevented the prepartum rise in plasma T3, whereas cortisol infusion increased T3 concentrations by stimulating tissue type I 5'-monodeiodinase activity and the production of T3 from T4 (40, 42). These observations indicate that T3 may mediate, at least in part, the maturational effects of cortisol on hepatic IGF-II gene expression in utero. Certainly, in the present study, suppression of hepatic IGF-II mRNA abundance only occurred in fetuses in which there were concurrent increases in plasma T3 and cortisol. Raising plasma T3 concentrations alone in immature fetuses with low circulating cortisol had no influence on hepatic IGF-II mRNA abundance, nor did the prepartum cortisol surge in hypothyroid fetuses. In addition, abolition of the rises in both plasma T3 and cortisol by fetal hypophysectomy has been shown to prevent the normal prepartum decline in plasma IGF-II concentrations (18, 30). Therefore, simultaneous increases in plasma cortisol and T3 appear to be needed to downregulate IGF-II gene expression in fetal ovine liver near term.

Although hepatic IGF-II mRNA abundance may be regulated by the increase in plasma T3 close to term, it does not appear to be modulated by thyroid hormones earlier in gestation. In the present study, hypothyroidism induced by thyroidec-tomy had little influence on hepatic IGF-II mRNA levels before the immediate prepartum period. Similarly, tissue content and plasma concentrations of IGF-II were normal during late gestation in sheep and pig fetuses made hypothyroid by fetal hypophysectomy (21, 30). Plasma T4, therefore, appears to have little, if any, direct role in the control of hepatic IGF-II gene expression in utero. Indeed, no correlation was observed between hepatic IGF-II mRNA levels and plasma T4 in fetuses in the present study. Regulation of hepatic IGF-II gene expression close to term, therefore, does appear to depend primarily on the cortisol-induced rise in plasma T3. These observations are consistent with previous studies in neonatal rats that show that maturational changes in hepatic IGF-II synthesis and plasma IGF-II concentrations are associated with an increase in circulating corticosterone and are severely attenuated by hypothyroidism (4, 16, 24, 31).
The ineffectiveness of exogenous T₃ alone in down-regulating hepatic IGF-II gene expression at 127–130 days may have been due to the relatively low number of nuclear thyroid hormone receptors present in fetal tissues at this gestational age (34). In sheep and pig fetuses, hepatic thyroid hormone binding increases progressively from 80 days of gestation to peak at birth in parallel with the rise in fetal plasma cortisol (10, 34). This ontogenic increase in hepatic thyroid hormone binding may contribute to the effects of the endogenous rise in plasma T₃ on hepatic IGF-II gene expression close to term. However, if T₃ mediates the effects of cortisol, the ontogenic change in thyroid hormone receptor density might be expected to make exogenous cortisol less effective at suppressing hepatic IGF-II mRNA abundance at 127–130 days than the endogenous rise in cortisol at term. However, in the present study, hepatic IGF-II mRNA levels in the cortisol-infused intact and TX fetuses were similar to those seen in intact fetuses at 142–145 days. Because the ontogeny of hepatic thyroid hormone binding is not affected by fetal thyroidectomy (34), these observations suggest that cortisol may act indirectly to suppress hepatic IGF-II gene expression not only via the production of T₃ but also by inducing thyroid hormone receptors in the fetal liver. This latter action of cortisol is in keeping with its other known maturational effects on hormone receptors in the ovine fetal liver (2, 27).

It was not possible to investigate the effect of increasing concentrations of cortisol early in gestation in the absence of a concomitant rise in plasma T₃ because plasma T₃ concentrations increased in response to exogenous cortisol infusion in the TX fetuses. This observation was surprising. In all TX fetuses, there was no evidence of thyroidal tissue at delivery, and plasma concentrations of T₃ were below the limit of detection of the radioimmunoassay. One source of plasma T₃ in TX fetuses may be the sulfated forms of thyroid hormones that are abundant in the circulation of the sheep fetus and can account for up to 80% of T₄ metabolism and clearance (35). In intact fetuses, plasma concentrations of T₄ sulfite (T₄S), reverse T₃ sulfite (rT₃S), and T₃ sulfite (T₃S) peak at 125–130 days and decrease thereafter when T₃ production becomes the major route of T₄ metabolism toward term (35). Although the sulfated thyroid hormones do not bind to nuclear thyroid hormone receptors and therefore have no biological activity, they may contribute to plasma and tissue concentrations of T₃, especially during fetal hypothyroidism (33, 43). Thyroidectomy in the sheep fetus at 110–113 days has been shown previously to cause a marked reduction in plasma concentrations of thyroid hormones and T₃S and rT₃S (43). Plasma T₃S concentrations, however, are maintained for at least 2 wk after the removal of the thyroid glands because of downregulation of type I monodeiodinase enzyme activity (36, 43). Therefore, slow clearance of T₃S in the TX fetuses may have provided a supply of T₃ to fetal tissues after thyroidectomy. Although the site and regulation of desulfation of T₃S to T₃ in the sheep fetus are unknown, sulfatase activity has been reported in microsomes from the liver and brain of fetal rats (38). In the present study, it is possible that exogenous cortisol infusion increased plasma T₃ concentrations in TX fetuses by desulfating T₃S in fetal tissues. Indeed, the cortisol-induced rise in plasma T₃ observed in intact fetuses in this and previous studies (14, 15) may have originated from activation of both sulfatase and 5'-monodeiodinase enzymes.

The endogenous cortisol surge close to term, however, did not appear to stimulate T₃ production in TX fetuses at 142–145 days. Plasma T₃ concentrations in TX fetuses did not show any ontogenic change and remained undetectable at term. The inconsistency between the effects of exogenous and endogenous cortisol on circulating T₃ concentrations in TX fetuses may have been due to the longer interval between thyroidectomy and tissue collection in the older group of animals and, hence, a greater degree of T₃S clearance (43). Although T₃S concentrations were not measured in the present study, the absence of a cortisol-induced rise in plasma T₃ in the TX fetuses close to term may have reflected deficiency of not only T₃ but also T₃S.

Regardless of the source of T₃, an increment in its bioavailability appears to be essential for the cortisol-induced downregulation of hepatic IGF-II gene expression. However, the mechanisms by which these hormones affect IGF-II mRNA abundance remain unknown. In the present study, the ovine IGF-II riboprobe used was specific to the main coding exon 8 (28), and, therefore, IGF-II mRNA levels measured represented total IGF-II gene expression in the fetal liver. In fetal and neonatal sheep, at least three leader exons, 5, 6, and 7, of the IGF-II gene are known to be alternatively spliced to the main coding exon, and, hence, a number of mRNA transcripts are derived from the single gene (25). Expression of each of these transcripts may be under developmental and tissue-specific control. Previous studies have demonstrated that cortisol downregulates IGF-II mRNA abundance in the liver of the sheep fetus specifically by suppressing expression of leader exon 7 (25). A region of 172 bases downstream of the first transcription site for leader exon 7 has been shown to respond to cortisol, but no consensus sequences for repressive glucocorticoid or thyroid hormone response elements have yet been identified in the regulatory regions of the IGF-II gene (25). Alternatively, cortisol and thyroid hormones may act indirectly on the IGF-II gene via transcription factors and their binding to DNA (5, 22).

The actions of cortisol and T₃ in suppressing hepatic IGF-II gene expression close to term may have major implications for growth and development in utero. They may provide an explanation for the decline in growth rate that occurs toward term and in response to cortisol infusion earlier in gestation (12). They may also have an important role in the prepartum maturation of the fetal liver (11). Furthermore, downregulation of hepatic IGF-II gene expression close to term forms part of a more general developmental change in the somatotropic axis. At birth, IGF synthesis switches from a local GH-independent production of predominantly IGF-II in utero to the adult GH-dependent production
of endocrine IGF-I (17). Many of the changes in gene expression associated with this transition have now been shown to be cortisol dependent, but the extent to which they may also rely on the concomitant change in plasma T₃ remains unknown. Recent preliminary findings suggest that the cortisol-induced upregulation of hepatic T₃ may, therefore, lead to developmental abnormalities in the somatotrophic axis and IGF production with adverse consequences for growth both before and after birth.

We are grateful to Paul Hughes, Malcolm Bloomfield, and Val Whittaker for technical assistance and to Sue Nicholas, Ivor Cooper, and Alan Graham for the care of the animals.

This work was supported by the Biotechnology and Biological Sciences Research Council.

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Received 24 December 1997; accepted in final form 13 April 1998.

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