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Effects of Maternal Thyroid Status on Thyroid Hormones and Growth in Congenitally Hypothyroid Goat Fetuses during the Second Half of Gestation*

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ABSTRACT

Congenital hypothyroidism in Dutch goats is due to a thyroglobulin (TG) synthesis defect that is inherited in an autosomal recessive manner. Minute amounts of mutated TG messenger RNA are translated into glycosylated TG fragments that contain the N-terminal hormonogenic site and are able to form T2, albeit less efficiently. We analyzed the effects of maternal thyroid status on fetal plasma thyroid hormones and growth during the second half of gestation (E90–E150).

Maternal hypothyroidism, present from midgestation, resulted in decreased brain and cerebellum weights of affected goitrous fetuses, most evident at term gestation (E150). Brain and cerebellum weights of affected fetuses from unaffected mothers were not decreased. T2 and FT4 levels in affected fetuses were dependent on the maternal phenotype, as was the degree of enlargement of the goiter at E150.

Newborn unaffected lambs from affected mothers had plasma T4 levels within the normal range.

The present data show that in late gestation, fetal goats have to rely on their own thyroid T4 production. The results suggest that affected fetuses are able to maintain sufficiently high T4 and T3 levels to prevent severe adverse effects of thyroid hormone deficiency on the brain if maternal iodide supply is adequate, although a possible increased transfer of maternal T4 to affected fetuses cannot be excluded. Under normal conditions, sufficient amounts of iodine are provided by the efficient iodine metabolism in euthyroid mothers. In affected mothers, much iodine is wasted because the thyroid also iodinates proteins other than the aberrant TG, resulting in insufficient iodine provision of the fetus and, consequently, in severe hypothyroidism.

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THE TRANSFER OF thyroid hormones, l-thyroxine (T4) and 3,5,3'-triiodo-l-thyronine (T3), from mother to fetus and the effects of maternal thyroid status on fetal development have been studied in various species. In man, embryonic tissues contain T3 and T4 before the onset of fetal thyroid function, giving evidence that the thyroid hormones present are of maternal origin (1–3). Also, during late gestation, substantial amounts of maternal T4 are transferred to the fetus, as was indicated by the presence of T4 in cord serum and shortly after birth in serum of neonates with severe congenital hypothyroidism due to a total iodide organification defect or thyroid agenesis (4). This might explain why most of the neonates who subsequently suffer from severe hypothyroidism show no clear features of the condition at birth. Because T4 has a relatively short half-life in neonates, treatment must be started immediately after detection of hypothyroidism (4). Untreated severe congenital hypothyroidism may lead to mental retardation and other neurological deficits (5).

Most data on maternal-fetal transfer of thyroid hormones and brain development were obtained in rats and sheep in which hypothyroidism was experimentally induced by thyroidectomy. In rats, both T4 and T3 are transferred from mother to fetus in early and late gestation. This was indicated by the presence of T4 and T3 in embryonic tissues before the onset of fetal thyroid function (6, 7). In addition, maternal thyroidectomy in rat resulted in undetectable T4 and T3 levels in the fetus before the onset of fetal thyroid function and reduced fetal body weight and the weights of organs such as brain, liver, and lung near term (8). T4 and T3 infusion experiments in hypothyroid rat mothers showed transfer of thyroid hormones to fetuses until term (9, 10). In sheep, maternal thyroid metabolism is also important for fetal sheep development during early gestation. Maternal thyroidectomy before conception caused a reduction in fetal brain and body growth at midgestation, which was not evident at term. Also, from midgestation on, fetal plasma T4 levels were not different from those of control fetuses, indicating that from midgestation onward, the fetus can provide its own T4 (11). Placental transfer of thyroid hormones in sheep seems to be absent or minimal in the second half of gestation. Fetal thyroidectomy decreased fetal serum T3 and T4 to undetectably low levels and caused severe somatic damage, retarded fetal brain development, and early postnatal death (12–14). Combined early maternal and fetal thyroidectomy (15) and iodine deficiency (16, 17) caused even more severe fetal brain retardation than only fetal thyroidectomy. Thus, in sheep, placental transfer of thyroid hormones is important during early gestation, but, in contrast to man and rat, seems to be absent or at least strongly diminished in the second half of gestation.
In an inbred strain of Dutch goats, congenital hypothyroidism and goiter due to a thyroglobulin (TG) synthesis defect have been studied extensively. The disease is inherited in an autosomal recessive way (18). The TG synthesis defect is due to a point mutation in exon 8 of the TG gene, which creates a premature stop codon (19). In the goiter, the mutated TG messenger RNA, which is present in a very low concentration (20), is translated into TG fragments of at most 40 kDa. Tg formation is possible because these fragments contain the N-terminal hormonogenic site (21, 22). High dietary iodine intake caused the affected animals to become euthyroid, although the goiter remained, suggesting inefficient thyroid hormone synthesis (23). The present study reports on the effects of maternal thyroid status on fetal plasma thyroid hormone levels and brain and body weights in fetal goats with genetically determined hypothyroidism during the second half of gestation.

**Materials and Methods**

**Animals**

Dutch goats with congenital hypothyroidism (24) were bred at the Academic Medical Center animal facilities. The maintenance and handling of the animals were as recommended by the Dutch guidelines on the care and use of laboratory animals. Fetal age was calculated from dated matings. In hypothyroid animals, term gestation is 153 ± 5 days; in euthyroid animals, it is 146 ± 3 days (25). Affected goats, which are homozygous for the TG synthesis defect are indicated by TG−, whereas unaffected, normal goats, which are either heterozygous for the defect or homozygously normal are indicated by TG+. Unaffected TG+ fetuses from unaffected TG+ mothers are indicated by TG+/TG+ (fetal phenotype/maternal phenotype in italics), affected TG− fetuses from unaffected TG+ mothers are indicated by TG−/TG+, and affected TG− fetuses from affected TG− mothers are indicated by TG−/TG−. No attempt was made to analyze the data with respect to sex or litter size.

**Experimental design**

TG+/TG+ fetuses, TG−/TG+ fetuses, and TG−/TG− fetuses were studied in the following periods: 90–96 days gestation (E90), 112–129 days of gestation (E120), and 144 days gestation-newborn (E150). The number of animals per group is indicated in Fig. 1 and Tables 1–4. In view of the low conception frequency and the high abortion rate in severely hypothyroid animals, supplementary iodine was administered to all ewes through their food until 60 days after conception, i.e. before the onset of fetal thyroid function (26). Increased iodine intake ameliorates the clinical state (23). The additional iodine supply to the TG− ewes resulted in plasma T4 levels comparable to those in normal goats until 3 months of gestation (data not shown). After this period, plasma T4 levels in TG− ewes decreased to below the levels in euthyroid TG+ ewes. Blood samples were taken from mothers and fetuses for T3, T4, free T4 (FT4), and insulin-like growth factor I (IGF-I) determinations. Maternal T3, T4, and FT4 values from E120 and E150 fetuses were pooled and represent the adult values shown in Fig. 1. The fetuses were killed by means of an injection of a lethal dose of pentobarbital sodium, after which they were weighed and dissected. Organs were removed and weighed.

In addition, blood samples were collected from six newborn unaffected TG+ lambs from affected TG− mothers (indicated by TG+/TG−) for plasma T4 determination.

**Plasma T3, T4, and FT4 determinations**

Plasma T3, T4, and FT4 values were determined by RIA (27). The lower limit of detection for T3 was 5 nmol/liter. The intraassay coefficient of variation was 5% or less (each sample was assayed twice). The interassay coefficient of variation was 7% or less. The lower limit of detection for T4 was 0.1 nmol/liter. The intraassay coefficient of variation was be-

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**Fig. 1.** Plasma T4, FT4, and T3 concentrations in normal fetuses from normal mothers (TG+/TG+ fetuses), affected goitrous fetuses from normal mothers (TG−/TG+ fetuses), affected fetuses from affected mothers (TG−/TG− fetuses) of 90, 120, and 150 days gestation (i.e. E90, E120, and E150), and adult goats. Adult represents the maternal values pooled from E120 and E150 fetuses. Vertical bars and lines represent the mean ± SEM. Statistical analysis was performed by ANOVA. Final significance (P < 0.05) was assessed by the two-sample t test and was performed when the number of animals per group was three or more. Within each bar the number of animals per group is indicated. Filled circles show the values of individual animals when n = 2. *, Significantly different from TG+/TG+ group at the same gestational age; +, significantly different from TG−/TG− group at the same gestational age; , significantly different from the E150 TG+/TG+ fetal group when comparing all TG+/TG+ groups. E, Prenatal days.
Plasma T4 and FT4 levels were significantly reduced in TG− mothers (affected) compared to those in TG+ mothers [T(12) = 4.57; P = 0.001 and T(11) = 5.76; P < 0.001, respectively; Fig. 1, A and B, adult values], whereas T3 levels were not significantly different [T(12) = 1.14; P = 0.28] (Fig. 1C).

Plasma T4 levels were significantly reduced in TG− fetuses from TG− mothers (TG−/TG− fetuses) compared to levels in TG+/TG+ fetuses at E120 [T(5) = 24.12; P < 0.001] and E150 [T(9) = 7.59; P < 0.001; Fig. 1A and Table 1]. At E90, the two TG−/TG− values (16.0; 18.0 nmol/liter) were below the 95% confidence interval for the mean plasma T4 level of E90 TG+/TG+ fetuses (72.2 to 119.8 nmol/liter). The T3 levels in TG− fetuses from TG− mothers (TG−/TG− fetuses) were significantly higher than levels in TG−/TG− fetuses at E150 [T(8) = 5.30; P = 0.001] and did not differ significantly from levels in TG+/TG+ fetuses at E150 (Fig. 1A and Table 1). The two TG−/TG+ values at E90 (85.0; 77.0 nmol/liter) and E120 (220.0; 240.0 nmol/liter) were within the 95% confidence interval for the mean plasma T3 level in E90 (see above) and E120 (217.0 to 264.0 nmol/liter) TG+/TG+ fetuses. The T3 levels in newborn TG+ lambs from affected TG− mothers (TG+/TG−) were significantly higher than levels in TG−/TG− animals [T(9) = 10.39; P < 0.001], whereas they were not significantly different from levels in TG+/TG+ and TG−/TG+ animals (Table 1).

At E150, plasma FT4 levels in TG−/TG− fetuses were significantly reduced compared to levels in TG+/TG+ fetuses [T(9) = 12.54; P < 0.001] and TG−/TG+ fetuses [T(8) = 9.0; P < 0.001; Fig. 1B]. The two TG−/TG− values at E90 (3.8; 3.4 pmol/liter) and E120 (5.4; 6.3 pmol/liter) were below the respective 95% confidence intervals for the mean plasma FT4 levels of TG+/TG+ fetuses (E90, 16.9–27.6 pmol/liter; E120, 38.1–64.8 pmol/liter). The FT4 levels in TG−/TG+ fetuses did not differ significantly from levels in TG+/TG+ fetuses at E150 (Fig. 1B). The two TG−/TG+ FT4 values at E90 (20.4 and 20.0 pmol/liter) and E120 (39.6 and 40.8 pmol/liter) were within the respective 95% confidence intervals for the mean plasma FT4 levels of TG+/TG+ fetuses (see above).

At E150, plasma T3 levels were significantly reduced in TG−/TG− fetuses compared to TG+/TG+ fetuses [T(9) = 5.43; P < 0.001] and TG−/TG+ fetuses [T(8) = 3.51; P = 0.008; Fig. 1C]. At E120, plasma T3 levels were significantly higher in TG−/TG− fetuses compared to levels in TG+/TG+ fetuses [T(5) = 2.57; P = 0.05]. At E90, the two TG−/TG− T3 values (0.55; 0.50 nmol/liter) were within the 95% confidence interval for the mean plasma T3 level of E90 TG+/TG+ fetuses (0.21–0.56 nmol/liter). The T3 levels in TG−/TG+ fetuses did not differ significantly from levels in TG+/TG+ fetuses at E150 [T(9) = 1.97; P = 0.08; Fig. 1C]. The two TG−/TG+ T3 values at E90 (0.4 and 0.32 nmol/liter) were within the 95% confidence interval for the mean plasma T3 level of E90 TG+/TG+ fetuses (0.45–0.66 nmol/liter).

### Table 1. Plasma T4 values on E150

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Plasma T4 conc. (nmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG+/TG+</td>
<td>6</td>
<td>187.0 ± 20.1*</td>
</tr>
<tr>
<td>TG−/TG−</td>
<td>5</td>
<td>18.0 ± 1.2</td>
</tr>
<tr>
<td>TG−/+TG+</td>
<td>5</td>
<td>227.2 ± 39.4*</td>
</tr>
<tr>
<td>TG−/+TG+</td>
<td>6</td>
<td>215.0 ± 17.1*</td>
</tr>
</tbody>
</table>

E150, All values pooled from 144 days of gestation-newborn. Values are the mean ± SEM. TG−, Affected goitrous goat, homzygous for the thyroglobulin (TG) synthesis defect; TG+, unaffected normal goat; TG+/TG−, TG+ goat from TG+ mother, TG−/TG−, TG− goat from TG− mother; TG−/+TG+, TG− goat from TG+ mother; TG+/TG+, TG+ goat from TG− mother. n, number of goats.

* P < 0.001 compared to TG−/TG− group, by ANOVA with post-hoc two-sample t test.

#### Plasma IGF-I determination

Aliquots of sera (250 μl) were acidified by the addition of 1 ml 0.5 m HCl containing 5 mM CaCl2 and incubated at room temperature for 1 h. Subsequently, IGFS were separated from IGF-binding proteins by Sep-Pak C18 (Waters Associates, Milford, MA) chromatography (28). IGF-I in the serum extracts was measured by RIA using 125I-labeled native human IGF-I (29) as tracer and a polyclonal antiserum (kindly provided by Dr. Gluckman) (30). The results were expressed in nanograms per ml, using recombinant IGF-I (International Reference Reagent 87/518 from the National Institute for Biological Standards and Control, Potters Bar, UK) as the reference peptide. The intra- and interassay coefficients of variation were 7.5% and 9.9%, respectively. The minimal detection limit (corrected for sample dilution) was 9 ng/ml.

#### Statistical analysis

Results are reported as the mean ± SEM. Data from all groups were submitted to ANOVA. Significance of differences between group means was assessed using the two sample t test and was performed only when the number of animals per group was three or more. A difference was considered significant when P ≤ 0.05. When only two values were available, these values were compared with the 95% confidence interval for the mean value of the TG+/TG+ group at the same gestational age.

### Results

#### Developmental changes in normal fetal plasma T4, FT4, and T3 concentrations

Plasma T4 and FT4 levels in normal fetuses from normal mothers (TG+/TG+ fetuses; for definition of the phenotype, see Materials and Methods) rise from 90 days of gestation (E90) until 120 days gestation (E120) and remain constant until term gestation (E150; Fig. 1, A and B). The T4 and FT4 values at E90 were significantly lower than those in the E150 animals [T(8) = 3.52; P = 0.008 and T(8) = 6.55; P < 0.001, respectively]. The adult values represent pooled maternal values from E120 and E150 fetuses. The T4 and FT4 values in the adult goats were significantly lower than the fetal E150 values [T(10) = 5.26; P < 0.001 and T(10) = 10.95; P < 0.001, respectively]. Plasma T3 levels increased progressively from E90 until E150 (Fig. 1C). The plasma T3 levels at E150 were significantly higher than levels in E90 [T(8) = 6.45; P < 0.001], E120 [T(8) = 6.20; P < 0.001], and adult animals [T(10) = 5.59; P < 0.001].
Effects of congenital hypothyroidism on fetal plasma IGF-I concentrations

In view of the proposed effect of thyroid status on IGF-I (31), we determined circulating levels of IGF-I in all goats (Table 2). At E150, the plasma IGF-I levels of TG+/TG+, TG−/TG−, and TG−/TG− fetuses did not differ significantly. At E120, no significant differences were found between IGF-I levels of TG+/TG+ and TG−/TG− fetuses. The two TG−/TG− plasma IGF-I values at E120 were within (101 ng/ml) and above (137 ng/ml) the 95% confidence interval for the mean IGF-I concentration of E120 TG+/TG+ fetuses (41.52–111.02 ng/ml). At E90, the two TG−/TG− plasma IGF-I values (38 and 47 ng/ml) were within the 95% confidence interval for the mean plasma IGF-I level of E90 TG+/TG+ fetuses (8.94–68.69 ng/ml). The two TG−/TG− IGF-I values at E90 (68 and 73 ng/ml) were within and above the 95% confidence interval, respectively.

Effects of congenital hypothyroidism on weights of fetuses and their organs

Thyroid glands of TG−/TG− fetuses were visibly and significantly enlarged compared to those of TG+/TG+ fetuses from 90 days of gestation until term [E90: T(5) = 4.98; P = 0.004; E120: T(7) = 5.23; P = 0.001; E150: T(7) = 3.95; P = 0.006; Table 3]. At E150, the thyroid glands of TG−/TG+ fetuses were significantly larger than those of TG+/TG+ [T(8) = 4.36; P = 0.002] and TG−/TG− fetuses [T(7) = 2.86; P = 0.024]. At E90 and E120, the two individual values of TG−/TG+ fetuses were above the 95% confidence interval for the mean thyroid gland weight of TG+/TG+ fetuses (E90, 0.5–2.0 g; E120, 0.42–0.73 g), whereas compared with the 95% confidence interval for the mean thyroid gland weight of TG−/TG− fetuses (E90, 0.06–4.67 g; E120, 12.4–33.24 g), no consistent increase in goiter weight was found. The enlargement of TG−/TG− goiters was approximately 50-fold at E90, 66-fold at E120, and 197-fold at E150, thus showing a progressive increase.

At E150, the body weight of TG− fetuses from either TG− or TG+ ewes was comparable to that of TG+/TG+ fetuses (Table 4). Also, no significant difference was found between body weights of TG+/TG+ and TG−/TG− fetuses at E120. The body weights of the two TG−/TG− fetuses were within the confidence interval for the mean body weight of E120 TG+/TG+ fetuses (1046.6–2412.55 g). At E90, the body weights of TG−/TG− and TG+/TG− fetuses were all, except for one TG−/TG+ value (368 g), below the 95% confidence interval for the mean body weight of TG+/TG+ fetuses (360.91–401.24 g).

At E150, brain and cerebellum weights of TG−/TG− fetuses were significantly reduced compared to brain and cerebellum weights of TG+/TG+ fetuses [T(9) = 3.23; P = 0.01 and T(9) = 3.35; P = 0.009, respectively; Table 4]. Total brain weights of E150 TG−/TG+ fetuses tended to be significantly different from weights of TG−/TG− fetuses [T(8) = 2.205; P = 0.059], and cerebellum weights were not significantly different from cerebellum weights of TG−/TG− fetuses [T(8) = 1.805; P = 0.109]. Also, no significant difference was found between brain and cerebellum weights of E150 TG−/TG+ and TG−/TG+ fetuses [brain: T(9) = 0.64; P = 0.54; cerebellum: T(9) = 0.60; P = 0.56]. At E120, no significant difference between brain and cerebellum weights of TG−/TG− and TG+/TG+ fetuses was found. The brain weights of the two E120 TG−/TG+ fetuses were within (37.3 g) and below (36.2 g) the 95% confidence interval for the mean brain weight of E120 TG+/TG+ fetuses (36.75–46.7 g). The cerebellum weights of these two TG−/TG+ fetuses were both within the 95% confidence interval for the mean cerebellum weight of TG+/TG+ fetuses at E120 (3.51–6.59 g). At E90, the number of observations was too low for statistical analysis. However, the brain weights of the two TG−/TG− fetuses and the two TG−/TG+ fetuses were below and within, respectively, the 95% confidence interval for the mean brain weight of TG+/TG+ fetuses at E90 (11.76–15.09 g). The cerebellum weights of TG−/TG− and TG−/TG+ fetuses were all, except for one TG−/TG− value (0.2 g), within the 95% confidence interval for the mean cerebellum weight of TG+/TG+ fetuses at E90 (0.29–2.11 g).

### TABLE 3. Effects of hypothyroidism on fetal thyroid weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Thyroid gland wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E90</td>
</tr>
<tr>
<td>TG+/TG+</td>
<td>0.12 ± 0.03 (4)</td>
</tr>
<tr>
<td>TG−/TG−</td>
<td>2.37 ± 0.54 (3)*</td>
</tr>
<tr>
<td>TG−/TG+</td>
<td>4.10/5.90 (2)</td>
</tr>
</tbody>
</table>

*Shown are the mean ± SEM; the number of goats is given in parentheses; individual values are given when n = 2. TG−, Affected goitrous goat, homozygous for the thyroglobulin (TG) synthesis defect; TG+: unaffected normal goat; TG+/TG+, TG− fetus from TG+ mother; TG−/TG−, TG− fetus from TG− mother; TG−/TG+, TG− fetus from TG+ mother. Gestational age is indicated as E (days). Statistical analysis was performed by ANOVA.

### TABLE 2. Effects of hypothyroidism on plasma IGF-I concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma IGF-I (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E90</td>
</tr>
<tr>
<td>TG+/TG+</td>
<td>59.75 ± 2.81 (4)</td>
</tr>
<tr>
<td>TG−/TG−</td>
<td>38/47 (2)</td>
</tr>
<tr>
<td>TG−/TG+</td>
<td>68/73 (2)</td>
</tr>
</tbody>
</table>

*Shown are the mean ± SEM; the number of goats is given in parentheses; individual values are given when n = 2. TG−, Affected goitrous goat, homozygous for the thyroglobulin (TG) synthesis defect; TG+: unaffected normal goat; TG+/TG+, TG− fetus from TG+ mother; TG−/TG−, TG− fetus from TG− mother; TG−/TG+, TG− fetus from TG+ mother. Gestational age is indicated as E (days). Statistical analysis was performed by ANOVA.

### Discussion

Effects of maternal thyroid status on fetal growth and thyroid parameters during the second half of gestation

In the present study, the ewes received iodine supplementation for the initial 2 months of gestation to avoid the severe hypothyroidism, low conception frequency, and high abortion rate in affected ewes. Thus, before the onset of fetal thyroid function at approximately 70 days gestation (26),
**TABLE 4.** Effects of hypothyroidism on weights of body, total brain, and cerebellum in fetal goats

<table>
<thead>
<tr>
<th>Age</th>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>Brain wt (g)</th>
<th>Cerebellum wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E90</td>
<td>TG+/TG+</td>
<td>4</td>
<td>381.1 ± 6.3</td>
<td>13.4 ± 0.5</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>TG−/TG−</td>
<td>2</td>
<td>267/296</td>
<td>11.5/11.5</td>
<td>0.5/0.2</td>
</tr>
<tr>
<td></td>
<td>TG−/TG+</td>
<td>2</td>
<td>303/368</td>
<td>12.0/12.3</td>
<td>0.9/1.4</td>
</tr>
<tr>
<td>E120</td>
<td>TG+/TG+</td>
<td>4</td>
<td>1729.6 ± 214.6</td>
<td>41.7 ± 1.6</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>TG−/TG−</td>
<td>3</td>
<td>1642.1 ± 29.4</td>
<td>34.6 ± 1.0</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>TG−/TG+</td>
<td>2</td>
<td>1614/1685</td>
<td>37.3/36.2</td>
<td>4.4/4.0</td>
</tr>
<tr>
<td>E150</td>
<td>TG+/TG+</td>
<td>6</td>
<td>2978.3 ± 302.7</td>
<td>54.8 ± 1.1</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>TG−/TG−</td>
<td>5</td>
<td>2611.0 ± 237.4</td>
<td>45.4 ± 2.9</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>TG−/TG+</td>
<td>5</td>
<td>3056.5 ± 452.4</td>
<td>57.6 ± 4.7</td>
<td>7.4 ± 0.6</td>
</tr>
</tbody>
</table>

Shown are the mean ± SEM. n, Number of goats; different number of goats are shown in parentheses; individual values are given when n = 2. TG−, Affected goat, homozygous for the thyroglobulin (TG) synthesis defect; TG+, unaffected normal goat. TG+/TG+, TG−/TG−, TG−/TG+ fetuses from TG+ mother; TG+/TG−, TG− fetuses from TG− mother; TG−/TG−, TG− fetuses from TG+ mother. Gestational age is indicated as E (days). Statistical analysis was performed by ANOVA. Final significance ($P \leq 0.05$) was assessed by the two-sample $t$ test and was determined when the number of animals per group was three or more.

*Significantly different from TG+/TG+ group at the same age.

TG− (for definition of phenotype, see Materials and Methods) mothers were comparable to TG+ mothers. Therefore, the influence of maternal thyroid status can only be related to the second half of gestation. This might explain why the most significant difference in total brain and cerebellum weight between affected goitrous TG− fetuses from TG− mothers (TG−/TG− fetuses) and normal TG+ fetuses from normal TG+ mothers (TG+/TG+ fetuses) is found at term gestation (E150). It might also explain why the observed effects in TG−/TG− goats appear to be less severe than the fetal brain retardation caused by combined maternal and fetal thyroidectomy in sheep (15), as in the latter, maternal hypothyroidism was already present from conception. At E120, no significant difference between brain and cerebellum weights of TG−/TG− and TG+/TG+ fetuses is found. At E90, the brain weights of the two TG−/TG− fetuses were below the 95% confidence interval of TG+/TG+ fetuses, but these results should be interpreted with caution.

In contrast to TG−/TG− fetuses, the brain and cerebellum weights of TG− fetuses from TG+ mothers (TG−/TG+ fetuses) at E150 were not affected compared to those of TG+/TG+ fetuses. Furthermore, the difference in mean brain weight between TG−/TG+ fetuses and TG−/TG− fetuses at E150 is quite large and tends to be significant, suggesting that the brain weight of TG− fetuses might depend on the maternal phenotype at E150. These findings are in contrast to the retarded brain development observed in thyroidectomized fetal sheep from normal mothers (12–14) and can be explained by the remaining thyroid function, albeit impaired, in the TG−/TG+ fetuses, resulting in plasma FT$_4$ and T$_4$ levels in the normal range, whereas perinatal plasma T$_4$ concentrations in thyroidectomized sheep are undetectable (12, 13, 15). No significant effect of maternal and fetal hypothyroidism on body weight at E120 and E150 was found, which is in agreement with previous findings in goitrous newborn goats (32). The finding that the body weights of the two TG−/TG− fetuses at E90 lie below the 95% confidence interval for the mean body weight of TG+/TG+ fetuses may suggest a transient effect of hypothyroidism on growth. However, more information is needed regarding E90 body weights before the present results can be adequately interpreted.

The present findings show that a goiter is present in TG− fetuses during all stages of gestation studied (E90, E120, and E150). Remarkably, the degree of enlargement of the goiter in TG− fetuses is dependent on the maternal phenotype at E150. At E90 and E120, no clear dependency is evident, although the number of observations is too low for statistical analysis. Two possible mechanisms might account for the difference in goiter increase at E150. Firstly, the goiter increase might reflect the progressive increase in pituitary TSH secretory capacity (33), which might be delayed in TG−/TG− fetuses due to a delayed maturation of the hypothalamo-pituitary-thyroid system. Secondly, IGF-I plasma concentrations might be diminished in malnourished hypothyroid TG−/TG− fetuses compared to TG−/TG+ fetuses, producing less thyroid growth ability in response to TSH (31, 34). However, our data show that this is not the case in this study, thus favoring the first possibility.

Plasma T$_3$ and FT$_4$ concentrations in TG− fetuses are dependent on the maternal phenotype, as studied during the second half of gestation (E90–E150). Two possible mechanisms could account for the normal plasma thyroid hormone levels in TG−/TG+ fetuses: increased iodine availability for the fetal thyroid and/or increased transfer of T$_3$ from the mother in TG−/TG+ fetuses compared to TG−/TG− fetuses. We favor the former possibility as a major source of thyroid hormones in the second half of gestation for the following reasons. First, newborn TG+ lambs from TG− mothers (TG+/TG− lambs) are indistinguishable from TG+/TG+ lambs and have plasma T$_3$ levels within the normal range. This demonstrates that in late gestation, fetal goats, like fetal sheep (11, 13, 35), have to rely on their own thyroidal T$_4$ production. In addition, TG−/TG+ fetuses have much higher plasma T$_3$ and FT$_4$ concentrations than their TG+ mothers, suggesting fetal thyroid hormone synthesis. Second, if in TG−/TG+ fetuses plasma T$_4$ would originate from the mother, the goiter size would be much smaller, as has been observed in human pathology (36). The selective effect of the maternal TG genotype on fetal T$_3$ levels can be explained by the former mechanism as follows. Due to the inefficient thyroid hormone formation in the TG− goats, an inefficient iodine metabolism occurs. Not only is the iodine rapidly released from the thyroid gland in the form of serum protein-bound iodine (21, 23, 37, 38), but a marked increase in urinary excretion of low mol wt iodinated ma-
terial (LOMWIOM), representing the break-down products of the iodinated proteins by the thyroid cells, is found in affected TG− goats (25, 39). This results in a negative iodine balance in a TG− goat (23), and as a consequence, the iodine supply to the TG−/TG− fetus is impaired. Only at a high dietary iodine intake are the TG− goats able to synthesize sufficient amounts of T4 and T3 to become clinically euthyroid (23). In TG−/TG+ fetuses, normal thyroid hormone levels can be explained by the availability of more iodine for the fetus due to an efficient iodine metabolism of the normal mother. The presence of a huge goiter in the TG−/TG+ fetus can be explained by continuous TSH stimulation due to the inefficient T4 synthesis by the TG fragments that are present in low amounts (20, 22, 38). Inefficient fetal T4 synthesis is also indicated by a high fetal-derived LOMWIOM excretion in maternal urine, which increases with gestational age (39).

It is not possible from the present data to evaluate the relative participation of both mechanisms, i.e. increased iodine availability for the fetal thyroid and/or increased transfer of maternal T4. However, data obtained in sheep, a species closely related to goat, where fetal thyroidectomy resulted in undetectable low plasma T4 and T3 levels, suggest that placental T4 transfer is virtually absent in the second half of gestation (13, 14), thus favoring the mechanism of increased iodine availability in the TG−/TG+ fetus.

**Developmental changes in fetal T₄, FT₄, and T₃ concentrations**

The developmental increase in T₄ and T₃ concentrations in TG+ fetuses is in agreement with data obtained in sheep (16, 40). The increase in plasma FT₄ concentrations parallels the increase in plasma T₄ levels, suggesting that there is no major developmental change in T₄-binding protein concentrations after E90. The low plasma T₃ concentrations at E90 and E120 probably reflect levels of type I deiodinase (ID-I) activity in fetal tissues (33, 41–44). At E150, the high T₃ concentrations are in agreement with studies in sheep describing a prenatal T₃ increase and a postnatal T₃ surge that occur within the first hour after caesarean section delivery as well as after spontaneous labor (41, 45, 46). This T₃ increase might result from increased ID-I activity and/or an increased TSH secretion immediately after birth (33).

The developmental increase in plasma T₃ levels in TG−/TG− fetuses is not progressive; T₃ levels are unchanged at E90, elevated at E120, and strongly reduced at E150 compared to those in TG+/TG+ fetuses. The increased plasma T₃ levels at E120 might be due to increased thyroidal ID-I activity and/or increased type II deiodinase (ID-II) activity. The former was increased in hypothyroid rats and humans (47, 48), and the latter was increased in brown adipose tissue and brain in hypothyroid rats (49, 50) and sheep (43, 44). The decreased plasma T₃ levels at E150, might result from decreased conversion of T₁ to T₃ by ID-I due to low T₄ availability and/or reduced hepatic and renal ID-I activity, as was found in the hypothyroid fetal sheep until late in the third trimester (43).

**Summary and conclusions**

The present data indicate that in late gestation fetal goats have to rely on their own thyroidal T₄ production. Most likely, affected goitrous TG− fetuses are able to produce sufficient T₄ and T₃ to maintain the euthyroid status, provided that the maternal iodine supply to the fetus is adequate, although a possible increased transfer of maternal T₄ to TG−/TG+ fetuses cannot be excluded. The fetal thyroid hormone production suffices for preventing severe adverse effects of thyroid hormone deficiency on the brain. TG+ mothers with a normal efficient thyroid function provide sufficient iodine to the fetus. However, in TG− mothers, much iodine is wasted, because the thyroid iodinates proteins other than the aberrant TG fragment, such as serum albumin (38, 51), and the goats excrete iodinated peptides in the urine, thus loosing iodine (25, 39). This results in iodine deficiency of the fetus that also inefficiently metabolizes the iodine supplied. Consequently, it results in severe hypothyroidism, with marked effects on brain development.

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**References**

severe dietary iodine deficiency in sheep. Neuropathol Appl Neurobiol 8:303–313
17. Potter BJ, Mano MT, Belling GB, Martin DM, Cragg BG, Chavadej J, Hetzel BS 1984 Restoration of brain growth in fetal sheep after iodined oil administra-
tion to pregnant iodine-deficient ewes. J Neurol Sci 66:15–26
20. Van Voorhuijzen WF, Dinsart C, Flavell RA, De Vijlder JMM, Vassart G 1978a Abnormal cellular localization of thyroglobulin mRNA associated with he-
reditary congenital goiter and thyroglobulin deficiency. Proc Natl Acad Sci USA 75:74–78
versity of Amsterdam, Amsterdam, The Netherlands
27. Wietersinga WM 1979 The Peripheral Conversion of Thyroxine (T4) into Tri-
oiodothyronine (T3) and Reverse Triiodothyronine (rT3). PhD Thesis, University of Amsterdam, Amsterdam, The Netherlands
mined by a radioimmunoassay specific for the synthetic 53–70 peptide region. Clin Endocrinol (Oxf) 25:67–74
30. Breier BH, Gallaher BW, Gluckman PD 1991 Radioimmunoassay for insulin-
32. Van Dijk JE 1981 Congenital Goitre in Dutch goats. A Pathological Charac-
35. Fisher DA, Dussault JH, Erenberg A, Lam RW 1972 Thyroxine and triodo-
37. Rijnenberk A, De Vijlder JMM, Van Dijk JE, Jorna TJ, Tegelaars WHH 1977 Congenital defect in iodothyronine synthesis. Clinical aspects of iodine me-
tabolism in goats with congenital goitre and hypothyroidism. Br Vet J 133:695–703
44. Wu SY, Merryfield ML, Polk DH, Fisher DA 1990 Two pathways for thy-
roxine 5'-monodesiodination in brown adipose tissue in fetal sheep: ontogen-
esis and divergent responses to hypothyroidism and 3,3',5'-triiodothyronine. Endocrinology 126:1950–1958
45. Nathanielz PW, Silver M, Comline RS 1973 Plasma triiodothyronine con-
46. Klein AH, Oddie TH, Fisher DA 1978 Effect of parturition on serum iodo-
thyronine concentrations in fetal sheep. Endocrinology 103:1435–1437
dinases in human thyroid gland by thyrotropin. J Clin Endocrinol Metab 57:500–505
50. Silva JE, Matthews PS 1984 Thyroid hormone metabolism and the source of plasma triiodothyronine in 2-week-old rats: effects of thyroid status. Endo-
crinology 115:2394–2405