Pediat. Res. 6: 894–899 (1972)

Fetuin
fetus
triiodothyronine
thyroxine
thyroxine-binding globulin

Thyroxine and Triiodothyronine Metabolism in Maternal and Fetal Sheep

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Extract

Kinetic studies of thyroxine (T4) and triiodothyronine (T3) and measurements of hormone turnover were conducted in chronically catheterized maternal and fetal sheep during the last trimester of gestation. Mean serum total T4 and free T4 (FT4) concentrations were higher in fetal than in maternal serum (7.5 *versus* 5.4 μg/100 ml and 5.1 *versus* 2.4 ng/100 ml, respectively), whereas total T3 and free T3 (FT3) concentrations were higher in maternal than in fetal serum (74 *versus* <18 ng/100 ml and 176 *versus* <90 pg/100 ml, respectively). Mean maximal thyroxine-binding globulin (TBG) binding capacity was higher in maternal than in fetal blood (16.5 *versus* 8.1 μg T4/100 ml, respectively). Fetal serum was observed to contain a T4-binding α-1-globulin, which probably is identical with fetuin, a glycoprotein unique to sera of fetuses and neonates of several species including the calf, foal, sheep, pig, and chick. Mean T4 clearance values were 0.614 and 0.107 liters/kg/24 hr, respectively, in fetal and maternal sheep and mean T3 clearance values were 8.52 *versus* 1.66 liters/kg/24 hr, respectively. Mean T4 turnover values were 46.1 and 5.59 μg/kg/24 hr, and mean T3 turnover values were <1.50 and 1.22 μg/kg/24 hr in fetal and maternal sheep, respectively. No placental transfer of T4 occurred and net maternal to fetal transfer of T3 amounted to only about 1% of total turnover of fetal thyronine.

These data confirm the autonomy of the fetal hypothalamic-pituitary-thyroid axis and they indicate that hormone utilization is much higher in fetal sheep (per kilogram body weight) than in maternal sheep. In addition, the higher T4/T3 serum concentration ratio and T4/T3 turnover ratios in the fetus (>471/1 *versus* 74/1 and >31/1 *versus* 4.6/1) indicate that the relative rates of T3 secretion and/or T3 conversion from T4 in peripheral tissues are less in fetal than in maternal sheep. Finally, the data indicate a poor correlation between free hormone concentrations and either hormone clearance or hormone turnover rates, which suggests tissue binding and/or metabolism are important determinants of thyroid hormone turnover in the fetus.

Speculation

The present data regarding thyroid hormone metabolism in fetal sheep are consistent with present evidence about thyroid hormone metabolism in the human fetus and suggest that the sheep can serve as a useful model for the human system. Furthermore, data in both species support the concept of autonomy of fetal thyroid function. The ob-

servation of high T4/T3 concentration and turnover ratios in the fetus was unexpected and might be explained on the basis of a high T4/T3 secretion ratio, a low rate of conversion of T4 to T3, or a high rate of biliary T4 excretion in the fetal sheep.

Introduction

Recent data in man indicate that the fetal hypothalamic-pituitary thyroid axis functions autonomously of the maternal system. There is no correlation between total thyroid stimulating hormone T4, free thyroxine (FT4) or TSH concentrations in fetal or maternal serum at any time during gestation [9, 12, 13, 17]. Concentrations of T4 and FT4 in serum in fetuses at term usually exceed the respective maternal values [12, 17]. Fetal thyroidal autonomy has also been demonstrated in the sheep, in which species it has been demonstrated that TSH and T4 do not cross the placenta in significant quantities [1, 5, 14]. Moreover, in this species, T4 turnover in the fetus during the last trimester of pregnancy exceeds turnover of maternal T4 by about 8 times on a microgram per kilogram basis [5]. To characterize fetal thyroid hormone metabolism further in the sheep and to relate hormone turnover to free hormone concentrations, we have measured simultaneously T4 and T3 kinetics, total and free T4, total and free T3 concentrations, and binding capacities of thyroxine-binding proteins in maternal and fetal sheep during the third trimester of normal pregnancy.

Materials and Methods

One- to four-year-old Columbia and Columbia Suffolk sheep were obtained from a local source. Animals were maintained at an environmental temperature of 57–85° F, were fed bailed alfalfa, and had free access to water. Uterotomies were performed with spinal anesthesia on ewes of 95- to 135-day gestation and indwelling exteriorized femoral artery catheters were placed in the fetuses. Jugular vein catheters were inserted into the dams to provide ready access to maternal blood. Blood specimens were drawn from the dam and fetus 48–96 hr after surgery for measurements of serum T4, T3, FT4, and FT3 concentrations and for assessment of the binding capacity of serum thyroid hormone-binding proteins. In one group of five animals, T4 kinetic studies were conducted using tracer hormones. ^{125}I-labeled T4 and ^{131}I-labeled T4 were injected into the dam and fetus, respectively, and serial blood specimens were drawn from each for periods of up to 96 hr. Potassium perchlorate (400 mg) was administered orally to the dam twice daily to prevent iodide recycling. In the second group of six animals, T3 kinetic studies were obtained by a similar dual label protocol. The volume of distribution and half-time of plasma disappearance of T4 and T3 in the dam and fetus were estimated from these data. Placental hormone transfer was studied by counting ^{125}I-labeled T4 or T3 in serum from the fetus after the dam had received injections, and ^{131}I-labeled T4 or T3 in serum from the dam after the fetus had received injections.

All labeled isotopes were checked for iodide contamination by high voltage electrophoresis and they were discarded if inorganic iodide exceeded 2%. Alkali-washed butanol extracts of 250-μl aliquots of each serum specimen were prepared in duplicate as described by Fisher *et al.* [11]. Specimens and standards were counted for ^{131}I and ^{125}I activities. From these data the disappearance of isotopes from plasma of dams and fetuses was determined. Data were recorded as percentage of injected dose per liter of plasma and were plotted semilogarithmically. From these plots the linear component of the disappearance curve was defined by the method of least squares and this was extrapolated to zero time. The volume of distribution (V_D) of isotopes was calculated from the extrapolated *zero time* concentrations by the usual dilution formula. The half-time ($t_{1/2}$) of hormone turnover was determined by least squares regression analysis of the linear portion of the disappearance curves. Hormone disappearance or clearance in liters/day was calculated as: clearance (liter/24 hr) = V_D (liters) $\times$ $0.693/[t_{1/2}$ (24 hr)].

Details of the kinetic studies and estimates of hormone turnover have been published separately [4, 5].

Placental transfer of butanol-extractable radioactivity was measured as fetal-maternal and maternal-fetal placental clearance of labeled hormone assuming exchange between two hormone compartments (fetal and maternal) from which irreversible clearance is ongoing simultaneously. These computational methods have also been published [4].

Total T4 was measured by the protein-binding method of Murphy [15] and total T3 by the radioimmunoassay (RIA) procedure of Chopra *et al.* [2, 3]. Dialyzable T4 and T3 were measured by a modification of the method of Sterling and Brenner [18]. Serum

Table I. Total thyroxine (T4), free thyroxine (FT4), and thyroxine-binding globulin (TBG) capacity in serum of maternal and fetal sheep

Sheep	Maternal				Fetal			
	T4, μg/100 ml	FT4, %	Absolute FT4, ng/100 ml	TBG, μg/100 ml	T4, μg/100 ml	FT4, %	Absolute FT4, ng/100 ml	TBG, μg/100 ml
O3	3.2	0.042	1.30	17.6	6.9	0.050	3.45	6.4
O4	6.2	0.059	3.65	12.8	8.7	0.076	6.60	5.8
O5	7.6	0.045	3.42	13.8	6.4	0.065	4.16	9.0
E6	4.4	0.032	1.41	25.6	4.5	0.060	2.70	
O9	5.4	0.042	2.27	15.4	10.8	0.080	8.60	6.4
Mean	5.36	0.044	2.4	17.0	7.5	0.066	5.1	6.9
SEM	0.75	0.004	0.5	2.3	1.1	0.005	1.1	0.7

Table II. Total triiodothyronine (T3), free triiodothyrone (FT3), and thyroxine-binding globulin (TBG) capacity in serum of maternal and fetal sheep

Sheep	Maternal				Fetal			
	T3 RIA,[1] ng/100 ml	FT3, %	Absolute FT3, pg/100 ml	TBG, μg/100 ml	T3 RIA, ng/100 ml	FT3, %	Absolute FT3, pg/100 ml	TBG, μg/100 ml
O23	110	0.30	330	25	15	0.55	83	11.0
O24	98	0.13	127	15	<15	0.39	<59	8.0
O25	96	0.22	211	16.5	<15	0.49	<74	10.5
O26	56	0.19	106	15.5	<15	0.61	<92	7.5
O27	26	0.46	120	15.4	<15	0.49	<74	9.4
O28	58	0.28	162	9.4	31	0.51	158	6.5
Mean	74	0.263	176	16.1	<18	0.506	<90	8.8
SEM	13.2	0.046	34.4	2.05	<2.7	0.029	<14	0.7

[1] RIA: Radioimmunoassay.

binding proteins were studied with reverse flow electrophoresis in a Beckman system [7]. Binding capacities were measured by loading sera with increasing quantities of T4 until saturation of binding proteins was assured.

Results

Data on total T4 and T3 and the binding capacities of TBG in the two groups of maternal and fetal pairs are presented in Tables I and II. The mean T4 concentration was higher in the fetuses than in the dams; whereas the mean T3 concentration was higher in the dams. The mean dialyzable T4 (Table I) also was significantly higher in the fetuses than in their dams (0.066 *versus* 0.044%, $P < 0.01$) as was the absolute FT4 (5.1 ng/100 ml *versus* 2.4 ng/100 ml, $P < 0.05$). Dialyzable T3 was higher in the fetuses (Table II) than in the dams (0.506 *versus* 0.263%, $P < 0.01$), however, absolute FT3 levels were lower in the fetuses than in blood samples from the paired dams (<90 *versus* 176 pg/100 ml $P < 0.05$).

The mean maternal and fetal T4 binding capacities of TBG were 17.0 and 6.9 μg/100 ml, respectively, for animals from the T4 study and 16.1 and 8.8 μg/100 ml, respectively, for animals from the T3 study. There is no thyroxine-binding pre-albumin in the sheep and no estrogen hypersecretion to increase TBG levels during pregnancy. However, a T4-binding protein was observed in serum of fetal sheep that under electrophoresis moved ahead (anodal) of α-1-globulin and behind albumin (see Fig. 1). We have referred to this T4 bind-

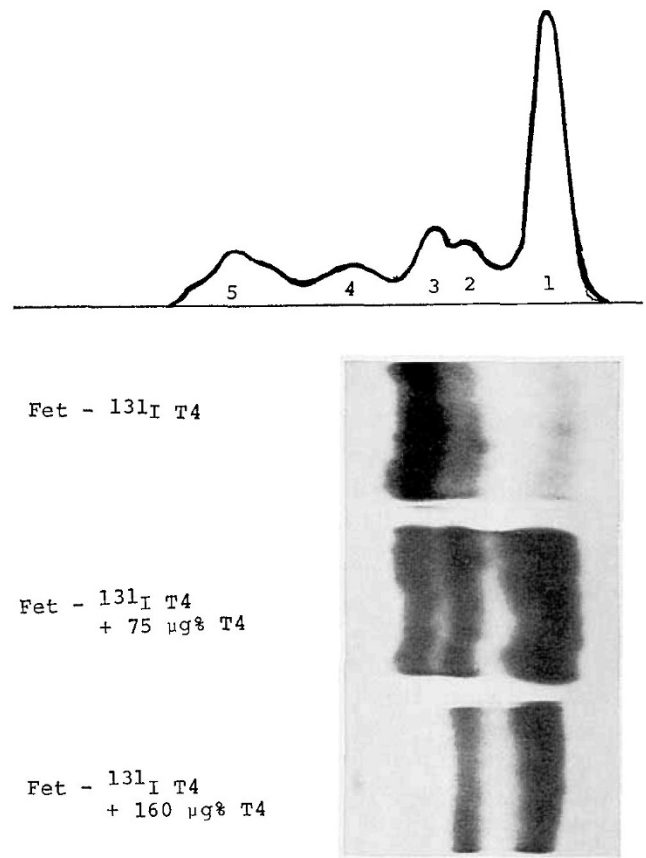

Fig. 1. Densitometer pattern and labeled thyroxine (T4) binding pattern in fetal sheep serum (130-day gestation). Peaks 1–5 represent albumin, fetuin, slow moving α-1-globulin, α-2-globulin, and β-globulin. There is little or no γ-globulin. The upper radioautogram was developed after electrophoresis of fetal serum with only tracer ^{125}I-labeled T4. Most of the radioactivity moves with inter-α-globulin (TBG) and lesser amounts with fetuin and albumin. When the serum is loaded with 75 μg/100 ml unlabeled T4, the T4 label is displaced from TBG to fetuin and albumin, but residual radioactivity is visible in the TBG area. When the serum is loaded with 160 μg/100 ml unlabeled T4, radioactivity is visible only in the fetuin and albumin areas.

ing protein as thyroxine binding fetal protein (TBFP) and preliminary studies indicate that it is, in fact, fetuin, an α-1-glycoprotein unique to fetal and neonatal serum of several species, including the calf, foal, sheep, pig, and chick [10]. Fetuin has a molecular weight of about 50,000 [16]. The T4-binding affinity of fetuin is less than that of TBG and the maximal binding capacity is very high (>600 μg/100 ml) [10]. The mean distribution of tracer ^{125}I-labeled T4 among TBG, TBFP, and albumin measured during paper electrophoresis at pH 8.6 in six paired sera from maternal and fetal sheep was: TBG 50%, TBFP 17%, and albumin 33%. Thus, although TBFP-fetuin binds both T4 and T3, the dialyzable fractions of these hormones are greater in the fetus than in the dam. Moreover, absolute concentration of fetal FT4 is greater than the level of maternal FT4. Thus it seems that TBG is the major T4-binding protein in the fetus as it is in the adult sheep; TBFP, because of its lesser binding affinity, is of secondary importance.

Table III summarizes the T4 and T3 clearance data in the two groups of maternal-fetal pairs. Mean rates of clearance of T4 and T3 in liters/kg/24 hr, in the fetus exceed maternal clearance rates by five- to sixfold (T4 = 0.614 *versus* 0.107 and T3 = 8.52 *versus* 1.66).

Table IV summarizes the mean clearance and mean hormone concentration data and includes mean rates of turnover of T4 and T3 in the dams and their fetuses. Mean rate of fetal turnover of T4 exceeds the maternal sheep turnover rate by eight- to ninefold (46.1 *versus* 5.59 μg/kg/24 hr). Mean T3 turnover is either similar in fetus and dam or lower in fetal sheep (<1.50 *versus* 1.22 μg/kg/24 hr) (Table IV).

Table III. Iodothyronine clearance in maternal and fetal sheep

Sheep	Maternal clearance				Fetal clearance			
	Thyroxine		Triiodothyronine		Thyroxine		Triiodothyronine	
	liters/24 hr	liters/kg/24 hr	liter/24 hr	liters/kg/24 hr	liters/24 hr	liters/kg/24 hr	liters/24 hr	liters/kg
O3	6.24	0.106						
O4	7.79	0.130			1.29	0.478		
O5	3.47				1.74	0.967		
E6	5.45	0.086			1.74	0.644		
O9	5.78	0.105			1.54	0.367		
O23			91.4	1.83			11.1	6.53
O24			73.3	1.55			13.7	9.13
O25			103.8	2.28			10.4	13.00
O26			66.6	1.38			15.2	6.61
O27			32.0	0.64			27.6	9.20
O28			103.8	2.28			16.6	6.64
Mean	5.43	0.107	78.7	1.66	1.58	0.614	15.8	8.52
SEM	0.65	0.009	11.2	0.25	0.10	0.130	2.56	1.03

Table IV. Turnover of thyroxine (T4) and triiodothyronine (T3) in maternal and fetal sheep

	Mean clearance, liters/kg/24 hr	Mean concentrations, μg/liter	Mean turnover, μg/kg/24 hr
Maternal			
T4	0.107	53.6	5.59
T3	1.66	0.740	1.22
Fetal			
T4	0.614	75.0	46.1
T3	8.52	<0.18	<1.50

Discussion

In the present study, total T4, percentage of dialyzable T4, and absolute free T4 concentrations were higher in fetal than in maternal serum. These findings are in agreement with those reported previously for the human fetus [6, 9, 12, 17]. The difference in absolute free T4 is more marked in sheep because the T4 binding capacity of TBG is lower and the percentage of dialyzable T4 is higher in the sheep fetus than in the human fetus. Total concentrations of T3 and FT3 are higher in maternal than in fetal serum, even though the dialyzable T3 fraction is greater in the fetus than in the dam (Table II). Data on total T3 concentration are not yet available for the human fetus; earlier data [6] were obtained at term using a paper chromatographic method which is not reliable [8].

The hormone turnover data (Table IV) were not corrected for placental transfer, since this is minimal in degree. No T4 transfer was detected in either the maternal to fetal (M-F) or the fetal to maternal (F-M) direction [5]. Some T3 transfer was observed, but it was minimal when compared with total fetal thyronine turnover [4]. Calculated F-M placental T3 clearance was 4.6 liters/24 hr [14], whereas total fetal T3 clearance was 15.8 liters/24 hr (Table III). Calculated M-F placental clearance was 3.56 liters/24 hr [4], whereas total maternal T3 clearance was 78.7 liters/24 hr (Table III). Calculated T3 transfer was 1.89 μg/24 hr in the M-F direction and 0.83 μg/24 hr in the F-M direction; net transfer was about 1 μg/24 hr in the M-F direction. Therefore, about 0.5 μg T3/kg/24 hr was contributed to the fetus, whereas total turnover of fetal hormone (T4 + T3, Table IV) was about 47 μg/kg/24 hr. These data support the autonomy of fetal hypothalamic-pituitary-thyroid function and they indicate that turnover of fetal hormone on the basis of body weight greatly exceeds maternal turnover.

Mean concentration of fetal serum T3 in the present study was much lower than the level of T3 in maternal

serum (Table II); thus the T4/T3 concentration ratio in maternal serum was 74/1, whereas, in fetal serum, the value was <417/1. The T4/T3 turnover ratios were 4.6/1 and >31/1, respectively. Possible explanations for the low level of T3 in fetal serum and the high fetal T4/T3 turnover ratio include: (*1*) a relatively high T4/T3 secretion ratio from the thyroid gland, (*2*) a relatively low rate of peripheral conversion of T4 to T3 and (*3*) a high rate of biliary T4 excretion by the fetus so that the T4 is not available for peripheral deiodination. Further studies are in progress to differentiate among these possibilities.

Finally, the present data indicate that disposal of T4 and T3 in the sheep correlates poorly with free hormone concentrations. An inverse correlation is seen between the T4-binding capacity of TBG and the percentage of dialyzable T4; mean TBG-binding capacity is lower in fetuses than in dams and the mean percentage of dialyzable T4 is higher (Table I). Thus, mean fetal clearance of T4, as might be expected, is higher than mean maternal clearance of T4 (Table III). It cannot be concluded, however, that FT4 concentration is the major determinant of disposal of T4; although the mean concentration of FT4 in the fetus exceeds that in the dam twofold (Table I), mean fetal clearance of T4 exceeds mean maternal clearance of T4 fivefold (Table III) and mean fetal turnover of T4 exceeds mean maternal turnover of T4 eightfold (Table IV). The T3 data are even more disparate. Although the mean dialyzable T3 in the fetus is twice the maternal value, mean fetal clearance of T3 is 5 times mean maternal clearance of T3 (Table III), and mean fetal and maternal turnover of T3 are nearly comparable (Table IV). Presumably, tissue binding and/or metabolism are more important determinants of thyroid hormone turnover than are the free hormone concentrations.

Summary

Studies of T4 and T3 metabolism were conducted in fetal sheep during the last trimester of gestation. Stable hormone concentrations were measured by radioimmunoassay; free hormone concentrations were estimated by equilibrium dialysis. Maximal binding capacity of TBG was measured by reverse flow paper electrophoresis. Kinetic studies of T3 and T4 were conducted using radioiodine labeled hormones. Mean concentrations of T4 and free T4 were 7.5 μg/100 ml and 5.1 ng/100 ml, respectively, in fetal serum and 5.4 μg/100 ml and 2.4 ng/100 ml, respectively, in maternal serum. Mean concentrations of T3 and free T3 were <18 ng/100 ml and <90 pg/100 ml, respectively, in fetal serum and 74 ng and 176 pg/100 ml, respectively, in maternal serum. The mean maximal T4-binding capacities of TBG in fetal and maternal sera were 8.1 and 16.5 μg/100 ml, respectively. In addition, labeled T4 binding was observed in the α-1-globulin area in fetal serum during electrophoresis, which indicates the existence of a fetal T4-binding glycoprotein other than TBG. The significance of this protein in fetal T4 metabolism is not clear.

The T4 and T3 kinetic studies revealed mean metabolic clearance rates (MCR) in fetuses and dams for T4 of 0.62 and 0.11 liters/kg/24 hr, respectively, and of 8.5 and 1.7 liters/kg/24 hr, respectively, for T3. Finally mean turnover values were 46 and 5.6 μg/kg/24 hr for T4 and <1.5 and 1.2 μg/kg/24 hr for T3 in the fetuses and dams, respectively. Since little or no placental transfer of T3 or T4 occurred during these studies, we conclude that the fetal hypothalamic-pituitary-thyroid axis is functioning autonomously of the maternal system.

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20. This work was supported by United States Public Health Service Grant no. AM04270 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.
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22. Accepted for publication August 9, 1972.

Printed in U.S.A.