ably occurred with height and weight growth, requires an increase in the body's protein content. The body's protein content in turn will increase if the net protein synthesis (S-B) is positive. Since protein synthesis is an energy expensive process (8), while protein breakdown is not, the enhancement of net protein synthesis by a reduction of protein breakdown relative to protein synthesis is clearly an energy efficient adaptation permitting increased growth at a minimal energy expenditure.

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A Differential Effect of Thyroxine and Glucocorticoids on Fetal Brain and Heart Insulin Receptor

SHERIN U. DEVASKAR, PAUL F. GRIM III, AND UDAY P. DEVASKAR

Division of Perinatal-Neonatal Medicine, Department of Pediatrics, Saint Louis University School of Medicine, Pediatric Research Institute, Cardinal Glennon Memorial Hospital for Children, St. Louis, Missouri 63104

ABSTRACT. We investigated the effect of thyroxine (T4), glucocorticoids, and T4 + glucocorticoids on the maturation of fetal rabbit brain and heart insulin receptors. Five doses of T4 over 10 days (50 μg/kg body weight per dose) were administered to the mother; significant amounts crossed the placenta (fetal serum free T4 = 0.75 ± 0.08 *versus* a control of 0.21 ± 0.02 ng/dl, $p < 0.02$) and increased the specific binding of [^{125}I]insulin to 30-day-old fetal heart membranes from a control of 3.6 ± 0.74% per 100 μg protein to 5.8 ± 0.19% ($p < 0.05$). Curvilinear Scatchard plots revealed an increase in receptor number × 10^7 μg protein^{-1} from 137 ± 4 to 244 ± 39 ($p < 0.05$) with no change in receptor affinity. No appreciable alteration by T4 in the [^{125}I]insulin-specific binding and receptor number of 30-day fetal brains was noted. Fetal heart glycogen content was decreased and there was a small increase in plasma glucose concentration in the T4-treated group (each $p < 0.02$). Betamethasone at 0.17 mg/kg did not affect the specific binding of [^{125}I]insulin to 27-day fetal heart or brain plasma membranes, although a decrease in heart glycogen content and an increase in plasma glucose concentration were observed (each $p < 0.02$). Also T4 + betamethasone did not alter the [^{125}I]insulin binding to 27-day fetal heart or brain plasma membranes, but resulted in an additive effect (a marked depletion) on cardiac glycogen ($p < 0.001$). Brain glycogen was undetectable in all

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Reprint requests Sherin U. Devaskar, M.D., Room No. 519, Glennon Hall, 1465, S. Grand Boulevard, St. Louis, MO 63104.
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fetuses, control or treated (regardless of the type of hormonal treatment). Thus T4 and glucocorticoids produce different effects on developing fetal brain and heart insulin receptors, along with a depletion of the myocardial glycogen stores. (*Pediatr Res* 19: 192–198, 1985)

Abbreviations

T4, thyroxine
T3, triiodothyronine

Glucose is an essential fuel for the metabolism of vital organs such as the brain and the heart (5, 36). Insulin promotes glucose uptake in various adult (9, 6, 28) and fetal tissues (30). Since the biologic effects of insulin are mediated by binding to specific tissue receptors, a change in the physical characteristics of these receptors as well as changes in postreceptor events may influence fetal anabolic processes (24). Hormones that antagonize the effects of insulin modulate insulin receptor characteristics in both the adult (2, 12, 16, 25) and fetus (11, 34). Insulin influences the growth and metabolism of fetal organs (18, 30). T4 and glucocorticoids may modify this effect of insulin, since both these hormones have been demonstrated to alter the insulin receptors (10, 11, 34).

In the human fetus, glucocorticoids are administered to stimulate surfactant synthesis (19) and the use of thyroid hormones is contemplated in situations where the effect of glucocorticoids is limited (14, 21). It is therefore important to define the effects of these hormones on other organs in the fetus. In this study, we investigated the influence of T4 and glucocorticoids on the maturation of the insulin receptor along with certain metabolic events in two vital organs, the fetal brain and heart.

MATERIALS AND METHODS

Animals. Pregnant New Zealand White rabbits of known gestation (term ~31 days) were assigned to one of the five groups: 1) T4 treatment (30 days gestation—$n = 7$; 27 days—$n = 3$); 2) control I ($n = 6$); 3) steroid treatment ($n = 6$); 4) control II ($n = 6$); and 5) T4 and steroid treatment ($n = 4$).

The T4 treatment group of animals ($n = 7$) received intramuscular injections of T4 at 50 μg/kg body weight every other day for a total of five injections from day 21 to 29 of gestation. This dose is approximately three times the dose reported to maintain a maternal euthyroid state in does rendered hypothyroid with propylthiouracil (11).

Another small group of pregnant rabbits ($n = 3$) received a total of five doses of T4 (50 μg/kg every other day) between days 18 and 26 of gestation. This group was included to compare the effects of T4 on 27- and 30-day-old fetuses. Animals in this small subgroup were sacrificed at 27 day of gestation. Control I animals received 0.1 ml of normal saline on alternate days between days 21 and 29 of gestation.

The steroid-treated animals received betamethasone (0.085 mg/kg) on days 25 and 26 of gestation. This dose has been shown to suppress endogenous maternal and fetal corticosteroid production and modulate fetal lung and liver insulin receptors without altering fetal plasma insulin concentrations (11, 34). Control II animals received two 0.1 ml intramuscular injections of normal saline on days 25 and 26 of gestation.

The T4 plus steroid treatment group of pregnant does ($n = 4$) received 50 μg/kg of T4 every other day (a total of five doses) from day 18 to 26 of gestation. In addition on days 25 and 26 of gestation these animals received 0.085 mg/kg/dose of betamethasone.

The T4 treated and control I animals were sacrificed on day 30 of gestation. The steroid treated, control II and T4 + steroid treated animals were sacrificed on day 27.

Immediately prior to sacrifice, maternal arterial blood was collected. Animals were sacrificed by intravenous pentobarbitone; fetal (free flowing) blood was collected after decapitation for measurements of plasma glucose, free T4, total T3, and insulin concentrations (11). Fetal blood collected in this manner yields results comparable to values reported previously by us (11, 34) and other investigators (23).

Plasma membranes. Brain plasma membranes were prepared by the method of Havrankova and Roth (15) and cardiac plasma membranes by the method of Rockson *et al.* (27). Briefly fetal brains from a litter were removed from the cranium and pooled prior to homogenization in a Dounce glass homogenizer containing 1 mM bicarbonate buffer (pH 8.0). The homogenate was centrifuged at 600 × *g* for 10 min at 4° C. The pellet was discarded and the supernatant centrifuged at 20,000 × *g* for 30 min. The final pellet was resuspended in 1 volume of 50 mM Tris buffer for every gram of original brain weight.

Hearts from a litter (two litters when there was insufficient tissue) were pooled, trimmed, minced, and homogenized in 10 vol of 0.25 M Tris, 1 mM $MgCl_2$, 5 mM EGTA using a Dounce glass homogenizer. After filtering through two layers of cheesecloth, the homogenate was centrifuged at 3000 × *g* for 10 min. The pellet was resuspended in 0.75 ml of buffer per gram of original heart weight.

Protein concentration of both the brain and heart homogenates and membranes was estimated by the method of Lowry (20). Organ DNA content was determined by Zamenof's modification (38) of Burton's technique (4). 5′-Nucleotidase activity in brain and heart homogenates, brain plasma membranes, and heart plasma membranes was measured as described before (1). The 5′-nucleotidase activity in all membranes studied was unchanged, regardless of T4 or steroid treatment. In comparison with the respective tissue homogenates, a 2-fold enrichment of the enzyme activity in the brain plasma membranes and a 1.6-fold enrichment in the heart plasma membrane was observed. Brain, heart, and liver glycogen was quantified by hydrolysis and estimation of glucose (26).

Insulin binding assay. The [^{125}I]insulin binding assay was performed as described previously (11), with the exception of the final assay pH (8.0 for the brain and 7.4 for the heart) (Fig. 1). In addition ~70,000 cpm of labeled insulin (specific activity ~100 to 150 mCi/mg) for the brain and ~35,000 cpm for the heart were used as the ligand. Membrane protein concentrations of 50 to 100 μg (heart) and 200 μg (brain) per tube were used. Scatchard plots of insulin binding data were used to determine total binding capacities (R0) and mean association constants (Ke) for receptors (29). The curvilinear Scatchard plots were resolved into two components, a high affinity low capacity component (R1) and low affinity high capacity component (R2). The association constants (K1 and K2) for these two components, respectively, also were calculated (35). Binding capacities in mol/liter were converted to number of receptors per microgram protein.

Other assays. Plasma glucose was measured by a glucose oxidase method (11). Plasma insulin (33) and serum-free T4 concentrations (33) were determined by specific radioimmunoassays as described previously. Serum total T3 concentrations were measured using a T3 immunophase RIA kit (Corning Medical, Medfield, MA). The sensitivity of the assay is 0.1 ng/ml. Interassay and intraassay coefficient of variation was less than 4%. The antibody cross-reacts 100% with L-triiodothyronine, 72% with D-triiodothyronine, 27% with triiodothyroacetic acid, 0.14% with D-thyroxine, and 0.12% with L-thyronine.

Statistics. All data are presented as a mean ± SEM. Statistically significant differences between the treated and the respective control groups was determined by the two-tailed Student's *t* test. Analysis of variance was employed when more than two groups were compared simultaneously. Correlation between the maternal and fetal plasma-free T4 concentrations in the T4-treated group of animals was determined by linear regression analysis.

RESULTS

Table 1 lists the fetal body weights and mean brain and heart weights. No differences in fetal body, brain, or heart weights were observed in the T4-treated and control I groups. The steroid-treated fetuses weighed (21.54 ± 1.34 g) less than the control II animals (28.85 ± 2.21 g, $p < 0.02$). The T4 + steroid-treated animals were intermediate in weight (25.39 ± 2.46) between the steroid-treated and control II groups; the mean weights were statistically similar in the three groups. As expected, the fetal body and organ weights were greater at 30 days than at 27 days of gestation. In addition, both the brain and heart DNA contents were similar in the T4-treated group and the T4 + steroid-treated group when compared to their respective controls. Heart DNA content decreased from 1.82 ± 0.06 to 1.43 ± 0.12 mg/g ($p < 0.02$), in response to steroid treatment; there was no change in brain DNA content in response to steroid treatment. The protein content as described in other organs (37) increased from 51.58 ± 1.98 to 56.63 ± 0.68 mg/g ($p < 0.05$) in the T4-treated fetal brain and from 89.17 ± 4.08 to 105.9 ± 3.5 mg/g ($p < 0.02$) in the T4-treated fetal hearts. A similar increase was observed in both organs in response to T4 + steroid treatment. Steroid treatment alone, however, did not alter brain or heart protein content.

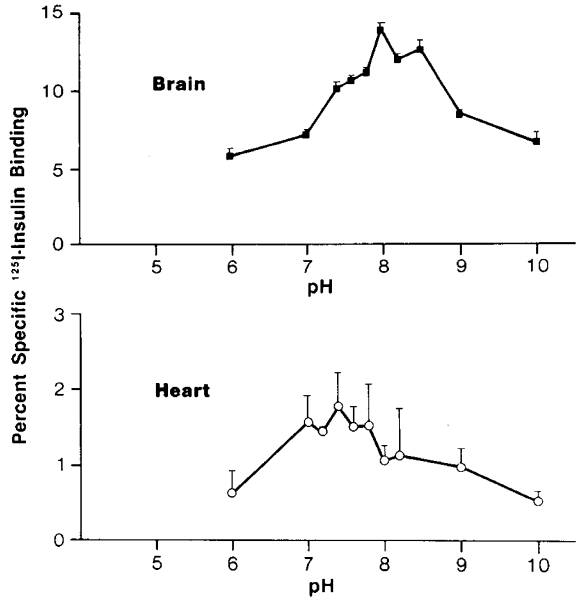

Fig. 1. Specific binding of [^{125}I]insulin to fetal brain and heart at various pH. The final pH used in the assays were peak values: 8.0 for the brain and 7.4 for the heart plasma membranes.

Plasma glucose and plasma/serum hormone concentrations are depicted in Table 2. T4 treatment increased fetal plasma glucose concentrations (47 ± 4.9 to 69 ± 4.7 mg/dl, $p < 0.02$) with no change in maternal concentrations. A marked increase in plasma glucose concentrations from 57 ± 4.8 to 124 ± 12.4 mg/dl ($p < 0.02$) and 101 ± 14.5 mg/dl ($p < 0.02$) in the steroid treated and T4 + steroid treated fetuses, respectively, was observed with no alteration in maternal values. Fetal plasma insulin concentrations were not altered by T4, steroid, or T4 + steroid treatment.

Serum-free T4 concentrations increased from a control value of 0.21 ± 0.02 to 0.75 ± 0.08 ng/dl ($p < 0.02$) in the 30-day gestation animals and from 0.06 ± 0.02 to 0.31 ± 0.07 ($p < 0.02$) in the 27-day gestation T4-treated fetuses (not shown in Table 2). The maternal serum-free T4 values were 5.41 ± 0.55 at 30 days (control I = 0.87 ± 0.33 ng/dl, $p < 0.001$) and 2.31 ± 0.27 at 27 days (control II = 0.53 ± 0.06) in the T4-treated animals. When fetal serum-free T4 values were plotted against maternal values in the T4-treated group (30 days), a linear correlation was noted ($y = 0.1248x + 0.0739$, $r = 0.86$). The maternal to fetal plasma-free T4 concentration ratio in the T4-treated group was 8:1. Serum total T3 increased from a control value of 0.76 ± 0.03 to 1.17 ± 0.02 ng/ml in the T4-treated and to 1.08 ± 0.25 ng/ml in the T4 + steroid-treated groups. Maternal serum total T3 was greater than 10 ng/ml in both groups (control = 2.31 ± 0.27). These data indicate significant transplacental transfer of free T4 to the fetus during the administration of a pharmacologic dose of T4 to the mother. Administration of steroid alone resulted in an increase in serum free T4 levels in the fetus from 0.06 ± 0.02 to 0.33 ± 0.03 ($p < 0.001$).

Figure 2 demonstrates the [^{125}I]insulin competition curves in both the fetal T4-treated and control I brain and heart membranes. The mean ± SEM are represented in these curves. In the inset Scatchard plots (mean values) are represented. The Scatchard plot data obtained are summarized in Table 3. Using brain plasma membranes, no difference in percent specific [^{125}I]insulin binding (Fig. 2, Table 3), R0, R1, and R2 is observed between the T4-treated and control I fetuses. Conversely in the T4-treated heart plasma membranes, an increase from 3.6 ± 0.74 to 5.8 ± 0.19% ($p < 0.05$) in [^{125}I]insulin binding is noted. In addition an increase in the total receptor number (R0) from 137 ± 3.9 to

Table 1. *Fetal body wt, organ wt, protein, and DNA content*

Groups (n)		Fetal body wt (g)	Fetal brain wt (g)	Fetal heart wt (g)	Protein content (mg/g)		DNA content (mg/g)	
					Brain	Heart	Brain	Heart
30-day								
T4-treated group	$\bar{X}$	44.05	1.08	0.23	56.63*	105.9†	1.24	2.28
($n = 6$)	SEM	2.89	0.05	0.02	0.68	3.5	0.04	0.24
Control I group		51.78	1.12	0.19	51.58	89.17	1.23	2.10
($n = 7$)		2.68	0.04	0.02	1.98	4.08	0.06	0.26
27-day								
Steroid-treated group		21.54†	0.51	0.13	61.70	47.50	1.26	1.43†
($n = 6$)		1.34	0.04	0.01	3.49	2.04	0.13	0.12
Control II group		28.85	0.62	0.14	63.12	54.70	1.43	1.82
($n = 6$)		2.21	0.06	0.03	1.76	3.02	0.05	0.06
T4 + steroid-treated group		25.39	0.53	0.13	71.63*	62.66*	1.49	2.53
($n = 4$)		2.46	0.03	0.04	4.85	5.20	0.02	0.49

* $p < 0.05$, when compared to controls.
† $p < 0.02$, when compared to controls.

Table 2. *Fetal plasma glucose and insulin, serum-free T4, and total T3 concentrations*

Groups (n)		Plasma glucose (mg/dl)	Plasma insulin (μU/ml)	Serum-free T4 (ng/dl)	Serum total T3 (ng/ml)
T4-treated	Mean	69*	20.1	0.75*	
group—30 days	SEM	4.7	3.0	0.08	
(n = 6)		(115 ± 6.8)†		(5.41 ± 0.55)†	
(n = 3)—27 days‡		67	53.4	0.76*	1.17*
		9.5	12.6	0.03	0.02
		(135 ± 6.5)†		(2.31 ± 0.27)†	(>10)†
Control I		47	26.2	0.21	
group—30 days		4.9	4.0	0.02	
(n = 7)		(111 ± 2.7)†		(0.87 ± 0.03)†	
Steroid-treated		124*	17.5	0.33*	
group—27 days		12.4	7.5	0.03	
(n = 6)		(166 ± 23.9)†			
Control II—27 days		57.3	37.5	0.06	0.76
(n = 6)		4.8	6.5	0.02	0.03
		(126 ± 4.4)†		(0.53 ± 0.06)†	(2.31 ± 0.27)
T4 + steroid—27 days		101*	22.9	0.36*	1.08*
treated group		14.5	5.2	0.01	0.25
(n = 4)		(142 ± 6.8)†		(3.51 ± 0.39)†	(>10)†

* $p < 0.02$, when compared to controls.
† Maternal values are represented in parentheses.
‡ The 27 days T4-treated subgroup was compared with control II.

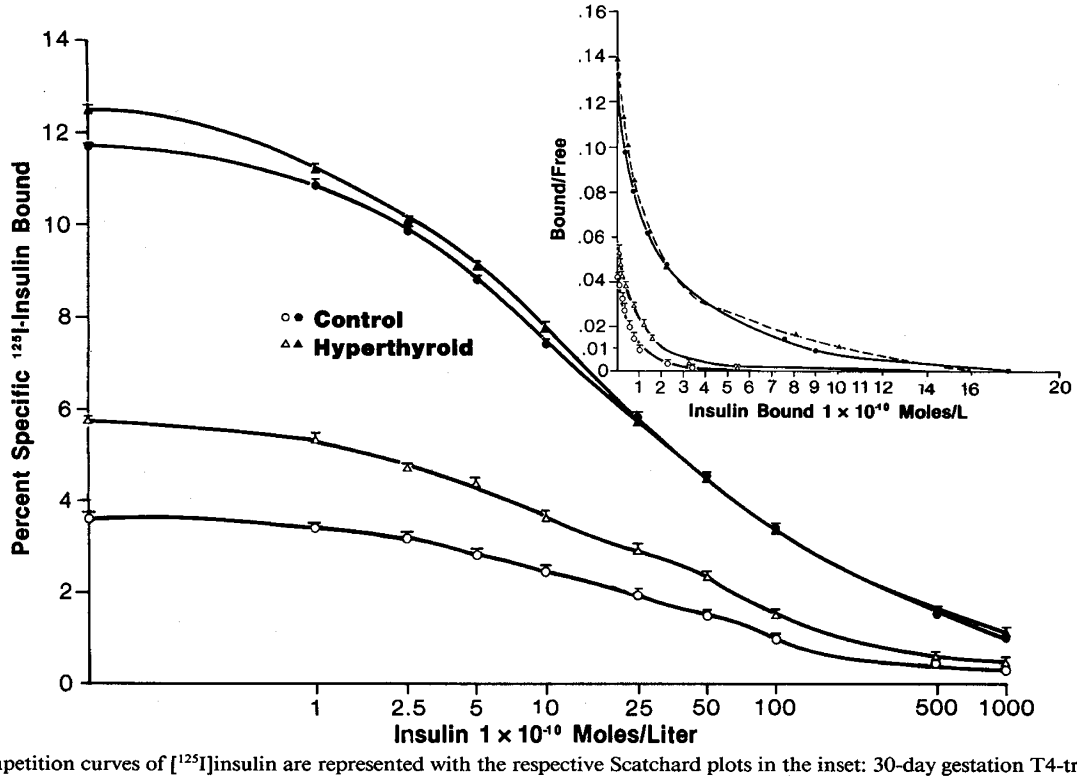

Fig. 2. Competition curves of [^{125}I]insulin are represented with the respective Scatchard plots in the inset: 30-day gestation T4-treated fetal brain (*solid triangles*) and heart (*open triangles*) plasma membranes versus control fetal brain (*solid circles*) and heart (*open circles*) plasma membranes.

244 ± 39.2 × 10^7 μg protein^{-1} ($p < 0.02$) and the low affinity, high capacity (R2) receptor sites is observed. No change in the high affinity, low capacity (R1) receptor number is demonstrated. In both the brain and heart the affinity constants remained unchanged. Similar [^{125}I]insulin binding results were observed in the T4-treated 27-day-old subgroup of fetuses (n = 3). No significant difference was observed between the two gestational ages.

Steroid treatment (Table 4) did not alter the [^{125}I]insulin binding to fetal brain (10.3 ± 0.41% *versus* a control of 9.2 ± 0.52%) and heart (3.4 ± 0.24% *versus* a control of 3.5 ± 0.33%). Similarly T4 + steroid treatment did not alter the [^{125}I]insulin binding to the fetal brain (9.3 ± 0.61%) and fetal heart (4.3 ± 0.13%) although in the latter binding was intermediate between the control and T4-treated values.

Figure 3 demonstrates the total glycogen content in μM glucosyl units/mg protein of heart in addition to the specific [^{125}I] insulin binding to myocardial membranes. Contrary to the change in [^{125}I]insulin binding and total receptor number, a decrease in fetal cardiac glycogen from 0.65 ± 0.04 to 0.46 ± 0.01 μM glucosyl units/mg protein ($p < 0.02$) is observed in

Table 3. *Insulin receptor characteristics in 30-day fetal brain and heart plasma membranes*

Groups (n)		% Specific binding of [^{125}I]insulin per 100/ 200 μg protein	Receptor no. $\times 10^7$ μg protein^{-1}			Affinity constant $\times 10^7$ M		
			Ro	R1	R2	Ke	K1	K2
T4-treated brains ($n = 6$)	$\bar{x}$	12.5	153	8.2	145	9.91	123	2.60
	SEM	0.5	10.0	1.0	9.9	0.61	12.1	0.41
Control I brains ($n = 7$)		11.7	186	15.2	171	7.87	86	1.53
		0.6	14.8	2.4	15.0	0.86	9.0	0.41
T4-treated hearts ($n = 4$)		5.8*	244*	10.8	233†	5.80	189	0.60
		0.2	39.2	5.7	36.7	1.29	85.0	0.10
Control I hearts ($n = 5$)		3.6	137	11	126	5.75	85	0.60
		0.7	3.9	4.0	2.7	0.81	16.3	0.10

* $p < 0.05$, when compared to controls.
† $p < 0.02$, when compared to controls.

Table 4. *% Specific binding of [^{125}I]insulin to 27-day fetal brain and heart plasma membranes*

Groups	Brain (per 200 μg)	Heart (per 100 μg)
Steroid treated	10.3 (6)*	3.4 (4)
	0.41	0.24
Control II	9.2 (6)	3.5 (5)
	0.52	0.33
T4 + steroid treated	9.3 (4)	4.3 (4)
	0.61	0.13

* The numbers in parentheses denote the number of pooled brains/ hearts.

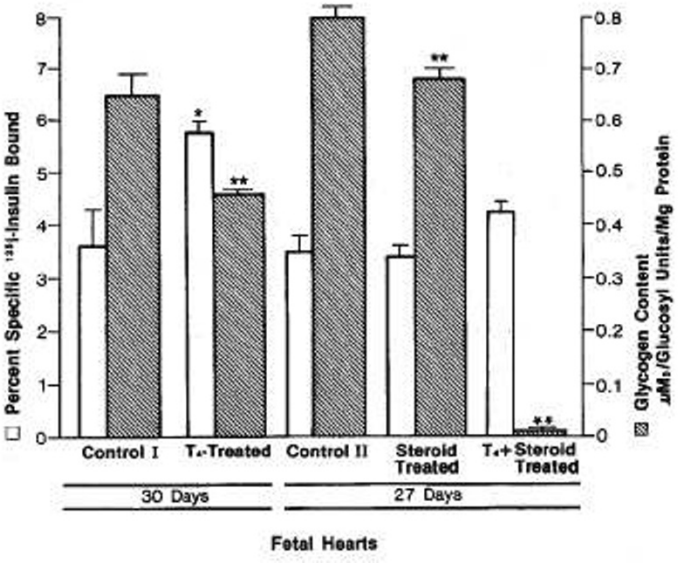

Fig. 3. Percent [^{125}I]insulin binding to heart plasma membranes and myocardial glycogen content in μmol glucosyl units/mg protein in control I and T4-treated 30-day gestation fetuses; and in control II, steroid-treated, and T4 + steroid-treated 27-day gestation fetuses. p * < 0.05, ** < 0.02, when compared to their respective gestational age-matched control group.

response to T4 treatment. Steroid treatment also decreased the fetal myocardial glycogen content from 0.80 ± 0.31 to 0.68 ± 0.02 μM glucosyl units/mg protein ($p < 0.02$). T4 + steroid treatment similarly decreased the glycogen content in the heart to 0.07 ± 0.005 ($p < 0.001$). Myocardial glycogen content, expressed as μM glucosyl units/μg DNA, decreased from 0.020 ± 0.0001 in the 30-day control fetus to 0.015 ± 0.001 ($p < 0.01$) in the T4-treated fetus. Similarly a decrease from 0.17 ± 0.01 in the 27-day control fetus to 0.12 ± 0.005 ($p < 0.02$) and 0.0016 ± 0.0002 ($p < 0.001$) in the steroid-treated and T4 + steroid-treated fetuses, respectively, was observed. Total brain glycogen content in all five groups was less than the sensitivity of the assay and thus undetectable. The sensitivity of the glycogen assay is 0.025 μM glucosyl units/100 μl or 0.1 g of brain tissue. Glycogen content in both organs was measured as an end product of insulin-regulated glucose uptake by myocardial and brain cells. In addition, fetal liver glycogen content was quantified and noted to be no different in the T4-treated and control I groups (215 ± 11.8 *versus* 210 ± 8.8 μM glucosyl units/g wet weight).

DISCUSSION

Previously we demonstrated that administration of T4 at 25 μg/kg every other day for a total of three doses (75 μg/kg) to hypothyroid pregnant does rendered them euthyroid, without affecting the low fetal-free T4 levels (11). However, in the present study, we observed that administration of a higher dose of T4 to euthyroid pregnant does (total of 250 μg/kg) results in high maternal and fetal-free T4 levels. The high serum T4 levels exert a biologic effect in the fetus. In addition we demonstrated an increase in cardiac insulin receptors with a decrease in glycogen content in the T4-treated fetuses. Our results are consistent with previous observations that T4 increased the insulin receptor number of adult adipocytes (16) and hypothyroidism was associated with a decrease in fetal lung and liver insulin receptors (11, 34).

Following maternal administration of a synthetic thyroxine preparation, 3,5, dimethyl 3′ isopropyl thyronine, which more readily crosses the placenta, a depletion in fetal myocardial glycogen has been observed (23). In the present study, no effect of the T4 treatment on fetal liver glycogen content was observed. In a preliminary experiment ($n = 6$), at twice the dose of T4 administered in this study (*i.e.* 500 μg/kg), we observed a depletion of fetal liver glycogen (7). These observations suggest that in the fetus, myocardial glycogen is more sensitive than liver glycogen to the effects of T4.

In the adult heart, insulin specifically promotes glycogen synthesis by increasing the glycogen synthase enzyme activity with little or no effect on phosphorylase activity (22). However, the phosphorylase enzyme system is hypersensitive to insulin antag-

onists, *e.g.* α and β adrenergic agonists and glucagon (17). Plasma insulin concentrations remaining constant and an increase in insulin receptor number with unchanging postreceptor events should increase the biologic effect of insulin and augment glucose uptake. This has been demonstrated in the adult (24). However, the fetus is relatively resistant to insulin; peripheral glucose uptake increases only when pharmacologic doses of insulin are administered (30), and glucose challenge produces a relatively obtunded insulin response (32). In addition, a delayed maturation of insulin-induced glucose uptake *versus* amino acid uptake has been demonstrated in fetal hepatocytes (31).

Besides an increase in the myocardial insulin receptor number, the effect of T4 on various enzymes regulating glycogen synthesis is not clear. An activation of myocardial phosphorylase and an acceleration of glycogen depletion coincides with the postnatal period of physiologic T4 surge in the neonate (13). T4 (3,5,dimethyl 3′ isopropyl thyronine) also evokes a depletion of myocardial glycogen in a hyperinsulinemic fetus of an alloxan-diabetic mother (23). This suggests that T4 alters the enzyme activity that controls glycogen metabolism. The influence of T4 on postreceptor events may be responsible for the depletion of myocardial glycogen. T4, by independently modulating the insulin receptor and postreceptor action(s), thus dissociates the physical characteristics of the receptor and the biologic function. In association with the decrease in cardiac glycogen stores, a slight increase in fetal plasma glucose concentration was noted in the present study. No similar change in maternal glucose values was observed possibly due to the dilutional effect in the large maternal pool. Again an increase in fetal plasma glucose values did not stimulate a rise in fetal insulin concentrations, due to an obtunded fetal pancreatic islet cell response to a glucose challenge (32).

During the same developmental period, no modulation by T4 of fetal brain insulin binding or receptor number was observed. Most biochemical events of physiologic significance in the brain, such as the synthesis of myelin lipids, occur postnatally (8). In the presence of a change in cardiac insulin receptor number, an absence of a change in brain insulin receptor characteristics signifies a difference in organ maturity or a different response by separate organs to the same stimulus.

Glucocorticoid treatment did not change fetal cardiac [^{125}I] insulin binding despite elevated serum-free T4 concentrations. Although the free T4 levels after steroid treatment were similar to the levels achieved with T4 treatment alone, no increase in the insulin binding akin to that observed in response to T4 treatment alone was demonstrated. On the contrary, the steroid-induced decrease in [^{125}I]insulin binding and receptor number observed in the fetal liver (34) was absent in the myocardium. The influence of high T4 concentrations was masked by the effect of the steroids in the fetus. In addition, a reduction in the heart glycogen content along with an increase in fetal glucose concentrations was noted in response to steroid treatment alone. Although during development changes in the fetal liver insulin receptor characteristics secondary to steroid treatment have been demonstrated (34), the lack of change in brain [^{125}I]insulin binding may again be due to organ maturational differences.

T4 + steroid treatment also resulted in an absence of a change in either the fetal myocardial or the brain [^{125}I]insulin binding. However, a marked depletion of myocardial glycogen with an elevation of plasma glucose levels was observed as an additive effect of T4 and steroids.

Our studies demonstrate that the fetal brain insulin receptor is not modulated by the systemic hormonal changes that modulate the cardiac receptor. This suggests a relative unresponsiveness on the part of fetal neural tissue. On the other hand, in the fetal myocardium, combined T4 and steroid treatment neutralize the effect of either hormone alone on insulin binding (an increase in number by T4 and an absence of a decrease by steroids).

Although the physiologic significance remains unclear, we have demonstrated an additive interaction between T4 and betamethasone with regard to myocardial glycogen depletion. The sensitive period for a T4 effect on human fetal insulin receptors has not been defined; besides 10 days of T4 therapy in the rabbit fetus cannot be related to the clinical situation. However, our present studies stress the need for more information about other effects, since combined thyroid hormone and glucocorticoid therapy has recently been proposed in the amelioration of respiratory distress syndrome in the impending delivery of a premature infant (21).

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Urinary Excretion of an Isomer of Bilirubin during Phototherapy

ISABELLA KNOX, JOHN F. ENNEVER, AND WILLIAM T. SPECK

Department of Pediatrics, Rainbow Babies and Childrens Hospital, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

ABSTRACT. Lumirubin, a water-soluble photoproduct of bilirubin formed *in vivo* during phototherapy, is excreted in the urine. In premature infants with little or no bilirubin conjugating activity, lumirubin is the principal yellow pigment found in the urine during phototherapy. The clearance rate of lumirubin in nine premature infants varied from 0.05 to 0.65 ml/min and increased with postconceptional age in parallel with increased creatinine clearance rate. The amount of lumirubin excreted per 24 h was estimated to be from 0.2 to 9.4 mg with a mean of 3.2 mg. The urinary excretion of lumirubin is a significant pathway for pigment elimination during phototherapy. (*Pediatr Res* 19: 198–201, 1985)

Visible light phototherapy has been used to treat neonatal hyperbilirubinemia for more than two decades (1, 2). Although precise data are not available, it has been estimated that between 2 and 5% of all newborn infants are treated with phototherapy (3). Despite this widespread use over a number of years, the mechanism by which phototherapy lowers serum bilirubin *in vivo* is not known. The purpose of this study was to determine whether urinary excretion is an important pathway for the elimination of bilirubin photoproducts.

Bilirubin, a metabolic product of heme degradation (4), is a highly lipophilic molecule (5); prior to excretion, bilirubin is made more water soluble by conjugation to glucuronic acid (6). Because newborn infants are deficient in the enzyme(s) responsible for this conjugation reaction they frequently develop hyperbilirubinemia which is most often treated with phototherapy. During phototherapy, bilirubin undergoes two principal photochemical reactions, which yield products that are more polar than the native molecule (7–10). The relative importance of these two reactions to the therapeutic response seen with phototherapy depends on both the rates of formation and the rates of excretion of the photoproducts.

The two principal photoproducts are 4Z,15E-bilirubin, a configurational isomer of the native 4Z,15Z-bilirubin (9), and lumirubin, a structural isomer which contains a seven-member ring (10) (Fig. 1). The relative rates of the two reactions are known from *in vitro* studies (9–11) and appear to be similar *in vivo* (12). The faster reaction is the configurational isomerization which is freely reversible. The formation of lumirubin occurs more slowly (11) but is essentially irreversible. Typically during phototherapy, 2 to 6% of the total bilirubin is present as lumirubin whereas 15 to 20% is present as the configurational isomer (12). A third type of reaction, the photooxidation of bilirubin to mono and dipyrroles (13) occurs at a much lower rate than either isomerization reaction (11) and is not thought to be a quantitatively important pathway for bilirubin elimination.

The decline in serum bilirubin during phototherapy requires not only formation of these bilirubin isomers but also their elimination. The principal route of photoproduct elimination is thought to be through the bile. Onishi *et al.* (8) have reported finding a bilirubin photoproduct, which they called "unknown pigment," in the bile and urine of infants treated with phototherapy. We have used a high pressure liquid chromatographic method to quantitate the urinary excretion of bilirubin isomers in nine preterm infants. We have identified the photoproduct in the urine as the configurational isomer of bilirubin, lumirubin. We have determined the rate of urinary excretion of lumirubin

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Address correspondence and requests for reprints to John F. Ennever, Ph.D., M.D., Department of Pediatrics, Rainbow Babies and Childrens Hospital, 2101 Adelbert Road, Cleveland, OH 44106.
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