Fetal Hyperinsulinemia and Protein Turnover in Fetal Rat Tissues

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The effects of fetal hyperinsulinemia on protein turnover in various tissues of fetal rats were determined after transuteral injection of insulin to rat fetuses at day 19 of gestation. Tissue protein content was measured on the subsequent days of gestation (days 20–22), and protein synthesis was determined at day 20 of gestation in fetal tissues after intravenous injection of [^{3}H]phenylalanine into the maternal circulation, followed by measurements of tissue free and protein-bound phenylalanine specific radioactivity in fetal diaphragm, brain, heart, and liver. Rates of protein degradation in these fetal tissues were calculated by subtracting protein accretion rates from rates of protein synthesis. The injection of insulin to rat fetuses at day 19 of gestation resulted in relative macrosomia versus saline-injected controls from the same litter (body wt at day 20 of gestation, 3.26 ± 0.15 g for saline-injected fetuses and 3.60 ± 0.25 g for insulin-injected fetuses, $P < 0.001$) and increased protein and RNA content of brain, heart, and liver. Although fractional rates of protein synthesis were not significantly elevated in tissues from the hyperinsulinemic fetuses, absolute rates of protein synthesis were increased in brain, heart, and liver of hyperinsulinemic fetuses. Hyperinsulinemia did not reduce calculated rates of protein breakdown in fetal brain, heart, or liver but did in fetal diaphragm. We conclude that the major effect of fetal hyperinsulinemia on protein turnover in rats is to increase protein synthesis in selected tissues without simultaneously affecting protein breakdown. *Diabetes* 39:541–48, 1990

Insulin is felt to be an important fetal growth factor by many investigators (1–5). The mechanisms by which fetal insulin augments fetal growth are unclear. We are aware of only one previous study that has investigated the effects of fetal hyperinsulinism on protein turnover in the fetus in vivo. Horn et al. (6) produced hyperinsulinism in chronically catheterized fetal sheep and determined parameters of protein turnover after [^{14}C]lysine infusion. They concluded that fetal hyperinsulinism had little effect on protein synthesis in fetal organs but retarded protein breakdown.

Studies of the effects of hyperinsulinism on protein turnover in young adult animals have demonstrated enhanced protein synthesis in some tissues and reduced protein breakdown in many tissues (7). In this study, we report the effects in rat fetuses of hyperinsulinism produced by transuteral injection of long-acting insulin at day 19 of gestation on tissue protein, RNA and DNA content, and parameters of protein turnover. Our findings suggest that the major effect of fetal hyperinsulinism in rats is to increase protein synthesis in selected tissues.

RESEARCH DESIGN AND METHODS

Virgin Sprague-Dawley rats were mated at 100 days of age (260–280 g body wt). Day 0 of gestation was defined as the day on which a vaginal plug was found (8). Throughout the mating period and gestation, rats were kept in a room controlled at 22 ± 1°C with a 12-h light-dark cycle. Animals were fed Wayne Lab Blox (24% protein; Allied Mills, Chicago, IL) ad libitum throughout gestation. At day 19 of gestation, pregnant rats were anesthetized lightly with diethyl ether, and both uterine horns were exteriorized after laparotomy. The contents of the uterine horns were rested on gauze pads soaked in 0.9% NaCl at 37°C. The number of fetuses in each uterine horn was recorded, and alternate fetuses were injected transuterally with either 2 U protamine zinc insulin or an equal volume of 0.9% NaCl into the subcutaneous tissue of the back with a 26-gauge hypodermic needle, similar to the procedure described by Catlin et al. (9). Protamine zinc insulin is a long-acting preparation with a duration of action of 24–36 h.

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The horns of the uterus were placed back into the abdominal cavity, the laparotomy incision was closed with suture material, and the animals were allowed to recover from the anesthetic. Subsequently, animals were killed at day 20, 21, or 22 of gestation. Fetuses were removed and weighed, blood was obtained from axillary incisions into heparinized pipettes, and brain, heart, diaphragm, and liver were carefully dissected, weighed, and frozen for subsequent chemical analyses. Another group of animals was utilized for determination of protein synthesis as described below. Fetal mortality was 14% for insulin-injected fetuses and 2% for saline-injected fetuses. Only live fetuses were employed for determinations of tissue composition and protein synthesis.

Determination of protein synthesis. We utilized the method of Garlick et al. (10) to determine protein synthesis in vivo. Massive concentrations of [^{3}H]phenylalanine were injected intravenously into pregnant rats to flood the precursor pool for protein synthesis. As we have reported previously, this technique results in a relatively constant specific radioactivity of free phenylalanine in fetal tissue for at least 30 min after injection and a linear rate of increase of tissue-bound [^{3}H]phenylalanine specific radioactivity over this period (11).

In this study, we injected pregnant rats with 150 μmol phenylalanine containing 65 μCi L-[4-^{3}H]phenylalanine (25 Ci/mmol)/100 g body wt via an internal jugular vein with the rats under light ether anesthesia on day 20 of gestation, 24 h after transuteral injection of insulin or saline to the fetuses. Pregnant rats were killed with an overdose of diethyl ether 20 min after injection of phenylalanine; fetuses were rapidly submerged in ice-cold 0.9% NaCl and separated into insulin- and saline-injected groups, and brain, heart, diaphragm, and liver were quickly dissected and frozen in liquid N_2.

Processing of tissue samples for determination of the specific radioactivity of free and protein-bound phenylalanine was conducted by the method of Garlick et al. (10). Fractional rates of protein synthesis in fetal tissues were calculated from the equation $K_s = S_b/S_a t$, where S_b is specific radioactivity (dpm/nmol) of protein-bound phenylalanine, S_a is specific radioactivity of tissue free phenylalanine, t is time in days, and K_s is the fractional rate of protein synthesis per day. Absolute rates of protein synthesis were calculated by multiplying K_s on day 20 of gestation by the tissue protein content on day 20 of gestation extrapolated from the regression analyses of protein content versus gestational age.

Determination of rates of protein accretion and degradation. The protein-balance equation for any tissue is expressed by the equation $K_g = K_s - K_d$, where K_g is the fractional rate of protein accretion per day and K_d is the fraction of protein in the total pool that is degraded per day. As described above, K_s was determined directly. K_g was determined by constructing growth curves for the protein content of individual tissues from a separate group of fetuses for both saline and insulin injections. Regression analysis was used to analyze protein-accretion data. The experimental design of the study dictates that the regression lines for both saline and insulin must go through the same intercept on the 1st day of the study (gestational age of 19 days). The mathematical model is similar to the one used in slope-ratio assays (12). The full model uses two curved lines to explain the data. The equation is

$$Y = \alpha + B_{s1} D_s + B_{s2} D_s^2 + B_{I1} D_I + B_{I2} D_I^2$$

where Y is protein content, D_s is study day as gestational age − 19 for saline samples and 0 for insulin samples, D_I is study day as 0 for saline samples and gestational age − 19 for insulin samples, and α, B_{s1}, B_{s2}, B_{I1}, and B_{I2} are coefficients estimated from the data with multiple regression procedures.

Hypothesis tests for curvature and equal slopes were F tests based on the differences between the variability explained by this full model and the variability explained by the appropriate reduced models. For example, the test for curvature tests the full model against the straight-line model $Y = \alpha + B_s D_s + B_I D_I$ to determine if the model that includes quadratic terms explains significantly more variability than the straight-line model.

Absolute growth rates at day 20 of gestation were obtained from the regression equations and fractional growth rates (K_g) from the relation $K_g = (dP/dt)/Pt$, where Pt is the protein content at day 20 of gestation. K_d was calculated by subtracting K_g from K_s.

Biochemical determinations. Protein content of fetal tissues was determined on tissue homogenates by the method of Lowry et al. (13) with bovine albumin as the standard. RNA and DNA from tissue samples were extracted as described by Munro and Fleck (14), and their concentrations were measured as previously described (14,15). Plasma glucose was determined by a glucose oxidase procedure (16), and plasma insulin was determined by radioimmunoassay with rat insulin as the standard (17).

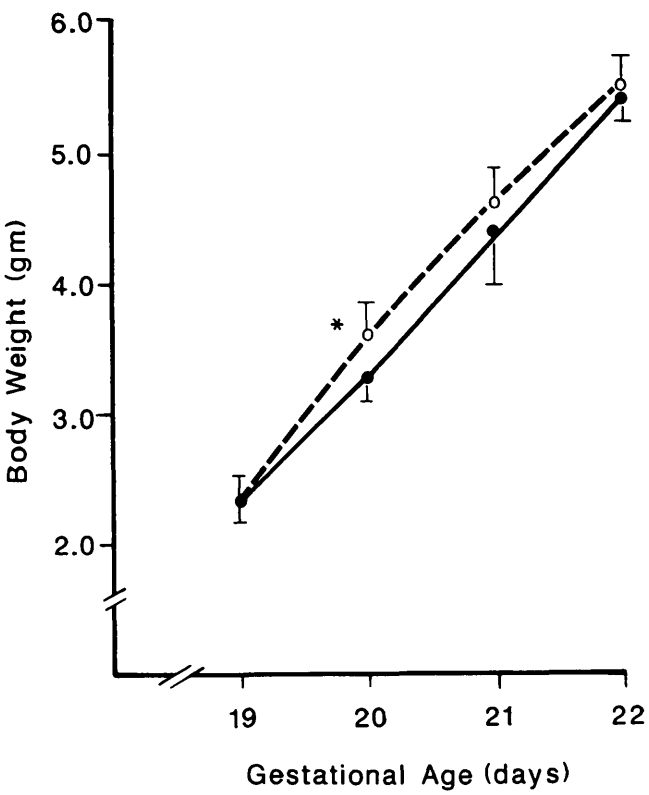

FIG. 1. Fetal body weight in insulin-injected (○) and saline-injected (●) fetuses. Values are means ± SD of pooled data from 9–13 litters from each treatment group at each gestational age. Statistical analysis was performed by paired *t* test. *$P < 0.001$.

For amino acid analyses, plasma samples were deproteinized with an equal vol of 5% sulfosalicylic acid containing 400 μM norleucine. Tissue samples were homogenized in 4 vol of 5% sulfosalicylic acid containing norleucine. Supernatants containing plasma and tissue free amino acids were stored at −70°C until analyzed. These plasma and tissue samples were derived from separate groups of fetuses 24 h after injection of 2 U protamine zinc insulin or saline on day 19 of gestation. Amino acid analysis was conducted by ion-exchange liquid chromatography with the Dionex amino acid analyzer (Sunnyvale, CA) with fluorometric detection with *o*-phthalaldehyde. Amino acid concentrations were calculated by integral area analysis compared with a standard amino acid mixture. The temperature and time programming of the procedure for these studies did not allow for accurate separation of the basic amino acids. All samples were coded so that the person performing the analyses was unaware of whether a given sample came from an insulin- or saline-injected fetus.

As previously indicated, regression analysis was used to evaluate protein-accretion data; paired Student's *t* tests were employed for comparison of variables between saline- and insulin-injected fetuses. No variances are shown for K_g and K_d because these variables are derived from other primary data.

RESULTS

General characteristics of fetal hyperinsulinism model. Plasma insulin concentrations in insulin-injected fetuses were markedly elevated versus saline-injected fetuses 24 h after the injections (mean ± SD 1712 ± 1774 vs. 116 ± 17 μU/ml on day 20 of gestation, $P < 0.05$) but thereafter were no different from the saline-injected controls. There were no significant differences in fetal plasma glucose concentrations between insulin- and saline-injected fetuses at any gestational age (39 ± 10 vs. 48 ± 4 mg/dl at day 20 of gestation, $P > 0.05$). Fetal body weight was significantly greater in the insulin-injected fetuses on day 20 of gestation. This difference in body weight persisted for the remainder of gestation but was not statistically significant on days 21 and 22 (Fig. 1). The protein content of fetal brain, heart, and liver was significantly increased in insulin versus saline-injected fetuses on some or all days after insulin injection, and a similar trend was exhibited by diaphragm (Fig. 2). RNA content similarly increased in the insulin-injected fetuses (Fig. 3). However, DNA content of these fetal tissues was not affected by insulin injection (data not shown).

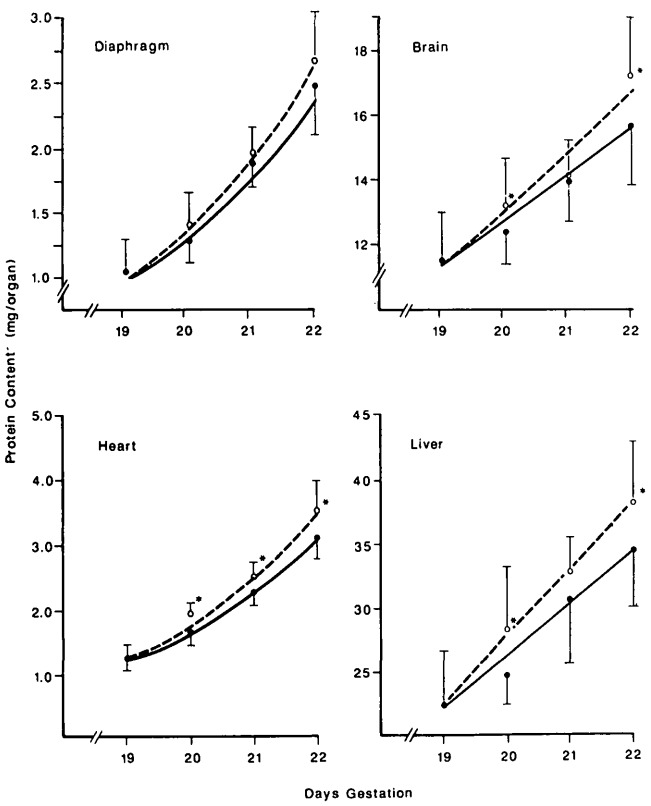

FIG. 2. Protein content of fetal tissues in insulin-injected (○) and saline-injected (●) fetuses. Values are means ± SD of fetuses from 6–10 litters at each gestational age. *$P < 0.05$ by paired *t* test.

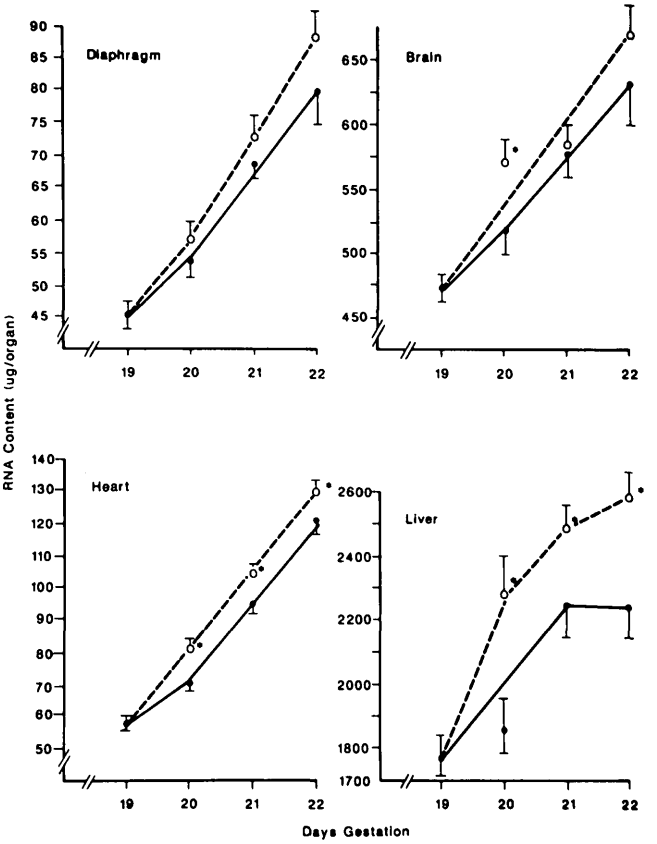

FIG. 3. RNA content of fetal tissues in insulin-injected (○) and saline-injected (●) fetuses. Values are means ± SD of fetuses from 6–10 litters at each gestational age. *$P < 0.05$ by paired *t* test.

Protein turnover in fetal tissues. Both quadratic and linear equations for tissue protein accretion are shown in Table 1. Only heart showed a significant effect for curvature, although diaphragm approached significance for curvature ($P = 0.06$), and quadratic equations for diaphragm placed the intercept at day 19 of gestation closer to the observed mean at that day than did the linear equations. Therefore, linear regressions for liver and brain but quadratic relationships for heart and diaphragm were used in Fig. 2. Tests for equality of the equations (slopes) were significantly different for saline- versus insulin-injected fetuses for liver and heart but did not reach statistical significance for brain ($P = 0.10$) or

TABLE 1
Regression equations and tests of hypotheses for fetal tissue protein accretion

Brain	
Test for curvature	$P = 0.10$
Quadratic equations	
Saline	$Y = 11.72 + 0.59D + 0.25D^2$
Insulin	$Y = 11.72 + 0.03D + 0.58D^2$
Test for equality	$P = 0.24$
Linear equations	
Saline	$Y = 11.06 + 1.49D$
Insulin	$Y = 11.06 + 1.77D$
Test for equality	$P = 0.18$
Heart	
Test for curvature	$P < 0.01$
Quadratic equations	
Saline	$Y = 1.28 + 0.27D + 0.12D^2$
Insulin	$Y = 1.28 + 0.43D + 0.11D^2$
Test for equality	$P < 0.001$
Linear equations	
Saline	$Y = 1.10 + 0.64D$
Insulin	$Y = 1.10 + 0.77D$
Test for equality	$P < 0.001$
Liver	
Test for curvature	$P = 0.83$
Quadratic equations	
Saline	$Y = 22.62 + 2.92D + 0.39D^2$
Insulin	$Y = 22.62 + 5.25D - 0.03D^2$
Test for equality	$P < 0.05$
Linear equations	
Saline	$Y = 22.34 + 4.03D$
Insulin	$Y = 22.34 + 5.29D$
Test for equality	$P < 0.05$
Diaphragm	
Test for curvature	$P = 0.06$
Quadratic equations	
Saline	$Y = 1.01 + 0.27D + 0.08D^2$
Insulin	$Y = 1.01 + 0.29D + 0.09D^2$
Test for equality	$P = 0.40$
Linear equations	
Saline	$Y = 0.87 + 0.53D$
Insulin	$Y = 0.87 + 0.57D$
Test for equality	$P = 0.18$

All regression analyses utilized experimentally determined protein contents over days 19–22 of gestation. The analyses conducted forced the intercept on day 19 of gestation to be the same for both saline- and insulin-injected fetuses. Y, protein content; D, study day (gestational age − 19).

diaphragm ($P = 0.18$). Rate constants for protein synthesis, protein accretion, and protein degradation are shown in Table 2. For all tissues except diaphragm, K_s tended to be greater in insulin- versus saline-injected fetuses, although none of the differences was statistically significant. K_g at day 20 of gestation was greater in all fetal tissues of insulin-injected rats versus saline-injected controls. K_d was very similar in brain, heart, and liver of both groups but was somewhat lower in diaphragm of insulin-injected fetuses versus saline-injected controls.

Values for absolute rates of protein turnover are shown in Table 3. Protein synthesis was enhanced in brain, heart, and liver of hyperinsulinemic fetuses versus controls, protein accretion was increased in all tissues from hyperinsulinemic fetuses, and protein breakdown was apparently reduced in diaphragm from insulin-injected fetuses.

Ratios of protein to RNA and protein to DNA were similar in tissues of saline- versus insulin-injected fetuses (Table 4). Similarly, there were little differences in total protein synthesis per unit of RNA and DNA in fetal organs of control versus insulin-treated groups (Table 4).

TABLE 2
Rate constants for protein synthesis (K_s), accretion (K_g), and degradation (K_d) in fetal tissues

Tissue	K_s	K_g	K_d
Brain			
Saline	0.411 ± 0.061	0.119	0.292
Insulin	0.437 ± 0.052	0.138	0.299
Heart			
Saline	0.532 ± 0.149	0.300	0.232
Insulin	0.566 ± 0.133	0.352	0.214
Liver			
Saline	0.719 ± 0.115	0.153	0.566
Insulin	0.772 ± 0.139	0.192	0.580
Diaphragm			
Saline	0.582 ± 0.146	0.315	0.267
Insulin	0.547 ± 0.296	0.337	0.210

Values for K_s are means ± SD in days^{-1} of 7–8 experiments conducted on day 20 of gestation. None of the differences between saline- and insulin-injected fetuses is statistically different by paired Student's t test. Values for K_g and K_d are calculated instantaneous numbers for day 20 of gestation.

Amino acid concentrations. Concentrations of fetal plasma amino acids are shown in Fig. 4. Threonine, glutamine, and glycine were significantly elevated in insulin- versus saline-injected fetuses, and free amino acids were higher for 12 of the 14 amino acids measured in the insulin-injected group. Total plasma free amino acids were significantly greater in insulin- versus saline-injected fetuses (5.66 ± 0.41 vs. 4.58 ± 0.07 mM). Free–amino acid concentrations for other fetal tissues are shown in Table 5. For all tissues except diaphragm, most individual amino acids and total amino acids were higher in the insulin-injected group, although not all of these differences reached statistical significance. Amino acids were approximately threefold more concentrated in placenta versus fetal plasma and even more so in the other fetal tissues. Note the very high concentration of free taurine in many fetal tissues, particularly brain and heart.

DISCUSSION

Mammalian prenatal growth is largely independent of growth hormone and thyroid hormone (18,19). However, consider-

TABLE 3
Absolute rates of protein synthesis, accretion, and breakdown in saline- and insulin-injected fetuses at day 20 of gestation

	Synthesis	Accretion	Breakdown
Brain			
Saline	5.16	1.49	3.67
Insulin	5.61	1.77	3.84
Heart			
Saline	0.884	0.499	0.385
Insulin	1.024	0.637	0.387
Liver			
Saline	18.96	4.034	14.93
Insulin	21.33	5.304	16.03
Diaphragm			
Saline	0.788	0.427	0.361
Insulin	0.759	0.467	0.292

Values (mg protein · tissue^{-1} · day^{-1}) were calculated by multiplying mean K values (Table 2) by protein content at day 20 of gestation calculated from the regression equations in Table 1.

TABLE 4
Tissue composition and relationship of protein synthesis to RNA and DNA content of fetal rat tissues at day 20 of gestation

Tissue	Protein/RNA	Protein/DNA	Protein synthesis/unit RNA (g protein · g RNA^{-1} · day^{-1})	Protein synthesis/unit DNA (g protein · g DNA^{-1} · day^{-1})
Brain				
Saline	24.2 ± 3.7	13.2 ± 3.4	9.8	5.1
Insulin	23.1 ± 3.0	14.1 ± 3.9	10.1	5.8
Heart				
Saline	23.7 ± 3.2	19.7 ± 6.1	12.6	9.7
Insulin	24.0 ± 2.6	20.6 ± 5.1	13.5	11.0
Liver				
Saline	13.6 ± 1.6	13.0 ± 4.1	9.6	9.1
Insulin	12.4 ± 1.5*	14.9 ± 5.6	9.6	10.6
Diaphragm				
Saline	23.9 ± 2.5	21.7 ± 4.8	13.8	12.2
Insulin	24.4 ± 4.0	22.8 ± 4.6	13.3	12.2

Protein/RNA and protein/DNA values are means ± SD of pooled tissues from 8–9 litters.
*$P < 0.05$ vs. saline-injected animals.

able evidence suggests that insulin plays a significant role in promoting fetal growth in mammals. The macrosomia seen in human infants of poorly controlled diabetic mothers is thought to result from fetal hyperinsulinism (1), and the rare case of pancreatic agenesis in the human fetus is associated with growth retardation (2).

Under some conditions, insulin administration to the fetus results in accelerated growth. Picon (3) demonstrated that daily insulin injections to fetal rats late in gestation result in increased weight and nitrogen and lipid content compared with littermate controls. Subsequently, others have demonstrated that one or two injections of insulin to rat fetuses late in gestation increase body and organ weight at term (9,20,21). Continuous infusion of insulin to rhesus monkey fetuses results in increased body and organ weight compared with age-matched controls (4,5), although insulin infusion to fetal pigs has no effect on fetal weight (22).

The metabolic actions of insulin in adult mammals are diverse and include stimulation of glucose uptake and lipogenesis and profound effects on protein metabolism. Insulin stimulates amino acid uptake in many tissues and protein synthesis in some and inhibits proteolysis in many. We know much less about the actions of insulin in fetal tissues. Few studies have addressed the effects of insulin on protein turnover in the fetus. Amino acid uptake by chick embryo heart is enhanced by insulin as early as 5 days of

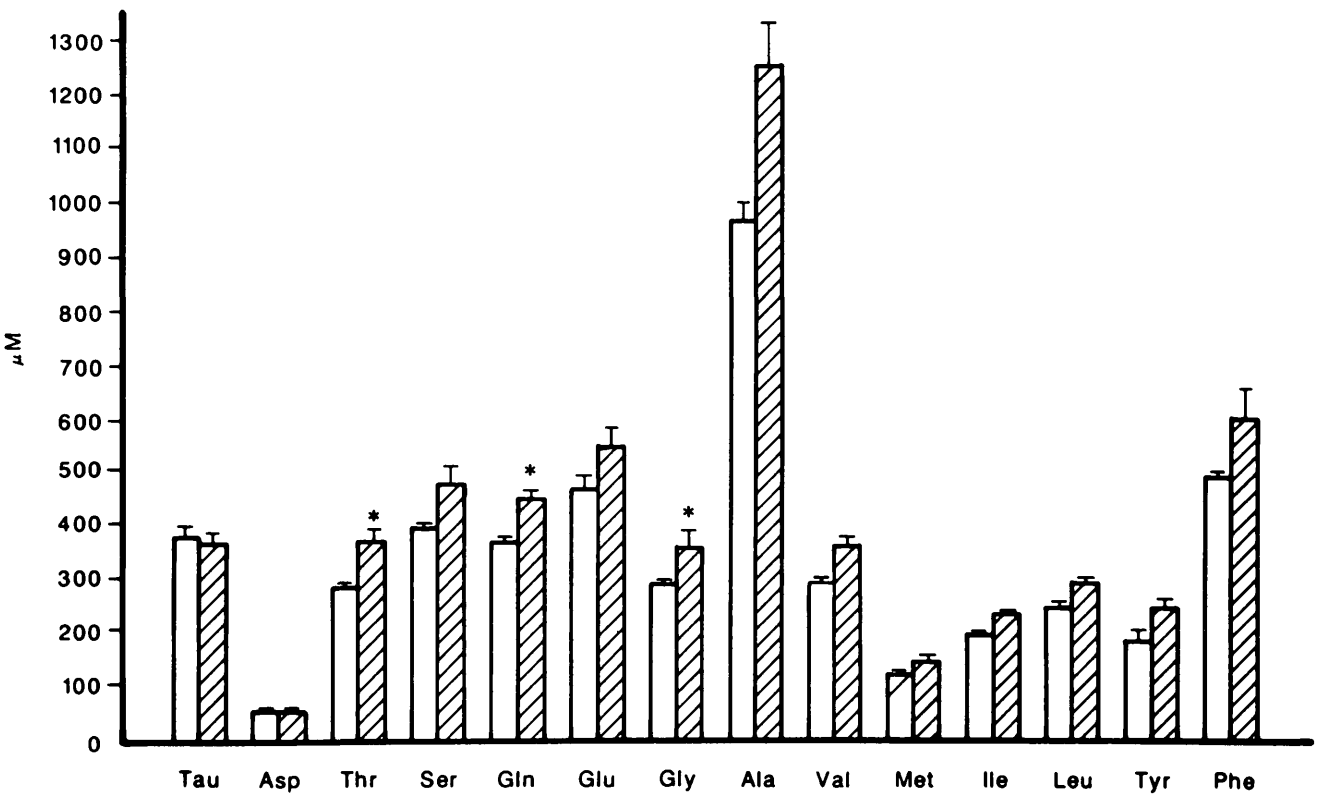

FIG. 4. Plasma free amino acids at day 20 of gestation in insulin-injected (*hatched bars*) and saline-injected (*open bars*) fetuses. Values are means ± SE of pooled samples from 4 litters. *$P < 0.05$ by paired *t* test.

TABLE 5
Tissue free amino acids in saline- and insulin-injected fetuses

Amino acid	Brain		Heart		Liver		Diaphragm		Placenta	
	Saline	Insulin	Saline	Insulin	Saline	Insulin	Saline	Insulin	Saline	Insulin
Taurine	15,468 ± 68	15,024 ± 205	13,767 ± 342	13,390 ± 316	8046 ± 616	7671 ± 566	6739 ± 370	6694 ± 354	3598 ± 146	3744 ± 180
Aspartic acid	1881 ± 47	2000 ± 40	2335 ± 76	2523 ± 72	707 ± 30	1038 ± 58	840 ± 46	823 ± 70	593 ± 18	690 ± 24*
Threonine	763 ± 10	895 ± 25*	894 ± 36	1033 ± 57	752 ± 107	960 ± 110*	843 ± 48	877 ± 57	788 ± 56	912 ± 31*
Serine	1202 ± 32	1273 ± 21*	1581 ± 42	1692 ± 122	1437 ± 150	1789 ± 148*	1727 ± 71	1545 ± 80*	1048 ± 26	1169 ± 43
Glutamine	1835 ± 48	2126 ± 72*	1809 ± 212	2130 ± 258*	1950 ± 385	2167 ± 424	2641 ± 234	2441 ± 226	949 ± 50	1106 ± 24*
Glutamic acid	7180 ± 157	7472 ± 190*	9959 ± 514	10,236 ± 296	6898 ± 690	8349 ± 694	5723 ± 304	5486 ± 231	2098 ± 61	2314 ± 70
Glycine	1419 ± 22	1804 ± 33*	1481 ± 124	1850 ± 158*	2570 ± 342	2806 ± 352	1468 ± 171	1633 ± 180*	1507 ± 98	1690 ± 80
Alanine	2931 ± 136	3329 ± 232*	4648 ± 186	5266 ± 302	2671 ± 220	3629 ± 326*	5127 ± 279	5187 ± 236	2614 ± 79	3103 ± 86*
Valine	224 ± 10	275 ± 16*	401 ± 6	438 ± 14	372 ± 28	413 ± 49	328 ± 10	373 ± 20	289 ± 10	327 ± 16
Methionine	25 ± 2	43 ± 6*	148 ± 23	183 ± 20*	64 ± 5	74 ± 12	150 ± 5	163 ± 6	38 ± 9	57 ± 7*
Isoleucine	159 ± 4	193 ± 12*	288 ± 4	323 ± 12*	238 ± 18	283 ± 32	221 ± 10	252 ± 20	200 ± 5	228 ± 12
Leucine	223 ± 11	265 ± 20*	373 ± 1	408 ± 10*	355 ± 32	402 ± 43	301 ± 9	333 ± 20	257 ± 13	286 ± 21
Tyrosine	213 ± 7	250 ± 8	289 ± 12	325 ± 20	269 ± 30	294 ± 30	307 ± 20	330 ± 25	198 ± 10	204 ± 6
Phenylalanine	469 ± 11	584 ± 16	672 ± 23	768 ± 62	572 ± 38	681 ± 56	662 ± 26	748 ± 52	441 ± 21	491 ± 27
Total (mM)	34.0 ± 0.3	35.5 ± 0.6*	38.7 ± 1.4	40.6 ± 1.5	26.9 ± 0.8	30.6 ± 1.6	27.1 ± 1.4	26.9 ± 1.4	14.6 ± 0.4	16.3 ± 0.4*
Total − taurine (mM)	18.5 ± 0.3	20.5 ± 0.6*	24.9 ± 1.1	27.2 ± 1.2*	18.9 ± 1.3	22.9 ± 1.6	20.3 ± 1.5	20.2 ± 1.6	11.0 ± 0.3	12.6 ± 0.3*

Individual amino acid concentrations (μM) are means ± SE of 4 experiments. For each experiment, tissues from saline- and insulin-injected fetuses were pooled by group before amino acid analysis. All experiments were conducted at day 20 of gestation, 24 h after saline or insulin injection.
*$P \leq 0.05$ vs. saline-injected fetuses by paired t test.

incubation (23,24). Physiological concentrations of insulin stimulate protein synthesis in cell cultures of skeletal and cardiac muscle cells from 8- to 11-day chick embryos (25). Insulin stimulates protein synthesis and retards degradation in organ cultures from hearts of fetal mice (26).

Horn et al. (6) studied the effects of short-term insulin infusion on protein turnover in fetal lambs at 130–140 days of gestation. They concluded that insulin enhanced protein synthesis in skeletal muscle and placenta but only if amino acids were infused simultaneously. No effects on protein synthesis were observed in brain, liver, or heart. Insulin also reduced muscle protein catabolism in these studies. Oddy et al. (27) reported the effects of insulin infusion on protein turnover in skeletal muscle and whole body of neonatal lambs. Insulin had no effect on protein metabolism in well-fed lambs but reduced both protein synthesis and degradation in fasting lambs.

Some studies in adult mammals also indicated reduced protein synthesis during insulin administration (28–31). Although some investigators reported that insulin enhances protein synthesis in fasted and diabetic rats (32–34), other studies suggest that insulin depresses muscle and whole-body protein synthesis in vivo unless hyperaminoacidemia is present (6,29,30,35).

Our studies demonstrate that a single injection of insulin to rat fetuses late in gestation enhances body weight and tissue protein and RNA content of brain, heart, and liver with lesser increases in diaphragm. We do not have an explanation of why insulin affects body weight only transiently (effect significant only on day 20 of gestation) other than a longer-term effect on the protein content of some tissues (e.g., heart in Fig. 2). This could relate to differential effects of insulin on various organs and/or to statistical considerations, because in all instances, the effects are relatively small. The apparent mechanism of increased organ protein content in brain, heart, and liver is elevated protein synthesis without a reduction in protein breakdown. Enhanced protein synthesis in brain, liver, and heart occurred without a change in protein synthesis per unit of RNA, thus suggesting normal translational efficiency with increased protein-synthesizing machinery, i.e., ribosomes.

In diaphragm, the slight increase in protein content appears to be the result of retarded protein degradation. We do not know how to explain this apparent discrepancy among tissues. The variability of measurements of protein synthesis is greatest in diaphragm. However, diaphragm is the only tissue in which free–amino acid concentration was not increased by insulin, so the findings may be significant. Dissociation of an effect of insulin on amino acid uptake and protein synthesis between diaphragm and heart has previously been described in the perinatal period. Although some investigators described insulin-stimulated amino acid uptake in fetal and neonatal rat diaphragm in vitro (36,37), others reported that amino acid transport into diaphragm of newborn rats is resistant to insulin (38), whereas insulin stimulates both amino acid uptake and protein synthesis in the isolated fetal rat heart (39). Asplund (37) found no stimulation of protein synthesis by insulin in diaphragm in vitro in fetal and newborn rats.

We have no explanation for the lack of effect of insulin injection to reduce protein breakdown in fetal brain, heart,

and liver. The ability of insulin to enhance protein synthesis in vivo in our experiments, in contrast to studies in fetal sheep, fasted neonatal lambs, and human adults, may be secondary to the interesting and surprising finding that insulin-injected rat fetuses had elevated tissue free–amino acid concentrations. It seems evident that the effects of insulin on protein synthesis in vivo are critically dependent on substrate availability (6,29,30,35).

Until recently, brain was considered to be an insulin-insensitive organ. However, over the past decade, it has become evident that insulin and insulin receptors are present in the CNS, and physiological effects of insulin on the CNS have been documented (40). Clarke et al. (41) showed that insulin stimulates protein, RNA, and DNA synthesis in cultured glial cells from neonatal rat brain. Our findings demonstrate an in vivo effect of hyperinsulinism on fetal rat brain protein and RNA content and protein synthesis.

The effect of fetal hyperinsulinism over 24 h to increase plasma and tissue free–amino acid concentrations in our studies was an unexpected finding. Acute insulin administration resulted in lowered plasma and tissue amino acid concentrations in several previous studies in various mammalian species (6,27,31,42). The effect we demonstrate represents a longer-term influence of insulin, i.e., studies conducted 24 h after administration of a long-acting insulin preparation. These findings raise the questions of whether fetal insulin might stimulate placental amino acid transport and, if so, whether this effect might be direct or indirect. Additional experiments are in progress to address these issues.

Finally, are the effects of insulin we observed on fetal protein metabolism the result of insulin acting on its own receptors or on somatomedin C receptors? Although the somatomedin C receptor has a low affinity for insulin, the pharmacological concentrations of insulin in fetal plasma in our experiments could stimulate the somatomedin C receptor to produce the effects of insulin on protein turnover that we observed.

In summary, fetal hyperinsulinism in the rat increases body weight and tissue protein and RNA content. Analysis of protein turnover suggests that fetal hyperinsulinism stimulates protein synthesis in brain, heart, and liver. Fetal plasma and tissue free–amino acid concentrations are increased by hyperinsulinism. The mechanisms by which these changes are accomplished deserve additional investigation.

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REFERENCES

1. Pederson J: *The Pregnant Diabetic and Her Newborn. Problems and Management*. 2nd ed. Baltimore, MD, Williams & Wilkins, 1977, p. 211–20.
2. Lemons JA, Redenour R, Orsini EN: Congenital absence of the pancreas and intrauterine growth retardation. *Pediatrics* 64:255–57, 1979
3. Picon L: Effect of insulin on growth and biochemical composition of the rat fetus. *Endocrinology* 81:1419–21, 1967
4. Susa JB, McCormick KL, Widness JA, Singer DB, Oh W, Admsons K, Schwartz R: Chronic hyperinsulinemia in the fetal rhesus monkey: effects on fetal growth and composition. *Diabetes* 28:1058–63, 1979
5. Susa JB, Neave C, Sehgal P, Singer DB, Zeller WP, Schwartz R: Chronic hyperinsulinemia in the fetal rhesus monkey: effects of physiologic hyperinsulinemia on fetal growth and composition. *Diabetes* 33:656–60, 1984
6. Horn J, Stern M, Young M: Influence of insulin and substrate concentration on protein synthetic rate in fetal tissues. *Res Vet Sci* 35:35–41, 1983
7. Jefferson LS, Flaim KE, Peavy DE: Effect of insulin on protein turnover. In *Diabetes Mellitus IV*. Brownlee M, Ed. New York, Garland, 1981, p. 133–77
8. Szabo KT, Free SM, Birkhead HA, Gay PE: Predictability of pregnancy from various signs of mating in mice and rats. *Lab Anim* 19:822–25, 1969
9. Catlin EA, Chung-Ja MC, Oh W: Postnatal growth and fatty acid synthesis in overgrown rat pups induced by fetal hyperinsulinemia. *Metabolism* 34:1110–14, 1985
10. Garlick PJ, McNurlan MA, Preedy VR: A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of ^{3}H-phenylalanine. *Biochem J* 192:719–23, 1980
11. Johnson JD, Dunham T, Skipper BJ, Loftfield RB: Protein turnover in tissues of the rat fetus following maternal starvation. *Pediatr Res* 20:1252–57, 1986
12. Finney DJ: *Statistical Methods in Biological Assay*. London, Griffin, 1952, p. 187–97
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–75, 1951
14. Munro HN, Fleck A: Analysis of tissue and body fluid for nitrogenous constituents. In *Mammalian Protein Metabolism*. Vol. 3. Munro HN, Ed. New York, Academic, 1969, p. 423–525
15. Giles KW, Myers A: An improved diphenylamine procedure for the estimation of deoxyribonucleic acid. *Nature* (Lond) 206:93, 1965
16. Huggett ASC, Nixon DA: Use of glucose oxidase, peroxidase and *o*-dianisidine in determination of blood and urinary glucose. *Lancet* 2:368–70, 1957
17. Hales CM, Randle PH: Immunoassay of insulin with insulin-antibody precipitate. *Biochem J* 88:137–46, 1963
18. Jost A, Picon L: Hormonal control of fetal development and metabolism. *Adv Metab Disord* 4:168–84, 1970
19. Robinson JS, Kingston EF, Thorburn GD: Physiological and endocrine factors in human fetal growth. *Postgrad Med J* 54 (Suppl. 1):51–61, 1978
20. Angervall L, Karlsson K, Martinsson A: Effects on rat fetuses of intrauterine injections of insulin. *Diabetologia* 20:558–62, 1981
21. Vileisis R, Oh W: Enhanced fatty acid synthesis in hyperinsulinemic rat fetuses. *J Nutr* 113:246–52, 1983
22. Garssen GF, Spencer GSG, Colenbrander B, Macdonald AA, Hill DJ: Lack of effect of chronic hyperinsulinemia on growth and body composition in the fetal pig. *Biol Neonate* 44:234–42, 1983
23. Guidotti GG, Borghetti AF, Gaja G, Loreti L, Ragnotti G, Foa PP: Amino acid uptake in the developing chick embryo heart: the effect of insulin on aminobutyric acid accumulation. *Biochem J* 107:565–74, 1968
24. Guidotti GG, Gaja G, Loreti L, Ragnotti G, Rottenberg DA, Borghetti AF: Amino acid uptake in the developing chick embryo heart: the effect of insulin on glycine and leucine accumulation. *Biochem J* 107:575–80, 1968
25. Airhart J, Arnold JA, Stirewalt WS, Low RB: Insulin stimulation of protein synthesis in cultured skeletal and cardiac cells. *Am J Physiol* 243:C81–86, 1982
26. Wildenthal K, Griffin EE, Ingwall JS: Hormonal control of cardiac protein and amino acid balance. *Circ Res* 38 (Suppl. 1):I138–42, 1976
27. Oddy VH, Lindsay DB, Barker PJ, Northrop AJ: Effect of insulin on hindlimb and whole-body leucine and protein metabolism in fed and fasted lambs. *Br J Nutr* 58:437–52, 1987
28. Tessari P, Nosadini R, Trevisian R, DeKreutzenberg SV, Inchiostro S, Duner E, Biolo G, Marescotti MC, Tiengo A, Crepaldi G: Defective suppression by insulin of leucine and alpha-ketoisocaproate metabolism in insulin dependent type I diabetes mellitus. *J Clin Invest* 77:1797–804, 1986
29. Fukagawa NK, Minaker KL, Rowe JW, Goodman MN, Matthews DE, Bier DM, Young VR: Insulin-mediated reduction of whole body protein breakdown. *J Clin Invest* 76:2306–11, 1985
30. Tessari P, Inchiostro S, Biolo G, Trevisan R, Fantin G, Marescotti MC, Iori E, Tiengo A, Crepaldi G: Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo. *J Clin Invest* 79:1062–69, 1987
31. Castello P, Luzi L, Simonson DC, Haymond M, DeFronzo RA: Effect of insulin and plasma amino acid concentrations on leucine metabolism in man. *J Clin Invest* 80:1784–93, 1987
32. Garlick PJ, Fern M, Preedy VR: The effect of insulin infusion and food intake on muscle protein synthesis in postabsorptive rats. *Biochem J* 210:669–76, 1983
33. Reeds PJ, Hay SM, Glennie RT, Mackie WS, Garlick PJ: The effect of indomethacin on the stimulation of protein synthesis by insulin in young post-absorptive rats. *Biochem J* 227:255–61, 1985
34. Pain VM, Albertese EC, Garlick PJ: Protein metabolism in skeletal muscle, diaphragm and heart of diabetic rats. *Am J Physiol* 245:E604–10, 1983
35. Garlick PJ, Grant I: Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. *Biochem J* 254:579–84, 1988

36. Fricke R, Clark CM Jr: Augmentation of glucose and amino acid uptake by insulin in the developing rat diaphragm. *Am J Physiol* 224:117–21, 1973
37. Asplund K: Protein synthesis and amino acid accumulation during development in the rat: dissociation of diaphragm and heart muscle sensitivity to insulin. *Horm Res* 6:12–19, 1975
38. Riggs TR, Wise HD, Motz KL: Amino acid transport in diaphragm from newborn rats: evidence for insulin resistance. *Am J Physiol* 235:E304–10, 1978
39. Clark CM Jr: The stimulation by insulin of amino acid uptake and protein synthesis in the isolated fetal rat heart. *Biol Neonate* 19:379–88, 1971
40. Baskin DG: Insulin in the brain. *Annu Rev Physiol* 49:335–47, 1987
41. Clarke DW, Boyd FT Jr, Kappy MS, Raizada MK: Insulin stimulates macromolecular synthesis in cultured glial cells from rat brain. *Am J Physiol* 249:C484–89, 1985
42. Johnson JD, Greenberg RE: Regulation of fetal growth. In *Prenatal and Perinatal Biology and Medicine*. Vol. 1. Kretchmer N, Quilligan EJ, Johnson JD, Eds. Chur, Switzerland, Harwood, 1987, p. 35–92