Protein anabolic effects of insulin and IGF-I in the ovine fetus

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Shen, Weihua, Paul Wisniowski, Lasker Ahmed, David W. Boyle, Scott C. Denne, and Edward A. Liechty. Protein anabolic effects of insulin and IGF-I in the ovine fetus. Am J Physiol Endocrinol Metab 284: E748–E756, 2003.—We determined the effect of insulin and/or recombinant human (rh)IGF-I infusion on ovine fetal phenylalanine kinetics, protein synthesis, and phenylalanine accretion. The chronically catheterized fetal lamb model was used at 130 days gestation. All studies were performed while fetal glucose and amino acid concentrations were held constant. Experimental infusates were 1) saline, 2) rhIGF-I plus a replacement dose of insulin (40 nmol), 3) insulin (890 mIU/h), and 4) IGF-I plus insulin (40 nmol IGF-I/h and 890 mIU insulin/h). Both hormones increased glucose and amino acid utilization, with insulin having a greater effect. The major effect on phenylalanine kinetics was a pronounced fall in phenylalanine hydroxylation, again with insulin having the greatest effect. Whole body protein breakdown was not significantly altered by either hormone; whole body protein synthesis was significantly increased during the combined infusion. Protein accretion was increased by both hormones, with the greatest increase during combined infusion. The fractional synthetic rate (FSR) of circulating albumin was increased by IGF-I but not by insulin. Both hormones significantly increased skeletal muscle FSR without a synergistic effect. The anabolic effects of insulin and IGF-I were more pronounced in these studies than in previous studies where amino acid concentrations were not maintained. The present data also suggest that insulin and IGF-I promote fetal growth through distinct, organ-specific mechanisms.

Fetal growth is regulated by a complex interaction between substrate supply and fetal hormonal milieu. Insulin and IGF-I are considered the most important anabolic hormones (23, 26), whereas glucose and amino acids are the substrates whose supply is most crucial for fetal growth (4).

Insulin and IGF-I have both been studied extensively in the fetus. Pharmacological hyperinsulinemia consistently decreases amino acid catabolism and, hence, increases fetal amino acid accretion (36, 41). Protein synthesis is generally enhanced, although the statistical power of several studies has not been adequate to prove this definitively. IGF-I infusion also decreases fetal amino acid catabolism and increases protein synthesis (34). In addition, IGF-I decreases fetal protein breakdown, further enhancing amino acid accretion (8, 35). The role of insulin in decreasing fetal protein breakdown has been inconsistent.

However, although most studies of hyperinsulinemia or IGF-I infusion have controlled glucose concentrations, they have not controlled the circulating concentrations of the other major fetal substrate, amino acids. Insulin infusion generally results in a fall of 20–40% in fetal amino acid concentrations (36, 41). Infusion of IGF-I also causes amino acid concentrations to fall, although to a lesser extent than does insulin (8, 44). In addition, studies of IGF-I infusion are further complicated by a somatostatin-like effect of IGF-I, resulting in 50% declines in circulation insulin concentration (34).

The present study was designed to address these shortcomings in previous experiments. In addition to glucose concentration clamping, amino acid concentrations were clamped in an analogous fashion by variable infusion of an amino acid solution. Furthermore, insulin was supplemented during IGF-I infusion in such a manner that the insulin concentrations would be comparable to those of the controls. The overall hypothesis of the experiment was that under these controlled conditions both insulin and IGF-I would increase fetal protein synthesis and protein accretion and that their actions would be synergistic.

MATERIALS AND METHODS

Animals and surgical procedures. Thirty-five ewes of 115–120 days of gestation were utilized for this study.1 Animal care was in strict compliance with National Institutes of Health guidelines within an American Association for Laboratory Animal Care certified facility, and the study protocols were not maintained. The present data also suggest that insulin and IGF-I promote fetal growth through distinct, organ-specific mechanisms.
were approved by the Institutional Animal Care and Use Committee.

Surgical procedures were performed aseptically while the animals were under general anesthesia. Anesthesia was induced with intravenous ketamine and maintained with isoflurane inhalation. Fetal catheters were placed in the inferior vena cava and abdominal aorta. Catheters were exteriorized and irrigated daily with 0.9% saline containing 50 U heparin/mL. All ewes consumed ad libitum a diet consisting of hay and pelleted alfalfa and had constant access to water and a salt lick.

Study design. The animals were allowed a minimum of 5 days of recovery from operative stress before the study. Complete recovery and fetal health were assessed by monitoring maternal food intake, fetal and maternal glucose concentrations, and acid-base status. The animals were fed ad libitum before and throughout the study.

On the day of the study, baseline blood samples were obtained for amino acid, glucose, and hormone concentration assays. Then, the fetus was infused with one of the following four infusates: 1) saline, 2) recombinant human (rh)IGF-I (gift of Eli Lilly Research Laboratories, Indianapolis, IN) plus a replacement dose of insulin (40 nmol IGF-I/h and 16 mIU insulin/h), 3) insulin (890 mIU/h), and 4) IGF-I plus insulin (40 nmol IGF-I/h and 890 mIU insulin/h). The infusions were advanced into the fetal inferior vena cava and continued for the duration of the 420-min study. The infusion rates of insulin and IGF-I are similar to those of our previous work and have been shown to result in pharmacological concentrations of insulin and IGF-I and have been shown to affect amino acid and protein kinetics in the ovine fetus (8, 19, 34, 36). The dose of insulin infused in study 2 was expected to maintain plasma insulin concentrations at the baseline level, counteracting the inhibition of insulin secretion during the rhIGF-I infusion. In addition, 1-[ring-2H2]phenylalanine (1.2 μmol/min), 1-[ring-2H3]tyrosine (0.12 μmol/min), and 1-[ring-2,6-3H]phenylalanine (0.66 μCi/min) were infused into the fetal inferior vena cava, and 1-[15C]phenylalanine (3.6 μmol/min) was infused into the maternal inferior vena cava throughout the study.

During the hormone infusion, fetal whole blood glucose was clamped at the baseline level by frequent glucose concentration measurement with a glucose analyzer (YSI 2300, Yellow Springs Instrument, Yellow Spring, OH) and a variable infusion of 20% glucose. Likewise, the total fetal plasma branched-chain amino acid (BCAA) concentrations were measured at 15-min intervals (55), and a balanced amino acid solution (AminoSyn II, Abbott Laboratories, Abbott Park, IL) was infused at a variable rate to maintain plasma BCAA concentrations at the baseline level. Blood samples for analysis of metabolic substrates were obtained at 240, 260, 280, 300, 360, 380, 400, and 420 min. After the final blood samples were obtained, the ewe was anesthetized, and fetal quadriceps biopsy samples were taken. Finally, the ewe underwent euthanasia, and fetal size and fetal weight were recorded. All catheter placements were confirmed at autopsy, and fetal autopsy samples were taken. Tissue samples were snap-frozen in liquid nitrogen and stored at −70°C until analysis for specific radioactivity.

Analytical methods. During the hormone infusion, total BCAA concentrations were monitored by spectrophotometry. The fetal plasma phenylalanine concentrations, tracer infusate phenylalanine, and tyrosine concentrations were determined by standard ion exchange chromatography methodology utilizing a Beckman 6300 automated amino acid analyzer (Beckman-Coulter, Palo Alto, CA).

Insulin concentrations in fetal plasma were determined in duplicate by a double-antibody radioimmunoassay using ovine insulin to construct the standard curve (kit no. SRI-13K, Linco Research, St. Charles, MO). Total IGF-I in fetal plasma was determined by a validated competitive RIA that employed formic acid-acetone as the IGF-I extraction procedure (34).

Glucose was analyzed in whole blood by the glucose oxidase method (Boehringer Mannheim kit no. 189197). Intracellular coefficients of variation were obtained at 46%. The whole blood oxygen content was determined in duplicate with an automatic, direct reading by photometer (OSM-3; Radiometer, Copenhagen, Denmark). The within-animal coefficient of variation was 13% for arterial or venous oxygen contents.

Phenylalanine and tyrosine isotopic enrichments were determined in plasma after derivatization to their tertiary butyldimethylsilyl (TBDMs) derivatives (48). These derivatives were injected into a Hewlett-Packard 5970 GC-MS (Hewlett-Packard, Palo Alto, CA) with electron impact ionization and selected ion monitoring of ions. Tracer-to-tracee ratios were calculated by the method of Rosenblatt and Wolfe (46).

Phenylalanine and tyrosine kinetics, including phenylalanine conversion to tyrosine, were estimated by the method of Clark and Bier (10), modified for the fetus as we have previously described (33, 34). Phenylalanine kinetic parameters estimated by this model include rate of appearance (R1), phenylalanine irreversible disposal by hydroxylation, unidirectional flux of phenylalanine from maternal to fetal plasma (F0), phenylalanine used for protein synthesis (PS), and phenylalanine appearance from protein breakdown (PB). Muscle fractional protein synthetic rate was estimated by the ratio of specific activities of protein bound to plasma [3H]phenylalanine. Muscle tissue was obtained from fetal quadriceps at study completion immediately before euthanasia and snap-frozen in liquid nitrogen. Samples were homogenized and protein precipitated. The protein pellet was hydrolyzed, and radioactivity from phenylalanine was isolated by HPLC. Phenylalanine concentration in the hydrolysate was determined by AccQTag HPLC methodology (Waters, Milford, MA). Plasma phenylalanine concentration and tritium radioactivity were measured on a single sample after separation by HPLC. For determining plasma specific activity curves, additional blood samples were taken at 10, 20, 40, 60, and 90 min after beginning the [3H]phenylalanine infusion. These data, in addition to the steady state, were fitted to a two-parameter exponential rise to maximum equation: f = a·(1 − e−b·x), using SigmaPlot (SPSS, Chicago, IL). The area under the curve (AUC) of the resulting fitted equation was then determined. To determine the fractional synthetic rate, the specific activity of the skeletal muscle protein-bound fraction was divided by the plasma AUC. The results are expressed as %/day.

Albumin fractional synthetic rate. Albumin was isolated by differential solubility in trichloracetic acid-absolute alcohol (32, 50). The sample was dried by vacuum distillation and hydrolyzed by adding 1 mL of 4 N HCl into the dried albumin (40). Phenylalanine from albumin was enzymatically converted to phenylethylamine by L-tyrosine decarboxylase (9). TBDMS-phenylethylamine was formed, and the enrichment of TBDMS-[3H2]phenylethylamine was determined by electron impact ionization and selected ion monitoring. The time-dependent slope of the albumin enrichment was determined using SigmaPlot. The fractional synthetic rate of albumin was calculated by dividing the increase (slope) in the enrichment of [3H2]phenylalanine in albumin by the steady-state
Table 1. Fetal weights, hormone concentrations, and physiological parameters during each study condition

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>IGF-I (n = 7)</th>
<th>Insulin (n = 10)</th>
<th>Both (n = 8)</th>
<th>P Ins</th>
<th>P IGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, kg</td>
<td>3.13 ± 0.089c</td>
<td>3.29 ± 0.08c</td>
<td>4.20 ± 0.05x,b,d</td>
<td>3.13 ± 0.078c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBF, ml·kg⁻¹·min⁻¹</td>
<td>170 ± 8d</td>
<td>192 ± 8</td>
<td>193 ± 8d</td>
<td>218 ± 6b</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>0.91 ± 0.03</td>
<td>1.01 ± 0.03c</td>
<td>0.84 ± 0.03b</td>
<td>0.89 ± 0.03</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>22 ± 22</td>
<td>40 ± 27c,d</td>
<td>254 ± 33b,c,d</td>
<td>422 ± 24x,b,c</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td>90 ± 7b,e,c,d</td>
<td>357 ± 9c,e</td>
<td>121 ± 8b,c,d</td>
<td>327 ± 8b</td>
<td>NS</td>
<td>0.001</td>
</tr>
<tr>
<td>O₂ Cons, µM·kg⁻¹·min⁻¹</td>
<td>260 ± 29</td>
<td>329 ± 28d</td>
<td>323 ± 29d</td>
<td>488 ± 23b,c,d</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Glc Util, µM·kg⁻¹·min⁻¹</td>
<td>101 ± 8c,d</td>
<td>154 ± 8</td>
<td>171 ± 8</td>
<td>163 ± 7</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. UBF, umbilical blood flow; O₂ Cons, fetal oxygen consumption; Glc Util, fetal net umbilical glucose uptake; NS, not significant. Statistical evaluation was by 2-way ANOVA, using insulin (Ins) and IGF-I as experimental factors. P values in right-hand columns refer to overall effect of insulin or IGF-I, respectively. Lowercase letters denote post hoc group differences (a, differs from Control; b, differs from IGF-I; c, differs from Insulin; d, differs from Both); P < 0.05, determined by Tukey’s honestly significant difference method.

plasma [H₂]phenylalanine enrichment over the same period (52).

Statistical analysis. The data were analyzed by two-way ANOVA, using insulin and IGF-I as independent factors (JMP, SAS, Cary, NC). All values are expressed as means ± SE. Main effects were taken to be significantly different if the F-test resulted in a P value of <0.05. In all figures, the P values for the main effects and interactions between main effects are given at the top. Tukey’s honestly significant difference (HSD) method was used for post hoc analysis of group differences. In the figures, the results of the post hoc analysis are given by letters a-d, denoting group differences as detected by post hoc analysis.

RESULTS

Fetal weight was measured at the conclusion of each study. Although animals were assigned randomly to a particular infusion group, the animals in the insulin group were larger, averaging 4.2 kg (Table 1). There were no other significant differences between groups in fetal weight.

Fetal whole blood glucose concentrations were not different between groups. Insulin and IGF-I concentrations varied between groups as expected. In particular, there was not a statistically significant difference in insulin concentrations between the control group and the IGF-I infusion group. Therefore, the replacement insulin infusion was sufficient to counteract the somatostatin-like effect of IGF-I. There was a significant difference in insulin concentrations between the insulin infusion and combined infusion groups, even though both groups received the same absolute rate of insulin infusion. The most likely explanation for this is that, as noted above, the fetuses assigned to the insulin group were larger; therefore, the weight-normalized insulin infusion rate was less.

Arterial oxygen concentrations were slightly lower in the combined infusion group; this is likely explained by a significantly higher oxygen consumption in this group. By ANOVA, both insulin and IGF-I significantly affected oxygen concentration and consumption.

Figure 1A shows the glucose concentrations in each group over the time course of the experiment. There were minor, statistically insignificant fluctuations in glucose concentration during the clamp procedure.

Likewise, phenylalanine concentrations are shown in Fig. 1B. There was a tendency for phenylalanine concentrations to rise in the latter time points; this was most pronounced in the insulin group and less so in the control group. It is clear, however, that the amino acid clamp procedure was effective at preventing the usual 25–50% fall in amino acid concentrations during insulin infusion.

Figure 2 depicts the infusion rates of glucose and phenylalanine required to maintain the clamp, reflective of their respective abilities to stimulate glucose and/or amino acid utilization. Both hormones stimu-
lated glucose utilization; insulin had a greater effect, and the combined infusion resulted in a significant increase over insulin infusion alone. Amino acid utilization was stimulated mainly by insulin infusion. There was a small increase in amino acid utilization during IGF-I infusion that did not reach statistical significance. In addition, there was no additional increase in amino acid utilization above insulin infusion alone when both hormones were infused simultaneously.

Phenylalanine kinetics were determined as previously described. Steady state was achieved and maintained throughout the experiment in the primary pool for all isotopes. Ra Phe was slightly but significantly increased during the combined infusion (Fig. 3A). F_m, the proportion of phenylalanine Ra resulting from flux from the maternal to the fetal compartment, was slightly increased during IGF-I infusion and slightly decreased during insulin infusion (Fig. 3B). These effects were balanced during the combined infusion, which was not different from the control. Phenylalanine hydroxylation, reflective of phenylalanine catabolism, was decreased significantly by insulin infusion (Fig. 3C). The percent decrease in hydroxylation during the combined infusion was nearly 50%.

The rates of whole body phenylalanine use for protein synthesis, appearance from protein breakdown, and accretion are shown in Fig. 4. Protein breakdown was least in the insulin group and greatest in the combined group. Protein synthesis changes paralleled the changes in breakdown. Phenylalanine accretion was significantly increased by both hormones. Muscle fractional synthetic rate was stimulated by both hormones; when the hormones were given simultaneously, an intermediate effect, not statistically different from either the control or the IGF-I group, was observed.

DISCUSSION

Insulin and IGF-I are both considered to be important anabolic hormones in the developing fetus. However, previous studies have inconsistently demonstrated an effect of either hormone on protein synthesis or protein breakdown, the two components of net protein accretion. These previous studies have not controlled amino acid concentrations. Hormone-induced declines in amino acid concentrations may have significant impacts on protein synthesis, breakdown, or both. In the present study, both glucose and amino acid concentrations were clamped, whereas insulin, rhIGF-I, or both were infused at pharmacological doses. The results of the present study indicate that, when amino acid concentrations are maintained, whole body protein accretion and muscle and liver protein synthesis rates are stimulated. However, there appear to be mechanistic and perhaps organ specificity differences in the manner in which each hormone affects amino acid kinetics, protein kinetics, and, ultimately, fetal growth.

The use of glucose and amino acid clamping enabled us to compare the ability of each hormone to stimulate the respective substrate utilizations. Clearly, insulin was most effective at stimulating amino acid utilization. The phenylalanine infused to maintain the basal concentration was significantly elevated above the control animals and nearly three times greater than in the animals that received rhIGF-I alone. In addition, there was no additive effect when rhIGF-I was administered with insulin.
A different pattern was observed with regard to glucose utilization. Both hormones stimulated glucose utilization, although insulin caused a twofold greater increase than did IGF-I alone. As opposed to amino acids, there was a synergistic response, with a 25% further increase in glucose utilization when IGF-I was given in conjunction with the insulin. These data would indicate that the hormones interact predominantly with their own receptor, thereby activating unique signaling cascades and different end results.

Different patterns were also seen in whole body amino acid kinetics. Insulin was more effective at decreasing whole body endogenous phenylalanine appearance, whereas IGF-I was more effective at stimulating phenylalanine use for protein synthesis. Insulin was greatly more effective at reducing phenylalanine catabolism. The net result of these changes in kinetics was a stepwise increase in phenylalanine accretion, progressing from IGF-I to insulin to a synergistic combination of the two.

Whole body protein synthesis represents the integrated sum of synthesis in all body tissues (6). There may be organ-specific responses to hormonal infusions that are more pronounced than the whole body response. Therefore, we also examined the fractional synthetic rate of albumin (representative of hepatic protein synthesis) and of skeletal muscle. We chose these tissues as being especially responsive to nutritional and hormonal perturbations, such as seen in clinical intrauterine growth retardation (24) or in infants of diabetic mothers (5, 42, 47).

IGF-I infusion induced a 40% increase in albumin fractional synthesis. To our knowledge, this is the first study to examine the effect of IGF-I on in vivo albumin...
synthesis. Therefore, we do not know whether this effect is species or developmental stage specific. However, this was not an unexpected finding, because fetal liver has been found to be significantly increased in weight after prolonged IGF-I treatment in late-gestation fetal sheep (38). Albumin synthesis during combined infusion of IGF-I and insulin was not different from that with IGF-I alone. Surprisingly, insulin had no apparent effect on albumin synthesis. This finding contrasts significantly with studies, in human volunteers and other species, demonstrating a stimulatory effect of insulin on albumin synthesis (7, 18). Furthermore, insulin has been shown in vitro to increase albumin mRNA transcription (30). Our data may indicate a developmental resistance to insulin stimulation of albumin synthesis. Alternatively, there could be species differences in the regulation of albumin synthesis. For instance, it has been shown in swine that insulin infusion alone actually results in diminished albumin synthesis, whereas albumin synthesis during insulin plus amino acid infusion is not different from control (1). To our knowledge, only one other study has measured ovine albumin synthesis in vivo, in adult sheep, and not in response to insulin (11). It should also be noted that enteral delivery of amino acids appears to be much more effective than parenteral delivery in the stimulation of albumin synthesis (2, 3). The amino acids in the present study were delivered via the parenteral route, which may have decreased their effectiveness. Finally, it should be noted that the albumin fractional synthetic rates found in the present study are approximately twofold greater than those found in previous studies of adult human volunteers. Therefore, fetal albumin synthesis may be at or near maximum under normal conditions and thus incapable of being further increased by hyperinsulinemia.

In contrast to albumin synthesis, both hormones increased fetal skeletal muscle fractional synthetic rate. In fact, the mean increase was greatest for IGF-I, although the difference between IGF-I and insulin alone was not statistically significant. Surprisingly, when both hormones were administered simultaneously, a decreased mean fractional synthetic rate, albeit not statistically significant, was observed.

It could be argued that the lack of an additive effect was due to the pharmacological dose of either hormone saturating its own receptors and cross-reacting with the other hormone’s receptors. We cannot exclude this possibility at the level of individual organs; however, at least at the whole body level, the glucose and amino acid utilization data would seem to exclude this possibility. As discussed above, clear differences were observed between the glucose utilization stimulated by insulin and that by IGF-I; furthermore, an additive effect was observed.

A second explanation may be that distinct signaling pathways regulating protein synthesis exist for each hormone. In addition, the responsiveness of these pathways may be organ specific. In particular, the mammalian target of rapamycin (mTOR) pathway is a likely candidate. It is well accepted that insulin’s signal is transduced via mTOR to stimulate phosphorylation of the translation inhibitor protein eukaryotic initiation factor-binding protein (eIFBP)-1, as well as to activate ribosomal protein p70 S6 kinase (p70S6K). We have previously shown in a companion manuscript that insulin but not IGF-I leads to phosphorylation of both eIFBP-1 and p70S6K in ovine fetal skeletal muscle (49). However, despite this fact, IGF-I did result in increased eIF4F formation, which is thought to be the critical complex for translation initiation (22). Therefore, in fetal skeletal muscle, both hormones IGF-I and p70S6K in ovine fetal skeletal muscle (49).

In fetal liver, only IGF-I enhanced eIF4F formation and only when given alone; when insulin was administered simultaneously, eIF4F content was not different from control. However, as in skeletal muscle, p70S6K phosphorylation was stimulated by insulin, yet albumin synthesis was stimulated by IGF-I alone. This indicates that, at least for albumin and possibly other liver derived proteins, translation initiation through eIF4F is important, possibly more so than gene transcription. In addition, it is likely that mRNA expression of specific insulin-responsive
genes also enhances protein synthesis during prolonged insulin infusion. The results of the present experiments are somewhat different than those of our previous experiments, where insulin or IGF-I were administered singly, but without controlling amino acid concentrations (8, 34, 36, 37). In these previous studies, either hormone reduced amino acid catabolism, although insulin did so with greater effectiveness; consequently, accretion generally increased also. However, in contrast to the present findings, IGF-I did not increase protein synthesis, and insulin did so only in animals that had been fasted for 5 days. Furthermore, protein breakdown was inhibited only by IGF-I, not by insulin. In addition, when rhIGF-I was infused directly into the fetal hind-limb, whole body leucine Ra (reflective of protein breakdown) was reduced, but no effect on hindlimb Ra, rate of disappearance, or amino acid balance was seen (8). Seemingly contradictory findings are observed in the present study, emphasizing the importance of circulat-in amino acid supply in the interpretation of hormone effects.

Other investigators employing other models have also noted that discrepant results are observed when insulin is infused with or without concomitant amino acids. Milley (41) found reduced protein breakdown in fetal lambs only during amino acid infusion. Similarly, amino acid infusions have been shown to suppress proteolysis in newborn human infants (45). Amino acid infusion alone is also able to stimulate ovine fetal protein synthesis (33). Amino acid infusion has also been shown to enhance insulin’s effect to stimulate protein synthesis in both immature (17) and mature animals (54) and in humans (27). Thus, whether the effects of the amino acids are independent of insulin remains to be clearly shown. Furthermore, the mechanisms by which amino acids augment protein synthesis remain to be clearly shown. Additionally, the mechanisms by which amino acids augment protein synthesis remain to be clearly defined. However, increases in ribosomal efficiency and augmentation of substrate supply are each likely to be important.

The present study is supportive of the concept that protein synthesis and its hormonal regulation are under developmental control. Basal and insulin- or IGF-I-stimulated protein synthetic rates in specific tissues and on a whole body level decline as development progresses through fetal life (29), sucking (12, 13, 15, 16), weaning (19, 43, 44), and adult life (20, 21, 28, 39). An extensive series of studies in piglets has documented that this decline is due to a diminished protein synthetic response to insulin and IGF-I. This decline is, at least in part, due to decreased insulin receptor density (51), decreased ribosomal number (14), and decreased activity of the key activators of mRNA translation (31).

There appear to be important species differences. The neonatal pig is extraordinarily sensitive to insulin, exhibiting increases in maximal insulin-stimulated glucose disposal rates four- to fivefold greater than those observed in the ovine fetus (25). Likewise, insulin-induced increases in amino acid disposal are significantly greater in the piglet (53). The pigs in those studies tripled their birth weight in the 19 days between the 7-day and 26-day studies. This rate of growth is significantly greater than would be expected for lambs or human infants. To the extent that insulin sensitivity is necessary for rapid growth, differences in growth rates may partially explain the species differences. Nevertheless, the cross-species data, taken as a whole, support the concept of a developmental decline in insulin-regulated protein synthesis as animals age.

In summary, we have shown that insulin and IGF-I are individually and in combination potent anabolic agents in the late-gestation fetus. However, they appear to exert effects through different mechanisms and with organ specificity. Furthermore, with the exception of net phenylalanine accretion, the effects on amino acid kinetics are not additive and may, in fact, be antagonistic. Further studies examining the signal transduction pathways, including possible crosstalk, in specific organs will be necessary to understand the unique roles that each hormone plays in modulating fetal growth.

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