Differential effects of insulin on peripheral and visceral tissue protein synthesis in neonatal pigs

TERESA A. DAVIS, MARTA L. FIOROTTO, PHILIP R. BECKETT, DOUGLAS G. BURRIN, PETER J. REEDS, DIANE WRAY-CAHEN, AND HANH V. NGUYEN

United States Department of Agriculture, Agricultural Research Service, Children’s Nutrition Research Center, and Endocrinology and Metabolism Section, Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030

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Differential effects of insulin on peripheral and visceral tissue protein synthesis in neonatal pigs. Am J Physiol Endocrinol Metab 280: E770–E779, 2001.—We recently demonstrated in neonatal pigs that, with amino acids and glucose maintained at fasting levels, the stimulation of protein synthesis in longissimus dorsi muscle with feeding can be reproduced by a physiological rise in insulin alone. In the current report, we determine whether the response of protein synthesis to insulin in the neonatal pig is 1) present in muscles of different fiber types, 2) proportional in myofibrillar and sarcoplasmic proteins, 3) associated with increased translational efficiency and ribosome number, and 4) present in other peripheral tissues and in viscera. Hyperinsulinemic-euglycemic-amino acid clamps were performed in 7- and 26-day-old pigs infused with 0, 30, 100, or 1,000 ng·kg^{-0.66}·min^{-1} of insulin to reproduce insulin levels present in fasted, fed, refeed, and supraphysiological conditions, respectively. Tissue protein synthesis was measured using a flooding dose of L-[4-3H]phenylalanine. Insulin increased protein synthesis in gastrocnemius muscle and, to a lesser degree, masseter muscle. The degree of stimulation of protein synthesis by insulin was similar in myofibrillar and sarcoplasmic proteins. Insulin increased translational efficiency but had no effect on ribosome number in muscle. All of these insulin-induced changes in muscle protein synthesis decreased with age. Insulin also stimulated protein synthesis in cardiac muscle and skin but not in liver, intestine, spleen, pancreas, or kidney. The results support the hypothesis that insulin mediates the feeding-induced stimulation of myofibrillar and sarcoplasmic protein synthesis in muscles of different fiber types in the neonate by increasing the efficiency of translation. However, insulin does not appear to be involved in the feeding-induced stimulation of protein synthesis in visceral tissues. Thus different mechanisms regulate the growth of peripheral and visceral tissues in the neonate.

neonate; insulin action; amino acids; protein synthesis; nutrition

THE RATE OF PROTEIN DEPOSITION is more rapid during the neonatal period than at any other stage of postnatal life, and it is driven by high rates of protein synthesis (22, 32). Fractional rates of growth and protein synthesis decline with the advancement of development. During the neonatal period, more rapid gains in protein mass occur in skeletal muscle than in the body as a whole (57). Fractional rates of protein synthesis in skeletal muscle are high immediately after birth and decline more rapidly than in other tissues of the body during the first month of life (13, 18). This decline in skeletal muscle protein synthesis varies with fiber type. Marked changes in the composition of muscle also occur during the neonatal period. The changes include a marked reduction in ribosome number and a specific accumulation of myofibrillar proteins (17, 24).

Protein synthesis in the neonate is maximally stimulated after eating (13, 18, 23). The feeding-induced stimulation of protein synthesis occurs in all measured tissues in the neonatal pig and rat, and the greatest increase occurs in skeletal muscle, particularly in those muscles that contain predominantly fast-twitch muscle fibers (7, 8, 13, 15). This stimulation of skeletal muscle protein synthesis by feeding decreases with developmental age. We have recently shown that the postprandial increase in the efficiency of the translation process in the neonate (13, 18) is due to a marked increase in the activation of translation initiation factors involved in the binding of mRNA to the 43S preinitiation complex (20) and is dependent on activation of a protein kinase termed the mammalian target of rapamycin (mTOR) (38). All of these responses to feeding decrease with development.

Insulin likely plays a key role in the regulation of growth in the neonate. Insulin stimulates the utilization of amino acids for protein deposition in the neonatal pig, and both the sensitivity and responsiveness of amino acid disposal to insulin decline markedly with development (55). This response suggests that the enhanced insulin sensitivity of whole body amino acid disposal may underlie the more efficient use of dietary amino acids for growth in the neonate (19). Studies in the ovine fetus (40) and in growing rats (28) show that insulin stimulates whole body and muscle protein syn-
thesis. However, most, but not all (4), studies in adult humans have shown little, if any, response of muscle protein synthesis to physiological concentrations of insulin (31, 33, 42). These observations are consistent with a decline in the response of muscle protein synthesis to insulin as maturation proceeds.

Experimentally induced systemic hyperinsulinemia, in the absence of amino acid administration, results in a fall in amino acid concentration (39) that is more profound and more rapid the younger the animal (55). A decrease in circulating amino acid levels with insulin infusion could limit the ability of insulin to stimulate protein synthesis. However, in adult humans, maintenance of basal amino acid concentrations, or the use of regional insulin infusions that do not affect plasma amino acid concentrations, has failed to reveal an effect of insulin on either whole body or forearm protein synthesis (27, 42). In contrast, we recently showed in the neonatal pig that the infusion of physiological concentrations of insulin, with amino acids and glucose clamped at fasting levels, increased the rate of protein synthesis in longissimus dorsi muscle, a muscle that contains predominantly fast-twitch fibers (56). This response to insulin decreased with development. However, the stimulation of protein synthesis by insulin in the longissimus dorsi muscle cannot be necessarily extrapolated to the entire musculature. The sensitivity of glucose uptake to insulin varies according to the metabolic and contractile properties of the individual muscles (35). It is possible, therefore, that the protein synthetic response to insulin also differs in those muscles with more oxidative and slower contractile properties than the longissimus dorsi muscle. Additionally, we have shown that the developmental patterns for the fractional rates of synthesis of myofibrillar and sarcoplasmic proteins differ from each other (24); whether this reflects differences in the response of these protein compartments to insulin remains to be established.

Although a number of studies have examined the effects of insulin on protein synthesis in skeletal muscle (28, 31, 33, 42, 56), there are surprisingly few reports of the effects of insulin on protein synthesis in visceral tissues, except in type I diabetes (11, 36, 49). Because whole body protein synthesis rates represent the compensation of basal amino acid concentrations, or the use of regional insulin infusions that do not affect plasma amino acid concentrations, has failed to reveal an effect of insulin on either whole body or forearm protein synthesis (27, 42). In contrast, we recently showed in the neonatal pig that the infusion of physiological concentrations of insulin, with amino acids and glucose clamped at fasting levels, increased the rate of protein synthesis in longissimus dorsi muscle, a muscle that contains predominantly fast-twitch fibers (56). This response to insulin decreased with development. However, the stimulation of protein synthesis by insulin in the longissimus dorsi muscle cannot be necessarily extrapolated to the entire musculature. The sensitivity of glucose uptake to insulin varies according to the metabolic and contractile properties of the individual muscles (35). It is possible, therefore, that the protein synthetic response to insulin also differs in those muscles with more oxidative and slower contractile properties than the longissimus dorsi muscle. Additionally, we have shown that the developmental patterns for the fractional rates of synthesis of myofibrillar and sarcoplasmic proteins differ from each other (24); whether this reflects differences in the response of these protein compartments to insulin remains to be established.

Although a number of studies have examined the effects of insulin on protein synthesis in skeletal muscle (28, 31, 33, 42, 56), there are surprisingly few reports of the effects of insulin on protein synthesis in visceral tissues, except in type I diabetes (11, 36, 49). Because whole body protein synthesis rates represent the compi- lation of the rates of synthesis of protein in all tissues and organs of the body, and rates of protein synthesis in visceral tissues exceed those in peripheral tissues, examination of insulin’s effect on visceral tissues is critical. In the current report, we examined in the neonatal pig whether the stimulation of protein synthesis by insulin, when amino acids and glucose are maintained at fasting levels, 1) varies with fiber type, 2) is specific to myofibrillar proteins, 3) is due to an increase in ribosome number and translational efficiency, and 4) occurs in other peripheral tissues and/or the viscera.

**MATERIALS AND METHODS**

**Animals and surgery.** Multiparous crossbred (Yorkshire × Landrace × Hampshire × Duroc) sows (n = 11; Agriculture Headquarters, Texas Department of Criminal Justice, Huntsville, TX) were housed in lactation crates in individual, environmentally controlled rooms and maintained on a commercial diet (5084, PMI Feeds, Richmond, IN) throughout a 28-day lactation period. After farrowing, piglets remained with the sow and were not given supplemental creep feed. Piglets were studied at 7 (n = 26; 2.2 ± 0.3 kg) and 26 days of age (n = 37; 7.3 ± 0.8 kg). Three to five days before the insulin infusion study, pigs were anesthetized, and catheters were surgically inserted into a jugular vein and a carotid artery as described previously (55). Piglets were returned to the sow until studied. The protocol, previously described by Wray-Cahen et al. (56), was approved by the Animal Care and Use Committee of Baylor College of Medicine. The study was conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

**Hyperinsulinemic-euglycemic-amino acid clamps.** The clamp procedure has been previously described by Wray-Cahen et al. (56). Briefly, pigs were placed in a sling restraint system after a 12-h fast. The average basal concentrations of blood glucose and plasma total branched-chain amino acids (BCAA) to be used in the subsequent euglycemic-amino acid clamp procedure were established during a 30-min basal period. Blood samples (0.2 ml) were acquired every 5 min during the clamp and immediately analyzed for blood glucose (YSI 2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH) and BCAA concentrations (3). Plasma insulin concentrations were elevated via a primed, constant (12 ml/h) infusion of insulin (Eli Lilly, Indianapolis, IN) at 0, 30, 100, or 1,000 ng·kg⁻⁰·⁶⁶·min⁻¹ (21). The infusion rates of dextrose (Baxter Healthcare, Deerfield, IL) and an amino acid mixture, TrophAmine 10% (McCaw, Irvine, CA), were adjusted as necessary to maintain blood glucose and BCAA concentrations within 10% of the average basal concentration for each pig. Blood samples were collected at intervals for measurements of plasma insulin and amino acid concentrations.

**Tissue protein synthesis in vivo.** Fractional rates of protein synthesis were measured with a flooding dose of L-[⁴-H]phenylalanine (29) injected 3.5 h after the start of the clamp procedure. This was 2–2.5 h after the attainment of constant glucose and amino acid infusion rates necessary to maintain stable, fasting blood concentrations. Blood samples for measurement of the specific radioactivity of the extracellular free pool of phenylalanine were taken at 5, 15, and 30 min after the injection. Afterward, pigs were killed, and samples of longissimus dorsi, gastrocnemius, masseter, and cardiac muscles and skin, liver, jejunum, pancreas, kidney, and spleen were collected. Tissue samples were rapidly frozen in liquid nitrogen and stored at −70°C.

Total protein was isolated from all tissues sampled, and sarcoplasmic and myofibrillar proteins were isolated in longissimus dorsi muscle as described previously (17, 24). The specific radioactivities of the protein hydrolysates, homoge-neate supernatants, and blood supernatants were determined (24). Tissue protein synthesis was calculated as

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K_s (\% / day) = \frac{[S_S/S_a] \times (1,440t)}{[S_S/S_a]} \times 100
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where $S_b$ is the specific radioactivity of the protein-bound phenylalanine; $S_a$ is the mean specific radioactivity of the tissue-free phenylalanine for the labeling period determined from the value of the animal at the time of the tissue collection, corrected by the linear regression of the blood specific radioactivity of the animal against time; and $t$ is the time of labeling in minutes. We have demonstrated that, after a flooding dose of phenylalanine is administered, the specific radioactivity of tissue-free phenylalanine is in equilibrium with the aminoacyl-tRNA specific radioactivity, and therefore the tissue-free phenylalanine is a valid measure of the tissue precursor pool specific radioactivity (16).

Analysis of variance for repeated measures (by SPSS) was used to assess the effects of insulin, age, and their interaction on protein synthesis. A nested design with pigs blocked by litter within age was used. When significant interactions were detected, the value at each insulin dose for each age was compared with the basal value by use of *t*-tests. Differences among tissues in the basal rate of protein synthesis and in the response to insulin were also determined using *t*-tests. Results are presented as means ± SE. Probability values of <0.05 were considered statistically significant and are reported in Figs. 1–6.

**RESULTS**

Plasma insulin, amino acid, and glucose concentrations. The insulin infusion rates (0, 30, 100, and 1,000 ng·kg⁻¹·min⁻¹) used during the hyperinsulinemic-euglycemic-amino acid clamps largely reproduced the plasma insulin levels of the fasting state, the fed steady state, the refeeding state immediately after a meal, and supraphysiological administration, respectively (13, 56). Plasma insulin concentrations achieved by the infusion of 0, 30, 100, and 1,000 ng·kg⁻¹·min⁻¹ in 7-day-old pigs were 2 ± 1, 10 ± 1, 26 ± 2, and 891 ± 72 μU/ml, respectively, and in 26-day-old pigs were 3 ± 1, 12 ± 2, 41 ± 7, and 808 ± 110 μU/ml, respectively.

The amino acid clamp procedure maintained the circulating concentrations of the branched-chain and essential amino acids in both 7- and 26-day-old pigs (Fig. 1). However, plasma nonessential amino acid concentrations decreased at the 30, 100, and 1,000 ng insulin·kg⁻¹·min⁻¹ insulin infusion rates in both 7- and 26-day-old pigs ($P < 0.05$). This resulted in a reduction in plasma total amino acids in 7-day-old pigs at the 100 ng insulin·kg⁻¹·min⁻¹ insulin dose and in 26-day-old pigs at the 30, 100, and 1,000 ng insulin·kg⁻¹·min⁻¹ insulin doses ($P < 0.05$). Although nonessential amino acid levels decreased by 35% and total amino acids by 20% at the highest insulin doses, the amino acid clamp procedure prevented the ~60% reduction in plasma branched-chain, essential, nonessential, and total amino acids that we have previously observed during hyperinsulinemic-euglycemic clamps in which amino acids were not infused in neonatal pigs (55). Plasma glucose concentrations were maintained at the basal fasting level during the infusion of insulin in 7- and 26-day-old pigs.

Peripheral tissue protein synthesis. We have previously demonstrated in neonatal pigs that physiological concentrations of insulin stimulate protein synthesis in the longissimus dorsi muscle, a muscle that contains primarily fast-twitch glycolytic muscle fibers, and that this response decreases as the muscle progresses toward maturation (56). To determine whether the response of protein synthesis to insulin varies in muscles of different fiber types and whether this effect changes with development, fractional rates of protein synthesis in gastrocnemius and masseter muscles were determined in 7- and 26-day-old pigs during hyperinsulinemic-euglycemic-amino acid clamps (Fig. 2). Previously reported data (56) in the longissimus dorsi muscle are also shown for comparison (Fig. 2). The fractional rate of protein synthesis in the gastrocnemius, a muscle that is largely fast twitch but more oxidative than the longissimus dorsi, was more than twofold higher in 7-than in 26-day-old pigs in the basal fasting condition, in which plasma insulin concentrations were ~3 μU/ml (0 ng insulin·kg⁻¹·min⁻¹ infusion rate). Insulin increased gastrocnemius muscle protein synthesis in both 7- and 26-day-old pigs ($P < 0.01$), and the increase in protein synthesis was greater in 7- than in 26-day-old pigs ($P < 0.05$). The maximum response of gastrocnemius muscle protein synthesis to insulin was achieved at a plasma insulin level of ~11 μU/ml (30 ng·kg⁻¹·min⁻¹ infusion rate) in both age groups. Supraphysiological concentrations of insulin, i.e., >600 μU/ml (1,000 ng·kg⁻¹·min⁻¹ infusion rate), did not stimulate protein synthesis in gastrocnemius muscle.

The fractional rate of protein synthesis in the masseter muscle, which on average has a slower phenotype than the gastrocnemius and also is oxidative, was only 36% greater in 7- than in 26-day-old pigs in the basal
fasting condition (P < 0.001; Fig. 2). Plasma insulin concentrations of ~11 and 33 μU/ml, but not >600 μU/ml, increased protein synthesis in masseter muscle of 7-day-old pigs (P < 0.01). In 26-day-old pigs, protein synthesis in masseter muscle was increased only at a plasma insulin level of ~11 μU/ml (P < 0.05).

Basal rates of protein synthesis were similar in longissimus dorsi, gastrocnemius, and masseter muscles in 7-day-old pigs, but they were higher in masseter muscle than in longissimus dorsi muscles in 26-day-old pigs (P < 0.05). The stimulation of protein synthesis by insulin was greater in longissimus dorsi than in masseter muscle in both 7- and 26-day-old pigs (P < 0.05).

To determine whether the stimulation of muscle protein synthesis by insulin is specific to myofibrillar proteins, and whether the response changes with development, protein synthesis rates in myofibrillar and sarcoplasmic proteins were determined in longissimus dorsi muscle of pigs infused with 0 and 100 ng·kg⁻¹·min⁻¹ of insulin (~3 and 33 μU/ml of insulin in plasma). Insulin stimulated protein synthesis in total, myofibrillar, and sarcoplasmic protein fractions in both 7- and 26-day-old pigs (P < 0.05; Fig. 3). Rates of protein synthesis did not differ among the three protein fractions at any insulin dose or age.

Ribosome number was estimated from the amount of 18S rRNA per unit protein, and translational efficiency was calculated as the amount of protein synthesized per unit 18S rRNA in longissimus dorsi muscle of 7- and 26-day-old pigs infused with 0 and 100 ng insulin·kg⁻¹·min⁻¹ (Fig. 4). The results showed that the ribosome number in muscle was unaffected by acute changes in plasma insulin concentration at ei-

**Fig. 2.** Fractional protein synthesis (Kₜ) rates in longissimus dorsi, gastrocnemius, and masseter muscles and skin of 7- and 26-day-old pigs during hyperinsulinemic-euglycemic-amino acid clamps. Insulin infusion rates of 0, 30, 100, and 1,000 ng·kg⁻¹·min⁻¹ in 7- and 26-day-old pigs achieved average plasma insulin concentrations of 3, 11, 33, and >600 μU/ml, respectively. Values are means ± SE; n = 6–10 pigs for each age and insulin dose. Protein synthesis values in longissimus dorsi muscle were previously published (56) and are presented here for comparison. Protein synthesis decreased with age in longissimus dorsi, gastrocnemius, and masseter muscles and skin (P < 0.001). Insulin increased protein synthesis in longissimus dorsi, gastrocnemius, and masseter muscles and skin (P < 0.01). There was an interaction of insulin infusion rate and age in longissimus dorsi, gastrocnemius, and masseter muscles (P < 0.05) and skin (P < 0.001). *Significantly different from basal value (P < 0.05); †significantly different from value in 7-day-old pigs (P < 0.05).

**Fig. 3.** Fractional synthesis rates in total (TP), myofibrillar (MP), and sarcoplasmic (SP) proteins in longissimus dorsi muscle of 7- and 26-day-old pigs during hyperinsulinemic-euglycemic-amino acid clamps. Insulin infusion rates of 0 and 100 ng·kg⁻¹·min⁻¹ in 7- and 26-day-old pigs achieved average plasma insulin concentrations of 3 and 33 μU/ml, respectively. Values are means ± SE; n = 6–10 pigs for each age and insulin dose. *Significantly different from basal value (P < 0.05); †significantly different from value in 7-day-old pigs (P < 0.05).

**Fig. 4.** Ribosome number (top) and translational efficiency (bottom) in longissimus dorsi muscle of 7- and 26-day-old pigs during hyperinsulinemic-euglycemic-amino acid clamps. Insulin infusion rates of 0 and 100 ng·kg⁻¹·min⁻¹ in 7- and 26-day-old pigs achieved average plasma insulin concentrations of 3 and 33 μU/ml, respectively. Values are means ± SE; n = 6–10 for each age and insulin dose. *Significantly different from basal value (P < 0.05); †significantly different from value in 7-day-old pigs (P < 0.05).
ner age. However, the ribosome number decreased by 60% between 7 and 26 days of age (P < 0.001). The ratio of 18S rRNA to total RNA was unaffected by insulin infusion or age (data not shown). Translational efficiency was similar in 7- and 26-day-old pigs in the basal fasting condition (Fig. 4). Insulin increased translation efficiency, and this increase was greater in 7- than in 26-day-old pigs (P < 0.05).

Because the skin is a large component of peripheral tissue mass in the neonatal pig, its fractional rate of protein synthesis also was determined in pigs at both ages. The fractional rate of protein synthesis in skin was 50% higher in 7- than in 26-day-old pigs in the basal state (P < 0.001; Fig. 2). The protein synthesis rate in skin was increased at plasma insulin concentrations of ~11 and 33 μU/ml in 7-day-old pigs (P < 0.01). However, in 26-day-old pigs, protein synthesis in skin did not respond to insulin. The protein synthesis rate in skin was higher than in muscle at both 7 and 26 days of age (P < 0.05).

**Visceral tissue protein synthesis.** To determine whether insulin also stimulates protein synthesis in the visceral tissues of the neonate, the fractional rate of protein synthesis was determined in heart, liver, jejunum, pancreas, kidney, and spleen in 7- and 26-day-old pigs during hyperinsulinemic-euglycemic-amino acids clamps. The fractional rate of protein synthesis in the heart did not differ between 7- and 26-day-old pigs in the basal fasting condition (Fig. 5). Infusion of insulin to achieve the plasma concentration observed with refeeding (~33 μU/ml) increased protein synthesis in the heart by 30% at 7 days and 15% at 26 days of age (P < 0.05). Basal protein synthesis rates were similar in skeletal muscle and heart at 7 days of age but were higher in the heart than in skeletal muscle by 26 days of age (P < 0.05). The stimulation of protein synthesis by insulin was greater in the longissimus dorsi muscle than in the heart muscle at 7 days of age (P < 0.05).

The fractional rate of protein synthesis in liver was 33% greater in 7- than in 26-day-old pigs in the basal state (P < 0.001; Fig. 5). Insulin did not stimulate protein synthesis in liver. Moreover, liver protein synthesis was suppressed at plasma insulin concentrations of ~33 and >600 μU/ml in 26-day-old pigs (P < 0.01). The fractional rate of protein synthesis in the jejunum was unaffected by age. Insulin infusion did not stimulate jejunal protein synthesis; jejunal protein synthesis was suppressed at plasma insulin concentrations of ~33 μU/ml in 26-day-old pigs (P < 0.05).

Fractional rates of protein synthesis in the pancreas and kidney were unaffected by age and insulin infusion (Fig. 6). Fractional rates of protein synthesis in the spleen were higher in 7- than in 26-day-old pigs (P < 0.001) and were unaltered by insulin. Fractional rates of protein synthesis were greater in liver, gut, pancreas, spleen, and kidney than in skeletal muscles, skin, and heart in both 7- and 26-day-old pigs (P < 0.05).

**DISCUSSION**

The results of the current study support our previous work, which showed that the infusion of physiological concentrations of insulin, while circulating levels of amino acids and glucose are maintained near fasting levels, stimulates protein synthesis in skeletal muscle of the neonatal pig, a response that decreases with development. Our results further demonstrate that the response to insulin is greater in muscles that are predominantly fast-twitch glycolytic compared with those that are more oxidative and slower to contract, and that the response is not specific to the myofibrillar proteins. This insulin-induced stimulation of muscle protein synthesis is due to an increase in translational efficiency, not an increase in ribosome number. Furthermore, insulin also stimulates protein synthesis in cardiac muscle and skin but not in visceral tissues, including liver, intestine, spleen, pancreas, and kidney. Thus the results are entirely consistent with the hypothesis that, in the neonate, insulin mediates the stimulation of protein synthesis by feeding in peripheral tissues but not in visceral tissues, with the exception of the heart.
Protein synthesis decreased with age in spleen (P 6). Values are means ± SE; n = 6–10 for each age and insulin dose. Protein synthesis decreased with age in spleen (P < 0.001). †Significantly different from value in 7-day-old pigs (P < 0.05).

Insulin stimulates protein synthesis in peripheral tissues. The rapid rate of muscle growth in the neonate is associated with an enhanced stimulation of muscle protein synthesis by feeding. The postprandial increase in protein synthesis has been observed in skeletal muscle of the young rat, lamb, and pig, and in whole body of the newborn human (13, 18, 23, 48, 54) and decreases with development (13, 18). The response is present to a lesser extent, if at all, in adult mammals (2, 44, 52, 53). Thus there appear to be fundamental differences between the mechanisms that regulate muscle protein deposition in the adult and the neonate. In the adult, protein mass is gained and lost equally between the postprandial and postabsorptive periods, and changes in protein synthesis with feeding and fasting are minimal. In the neonate, protein is deposited more rapidly in the fed state than is lost between meals, and thus high postprandial rates of protein synthesis are required.

The results of the current study, together with previous work (14, 28, 56), suggest that the feeding-induced stimulation of skeletal muscle protein synthesis in the neonate is regulated by insulin. Postprandial changes in protein synthesis in neonatal pigs are correlated with changes in circulating insulin concentrations (14). Recently we reported that raising insulin concentrations in neonatal pigs to reproduce the levels in the fed state, while amino acids and glucose were maintained near fasting levels, increased the rate of skeletal muscle protein synthesis to that present in the fed state (56). This response to insulin is attenuated as the pig matures, in parallel with the developmental change in the stimulation of muscle protein synthesis by feeding. The developmental changes in insulin-stimulated protein synthesis in the neonatal pig are consistent both with the ability of insulin to stimulate protein synthesis in whole body of the fetal sheep (40), hindlimb of the young lamb (54), and skeletal muscle of the weaned rat (28) and with the failure of insulin to stimulate protein synthesis in skeletal muscle of the adult rat (2, 43) and forearm of the adult human (31, 33, 42). Collectively, these findings suggest that the response of muscle protein synthesis to insulin declines progressively with development.

In contrast to the stimulatory effect of physiological concentrations of insulin on muscle protein synthesis, supraphysiological concentrations of insulin did not stimulate skeletal muscle protein synthesis in the current study. We postulate that the reduced response to supraphysiological insulin doses was due to a limitation of substrate, i.e., amino acids or nitrogen. During the hyperinsulinemic-euglycemic-amino acid clamps, we infused an amino acid mixture, TrophAmine, which was designed for use in human neonates. Nonetheless, the circulating concentrations of some of the nonessential amino acids, particularly glutamine and glycine, were reduced at the highest insulin concentrations. Future studies will be required to determine whether the maintenance of nonessential amino acids with a modified amino acid mixture will sustain the stimulatory action of insulin on muscle protein synthesis at high insulin doses.

We (17) and others (2, 37) have shown that the response of muscle protein synthesis to anabolic agents is greater in muscles of predominantly fast than in those of the slow fiber type. Because our previous report (56) examined the effect of insulin on the longissimus dorsi muscle, which contains primarily fast-twitch glycolytic fibers, we wished to determine whether insulin-stimulated protein synthesis occurs in other muscles as well. In the current study, we found that insulin, in the presence of near euaminoacidemic and euglycemic conditions, increased protein synthesis in the gastrocnemius muscle, which is comprised of mixed fiber type and is more representative of the musculature as a whole than the longissimus dorsi. Insulin also stimulated protein synthesis in the masseter muscle, which has more slow-twitch, oxidative properties. The response to insulin was greater in the longissimus dorsi than in the masseter muscle and was intermediate in the gastrocnemius muscle, indicating that protein synthesis is more responsive to insulin in fast-twitch muscle fibers in the neonate. Furthermore, the developmental decline in fractional protein synthesis was also greater in the longissimus dorsi than in the masseter muscle and was intermediate in gastrocnemius muscle, consistent with our previously reported finding of a greater age-associated decline in...
protein synthesis in neonatal rat muscles containing more white than red muscle fibers (17).

The results demonstrate that the stimulation of myofibrillar and sarcoplasmic protein synthesis by insulin was proportional. Both proportional changes in myofibrillar and mixed muscle protein synthesis (45) and disproportional changes in myofibrillar and sarcoplasmic protein synthesis (51) have been reported in response to feeding in adult rodents. In newborn pigs, the feeding of milk or formula increases the synthesis of both protein fractions similarly; however, the ingestion of colostrum further stimulates the synthesis of myofibrillar proteins (25). In adult humans, insulin deprivation in type 1 diabetes has no effect on the synthesis of myofibrillar and mixed muscle proteins (12), as might be expected on the basis of previous studies showing that insulin has little effect on total muscle protein synthesis in adulthood (31, 33, 42).

Our results also demonstrated that myofibrillar and sarcoplasmic muscle protein synthesis rates were similar in the neonatal pig and that the age-associated decline in the synthesis rates of the two protein fractions was proportional during this stage of development. In our previous studies, we showed that myofibrillar protein synthesis rates are elevated in newborn rats and that they decline more rapidly than sarcoplasmic protein synthesis rates during early postnatal life until rates in both fractions are similar during the late suckling period (24). The differences between the two studies likely can be explained by the more mature phenotype of the pig than the rat at birth.

The rate of protein synthesis is dependent on both the capacity and the efficiency of the translation process (37). The capacity of the tissue for protein synthesis is determined by the number of ribosomes, and because the majority of RNA in tissues is ribosomal RNA, ribosomal capacity is generally estimated from the ratio of total RNA to total protein. In the current experiment, ribosomal capacity in longissimus dorsi muscle was determined more precisely from the amount of 18S rRNA expressed per unit protein, and the efficiency of the translation process was calculated from the amount of protein synthesized per unit 18S rRNA. Our results show that the stimulation of muscle protein synthesis by insulin in the neonate is due to an increase in translational efficiency, but the overall developmental decrease in protein synthesis is due to a reduction in ribosome number. These results are consistent with previous results of a feeding-induced stimulation of translational efficiency (13, 18) and the age-associated decline in ribosomal abundance in skeletal muscle of neonatal rats and pigs (13, 17, 18). We recently found that the developmental changes in the stimulation of muscle protein synthesis by feeding are regulated by changes in the activation of translation initiation factors that regulate the availability of eukaryotic initiation factor 4E for 48S ribosomal complex formation (20). Furthermore, preventing the formation of this active ribosomal complex by use of an inhibitor of the protein kinase, mTOR, attenuates the postpran-

dial increase in muscle protein synthesis in the neonatal pig (38).

Previous studies have reported rates of protein synthesis to be either higher or similar in skin compared with muscle (1, 5, 41, 47). In the current study, protein synthesis rates in skin were higher than those in skeletal muscle, and skin protein synthesis rates decreased with age, like those in skeletal muscle. Insulin increased protein synthesis in skin of the 7-day-old pig, similar to its effect on muscle. However, there was no effect of insulin on protein synthesis in skin at 26 days of age, an age in which the stimulatory effect of insulin on muscle protein synthesis was waning. Skin accounts for a large proportion of whole body protein synthesis (10–25%), and this proportion varies with stage of development (47). Hindlimb or forearm preparations, used by numerous investigators (31, 33, 42, 54), also contain skin, as well as bone and adipose tissue. Thus these results in skin, as well as those in other tissues (to be described), emphasize the importance of considering the influence of the individual tissues when examining the effects of anabolic or catabolic agents on protein synthesis in the hindlimb and, indeed, the whole body.

Effects of insulin on visceral tissue protein synthesis. Our previous studies showed that feeding increased protein synthesis in all tissues of the neonatal pig (7, 8, 13, 15). Therefore, we wished to determine whether insulin regulates the protein synthetic response to feeding in visceral tissues of the neonate, as it does for skeletal muscle (56). Our results showed that insulin stimulates protein synthesis in cardiac muscle of 7-day-old pigs, although not quite to the level of that in skeletal muscle composed of primarily white fiber type. In contrast to skeletal muscle, there was no effect of age on insulin-stimulated protein synthesis in the heart. In the young diabetic rat, insulin withdrawal reduces protein synthesis in both heart and skeletal muscle (49), but in normal adult animals, insulin infusion has no effect on protein synthesis in either heart or skeletal muscle (43). This suggests that an age-associated decline in insulin-stimulated protein synthesis in the heart may occur postweaning.

Liver protein synthesis rates can be modulated by changes in food intake in both growing and adult animals (7, 10, 13, 15, 45, 50). Studies in diabetic rats and in hepatocytes are suggestive of a role for insulin in the regulation of liver protein synthesis (34, 36). In the current study, however, insulin infusion did not stimulate protein synthesis in the liver, even though amino acids and glucose were maintained near fasting levels. We have previously shown that insulin has no effect on liver protein synthesis in 7-day-old pigs, even when aminocacyl-tRNA is used as the precursor pool for the calculation of protein synthesis rates (16). Thus the lack of stimulation of liver protein synthesis by insulin was not due to treatment-induced alterations in the various precursor pools for protein synthesis. Moreover, in the current study, liver protein synthesis decreased at the highest doses of insulin in the 26-day-old pig. These results indicate that anabolic factors other
than insulin must be responsible for the feeding-induced stimulation of liver protein synthesis in the neonate (10, 13). Because amino acids have been shown to stimulate protein synthesis in perfused liver (26), we speculate that liver protein synthesis may be responsive to the postprandial rise in amino acid concentrations. In addition, the suppression of liver protein synthesis in 26-day-old pigs at the highest insulin doses, when circulating nonessential amino acids were not completely maintained at fasting levels, is consistent with a role of amino acids in the regulation of liver protein synthesis.

Feeding modestly stimulates intestinal protein synthesis in the growing animal (9, 13, 50) but has little effect in the adult human (6). Insulin deprivation in adults with type 1 diabetes modestly reduces mucosal protein synthesis in the small intestine (11), suggesting that insulin may be required for the maintenance of mucosa protein synthesis rates. Our current study demonstrated that protein synthesis in the jejunum of the neonatal pig was unaffected by insulin infusion in the presence of near-fasting levels of amino acids and glucose and did not change with development. This suggests that the feeding-induced stimulation of intestinal protein synthesis in the neonatal pig is not mediated by insulin.

Protein synthesis rates in the pancreas and kidney were constant during the neonatal period. However, rates of protein synthesis in spleen were greater in 7- than in 26-day-old pigs, likely related to the development of immunocompetence during the early neonatal period (30). Although feeding stimulates protein synthesis in the pancreas, spleen, and kidney of the neonatal pig (7, 8, 13, 15), we found that insulin infusion, in the presence of near-fasting levels of glucose and amino acids, did not alter protein synthesis rates in any of these tissues. This suggests that insulin does not mediate the feeding-induced stimulation of protein synthesis in the pancreas, spleen, and kidney of the neonate.

Perspectives. Ethical considerations preclude the measurement of tissue protein synthesis in the human infant; therefore, we utilized the neonatal pig as an animal model because of its similarity to the human infant in anatomy, developmental physiology, and metabolism (46). Our results indicate that a principal mechanism regulating the magnitude of the response of muscle protein synthesis to food intake in the neonate is the magnitude of the response to insulin. The stimulation of muscle protein synthesis by insulin occurs even when amino acids and glucose are maintained near fasting levels. This response to insulin is not specific to muscles that contain primarily white fibers or to myofibrillar proteins, but it is specific to skeletal muscle, cardiac muscle, and skin. The insulin-induced stimulation of muscle protein synthesis is due to an increase in the efficiency of the translational process rather than an increase in the number of ribosomes available for translation. Given our recent results on the regulation of translation initiation by feeding in the neonatal pig (20), it seems likely that insulin may stimulate muscle protein synthesis by increasing the availability of eIF4E for 48S ribosomal complex formation, although this remains to be determined.

Our results further demonstrate that insulin does not stimulate protein synthesis in visceral tissues, despite the maintenance of near-fasting circulating amino acid and glucose concentrations, which suggests that different mechanisms regulate the synthesis of skeletal muscle and visceral tissues in the neonate. Further study is required to determine whether the postprandial rise in amino acids is the anabolic factor that promotes the feeding-induced stimulation of protein synthesis in visceral tissues, and whether the sensitivity of muscle protein synthesis to amino acids, as well as to insulin, contributes to the enhanced growth of muscle in the neonate.

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Present address of D. Wray-Cahen: US FDA/CDRH/OST/DLS/HSB Laboratory of Large Animal Research, MOD2, 8401 Muirkirk Rd., Laurel, MD 20708.

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