Somatotropin-induced protein anabolism in hindquarters and portal-drained viscera of growing pigs

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A PRIMARY GOAL OF EXOGENOUS SOMATOTROPIN (ST) treatment is to increase lean body mass. This is, in part, accomplished by the ST-induced increase in the overall efficiency with which dietary amino acids are used for protein deposition (18, 20). ST administration also decreases blood urea nitrogen concentrations (4, 15, 45, 48) and whole body leucine oxidation (45), suggesting a reduction in amino acid catabolism.

Most research in ST-deficient (2, 19, 37), as well as normal, mature animals and humans (1, 7, 18, 24, 25, 35) suggests that ST treatment increases protein deposition by stimulating whole body and muscle protein synthesis. For example, acute ST infusion in adult humans increases limb protein synthesis (24, 25), although differing results have also been reported (11).

Chronic ST treatment in cattle increases amino acid uptake by the hindquarter (4) and protein synthesis in skeletal muscle (18). Less is known about its effects on protein degradation, particularly at the tissue level. The equivocal findings of the ST-induced effects on protein synthesis and protein degradation in previous studies may be due to different developmental stages of the subject population (i.e., growing vs. mature), species studied, nutrient status of the subject (i.e., fasted or fed), tissues analyzed, and length and/or mode of ST treatment (4, 5, 17, 24, 25, 30, 37, 38, 42, 45).

Recently, studies in our laboratory have suggested that ST treatment for 7 days in young, growing swine enhances metabolic efficiency by minimizing protein loss during fasting and maximizing protein gain during meal absorption (45, 46). These studies demonstrated that ST treatment improves protein balance by maintaining higher rates of whole body protein synthesis in the postabsorptive condition (45). Feeding increases whole body protein synthesis, but the feeding-induced increase in protein synthesis is not significantly greater in ST-treated than in control pigs (46). Whole body protein degradation rates in the fed condition are

growth hormone; protein synthesis; protein degradation; amino acid kinetics; muscle

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reduced with ST treatment, thereby enhancing whole body protein balance. Thus the results suggest that ST treatment attenuates the reduction in whole body protein synthesis that occurs with fasting, further reduces whole body proteolysis that occurs with feeding, and reduces amino acid catabolism in both the postabsorptive and postprandial states. The conservation of amino acids results in an improvement in protein balance and likely contributes to the reduction in circulating amino acid concentrations that we have observed in fully fed ST-treated pigs.

In the current studies, we wished to identify the tissue-specific responses of protein synthesis and protein degradation to 7 days of exogenous ST treatment in fully fed, young, growing swine. Amino acid kinetics were measured in vivo in the hindquarter (HQ) and portal-drained viscera (PDV) with a dual stable isotope tracer/mass transorgan balance technique. Studies were performed in rapidly growing pigs (~25 kg) in which protein intake and ST treatment were rigorously controlled over a 7-day treatment period and during a 6-h isotope infusion study to ensure fully fed and steady-state conditions. The effects of ST treatment on whole body phenylalanine oxidation were reported in a recent paper on ureagenesis (6), but for completeness, oxidation data are presented herein together with data for whole body phenylalanine turnover.

**MATERIALS AND METHODS**

*Animals and dietary intake.* The protocol, previously described by Bush et al. (6), was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals.* Housing and care of the animals conformed to US Department of Agriculture guidelines. Twenty crossbred (Landrace × Yorkshire × Hampshire × Duroc) female pigs were purchased from the Agriculture Headquarters at the Texas Department of Criminal Justice, Huntsville, TX. The pigs were received at the Baylor College of Medicine Animal Facility at 8–10 wk of age, weighing ~10 kg, and were housed in individual cages. During the 2-wk acclimation period, pigs were fed a 24% high-protein dry diet (Producers Cooperative Association, Bryan, TX) at a rate of 6% of body weight per day. Pigs were weighed every other day to calculate feed intake at 6% body weight, thus ensuring that ~90% of ad libitum intake for pigs of this age was consumed. Pigs were offered food in two equal amounts (one-half the total amount of feed, twice daily) each day at 0800 and 1500. Pigs generally consumed all of the food presented to them; any unconsumed food was accounted for when estimating daily food intake and feed efficiency. Water was continuously available. The pigs received ~1,750 kJ·kg⁻¹·day⁻¹ metabolizable energy in the dry matter diet, which consisted of protein (263.5 g/kg from soybean meal), carbohydrate (470.9 g/kg from crude fiber), fat (69.6 g/kg from soybean oil, rice, and corn), vitamins (0.436 g/kg), minerals (17.264 g/kg), ash (76.010 g/kg), and moisture (59.201 g/kg).

*Surgery.* After the 2-wk orientation period, pigs were fasted overnight, and the carotid artery, jugular vein, hepatic portal vein, inferior caudal vena cava (inferior to the renal veins and superior to the common iliac vein), and duodenum were catheterized using sterile techniques and general anesthesia, as previously described (13). The catheters were flushed with 100 U heparin/ml saline and tied off to prevent discharge. Perivascular flow probes (Transonic Systems, Ithaca, NY) were secured around the hepatic portal vein and inferior caudal aorta, adjacent to the vena cava catheter insertion site, for measurement of blood flow. Catheters and flow probes were externalized and enclosed within a pocket of a specialized swine jacket (Lomir Biomedical, Harvard Apparatus, Holliston, MA).

Postoperatively, pigs received intravenous nutrition (a nutrient solution of 100 ml·kg⁻¹·day⁻¹ during the 2- to 3-day recovery period before returning to their normal dietary regimen (dry matter diet). The elemental nutrient solution consisted of glucose (104 g/l), lipid (21 g/l); Intralipid, Baxter Healthcare, Deerfield, IL), a complete amino acid mixture (55 g/l; Ajinomoto, Tokyo, Japan), electrolytes, and trace minerals sufficient to meet or exceed the requirements for young, growing pigs (5, 33, 34, 39). Intravenous antibiotics (enrofloxacin, 2.5–5.0 mg/kg) were administered daily to prevent infection. Intramuscular injections of a mild pain reliever (butorphanol tartate, 0.01 mg/kg) were given 1 day after surgery to reduce the sensation of pain from surgical intervention.

*Experimental design.* Pigs were weight-matched and randomly assigned to either the control (saline, n = 10) or recombinant porcine ST group (n = 10) (Southern Cross Biotech, Australia). The ST was administered at a concentration of 150 µg·kg⁻¹·day⁻¹ for a 7-day period. This dose has been shown to be effective in increasing protein deposition and reducing blood urea nitrogen in domestic animals (4, 9, 26, 45, 46, 48). The dose of ST was divided into two equal daily injections and administered into alternating HQ musculature concurrently with the feeding sessions. Control pigs received equal volume injections of saline. To minimize the confounding effect of differences in feed intake, control pigs were pair-fed to the level of their respective weight-matched ST-treated counterparts during the 7-day treatment period.

*Infusions.* On the morning of infusions, overnight-fasted pigs were given their final injection of ST (150 µg·kg⁻¹·day⁻¹) and secured in a swine hammock (Walter Terry Distributor, Houston, TX). To ensure that pigs were in the fully fed state throughout the infusion period, control and ST-treated pigs were infused intraduodenally for 7 h with the nutrient solution (11 ml·kg⁻¹·h⁻¹; 37.5 µmol·kg⁻¹·h⁻¹, 0.54 g amino acid-kg⁻¹·h⁻¹) beginning 1 h before the onset of the tracer infusion. To estimate CO₂ production rate, a primed (7.5 µmol/kg), continuous (10 µmol·kg⁻¹·h⁻¹) intravenous infusion of NaH¹³CO₃ (Cambridge Isotope Laboratories, Andover, MA) was performed from 0 to 120 min. Arterial whole blood (1.0 ml) and breath samples were obtained at baseline and every 15 min throughout the 2-h NaH¹³CO₃ infusion for analysis of steady-state CO₂ production rate.

To quantify phenylalanine kinetics in the HQ and PDV, a primed (10 µmol/kg), continuous (10 µmol·kg⁻¹·h⁻¹) intravenous infusion of [¹³C]phenylalanine (Cambridge Isotope Laboratories) and a primed (20 µmol/kg), continuous (20 µmol·kg⁻¹·h⁻¹) intraduodenal infusion of [¹²C]phenylalanine (Cambridge Isotope Laboratories) were performed from 120 to 360 min. Volume blood flow rate (ml/min) measurements were obtained by using simultaneous transit-time ultrasound and blood sampling by a dual-channel flowmeter (T206, Transonic Systems).

Arterial portal venous, and vena cava whole blood samples (1.0 ml) were obtained at baseline and every 30 min throughout the 4-h phenylalanine tracer infusions for analysis of steady-state isotopic enrichment of [¹¹C]- and [¹³C]phenylalanine and concentrations of whole blood amino acid, glucose, and CO₂. Arterial blood (1.0 ml) was also taken...
at 0, 240, and 360 min for measurement of plasma urea nitrogen (PUN) and insulin-like growth factor I (IGF-I). Breath samples were obtained at baseline and every 30 min throughout the 7-h infusion study for isotopic enrichment of expired $^{13}$CO$_2$. At the end of the 7-h infusion study, pigs under pentobarbital sodium anesthesia were killed via exsanguination.

**Hormone and substrate concentrations.** Heparinized blood (1.0-ml) samples were obtained and centrifuged at 2,500 g for 15 min at 4°C, and the plasma was stored at −80°C until analyzed for IGF-I and PUN concentrations. Plasma IGF-I concentrations were analyzed in duplicate via two-site immunoradiometric assay with prior extraction (Diagnostic Systems Laboratories, Webster, TX). PUN concentrations were analyzed in duplicate via an end-point colorimetric assay in which urease reacts to generate ammonia, which then reacts with bromophenol blue (Vitros Chemistry Products, Johnson & Johnson Clinical Diagnostics, Rochester, NY). Blood glucose concentrations were rapidly analyzed by a glucose oxidase reaction (YSI 2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH).

Heparinized whole blood (1.0 ml) was obtained for analysis of amino acid concentrations via reverse-phase HPLC, as previously described (14). Briefly, whole blood spiked with methionine sulfone (internal standard) was filtered through a 10,000 molecular weight filter. Phenylalanine and tyrosine concentrations were precolumn-derivatized with phenyl isothiocyanate, separated on a PICO-TAG reverse-phase column (Waters, Milford, MA), and detected on-line by spectrophotometry. Concentrations were calculated with the use of an amino acid standard (Pierce Chemical, Rockford, IL).

**Analysis of tracer enrichment.** Blood and breath samples for $^{13}$CO$_2$ production were analyzed using isotope ratio mass spectrometry (IRMS; ANCA, RoboPrep-G, Europa Instruments, Crewe, UK). Briefly, to estimate $^{13}$CO$_2$ production rate, an aliquot of whole blood (1.0 ml) was placed in a 10-ml vacutainer (Becton Dickinson, Franklin Lakes, NJ) containing 1.0 ml of perchloric acid (10% wt/wt), gently mixed, and placed on ice for 60 s. Room air was filtered via a soda lime filter (Sodasorb, Grace Container Products, Lexington, MA), and detected on-line by spectrophotometry. Concentrations were calculated with the use of an amino acid standard (Pierce Chemical, Rockford, IL).

**Mass spectrometric analysis of whole blood [1-13C]- and [3H$_5$]-phenylalanine was conducted via heptafluorobutyric anhydride derivative (12). Phenylalanine was isolated via cation exchange chromatography (AG-50W resin, Bio-Rad, Hercules, CA). The isotopic enrichment of derivatized [1-13C]- and [3H$_5$]-phenylalanine was determined by negative chemical ionization GC-MS (Hewlett-Packard 5890 Series II GC equipped with a Europa Orchid 20/20 stable isotope analyzer; Hewlett-Packard, Palo Alto, CA) by monitoring the mass-to-charge ratio of ions at 383/384 and 383/388, respectively.

**Calculations.** A schematic model of phenylalanine kinetics across the HQ and PDV is shown in Fig. 1. Calculations for whole body phenylalanine turnover and phenylalanine kinetics across the HQ and PDV are provided in the APPENDIX.

**Statistics.** Individual $t$-tests were performed on the data to detect significant differences among treatment groups for phenylalanine kinetics across the HQ and PDV. ANOVA with repeated measures was used to detect changes with treatment during sampling over the 7-h infusion for hormone and substrate concentrations and isotopic enrichments. Three control pigs and one ST-treated pig died during the treatment period because of complications following surgery; therefore, the resulting sample size was seven for the control group and nine for the ST-treated group. Results are presented as means ± SD. Probability values of $P < 0.05$ were considered statistically significant.

**RESULTS**

**Animal growth.** Body weight did not differ significantly ($P = 0.2$) between ST-treated and control pigs before the treatment period began (17.1 ± 1.6 vs. 15.6 ± 1.7 kg, respectively). At the end of the 7-day treatment period, the body weight of the ST-treated pigs was significantly ($P < 0.05$) greater than that of the control pigs (22.1 ± 2.2 vs. 19.5 ± 2.3 kg, respectively). The weight-scaled average daily gain tended to be higher ($P = 0.1$) in the ST-treated than in the control pigs during the 7-day treatment period (33.4 ± 6.2 vs. 27.2 ± 11.5 g·kg$^{-1}$·day$^{-1}$, respectively). Feed efficiency, as reflected by the gain-to-feed ratio, during the 7-day treatment period was not statistically different ($P = 0.2$) between ST-treated (0.56 ± 0.10 g gain/g intake) and control pigs (0.48 ± 0.18 g gain/g intake).

**Hormone and substrate concentrations.** To verify the effectiveness of ST treatment in growing pigs, circulating concentrations of IGF-I and PUN were determined.
As expected, IGF-I concentration was significantly (P < 0.001) higher (+464%) in the ST-treated group than in controls (352.0 ± 126.2 vs. 62.3 ± 24.1 ng/ml, respectively). There was a significant (P < 0.001) decrease (-46%) in PUN concentrations after 7 days of ST treatment compared with controls (9.7 ± 2.3 vs. 18.2 ± 2.7 mg/dl, respectively). Blood glucose concentrations increased (+25%) significantly (P < 0.04) in the ST-treated group compared with controls (151.4 ± 31.3 vs. 120.2 ± 9.2 mg/dl, respectively).

**Phenylalanine and tyrosine concentrations.** Phenylalanine concentrations were lower in ST-treated than in control pigs in the arterial (-8%), portal venous (-9%), and vena cava (-11%) circulation (Table 1). Tyrosine concentrations were also lower in ST-treated than in control pigs in the arterial (-150%), portal venous (-170%), and vena cava (-176%) circulation.

**Whole body phenylalanine turnover.** Isotope-labeled tracers (i.e., [1-13C]- or [2H5]phenylalanine and NaH13CO3) were utilized to estimate whole body phenylalanine turnover under steady-state conditions. There was no significant difference in whole body phenylalanine flux when calculated with either the [1-13C]- or the [2H5]phenylalanine tracers. Therefore, arterial [1-13C]phenylalanine was used to estimate whole body phenylalanine flux. There was no significant difference (P > 0.1) between the isotopic enrichment of 13CO2 in expired breath and that of 13CO2 in arterial blood in either treatment group during metabolic steady state of NaH13CO3 infusion (75–120 min) (6). Therefore, we used 13CO2 in expired breath to estimate whole body CO2 production rate (see APPENDIX) during metabolic steady state of NaH13CO3 infusion (75–120 min). Isotopic enrichments of 13CO2 in expired breath and [1-13C]phenylalanine in arterial blood during the phenylalanine infusion (270–360 min) plus the CO2 production rate were used to estimate whole body phenylalanine oxidation (see APPENDIX).

ST treatment significantly (P < 0.05) reduced (-10%) whole body phenylalanine flux (Fig. 2). There was a significant (P < 0.01) decrease (-22%) in whole body phenylalanine oxidation with ST treatment vs. control, indicative of a reduction in the loss of carbon from the amino acid pool. ST treatment significantly (P < 0.01) increased (+34%) whole body protein deposition, largely due to a significant (P < 0.05) decrease (-23%) in whole body protein degradation without a change in whole body protein synthesis. The efficiency with which dietary phenylalanine was utilized for protein deposition was significantly (P < 0.01) increased (+35%) with ST treatment (ST, 0.56 ± 0.07 vs. control, 0.36 ± 0.08 μmol·kg⁻¹·h⁻¹ deposited for μmol·kg⁻¹·h⁻¹ intake). Both groups exhibited an increased retention of amino acids, as indicated by higher whole body protein synthesis rates than whole body proteolysis rates, as would be expected in the fed state.

**Phenylalanine kinetics across the HQ.** Isotope-labeled tracers (i.e., [2H5]phenylalanine and NaH13CO3) and caudal aorta blood flow measurements were utilized to estimate phenylalanine kinetics across the HQ during isotopic steady state (Fig. 1). Isotopic steady state was achieved in the artery and vena cava for [2H5]phenylalanine (Fig. 3). Blood flow to the HQ was significantly (P < 0.01) increased (+63%) with ST treatment compared with controls (Table 2). ST treatment increased (P < 0.03) mass balance in the HQ, and thus the utilization of phenylalanine by the HQ was significantly (P < 0.01) increased (+44%). The extraction of phenylalanine by the HQ was similar between treatment groups (ST, 0.17 ± 0.04 vs. control, 0.23 ± 0.03 μmol·kg⁻¹·h⁻¹ uptake for μmol·kg⁻¹·h⁻¹ input). The majority of the phenylalanine extracted by the HQ was retained for protein synthesis and deposition, mediated largely by the significant increase in blood flow, not extraction rate. Although we did not quantify the rate of phenylalanine conversion to tyrosine, the net release of 13CO2 by the HQ was negligible. The rate of utilization of phenylalanine for protein synthesis in the HQ was significantly (P < 0.01) greater (+43%) in the

### Table 1. Phenylalanine and tyrosine concentrations in arterial, portal venous, and vena cava circulation in fed ST-treated and control pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial</th>
<th>Portal Vein</th>
<th>Vena Cava</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>Control</td>
<td>255.8 ± 31.7</td>
<td>295.9 ± 36.1</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>237.7 ± 21.2*</td>
<td>270.1 ± 20.8*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Control</td>
<td>144 ± 25</td>
<td>143 ± 28</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>57 ± 29*</td>
<td>53 ± 27*</td>
</tr>
</tbody>
</table>

Data are means ± SD expressed in μmol/l in control (n = 7) and somatotropin-treated (ST; n = 9) pigs. *Different from corresponding control, P ≤ 0.05.
assumption of proportionality of phenylalanine uptake of the different tracers.

Phenylalanine kinetics in the HQ and PDV of fed ST-treated and control pigs

Table 2. Phenylalanine kinetics in the HQ and PDV of fed ST-treated and control pigs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood flow</td>
<td>1.4 ± 0.1</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>Mass balance</td>
<td>36.3 ± 15.4</td>
<td>77.6 ± 33.1*</td>
</tr>
<tr>
<td>Utilization</td>
<td>86.2 ± 10.9</td>
<td>113.0 ± 35.0*</td>
</tr>
<tr>
<td>Oxidation</td>
<td>0.3 ± 0.5</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Synthesis</td>
<td>85.9 ± 10.9</td>
<td>112.8 ± 11.8*</td>
</tr>
<tr>
<td>Proteolysis</td>
<td>49.9 ± 24.4</td>
<td>37.2 ± 9.9</td>
</tr>
<tr>
<td>Deposition</td>
<td>35.9 ± 15.2</td>
<td>75.6 ± 32.1*</td>
</tr>
<tr>
<td>PDV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood flow</td>
<td>2.4 ± 0.4</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>Mass balance</td>
<td>99.1 ± 54.1</td>
<td>76.4 ± 56.1</td>
</tr>
<tr>
<td>Total utilization (enteral and arterial)†</td>
<td>130.3 ± 14.6</td>
<td>167.8 ± 23.6*</td>
</tr>
<tr>
<td>Arterial input of phenylalanine</td>
<td>620.4 ± 161.6</td>
<td>595.9 ± 108.7</td>
</tr>
<tr>
<td>Uptake from arterial source</td>
<td>63.7 ± 23.6</td>
<td>64.3 ± 35.5</td>
</tr>
<tr>
<td>Enteral input of phenylalanine</td>
<td>205.8 ± 0.0</td>
<td>205.8 ± 0.0</td>
</tr>
<tr>
<td>Uptake from enteral source</td>
<td>66.1 ± 43.6</td>
<td>103.5 ± 46.5</td>
</tr>
<tr>
<td>Absorption (from enteral source)‡</td>
<td>145.0 ± 39.7</td>
<td>111.4 ± 42.4</td>
</tr>
<tr>
<td>Oxidation</td>
<td>59.1 ± 15.9</td>
<td>53.9 ± 8.9</td>
</tr>
<tr>
<td>Synthesis</td>
<td>71.2 ± 42.6</td>
<td>113.9 ± 22.7*</td>
</tr>
<tr>
<td>Proteolysis</td>
<td>21.2 ± 12.1</td>
<td>29.3 ± 5.0</td>
</tr>
<tr>
<td>Deposition</td>
<td>46.8 ± 23.1</td>
<td>92.7 ± 11.4</td>
</tr>
</tbody>
</table>

Data are means ± SD in control (n = 7) and ST-treated (n = 9) pigs. Blood flow is in l·kg⁻¹·h⁻¹, and phenylalanine kinetics are in μmol·kg⁻¹·h⁻¹. HQ, hindquarters. *P < 0.05, different from corresponding control. †Total utilization of phenylalanine by the portal-drained viscera (PDV) is uptake of phenylalanine by combined arterial and enteral sources. ‡Absorption of enterally infused [1-13C]phenylalanine across the PDV was corrected for recycling of phenylalanine by use of the fractional uptake of the [2H5]phenylalanine tracer to the PDV, with assumption of proportionality of phenylalanine uptake of the different tracers.
Total utilization of phenylalanine, as reflected by combined enteral and arterial influx, by the PDV was increased (+23%) by ST treatment \((P < 0.05)\). In both treatment groups, \(\sim 42\%\) of total phenylalanine uptake was oxidized in the PDV and \(58\%\) was used for protein synthesis. This estimate represents total phenylalanine oxidation by the PDV, because we were not able to differentiate phenylalanine oxidation derived from arterial vs. luminal input. ST increased (+41%) the amount of phenylalanine utilized for protein synthesis by the PDV \((P < 0.02)\). Release of phenylalanine from protein degradation in the PDV was unaffected by ST treatment. Because protein synthesis rates were higher than protein degradation rates in all pigs, there was a positive net protein balance and, hence, dietary phenylalanine was deposited as protein in the PDV. The increased utilization of phenylalanine for protein synthesis, and therefore protein deposition, in the PDV of ST-treated pigs may have contributed to the significantly \((P < 0.02)\) increased (+21%) small intestinal weight-to-length ratio observed in ST-treated pigs \((ST, 0.70 \pm 0.11 \text{ vs. control, } 0.58 \pm 0.05 \text{ g weight/cm length})\), despite no significant difference in small intestinal mass per kilogram body weight with ST treatment \((ST, 37.7 \pm 3.5 \text{ vs. control, } 36.1 \pm 3.2 \text{ g weight/kg body wt})\).

**DISCUSSION**

The results of this study indicate that 7 days of exogenous ST administration in growing pigs enhanced protein anabolism in the fed state by altering protein turnover in the whole body and amino acid kinetics in the HQ and PDV. ST treatment in growing pigs reduced whole body phenylalanine flux, oxidation, and protein degradation without altering whole body protein synthesis. In the HQ and PDV, ST treatment increased protein deposition by increasing the utilization of phenylalanine for protein synthesis, but it had no effect on protein degradation or oxidation rates. Given the fact that protein synthesis rates exceeded proteolysis rates, a positive net protein balance was attained in both treatment groups at both the whole body and tissue-bed level in young, growing swine in the fed state.

**Whole body protein turnover effects of ST.** In the current study, ST treatment for 7 days in growing swine elicited the metabolic responses that are characteristic of ST treatment in domestic animals. ST treatment increased body weight and improved the efficiency \((+35\%)\) with which dietary phenylalanine was utilized for whole body protein deposition and growth, as demonstrated in numerous studies \((7, 9, 20, 21, 38, 45, 47)\). ST treatment also produced the characteristic stimulation of the somatotropic axis \((4, 9, 19, 45, 47, 49)\), as indicated by a fivefold elevation in IGF-I concentration, and a diabeticogenic response \((10, 16, 46, 48)\), as indicated by the rise in plasma glucose concentration.

ST administration reduced PUN concentration, indicating that ST administration reduced amino acid catabolism in the fed state. Our previous studies showed that the decrease in PUN is associated with a reduction in liver urea cycle enzyme activity, ureagenesis, leucine oxidation, and thus amino acid catabolism \((6, 45)\). The reduction in amino acid concentration in the systemic, PDV, and HQ circulation and the decrease in whole body phenylalanine oxidation in the current and previous \((6, 40, 41)\) studies are consistent with a reduction in substrate availability for both urea production and amino acid oxidation. The reduction in amino acid concentration with ST treatment suggests that ST induced an increase in net removal of amino acids from the plasma pool, a reduction in net release of amino acids from body protein into the plasma, or an increase in net absorption of dietary phenylalanine. In fact, all three are suggested from whole body and tissue kinetic data in ST-treated pigs. Together, the results demonstrate that ST treatment in a growing animal results in a more efficient use of dietary amino acids for growth.

Our current findings of the effects of ST on whole body accretion rates and amino acid oxidation are in accord with the majority of previous studies in animals and humans \((4, 6, 17, 24, 25, 29, 37, 38, 45, 46)\). However, less is known about the specific effects of ST on whole body proteolysis rates in the postabsorptive and postprandial states. In fact, conflicting results of an increase \((42)\), decrease \((45)\), and/or no change \((24, 29)\) in whole body proteolysis rates have been reported with ST treatment in either postabsorptive \((24, 29)\) or postprandial states \((42, 45)\). In the current study with phenylalanine tracers, as in our previous study using a leucine tracer \((45)\), we found that ST treatment in fed growing swine reduced whole body proteolysis.

**Evaluation of the dual stable isotope tracer/mass transorgan balance technique.** Several assumptions were made with this study design. First, we assumed that the metabolism throughout the body of the \([1-^{13}C]\)- and \([^{2H}_5]\)phenylalanine tracers would not be different simply because of their isotopic label \(i.e., \text{no isotope effect})\). Second, we have assumed that all \([1-^{13}C]\)phenylalanine taken up by the tissues \(\text{either HQ or PDV}\) and not oxidized to CO2 was incorporated into protein. A consequence of this assumption was that we might have underestimated the rate of irreversible loss of phenylalanine, since the rate of conversion to tyrosine was not quantified.

**Amino acid kinetics in the HQ.** A major objective of this study was to identify the responses of protein synthesis and degradation in the HQ to 7 days of exogenous ST administration. In the process of measuring the phenylalanine kinetics by the HQ, we observed that ST markedly increased blood flow to the HQ by \(80\\%). The substantial increase in blood flow contributed to the increase in the amount of phenylalanine utilized by the HQ in ST-treated pigs. This finding is consistent with previous studies and is believed to be a function of local tissue metabolism \((4, 16, 22, 24, 25, 33, 45)\). The localized increase in blood flow to the HQ may be mediated also by the vasodilator properties of IGF-I \((28)\), which can stimulate the pro-
duction of endothelial nitric oxide and induce endothelium-dependent vasodilation (3, 23, 28).

The phenylalanine uptake by the HQ was partitioned largely for protein synthesis in both control and ST-treated pigs. There are potentially three main metabolic fates of phenylalanine within the HQ: 1) incorporation into protein, 2) conversion to tyrosine, and 3) further metabolism of tyrosine to CO₂. Based on evidence of the absence of phenylalanine hydroxylase in muscle tissue, it is generally assumed that oxidation of phenylalanine does not occur in muscle tissue. We observed in our study no significant net release of ¹³CO₂ by HQ, thus supporting the assumption that oxidation of phenylalanine is negligible by the tissues of the HQ in vivo. This finding is similar to results obtained by Harris et al. (27), in that they observed no net release of ¹⁴CO₂ across the HQ and no venous accumulation of p-hydroxy-phenylpyruvate formed during the hydroxylation of phenylalanine to tyrosine. As a result of our observation in this respect, we have assumed that all [¹³C]phenylalanine taken up by the HQ is incorporated into protein, because the rate of conversion to tyrosine was not quantified. Thus the marked increase in phenylalanine utilization in response to ST was used for protein synthesis. This increase in phenylalanine utilization was due largely to increased blood flow (+63%) to the HQ with ST treatment and not to an increase in the extraction of phenylalanine by the HQ. Moreover, nearly 47% of whole body protein synthesis in ST-treated pigs was directed toward enhancing protein synthesis in the HQ. Several studies in both the postabsorptive (18, 24, 25, 38) and postprandial states (4, 46) have observed similar ST-induced increases in muscle protein synthesis in the mature, adult human (24, 25) and growing intestines (6).

Amino acid kinetics across the PDV. A secondary objective of this study was to compare amino acid kinetics in the HQ with those in the PDV. To determine amino acid metabolism in the PDV, we infused two separate isotope tracers of phenylalanine via the intraduodenal ([¹³C]phenylalanine) and intravenous ([²H₃]phenylalanine) routes. We did this because the intraduodenally infused [¹³C]phenylalanine tracer, once absorbed through the small intestine, is recycled back to the PDV through the systemic circulation. In contrast to the results in the HQ, blood flow in the hepatic portal vein was not significantly altered by ST treatment. After accounting for recycling of the phenylalanine to the PDV through the systemic circulation, total utilization, as reflected by the combined enteral and arterial sources of amino acid during metabolic steady state, was significantly (P < 0.05) greater (+22%) in ST-treated pigs than in controls. Of the enteral phenylalanine (206 μmol·kg⁻¹·h⁻¹) provided to the mucosa, −33–45% (control and ST, respectively) was utilized for metabolic processes within the mucosa (i.e., protein synthesis or oxidation), with no difference between treatment groups. However, there was a trend (P = 0.1) for an increase (+35%) in utilization of phenylalanine from enteral sources with GH treatment. Of the arterial phenylalanine available to the PDV, only a small percentage was extracted (~10%) by the PDV, regardless of treatment. However, a significantly (P < 0.01) greater percentage of enteral (~40%) vs. arterial (~10%) input of phenylalanine was utilized by the mucosa for metabolic processes, thus implying a preferential use of dietary phenylalanine by the gut. This may be a consequence of the transport capacity for amino acids on the basolateral vs. brush border (apical membrane) of mucosal epithelium, although in absolute terms, the amount of phenylalanine utilized by the PDV from the arterial and enteral input was similar.

Interestingly, ~40% of the total uptake of phenylalanine (arterial and enteral sources) by the mucosa was oxidized in control and ST-treated pigs. Oxidation of essential amino acids by the PDV has been reported in studies with young animals (i.e., lysine, leucine) (43, 44, 50, 51). Phenylalanine oxidation by the PDV accounted for nearly 40% of whole body phenylalanine oxidation in the current study. The values reported herein (~40% of whole body oxidation) are slightly higher than those reported for lysine (~30%; Ref. 43) and slightly lower than (~50%; Ref. 42) or equal to (~40%; Ref. 50) those reported for leucine. This raises further questions about the irreversible loss of phenylalanine. These values for phenylalanine oxidation in the present study may be underestimated, because the first irreversible loss of phenylalanine (i.e., its conversion to tyrosine) was not measured in this study. To our knowledge, this is the first report in the literature that the small intestine oxidizes phenylalanine to any significant degree in vivo. However, ST treatment had no effect on phenylalanine oxidation by the PDV, consistent with our previously reported finding of a lack of effect of ST treatment on urea cycle enzymes in the intestines (6).

Approximately 58–66% of the total phenylalanine extracted by the PDV was utilized for protein synthesis, with a significantly (P < 0.02) greater (+66%) rate of protein synthesis observed with ST treatment. Similar to the amino acid metabolism in the HQ, ST treatment did not affect protein degradation rates. Regardless of treatment and as observed in the HQ, a positive net protein balance was observed in the PDV, owing to higher protein synthesis vs. protein degradation rates. The increased utilization of phenylalanine for protein synthesis, and therefore protein deposition, in the PDV of ST-treated pigs may have contributed to the significantly increased small intestinal weight-to-length ratio observed with ST administration. Study findings suggesting increased amino acid utilization by the intestine (5) and an increased intestinal protein synthesis rate (18) in steers chronically treated with ST are consistent with those of our current study. The in-
creased mucosal weight-to-length ratio is consistent with previous studies indicating that ST has the capacity to stimulate enterocyte growth and differentiation in rats (39) and humans (8).

**Perspectives.** Few in vivo studies have been performed to estimate amino acid kinetics in different areas of the body by use of the dual stable isotope tracer/mass transorgan balance technique that was utilized in this current study. The technique allowed us to investigate the tissue-specific metabolism of an individual essential amino acid in the fed state in pigs administered ST for 7 days. On the whole body level, we observed a decrease in total phenylalanine flux, oxidation, and protein degradation with ST treatment that was similar to our previous results with a leucine tracer (45). However, at the individual tissue level, these observations were somewhat different. The results show that ST increased the utilization of phenylalanine for protein synthesis by the HQ and PDV. The increase in amino acid utilization and protein synthesis in the HQ, but not in the PDV, was mediated largely by the regional increase in blood flow. Interestingly, recent studies have shown that adipose tissue protein synthesis is highly responsive to nutrient stimulation (31, 32). Because ST induces a repartitioning of nutrients away from adipose tissue and toward lean tissue deposition (19), we postulate that the lack of ST-induced suppression of the feeding-induced stimulation of protein synthesis in adipose tissue. However, other mechanisms may be involved. We further found that ST treatment had no effect on protein degradation in either the HQ or PDV. Whether the reduction in whole body degradation with ST treatment can be accounted for by a reduction in proteolysis in liver and other visceral tissues remains to be determined. Phenylalanine was not oxidized by the HQ and thus was unaffected by ST treatment, and although a significant portion of phenylalanine utilized by the mucosa was oxidized, this was unaffected by ST treatment. Because ST treatment does not alter urea cycle enzyme activity in the intestine but reduces urea cycle activity in the liver (6), we postulate that the reduction in whole body phenylalanine oxidation is due largely to a reduction in phenylalanine oxidation by the liver.

Thus the fact that the results in the PDV and HQ differ from those in the whole body with regard to the effect of ST treatment on protein synthesis, degradation, and amino acid oxidation suggests that ST must have affected these processes in other tissues of the body differently. This suggests that the effects of ST treatment on the processes that regulate protein turnover, similar to those that regulate urea production (6), are tissue specific. Furthermore, because protein metabolism by the HQ represents that of individual skeletal muscles, skin, bone, and adipose tissue and that by the PDV includes the small intestine, large intestine, stomach, spleen, and pancreas, the role of these individual tissues in the observed responses to ST in the two tissue beds requires further study.

**APPENDIX**

**Whole body protein turnover.** Whole body phenylalanine kinetics were determined by the following standardized equations

\[
\text{total flux } Q (\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = \frac{(E_i \cdot [\text{1-13C}]\text{Phe}/E_p \cdot [\text{1-13C}]\text{Phe}) - 1} {\text{IR} \cdot [\text{1-13C}]\text{Phe}} \tag{A1}
\]

phenylalanine oxidation (\(\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[
\frac{(\text{CO}_2 \text{ production rate} \times E_i \cdot [\text{13CO}_2])}{(E_p \cdot [\text{1-13C}]\text{Phe})} \tag{A2}
\]

\[
\text{CO}_2 \text{ production rate} (\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = \left[ (E_i \cdot \text{NaH}^{13}\text{CO}_3/E_p \cdot [\text{13CO}_2] - 1) \times \text{IR} \cdot \text{NaH}^{13}\text{CO}_3 \right] \tag{A3}
\]

\[
Q = \text{intake} + \text{degradation} = \text{synthesis} + \text{oxidation} \tag{A4}
\]

where \(E_i\) is the isotopic enrichment of the infusate, \(E_p\) is the plasma isotopic enrichment during metabolic steady state of the phenylalanine infusion (270–360 min), \(E_p\) is the \(\text{13CO}_2\) isotopic enrichment in expired breath during metabolic steady state of the \(\text{NaH}^{13}\text{CO}_3\) infusion (75–120 min), IR is the infusion rate, and \(Q\) is the total flux of phenylalanine. Intake is a function of the enteral nutrient solution plus phenylalanine infusion rate. Degradation is equal to total flux minus intake. Synthesis is equal to total flux minus oxidation. Deposition is the difference in degradation and synthesis rates. Total flux was calculated using the arterial isotopic enrichment of \([1-13\text{C}]\text{phenylalanine tracer}\) (Eq. A1), with no significant difference observed in the flux when calculated using the arterial isotopic enrichment of \([^{2}\text{H}_5]\)-phenylalanine tracer.

**HQ phenylalanine kinetics.** Phenylalanine kinetics in the HQ were determined via incorporation of an arteriovenous phenylalanine tracer (Eq. A5). By calculating using the arterial isotopic enrichment of \([1-13\text{C}]\)phenylalanine tracer (Eq. A1), with no significant difference observed in the flux when calculated using the arterial isotopic enrichment of \([^{2}\text{H}_5]\)-phenylalanine tracer.

\[
\text{hindquarter mass balance} (\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) = ([C_A - C_V] \times BF_H) \tag{A5}
\]

utilization of phenylalanine (\(\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[
\frac{(C_A \times E_{A3}) - (C_V \times E_{V2}) \times BF_H} {(C_A \times E_{A2}) \times BF_H} \times BF_H \tag{A6}
\]

phenylalanine oxidation (\(\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[
\frac{([C_A \times E_{A3}) - (C_V \times E_{V2}) \times BF_H]}{([C_A \times E_{A2}) - (C_V \times E_{V2})]} \times BF_H \times BF_H \tag{A7}
\]

phenylalanine used for protein synthesis (\(\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[\text{Eq. A6} - \text{Eq. A7}\] (A8)

phenylalanine release from protein degradation (\(\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\))

\[\text{Eq. A8} - \text{Eq. A9}\] (A9)

net protein balance (protein deposition)

\[\text{Eq. A8} - \text{Eq. A9}\] (A10)

where \(C_A\) is arterial phenylalanine concentration, \(C_V\) is phenylalanine concentration in the vena cava, \(C_{A3}\) is arterial \(\text{CO}_2\) gas concentration, \(C_{V2}\) is \(\text{CO}_2\) gas concentration in the vena cava, \(E_{A3}\) is isotopic enrichment of arterial \(\text{13CO}_2\), \(E_{V2}\)
is isotopic enrichment of venous $^{13}$CO$_2$. $E_{A2}$ is isotopic enrichment of arterial $[^{2}\text{H}_5]$phenylalanine, $E_{V2}$ is isotopic enrichment of vena cava $[^{2}\text{H}_5]$phenylalanine, and BF$_t$ is blood flow in the caudal aorta.

**PDV phenylalanine kinetics.** Phenylalanine kinetics across the PDV were determined via incorporation of an arterio-venous difference calculation of the intravenous $[^{2}\text{H}_5]$-phenylalanine and enteral $[1-^{13}\text{C}]$phenylalanine tracers into the equations to follow. For estimations of PDV phenylalanine kinetics across the PDV, a $[1-^{13}\text{C}]$phenylalanine tracer was infused enterally. The potential for recycling of the enteral $[1-^{13}\text{C}]$phenylalanine tracer in PDV from arterial sources exists during steady-state conditions; thus the PDV phenylalanine kinetics were corrected for the recycling of this tracer. We estimated the percentage of $[^{2}\text{H}_5]$phenylalanine tracer utilization across the PDV to correct for the recycling of the enteral $[1-^{13}\text{C}]$phenylalanine tracer reentering the PDV through the arterial source, assuming that the kinetics of the two different isotope-labeled tracers of phenylalanine would be similar.

**PDV mass balance (μmol·kg$^{-1}$·h$^{-1}$)**

$$\text{PDV mass balance} = [(C_A - C_V) \times BF_p] \quad (A11)$$

utilization of phenylalanine

$$(\text{enteral and arterial sources}) = IR_0 \times \text{corrected uptake of arterial}[1-^{13}\text{C}]\text{phenylalanine} \quad (A12)$$

corrected uptake of arterial $[1-^{13}\text{C}]$ phenylalanine

$$= (\text{arterial}[1-^{13}\text{C}]\text{Phe uptake by PDV}) + [(C_p \times EP_p - C_A \times E_{A3} \times BF_p)]/IR_p \quad (A13)$$

where the uptake of arterial $[1-^{13}\text{C}]$phenylalanine by PDV is corrected for arterial recycling of the $[1-^{13}\text{C}]$phenylalanine tracer by using the percent uptake of the $[^{2}\text{H}_5]$phenylalanine tracer by the PDV.

**arterial $[1-^{13}\text{C}]$phenylalanine uptake by PDV**

$$\text{(μmol·kg}^{-1} \cdot \text{h}^{-1}) = (C_A \times E_{A1} \times BF_p) \quad (A14)$$

$$\times [(E_{A1} - E_{P1})/(C_A \times E_{A1} \times BF_p)]$$

**enteral utilization of phenylalanine by PDV**

$$\text{(μmol·kg}^{-1} \cdot \text{h}^{-1}) = (IR_0 + IR_p) \times \text{Eq. A13} \quad (A15)$$

**absorption of phenylalanine (μmol·kg$^{-1}$·h$^{-1}$)**

$$= IR_0 \times \text{Eq. A13} \quad (A16)$$

where absorption of phenylalanine is defined as the remaining phenylalanine not utilized by the PDV and thus absorbed into arterial sources, becoming available for utilization by the remainder of the body.

**phenylalanine oxidation (μmol·kg$^{-1}$·h$^{-1}$)**

$$=[((C_{A1} \times E_{A1}) - (C_{P1} \times E_{P1}) \times BF_p)/\text{IR}_p$$

$$- (C_p \times E_{P2} - C_A \times E_{A2} \times BF_p)] \times (\text{external and arterial trace uptake}) \quad (A17)$$

**enteral and arterial trace uptake**

$$= [100 - \text{corrected enteral absorption/IR}_p] \times (\text{IR}_0 + \text{IR}_P) \quad (A18)$$

**corrected (for $[1-^{13}\text{C}]$phenylalanine recycling) enteral absorption**

$$= \left(\frac{[(C_A \times E_{A2}) - (C_p \times E_{P2}) \times BF_p]}{(C_A \times E_{A2} \times BF_p) \times (C_A \times E_{A3} \times BF_p)} + [C_p \times E_{P3} - (C_A \times E_{A3} \times BF_p)]\right) \quad (A19)$$

**phenylalanine used for protein synthesis (μmol·kg$^{-1}$·h$^{-1}$)**

$$= \text{Eq. A12} - \text{Eq. A17} \quad (A20)$$

**phenylalanine release from protein degradation (μmol·kg$^{-1}$·h$^{-1}$)**

$$= \text{Eq. A20} - \text{Eq. A21} \quad (A21)$$

**net protein balance (protein deposition)**

$$= \text{Eq. A20} - \text{Eq. A21} \quad (A22)$$

where $IR_0$ is the infusion rate of the enteral nutrient solution, $IR_p$ is the infusion rate of enteral $[1-^{13}\text{C}]$phenylalanine, $C_A$ is arterial phenylalanine concentration, $C_{P1}$ is phenylalanine concentration in the portal vein, $E_{A1}$ is isotopic enrichment of arterial $[^{13}\text{C}]$phenylalanine, $E_{P1}$ is isotopic enrichment of portal venous $[^{13}\text{C}]$CO$_2$, $E_{A2}$ is isotopic enrichment of arterial $[^{2}\text{H}_5]$phenylalanine, and BF$_t$ is blood flow in the portal vein.

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