Rates of Lysine Catabolism Are Inversely Related to Rates of Protein Synthesis When Measured Concurrently in Adult Female Rats Induced to Grow at Different Rates1,2,3

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ABSTRACT To test the effect of changes in the rate of protein synthesis on amino acid oxidation, both were studied concurrently in individual 200-g female Sprague-Dawley rats. In a growth trial (Experiment 1), recombinant bovine somatotropin (rbST) was injected subcutaneously (0, 2 or 12 mg/d) over 6 d (n = 4 rats per rbST level). Weight gain increased with rbST level (P < 0.01); 1.96 ± 0.8, 4.24 ± 0.8 and 8.67 ± 0.8 g/d, respectively. After treatment with rbST (0 or 12 mg/d) for 4 d (Experiment 2), rats were injected via a tail vein catheter with valine (400 mmol; 4.07 mBq L-3,4(n)-3H(valine)) at 0, 4, 10, 13 or 16 h after the daily rbST injection and killed 20 min later. This flooding dose was 5 to 6 times, not 10 times, the free pool as hoped. Protein synthesis in rbST-treated rats increased 46% in muscle (P < 0.001) and 36% in liver (P < 0.01). The kS of free valine was constant over 20 min and was 94 ± 4% of that injected. Finally, in Experiment 4, protein synthesis and amino acid oxidation rates measured in the same rat revealed a 35% increase (P < 0.01) in protein synthesis in hind leg muscle and a 29% increase in liver (P < 0.01) from rbST-injected (12 mg/d) rats (n = 6). Lysine oxidation was estimated by continuous (12 h) infusion of L-[1-14C]lysine via the opposite tail vein catheter. Expired CO2 was collected over 20-min intervals and SA at plateau was estimated by fitting an exponential model. Lysine oxidation was reduced (P < 0.05) by 44% in rbST-treated rats. The idea that an increase in protein synthesis results in decreased amino acid oxidation remains tenable. J. Nutr. 128: 1503–1511, 1998.

KEY WORDS: rats · protein synthesis · lysine oxidation · somatotropin

The efficiency of amino acid retention in an animal may be directly related to the rate of protein synthesis, because changes in amino acid oxidation are inversely related to changes in protein synthesis (Benevenga et al. 1993). Therefore, the efficiency of amino acid retention should be improved in food animals by increasing the rate of protein synthesis, assuming that the treatment does not increase endogenous protein degradation to a greater extent. Assessments of protein synthesis or amino acid oxidation have been reported previously but none have made measurements of both synthesis and oxidation concurrently in the same animal. To clarify whether increased synthesis can result in a reduction in oxidation, concurrent measurements in the same animal are required. The objective of this investigation was to develop methods to measure protein synthesis (liver and muscle) and lysine oxidation rates simultaneously in the same animal. These parameters of amino acid metabolism were evaluated in adult female rats treated with bovine somatotropin, a method to increase protein synthesis independently of diet manipulation. Growth rates of female rats can be increased up to five times with daily injections of bovine somatotropin (Azain et al. 1993). With this model, inferences could be made about effects of protein synthesis on efficiency of amino acid use.

The methods selected to measure amino acid oxidation and protein synthesis will critically influence the outcome. Protein synthesis in whole animals has been measured using either flooding dose or continuous infusion methods, whereas amino acid oxidation is measured using continuous infusion with collection of total expired CO2. In these experiments, methods to measure amino acid oxidation were based on methods developed in neonatal pigs. In the pig model, amino acid oxidation was measured using a continuous infusion of labeled methionine or valine with collection of total expired CO2 (Benevenga et al. 1992). A similar metabolism chamber system was used to measure lysine oxidation in this study. Lysine was chosen as the indicator for amino acid oxidation because the first step in lysine degradation is irreversible and hence, commits lysine carbon to irreversible loss from the free amino acid pool. The
lysine molecule was labeled in the carboxyl-carbon, which is the first carbon lost in degradation. In addition, lysine can be separated from other amino acids by using a reverse-phase HPLC system (Bidlingmeyer et al. 1987) so that its specific activity could also be determined.

Two prevalent methods used to measure protein synthesis rates include continuous infusion and flooding dose methods (Waterlow et al. 1978). Continuous infusion techniques involve infusing tracer amounts of a labeled amino acid at a steady rate until a plateau in amino acid specific activity (or atom percent excess) is reached in plasma or tissue free amino acid pool (usually requires 6 h; Garlick et al. 1973). Flooding dose techniques involve injecting a large amount of unlabeled amino acid in association with labeled amino acid. The flooding dose experiment is conducted over 10–20 min compared with 6 h for continuous infusion experiments (Garlick et al. 1980; McNurlan et al. 1979).

We elected to use the flooding dose method to measure protein synthesis. Key factors that influence the validity of flooding dose techniques for animals include the amount of amino acid infused and the specific amino acid used. Generally, with rats, the size of the flooding dose administered has been based on 100 mmol leucine/100 g body weight or 150 mmol phenylalanine/100 g body weight. Both doses approach five to eight times the body free pool (Garlick et al. 1980; McNurlan et al. 1979). However, a significant decline in free amino acid specific activity was observed over the time course of these experiments, indicating that amino acids from protein degradation diluted specific activity of free amino acid. The flooding dose should be at least 10 times and probably 15 times the free amino acid pool in the animal (Attaix et al. 1986 and 1988) to maintain a constant specific activity of amino acid during the experiment.

In developing the model for our studies, the choice of amino acid for flooding dose was also a concern. The amino acid should not affect the metabolism of other amino acids in terms of protein synthesis or amino acid oxidation. Leucine is commonly used in flooding dose experiments. However, leucine appears to influence the metabolism of other amino acids such as isoleucine and valine (Shinnick and Harper 1977). Oxidation of valine was increased 60% when rats were fed a 5% isoleucine diet, and consumption of a 5% isoleucine diet decreased valine oxidation (Block and Harper 1984). Advantages of a valine flooding dose include no effect on the metabolism of the other branched-chain amino acids (Schinneck and Harper 1977), no apparent production of an insulin spike (Sève et al. 1993) and no influence on protein synthesis rates in a liver perfusion system (Khairallah and Mortimore 1976). Another advantage to using valine is that valine can be clearly separated from lysine by using a reverse-phase HPLC system (Bidlingmeyer et al. 1987) as a method to determine amino acid specific activity. Finally, valine is one of the more soluble amino acids, thus volume of the dose could be minimized.

In this experiment, we have been able to measure protein synthesis and amino acid oxidation in the same rat. We believe that this is a unique approach to address questions on relationships between protein synthesis and amino acid oxidation.

### MATERIALS AND METHODS

**Animals.** Female Sprague-Dawley rats (175–200 g; Harlan Sprague Dawley, Indianapolis, IN) were obtained ~1 wk before the start of an experiment. In protein synthesis and/or lysine oxidation experiments, extra rats were assigned to treatment groups because successful insertion of two separate tail vein catheters was not always possible. Rats were housed individually in suspended wire-bottomed cages in a room at 24°C (12-h light:dark cycle) and had unlimited access to food and water during the adaptation period (unless otherwise noted). Rats were offered a 20% casein diet (Table 1) and were randomly assigned to rbST treatments. Care and handling of animals was reviewed and approved by the University of Wisconsin Research Animal Resource Committee and Biological Safety Committee.

**Somatotropin.** Recombinant derived bovine somatotropin (rbST; Monsanto, St. Louis, MO) was reconstituted by lyophilized powder as previously described (Azain et al. 1993). Briefly, each vial of 150 mg was treated as follows: 2.405 mL of water was added and protein was partially solubilized; then 0.032 mL of 1 mol/L of NaOH was added to completely solubilize protein and, finally, 0.063 mL of 1 mol/L NaHCO₃ was added as a buffer. One-milliliter syringes were filled and frozen for later use. Each rat received 0.2 mL/d (12 mg) by subcutaneous injection (16 mm, 25-ga needle) over the mesocapular region. Control rats were given a 0.2 mL/d injection of a solution prepared as above but without rbST.

**Isotopes, amino acids and chemicals.** The L-[1-14C]lysine, graciously donated by Purina Mills, St. Louis, MO, was from American Radiolabeled Chemicals, St. Louis, MO, and the L-[3,4(n)-3H]valine was from American Radiolabeled Chemicals, Arlington Heights, IL. Radiochemical purity of compounds was checked using HPLC (Beckman Ultrasphere C-18 column, Palo Alto, CA) and a radioactive flow detector (Flo 1/8, Radiomatic Instruments, Tampa, FL). Radiochemical impurities were not detected. Sources of “cold” valine and lysine (U.S. Biochemical, Cleveland, OH) were also found to be free from detectable impurities based on PICO-TAG methods described below. Peroxidase is (PCA) was from MCB Reagents (Cincinnati, OH). All other reagents were from Sigma (St. Louis, MO) unless otherwise noted.

**Tail vein catheter placement.** A topical anesthetic (Lidocaine) was applied to the tail surface, and two tail vein catheters were placed as described by Rhodes and Patterson (1979). One catheter was inserted into one of the lateral tail veins and advanced to the vena cava if possible but usually only as far as the pelvis. This catheter was used for the flooding dose. When needed, another catheter was inserted in the opposite lateral tail vein, advanced to the vena cava and used for the continuous infusion. Catheter placement was verified by injection after animals were killed and tissue samples were collected.

**Tissue preparation.** Animals were killed by cervical dislocation. If needed, blood was collected by heart puncture and the entire liver was removed and immediately submerged in liquid nitrogen. After

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<tr>
<th>Ingredient</th>
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<tr>
<td>Casein³</td>
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<td>Corn starch²</td>
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<td>Corn oil⁴</td>
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<tr>
<td>Vitamin mix (AIN 76A)⁵</td>
<td>10</td>
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<tr>
<td>Mineral mix (AIN 76)⁶</td>
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<td>Choline Cl⁷,⁸</td>
<td>2</td>
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<tr>
<td>L-Methionine⁷</td>
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¹ Vitamin-free, Teklad, Madison, WI.
² Teklad, Madison, WI.
³ 333-Dextrose, A. E. Stahly, Decatur, IL
⁴ Contained 0.78 g butylated hydroxyanisole/3.7 L, Archer Daniels Midland, Decatur, IL.
⁵ Teklad, Madison, WI (AIN 1980).
⁶ Teklad, Madison, WI (AIN 1977).
⁷ U.S. Biochemical, Cleveland, OH.
⁸ Added as a 50% solution.
FIGURE 1 HPLC separation of phenyl isothiocyanate amino acid derivatives. Retention times for valine (peak 1) and lysine (peak 2) are identified in a Pierce Standard elution profile. A profile for amino acids in a perchloric acid supernatant from a liver homogenate (liver super.) is shown. An example profile after a SEP-PAK fractionation to obtain a “valine” fraction and a “lysine” fraction is also shown (see Materials and Methods). Retention times were verified by the addition of valine or lysine to the valine and lysine fractions. Specific radioactivity of valine or lysine in the fraction was calculated by measuring radioactivity in a 2-mL aliquot and relating it to amounts of valine or lysine in the sample based on a 10 μL PICO TAG assay.

liver removal, a hind leg muscle sample was removed and immediately submerged in liquid nitrogen. Time of tissue collection was noted for use in protein synthesis calculations. Tissues were stored at −80°C until further processing. Tissues were cooled in liquid nitrogen and fractured using a liquid nitrogen cooled sleeve and piston. The resulting powder was homogenized in 3 mol/L PCA using a Polytron (Kinematica GMBH, Luzern, Switzerland) and centrifuged (10,000 × g for 10 min) to separate protein-bound amino acids (pellet) from free amino acids (supernatant). The supernatant was precipitated by centrifugation at 10,000 × g. The homogenate pellet was washed 5 times with 0.01 mol/L PCA to remove radioactive free amino acids (radioactivity in the final wash was <1% of radioactivity in the pellet). The pellet was hydrolyzed in 20 or 40 mL (for muscle and liver, respectively) of 6 mol/L HCl for 55 min in a microwave (MDS 2000, CEM, Mathews, NC) at 275 kPa.

Amino acid analysis. After derivitization with phenyl isothiocyanate (Bidlingmeyer et al. 1987), a C_18 cartridge (SEP PAK, Millipore, Milford, MA) was used to partially purify and separate amino acids in supernatants and hydrolysates so that “valine” or “lysine” fractions could be obtained. By using C_18 cartridges, amino acids through the 10-min retention time (Liver super., Fig. 1) were discarded, and valine and lysine fractions were collected for measurement and counting. Amino acids in 10-μL fractions (Fig. 1, valine and lysine fractions) were quantified by reverse-phase HPLC (PICO-TAG method, Millipore). These samples were checked using the PICO-TAG system and the Flo 1/8 system to insure that all H was in the valine peak and all 14C was in the lysine peak in the respective fractions. Specific activity of valine and lysine (Bq/nmol) was calculated by dividing the counts in a larger (2 mL) aliquot determined by liquid scintillation spectrometry (model #LS3801, Beckman Instruments) by the amount of amino acid in the 2-mL sample. Dual channel (1H, 14C) liquid scintillation spectrometry was used in each case to correct counts for contamination. For example, lysine (14C) counts were discarded if some were a contaminant in the valine aliquot. Small contaminants were detected in both the valine and lysine fractions.

CO2 analysis. Specific activity and total expired CO2 was quantified as previously described (Benevenga et al. 1992). Briefly, expired CO2 collected in a 2 mol/L NaOH trap from the metabolism chamber was precipitated as BaCO3, filtered, dried and weighed. The entire filter paper and BaCO3 were sealed in a 22 × 60 mm glass vial. One milliliter of 300 g/L trichloroacetic acid was injected into the vial to volatilize the CO2 that was trapped in 0.46 mL of ethanol amine. After at least 2 h in a shaker, the entire ethanol amine trap was transferred to a scintillation vial; 1 mL of methylcellosolve (U.S. Biochemical) and 15 mL of BioSafe II Research Products International, Mount Prospect, IL) were added. Radioactivity was determined by liquid scintillation spectrometry. See results section for estimates of recovery.

Experiment 1. A growth trial was conducted to determine an effective dose of rbST to be used in subsequent experiments. Rats were allowed to adapt to a 20% casein diet for 8 d and then were injected (at the start of the light cycle, 1900 h) with vehicle, 2 or 12 mg of rbST daily for 6 d. The 2 mg/0.2 mL solution was prepared by diluting 12 mg/0.2 mL solution with vehicle. To calculate growth rate and food consumption, rats and food cups were weighed daily at the start of the light cycle. A 7-d postinjection period was included to monitor weight gain during recovery from rbST injections.

Experiment 2. This study was conducted to determine effects of diurnal variation on protein synthesis rates in both control and rbST-treated rats. Rats were randomly assigned to one of two rooms with a normal or reverse light cycle (0700–1900 h or 1900–0700 h). Both light cycles were used so that protein synthesis experiments could be
done during the day. Rats were injected (4 d) with vehicle or rbST (12 mg/d) 8 h after lights off (0300 or 1500 h). To improve the potential to show diurnal variation, rats were fed only during the dark period, because Potter et al. (1968) showed that limiting food intake to a specific time of day enhances the ability to detect diurnal effects. Protein synthesis experiments were performed at 0, 4, 7, 10, 13 and 16 h after the rbST injection (n = 1 rat for each rbST x time unit). A tail vein catheter was inserted and the rat was infused with 1.5 mL/h saline for 3–4 h to simulate continuous infusion, which was proposed for later experiments. To measure protein synthesis, a valine flooding dose (400 mmol valine and 4.07 mBq [1-3,4(n)-3H]-valine in 1.0 mL) was infused via the tail vein catheter. After the flooding dose, the catheter was flushed with 0.2 mL saline. Twenty minutes later, the rat was killed by cervical dislocation. Heparini- zed blood, the entire liver and then two muscle samples were collected, and actual time of freezing was noted for use in calculation of fractional synthesis rates. Hind leg muscle (~2 g, gastrocnemius) was collected from each limb 5 min apart so that effect of time after cervical dislocation on valine specific activity could be determined. Blood was centrifuged at 15,600 x g for 5–10 min and plasma was used to determine plasma free valine specific activity (SAf). The SAf and protein-bound valine specific activity (SAb) were determined as described in the amino acid analysis section above. Fractional rates of protein synthesis were calculated as follows (Garlick et al. 1980):

\[ k = \frac{SA_b}{SA_f} \]

where k is the fractional rate of protein synthesis (%/d), SAf is the specific activity of protein-bound valine (Bq/mmol), SAf is the specific activity of free valine (Bq/mmol) and t is the elapsed time between start of flooding dose infusion and freezing of tissue (~21–26 min but expressed in days).

**Experiment 3.** The assumptions of constant SAf and linear increase in SAf time course were investigated in three control and three rbST-treated rats. During a 5-d adaptation period, rats were fed only during the dark period (0700–1900 h). The feeding schedule continued throughout the 5-d treatment period, and rats were euthanized daily with vehicle or 12 mg/d rbST at 1500 h. The protein synthesis experiments were conducted 4 h after rbST injection. A tail vein catheter was placed in each of six rats (3 control and 3 rbST) and a valine flooding dose was infused (600 mmol valine and 7.33, 5.50 or 3.67 MBq [1-3,4(n)-3H]-valine for rats killed at 5, 10 or 20 min, respectively). The catheter was flushed with 0.2 mL of saline after the flooding dose. At 5, 10 or 20 min, a rat was killed by cervical dislocation and blood, liver and muscle tissues were collected. Again, muscle samples were taken 5 min apart to determine effects of time after death on SAf. Tissues were prepared and analyzed as described above. The specific activity of the injected dose was determined and values for tissues were expressed relative to the injected dose, which was set equal to 1.

**Experiment 4.** Lysine oxidation and protein synthesis were measured in rats injected with vehicle or 12 mg/d rbST for 5 d (0800 h). Two trials were conducted with three control and three rbST-treated rats in each trial. Rats had free access to food only during the dark period (2300–1100 h). Because a 12-h continuous infusion was planned, rats were trained (during the dark period, every other day) to be housed and fed in tubular cages designed to prevent coprophagy (Metta et al. 1961, Will and Suttie 1992). Thus, two tail vein catheters could be inserted and maintained for 1 d before the experiment. Tubular cages prevented rats from consuming the catheters while allowing access to food and water.

Immediately after rbST injection, rats were placed in a tubular wire-mesh restraining cage (5.5 cm x 15 cm) before being placed in metabolism chambers made from Plexiglass tubes (6.4 mm wall, 7 cm diameter and 33 cm long). A square Plexiglass plate was glued to the front of each chamber; a hole fitted with a rubber stopper and tubing provided the air outlet. The back of the metabolism chamber was fitted with a large rubber stopper. The air inlet tube, continuous infusion catheter, flooding dose catheter and a urine collection tube exited through the rear stopper. Room air was drawn through the metabolism chamber system at 1 L/min to provide an adequate oxygen supply. Carbon dioxide from incoming room air was removed by passage through a column of Sodasorb (Bennett Industries, Peotone, IL) before entering the rear of the chamber. Air and expired gases exited the front of the chamber and were drawn through 18 mL of 2 mol/L NaOH in 30-cm glass towers fitted with a fluted disk, which produced a bubble size of ~1 mm. Expired air CO2 was collected continuously, and NaOH was removed at intervals of 20 min over the 12-h experiment. Each chamber was connected to two towers, and air flow was alternated between towers so that continuous air flow was maintained during sample collection.

Because this experiment involved amino acid oxidation, the metabolism chamber system was checked for ability to remove all CO2 and 14CO2 from an air stream flowing through the chamber and tower at 1 L/min. A 220-g rat would be expected to expire 165 µmol CO2/min. Therefore, radiolabeled NaH13CO3 was infused for 2 h into a chamber in 2 mol/L NaOH and mixed with 6 mol/L HCl to produce CO2 at the expected rate. The CO2 was collected in 15-min samples in 15 mL of 2 mol/L NaOH and continued for 45 min after the end of infusion to monitor “washout” of CO2 in the chamber. Specific activity of CO2 was constant over the entire 165 min and was not different (P > 0.20) from infused specific activity, indicating no detectable dilution by outside air CO2. Recovery of CO2 and radioactivity was 98.8 and 98.5%, respectively, through the entire collection and analysis system.

For lysine oxidation experiments, Rainin Rabbit-Plus peristaltic pumps (Rainin, Woburn, MA) were used for infusion of [1-14C]-lysine at 1.5 mL/h (24 KBg/mL). An addition of “cold” [1-lysine-HCl was made to the infusion solution to compete for binding on tubing and glassware (1.8 µmol/h). The “cold” lysine represented 99% of infused lysine and 3% of free lysine in the rat. Approximately 60 µmol/h would be absorbed from diet assuming a constant rate of absorption over 24 h. Air was allowed to flow through the system for 10 min before infusion pumps were started to allow the rat to adjust to the chamber.

During lysine continuous infusion, the flooding dose catheter was flushed with 0.05 mL of heparinized saline every 1–2 h to prevent clots. Twenty minutes before the end of the experiment, a valine flooding dose was infused (600 mmol valine and 4.40 MBq [1-3,4(n)-3H]valine so that protein synthesis rates could be estimated. Catheters were flushed with 0.2 mL of saline after the flooding dose. Continuous infusion was maintained until liver and muscle samples were collected. Rats remained in metabolism chambers until 2 min before cervical dislocation. In addition to determination of valine SAf and SAf, the SAf of liver lysine was determined so that lysine oxidation could be calculated. Lysine oxidation (based on the 1 carbon) was calculated as follows: lysine oxidation (µmol/d) = expired CO2 SAf, [Bq/kg injected lysine SAf (Bq/mmol) x CO2 expiration rate (µmol/d)]. The CO2 expiration rate was used as the mean of 20-min samples over 12 h. Expired CO2 specific activity at equilibrium (SAf) was estimated as the value at plateau (time = infinity) derived from nonlinear regression and the fit of an exponential equation. The exponential equation used to describe the rise in expired CO2 specific activity vs. time was SAf = SAf0 (1-e^-kt).

**Statistics.** Data were analyzed by either linear or nonlinear regression procedures using SAS (version 6.09, Carey, NC). Effects of rbST were tested by using the General Linear Models Procedure and trends with time were evaluated using linear regression. Nonlinear regression was used to estimate the plateau in CO2 specific activity using a single exponential equation.

**RESULTS**

**Experiment 1.** Injection of rats with 2 or 12 mg rbST/d for 6 d increased weight gain 2.2 or 4.4 times, respectively, that of vehicle-injected controls (P < 0.01, Fig. 2). Increased gain associated with rbST injection ceased on the first day after injections were terminated. As expected, food intake during the injection period was greater (P < 0.05) for rats injected with 12 mg/d (16.8 + 0.5 g/d) compared with controls (12.8 + 0.5 g/d) or rats injected with 2 mg/d (12.7 + 0.5 g/d).

**Experiment 2.** Results from preliminary trials suggested that increases in protein synthesis were not detectable (P > 0.065) after ~2 d of rbST treatment (muscle k = control,
by rbST. Similar to Experiment 1, 12 mg rbST/d increased fractional protein synthesis rates from 0 to 16 h after the last injection of rbST. However, a decrease in protein degradation could be responsible for enhanced weight gain. Differences could also be masked if diurnal variation occurred in protein synthesis. The flooding dose technique provides estimates of protein synthesis only over a 20-min experiment. Protein synthesis measurements were conducted after 4 d of rbST treatment to determine if body weight changes returned to preinjection rates.

4.5 ± 0.5 vs. rbST, 5.5 ± 0.1, unpublished results). Greater differences were anticipated based on an expected rate of gain that was four times that of vehicle-injected controls (Experiment 1, Fig. 2). However, a decrease in protein degradation could be responsible for enhanced weight gain. Differences could also be masked if diurnal variation occurred in protein synthesis. The flooding dose technique provides estimates of protein synthesis only over a 20-min experiment. Protein synthesis measurements were conducted after 4 d of rbST treatment to determine if rbST had an effect on muscle and liver fractional protein synthesis rates from 0 to 16 h after the last injection of rbST. Similar to Experiment 1, 12 mg rbST/d increased (P < 0.001) growth rate (7.5 ± 0.5 vs. 2.0 ± 0.5 g/d) and food intake (15.4 ± 0.5 vs. 12.5 ± 0.5 g/d) compared with vehicle-injected controls. Somatotropin treatment increased (P < 0.005) protein synthesis rates (%/d) in muscle (6.52 ± 0.14 vs. 4.48 ± 0.14) and liver (78.5 ± 2.8 vs. 57.7 ± 2.8) compared with vehicle-injected controls. The kₙ in liver or muscle (Fig. 3) was not altered with time after rbST injection. In addition, kₙ in the second muscle taken after a 5-min sampling delay was not different (P > 0.10) from that of the first. Valine SAf in blood, liver or muscle was not affected by rbST or time. However, specific activity in liver was lower (P < 0.05) than in other tissues, especially in rbST-treated rats (Fig. 4). On average, the SAf was 85% of the infused dose, suggesting that the flooding dose was five to six times the endogenous free pool.

with a valine flooding dose, and 100 μL of blood was sampled 2, 5, 10, 15, 20 and 30 min later from the opposite tail vein catheter (data not shown). Valine SAf was equal to the infused dose by 2 min and fell <10% by 20 min. In this experiment, assumptions of constant SAf in liver, muscle and blood, and a linear increase in time course of SAf were investigated in control and rbST-treated rats. Twelve milligrams rbST/d increased (n = 6, P < 0.001) growth rate (6.7 ± 0.3 vs. 1.2 ± 0.3 g/d) and food intake (14.7 ± 0.3 g/d vs. 12.2 ± 0.3 g/d) compared with vehicle-injected controls. Valine SAf was constant over 20 min in vehicle- and rbST-injected rats (Figs. 5 and 6). Muscle, however, was an exception because an 8% increase in valine SAf was seen but was not considered to be biologically important considering the scope of this experiment (see Fig. 5). The SAf time course for plasma and liver is shown in Figure 6. A linear increase in SAf was observed for muscle (Fig. 5) and liver (Fig. 6) with a significantly greater slope being attributed to rbST-treated rats. No difference in SAf or SBf was detected in the two muscle samples taken ~5 min apart. On average, the SAf was 94 ± 4% of injected dose, indicating that the flooding dose of valine was at least 10 times the endogenous free pool.

**Experiment 3.** After finding that the flooding dose in Experiment 2 increased the free valine pool only five to six times, the flooding dose was increased to 600 mmol. In a preliminary experiment, one rat with two tail vein catheters was infused
**FIGURE 4** Experiment 2. Valine specific activity (SA) of the injected dose (horizontal line) compared with specific activity of free valine in muscle, liver and plasma of one control or one recombinant bovine somatotropin (rbST)-injected rat (n = 1 for each treatment time unit). Each group of four bars represents muscle samples (A = 0; B = 5-min delay), liver and plasma, respectively. Two muscle samples were taken from opposite hind limbs 5 min apart to test effects of sampling time. No significant differences were detected as a result of the 5-min time differential (P > 0.05). Specific activity of valine was not altered by treatment (P > 0.05) or time after rbST injection (P > 0.05). Specific activity of liver valine was lower than that of other tissues (P < 0.05). The apparent error in the dose administered to the control rat at time 0 could not be identified.

**Experiment 4.** Results from a preliminary experiment in which radiolabeled lysine was infused for 6 h suggested that 9–10 h would be required to achieve plateau specific activity in expired CO₂ on the basis of the predicted exponential curve. Liver free lysine specific activity in rbST-treated rats compared with controls also indicated that free lysine was not at steady state. Therefore, subsequent experiments were conducted for 12 h.

A summary of protein synthesis and lysine oxidation results is shown in Table 2. Growth and food intake were increased (P < 0.05) with rbST as observed in previous experiments. A 29% increase in liver fractional protein synthesis rate and a 35% increase in muscle fractional protein synthesis rate were observed. Injection of rbST decreased lysine oxidation from 293 to 163 μmol/(d·100 g body weight). An assumption is made that the 1-carbon of lysine represents the fate of the entire molecule. Lysine oxidation is compared with rate constants for protein synthesis in liver and muscle of control and treated rats in Figure 7. Lysine oxidation decreased by half as rate constants for protein synthesis increased 1.3–1.4 times. A similar relationship is not shown within control and treated.

**FIGURE 5** Experiment 3. Time course of valine specific activity (SA) in muscle free valine and muscle protein-bound valine of one control or one (rbST)-recombinant bovine somatotropin injected rat (n = 1 rat for each treatment × time unit). Specific activity of tissue free and protein-bound valine is expressed relative to that of the injected dose, which was set equal to 1. The actual time until the tissue was frozen in liquid nitrogen was used in calculations but time was standardized in the plot. Pairs of control and rbST-injected rats were killed at 5, 10 or 20 min after receiving a 600-mmol valine flooding dose with 3H-valine. As done previously, muscle samples were taken 5 min apart, but no differences (P > 0.05) in the specific activity of free or protein-bound valine were detected as a result of the 5-min sampling delay. With respect to sampling time within rat, results are pooled across muscle samples (mean ± SD; n = 2 muscle samples). Specific activity of muscle protein bound valine increased linearly with time (P < 0.01) and the slope was significantly greater in rbST-treated rats (P < 0.05), indicating a higher $k_s$.

**FIGURE 6** Experiment 3. Time course of valine specific activity (SA) in plasma and liver free valine and liver protein-bound valine of one control or one (rbST)-recombinant bovine somatotropin injected rat (n = 1 rat for each treatment × time unit). Specific activity of tissue free and protein-bound valine is expressed relative to that of the injected dose, which was set equal to 1. The actual time until the tissue was frozen in liquid nitrogen was used in calculations but time was standardized in the plot. Pairs of control and rbST-injected rats were killed at 5, 10 or 20 min after receiving a 600-mmol valine flooding dose with 3H-valine. As done previously, muscle samples were taken 5 min apart, but no differences (P > 0.05) in the specific activity of free or protein-bound valine were detected as a result of the 5-min sampling delay. With respect to sampling time within rat, results are pooled across muscle samples (mean ± SD; n = 2 muscle samples). Specific activity of muscle protein bound valine increased linearly with time (P < 0.01) and the slope was significantly greater in rbST-treated rats (P < 0.05), indicating a higher $k_s$. 

A summary of protein synthesis and lysine oxidation results is shown in Table 2. Growth and food intake were increased (P < 0.05) with rbST as observed in previous experiments. A 29% increase in liver fractional protein synthesis rate and a 35% increase in muscle fractional protein synthesis rate were observed. Injection of rbST decreased lysine oxidation from 293 to 163 μmol/(d·100 g body weight). An assumption is made that the 1-carbon of lysine represents the fate of the entire molecule. Lysine oxidation is compared with rate constants for protein synthesis in liver and muscle of control and treated rats in Figure 7. Lysine oxidation decreased by half as rate constants for protein synthesis increased 1.3–1.4 times. A similar relationship is not shown within control and treated.

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TABLE 2

Comparison of control and recombinant bovine somatotropin–treated rats

<table>
<thead>
<tr>
<th>Item</th>
<th>n</th>
<th>Control</th>
<th>Treated</th>
<th>Treated:Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain, g/d</td>
<td>11.13</td>
<td>0.45 ± 0.3</td>
<td>5.28 ± 0.3*</td>
<td>11.73</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>11.13</td>
<td>12.3 ± 0.4</td>
<td>13.8 ± 0.4*</td>
<td>1.12</td>
</tr>
<tr>
<td>Liver $k_s$, %/d</td>
<td>5.6</td>
<td>73.4 ± 2.6</td>
<td>94.6 ± 2.3*</td>
<td>1.29</td>
</tr>
<tr>
<td>Muscle $k_s$, %/d</td>
<td>6.6</td>
<td>5.0 ± 0.2</td>
<td>6.74 ± 0.2*</td>
<td>1.35</td>
</tr>
<tr>
<td>Lysine oxidation, [$\mu$mol/(d · 100 g BW)]</td>
<td>6.6</td>
<td>293.0 ± 15.3</td>
<td>163.1 ± 15.3*</td>
<td>0.56</td>
</tr>
</tbody>
</table>

1 Number of observations for control and treated groups, respectively.
2 Each value is the mean ± SEM.
* Different from control, (P < 0.05). $k_s$, fractional protein synthesis rates; BW, body weight.

animals possibly due to the narrow range of $k_s$ within treatment. This figure supports the concept of an inverse relationship between amino acid (lysine) oxidation and protein synthesis.

The time course for expired CO$_2$ and CO$_2$ specific activity for a single control and a single rbST-treated rat is shown in Figure 8. Plateau specific activity derived from the exponential equation (see Materials and Methods) was estimated from curves fit for each rat. Average CO$_2$ expiration rate was 551 ± 10 $\mu$mol/(min · kg$^{0.75}$) and treatment or trial effects were not detectable. Lysine oxidation was calculated as described in Materials and Methods. Expected lysine disappearance by oxidation or protein accretion was calculated to compare lysine intake with daily lysine oxidation shown above. Lysine intake was 1320 $\mu$mol/d for rbST-treated rats (13.8 g food intake/d). Assuming that weight gain is 18% protein and protein is 6% lysine, rats would retain 400 $\mu$mol lysine/d. Based on measured rates of lysine oxidation, a 225-g rat would oxidize 370 $\mu$mol of lysine/d. Lysine intake of control rats was 1160 $\mu$mol/d, lysine retention was 40 $\mu$mol/d and oxidation was 615 $\mu$mol/d. Thus, estimated rates of retention and oxidation account for 55–60% of lysine intake in control and rbST-treated rats.

DISCUSSION

Our selection of the flooding dose method to estimate protein synthesis in this work was based, in part, on turnover rates of different populations of proteins in relation to length of incorporation allowed for the isotope. At least three populations of proteins with markedly different turnover rates exist in rat liver. Turnover rates for three populations of proteins were 2.28, 0.27 and 0.062 times per day, making contributions of 9.5, 66.4 and 24.1% of total turnover, respectively (Garlick et al. 1976). For all proteins to contribute to the fractional protein synthetic rate of a tissue, each protein must be able to contribute in proportion to its mass and turnover rate throughout the experiment. In continuous infusion experiments that are 10–20 times longer than the 20-min flooding dose experiment, specific activity (SA$_b$) of the amino acid in rapid turnover proteins may approach specific activity (SA$_f$) of the amino acid in the free pool before the end of the experiment. Thus, rapid turnover proteins will not contribute to the calculation of protein synthesis based on specific activity of the amino acid in total tissue protein. Hence, with longer durations of measurements, the calculated rate of protein synthesis would decrease even though protein synthesis was still occurring at the same rate. Additional time in the divisor without a proportionate accumulation of radioactivity in the numerator would decrease the calculated estimate of synthesis. Higher rates of protein synthesis would be expected when the
flooding dose method is used because essentially all proteins would be in the linear phase of isotope incorporation (Samarel 1991). In summary, higher rates of protein synthesis measured in flooding dose experiments are expected on the basis of greater representation of short half-life proteins. Synthesis rates derived from flooding dose techniques provide a more accurate representation of total organ protein synthesis.

Young adult female rats treated with recombinant-derived bovine somatotropin (rbST) proved to be a valuable tool in the current experiment. Weight gain was increased up to 10 times (Table 2) by daily injections of rbST compared with vehicle-injected controls and was consistent with previous studies (Azain et al. 1993, Byatt et al. 1993). The marked increase in growth was associated with a significant increase (~30–40%) in protein synthesis and a 40% reduction in lysine oxidation.

An increase in protein synthesis (13% whole body, 30% in liver and muscle) has been observed when exogenous bovine somatotropin was injected in dwarf mice for 4 or 8 d (Bates and Pell 1991). After 7 or 14 d of human somatotropin injection, muscle, liver and heart protein synthesis rates were increased 67, 74 and 36%, respectively, in dwarf mice (Bates et al. 1992). Similarly, after twice daily injections of human growth hormone for 7 d, muscle and liver protein synthesis rates were increased in dwarf mice from 45 to 109% and from 57 to 77%, respectively (Pell and Bates 1992). Somatotropin also increased protein synthesis by 30% in biceps femoris muscle of lambs (Pell and Bates 1987); it increased protein accretion 40% and modestly increased protein synthesis (10%; P < 0.14) in the hind limb of Holstein steers (Boisclair et al. 1994). In addition, protein synthesis was increased ~50% in four muscles in beef steers (Eisemann et al. 1989a). In pigs, protein synthesis was increased 24% in longissimus muscle, 16% in liver (Steve et al. 1993) and 56% in whole body (Tomas et al. 1992).

Although an increase in fractional rates of protein synthesis is expected as a result of exogenous growth hormone treatment, the effects of growth hormone on amino acid catabolism have not received much attention. Experiments in Hereford heifers indicate that bovine growth hormone has a sparing action on amino acids by decreasing urinary nitrogen excretion 12% (Eisemann et al. 1986b). Additional experiments were conducted to characterize the effects on amino acid metabolism. Leucine irreversible loss was not changed by bovine growth hormone administration (Eisemann et al. 1986a). However, there was a 32% decrease in leucine oxidation (Eisemann et al. 1986a and 1989b) and, by difference, an 11% increase in whole-body protein synthesis (Eisemann et al. 1989b). Similarly, a 25% reduction in leucine oxidation was observed in human subjects receiving recombinant human growth hormone (Copeland and Nair 1994). Consistent with the decrease in leucine oxidation is the 60% reduction in system A amino acid transport V_{max} in hepatic plasma membrane vesicles after administration of human growth hormone (Pacitti et al. 1992).

Accordingly, as reported in this paper, the observed 40% reduction in oxidation of lysine and 30% increase in fractional protein synthesis rates in liver and muscle are consistent with our notion that protein synthesis, and not amino acid degrading enzyme activity, directs the fate of free amino acids. The literature basis of this idea (Benevenga et al. 1993) was as follows: 1) inhibition of liver protein synthesis with puromycin resulted in an immediate increase in lysine oxidation; 2) a decrease in the presence of an amino acid to support protein synthesis affects the immediate oxidative destruction of other amino acids; 3) an increase in amino acid–degrading enzyme activity by as much as 10-fold resulted in an increase of only 10–35% in amino acid oxidation, suggesting that massive increases in activity of amino acid–degrading enzymes are not necessarily translated into massive increases in amino acid catabolism; and 4) a review by Rogers (1976) showed that, on average, the k_{m} for amino acid–degrading enzymes were two to three orders of magnitude higher than those involved in activating the amino acid to its RNA derivative. These observations led to the idea that enzymes involved in activating amino acids for use in protein synthesis would be more fully saturated, whereas those involved in amino acid catabolism are not. If V_{max} potentials for protein synthesis and amino acid catabolism are “similar” in magnitude, then results obtained from the work reported in this paper support the idea that an increase in protein synthesis should automatically result in a decrease in oxidative catabolism of amino acids.

However, in a limited investigation on the same rats treated with rbST in this study, Blemings et al. (1996) found that lysine and valine oxidation were depressed 35% and methionine 20% in a liver homogenate system. Activity of lysine α-ketoglutarate reductase was also reduced 35%. The observation that treatment of rats with rbST decreases the activity of liver amino acid–degrading enzymes and oxidation of the three amino acids tested undermines our notion that protein synthesis and not amino acid–degrading enzyme activity is instrumental in determining the fate of free amino acids. If it can be shown in animals treated similarly to those used in the work reported here, that limitation of another indispensable amino acid results in an increase in the oxidation of amino acids as it did earlier in the work of Stripanuk and Benevenga (1977), then the original notion on the role of protein synthesis controlling amino acid catabolism would seem to be worthy of strong support.

**LITERATURE CITED**


Attaix, D., Aurossoue, E., Manget, H., Arnal, M. & Arnaud, G. (1995) Contribution of in vivo amino acid results in an increase in the oxidation of amino acids as it did earlier in the work of Stripanuk and Benevenga (1977), then the original notion on the role of protein synthesis controlling amino acid catabolism would seem to be worthy of strong support.


