Tissue protein synthesis and nucleic acid concentrations in steers treated with somatotropin

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(*Received 7 December 1988 – Accepted 3 July 1989*)

The effect of injection with bovine somatotropin (bST) on the fractional rate of protein synthesis (FSR) in tissues of beef steers was studied using a continuous infusion of [1-^{14}C]leucine. Minimum and maximum FSR were calculated from free leucine specific radioactivity (SRA) in plasma or tissue homogenate respectively. Tissue nucleic acid concentrations were also quantified. Tissue samples were obtained from several muscles, sections of the small intestine and liver. In response to bST, both minimum and maximum FSR increased in muscle but not liver or intestinal tissues. Absolute synthesis rate increased in several muscles and small intestine tissues. Treatment with bST increased the relative SRA of protein-bound leucine in muscles compared with liver; increased the amount of protein synthesis per unit empty body-weight (EBW) in most muscles; and increased weight of small intestine relative to EBW, suggesting a differential response between liver and the other tissues measured. Compositional changes in response to bST occurred only in muscles. DNA concentration increased while protein:DNA decreased in the gastrocnemius muscle and RNA:DNA increased in the longissimus dorsi. The maximum percentage contribution of tissue protein synthesis to whole-body protein synthesis was 12·6, 25·7 and 20·5, and 13·0, 29·4 and 25·8 for liver, muscle, and small intestine in placebo-treated and bST-injected steers respectively.

Protein synthesis: Somatotropin: Cattle

Exogenous somatotropin (ST) increases nitrogen retention in growing ruminants (Moseley *et al.* 1982; Grantley-Smith *et al.* 1983; Eisemann *et al.* 1989). Positive N retention in tissues occurs when synthesis of nitrogenous compounds exceeds degradation. An increase in retention can result from a combination of changes in both synthesis and degradation. Previous studies in cattle, using a continuous infusion of [1-^{14}C]leucine to quantify whole-body protein synthesis in response to bovine ST (bST), indirectly demonstrated an increase in protein synthesis (Eisemann *et al.* 1986*a*, 1989). Whole-body measurements are a composite of the net effect on a variety of tissues, but may hide interesting and important differential effects on specific body tissues. Information on specific tissues is necessary to understand the coordination of metabolism necessary for metabolic control.

Because of the potential for use of ST in production systems and its ability to alter nutrient partitioning in growing animals, it is important to study further its mechanism of action on body tissues. The objectives of the present study were to quantify the effects of exogenous bST on fractional rate of protein synthesis (FSR) in several body tissues and on tissue concentrations of protein and nucleic acids in cattle. The continuous infusion approach was used to estimate FSR because of the size of the animals involved. This technique has been widely used (for example, Simon *et al.* 1978, 1982, 1983; Lobley *et al.* 1980; Davis *et al.* 1981) and the limitations have been discussed relative to uncertainty of the specific radioactivity (SRA) of the precursor pool for protein synthesis (for example, Airhart *et al.* 1974; Waterlow *et al.* 1978).

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Table 1. *Composition of diet (g/kg dry matter*)*

Ingredient	
Cracked maize	369·0
Wheat straw	199·0
Cottonseed hulls	207·9
Soya-bean meal	125·0
Molasses	77·0
Dicalcium phosphate	11·1
Limestone	5·5
Trace mineralized salt with selenium	5·5
Vitamins A, D and E	†

* Dry matter content of 904 g/kg.
† Added at 600 μg, 7·5 μg and 11 mg/kg respectively.

MATERIALS AND METHODS

Animals and diets

Ten Hereford × Angus steers (289 (SE 2) kg body-weight (BW)) were used in the experiment. They were fed on a completely mixed, pelleted diet containing 10·42 MJ metabolizable energy (ME)/kg dry matter (calculated; National Research Council, 1976) and 127 g crude protein/kg dry matter (Table 1). The diet was given at a daily rate of 0·98 MJ ME/kg $BW^{0.75}$. Feeding was by hand every 4 h. The amount of diet offered was adjusted weekly according to BW.

Experimental design

Five steers received daily subcutaneous injections of bST and five steers received placebo injections for 19 or 20 d before infusions. Sterile injections were given daily at 09.00 hours in a volume of 14 ml. The bST dose was 29·2 IU/d (Miles Lot 14; Miles Laboratories, Naperville, IL; 1·3 IU/mg protein). The bST was dissolved in buffer as previously described (Eisemann *et al.* 1986*b*). For logistical reasons the steers were paired across treatments and the injection sequence was staggered for each pair of steers. Pairing was based on BW and rate of gain throughout a 6-month preliminary period.

Leucine infusion and sampling

On day 18 of the treatment period, sterile catheters were inserted into both jugular veins of each steer. The catheter used was a 300 mm section of tygon tubing (i.d. 1·27 mm, o.d. 2·29 mm) previously treated with TDMAC–heparin complex (Polysciences Inc., Warrington, PA). On day 19 or 20, one steer of each pair was moved to a metabolism crate for leucine infusion (only one steer could be infused/d). Sodium chloride was added to a stock solution of L-[1-^{14}C]leucine (SRA 57 mCi/mmol; Amersham Corporation, Arlington Heights, IL) to provide an infusate that had a final concentration of 0·15 M-NaCl. A syringe infusion pump was used for a 380 min continuous isotope infusion (unprimed; 5·7 μCi/min) into one jugular vein. Blood was collected at 20 min intervals from the catheter in the contralateral jugular vein. Protein-free plasma filtrates were prepared and analysed for radiolabelled and total leucine to determine leucine SRA. These findings on whole-animal leucine flux were previously reported (Eisemann *et al.* 1989).

After 380 min of isotope infusion, each steer was anaesthetized with sodium thiamylal (Bio-Ceutic Laboratories Inc., St Joseph, MO) and samples from liver, duodenum,

jejunum, ileum, biceps femoris, longissimus dorsi, triceps brachii, gastrocnemius and semitendinosus were taken within an average of 7 min. Two sets of tissue samples were taken, one for protein synthetic measurements and one for analysis of nucleic acid and protein concentrations. Excised tissues were immediately washed with 0·15 M-NaCl, wrapped in aluminium foil and frozen in liquid N_2. For the jejunum, only protein and nucleic acid concentrations were determined. For one steer on the bST treatment, there was no sample of ileum for protein synthetic measurements. The isotope infusion was maintained during the sampling period. At the end of sampling, the isotope infusion was stopped and each steer was killed by intravenous injection of solution T-61 (containing 200 mg embutramide, 50 mg mebezonium iodide and 5 mg tetracaine hydrochloride/ml with 0·6 ml dimethylformamide in distilled water).

Tissue SRA analysis

For liver and small intestine SRA measurements, duplicate samples were homogenized (Polytron) in cold water (2 g tissue + 15 ml water). Cold trichloroacetic acid (300 ml/l; TCA) was added to a final concentration of 128 ml/l and the sample was homogenized again. Sample tubes were placed in ice for 20 min before centrifugation at 12000 ***g*** for 20 min at 4°. The supernatant fraction was transferred to a separatory funnel, and the precipitate was washed twice with 10 ml TCA (50 ml/l) and centrifuged as described previously. All supernatant fractions were combined and norleucine was added as an internal standard.

For muscle tissues, a 2 g sample in duplicate was initially homogenized in 35 ml 0·01 M-phosphate buffer, pH 7·4. Sample tubes were placed in ice for 20 min before centrifugation at 1400 ***g*** at 4° for 20 min. The precipitate was designated the insoluble (myofibrillar and collagenous) protein fraction (Helander, 1957; Goldberg, 1968). Cold TCA (300 ml/l) was added to the supernatant fraction and it was treated as previously described for liver and small intestine. The protein precipitate from the supernatant fraction was designated the soluble (sarcoplasmic) protein fraction. Both protein precipitates were washed twice with 10 ml TCA (50 ml/l) as previously described. All supernatant fractions following TCA precipitation were combined.

Diethyl ether (3 vol.) was mixed with the combined supernatant fractions in a separatory funnel and the aqueous phase was removed and applied to a cation-exchange column (Bio-Rad Cation Exchange, AG 50W-X8, 100–200 mesh, H^+ form) to separate [1-^{14}C]leucine from other radiolabelled compounds. The sample was sequentially followed by 8 ml 0·01 M-hydrochloric acid and 3 ml water. Amino acids were eluted sequentially with 2 ml 1 M-ammonium hydroxide, 7 ml 2 M-NH_4OH and 2 ml water. This procedure separates leucine from its keto acid derivative (Eisemann *et al.* 1986*a*). The eluate was evaporated to dryness at 85° under N_2 and reconstituted in 2 ml 0·1 M-HCl. After filtering, a 1 ml portion was used to quantify radioactivity by liquid-scintillation counting; it was assumed that all ^{14}C in the portion was associated with leucine. An external standard was used to correct for counting efficiency. A second portion was used for quantification of leucine and norleucine by ion-exchange chromatography using a five buffer analysis method (Beckman Instruments Inc., Palo Alto, CA).

Cold ethanol (950 ml/l; 25 ml), containing potassium acetate (10 g/l), was added to the protein precipitate. The precipitate was homogenized, and sample tubes were placed in ice for 10 min before centrifugation for 10 min at 1000 ***g***. The supernatant fraction was discarded. The precipitate was sequentially washed with 25 ml ethanol–diethyl ether (1:1, v/v), 25 ml trichloromethane–ethanol–diethyl ether (1:2:2, by vol.), 25 ml diethyl ether and 10 ml diethyl ether twice. The protein precipitate was air-dried and stored at −20° until leucine analysis.

Approximately 15 mg protein precipitate were added to 11 ml 6 M-HCl with 10 μmol norleucine added as an internal standard. Samples were frozen in a methanol–dry-ice bath (−80°), tubes were evacuated and flushed with N_2 twice, and evacuated a final time. The protein was hydrolysed for 24 h at 105°. After hydrolysis, samples were evaporated at 60° under reduced pressure. Sample tubes were rinsed with water and evaporated twice more. The final residue was reconstituted in 2 ml 0·1 M-HCl and filtered. A 1 ml portion was used for liquid-scintillation counting. A second portion was diluted and analysed for leucine and norleucine contents by ion-exchange chromatography (Beckman Instruments).

Tissue protein and nucleic acid analysis

For tissue protein and nucleic acid concentrations, a homogenate (100 g/l) was prepared in 0·01 M-phosphate buffer containing 0·15 M-NaCl, 0·001 M-EDTA, and sodium azide (0·2 g/l). Protein was analysed in the homogenate by the method of Lowry *et al.* (1951).

For nucleic acid analysis, a portion of homogenate (100 g/l) was transferred to a siliconized glass tube, perchloric acid (PCA) was added to a final concentration of 0·4 M. Sample tubes were placed in ice for 15 min before centrifugation at 1000 ***g*** for 10 min at 4°. The pellet was washed twice with 2 ml cold 0·4 M-PCA. The final pellet was resuspended in 0·4 M-PCA and hydrolysed for 20 min at 100°. Sample tubes were placed in ice for 15 min, centrifuged at 1000 ***g*** for 10 min at 4° and the supernatant fraction was used for analysis of DNA (Burton, 1956) and RNA (Ceriotti, 1955) concentrations. Standards for DNA and RNA concentrations were hydrolysed as described previously.

Calculations

For plasma leucine SRA, the plateau value was determined by fitting values for each steer to a single-component negative exponential function:

$$S_p = S_{pmax}(1-e^{-\lambda_p t}), \tag{1}$$

where S_p is the SRA of plasma amino acids, S_{pmax} is the value of S_p at plateau, λ_p is the rate-constant for the increase in plasma SRA during infusion and t is time.

The equation described by Garlick *et al.* (1973) was solved using an iterative procedure to calculate both minimum (k_{smin}) and maximum (k_{smax}) tissue rates of protein synthesis.

$$\frac{S_B}{S_i} = \frac{\lambda_i}{\lambda_i - k_s} \cdot \frac{(1-e^{-k_s t})}{(1-e^{-\lambda_i t})} - \frac{k_s}{(\lambda_i - k_s)}, \tag{2}$$

where S_B and S_i are the SRAs of protein-bound and free leucine in tissues respectively, at the end of infusion, k_s is the FSR, and t is time. For k_{smax}, the value of λ_i, the rate-constant for the increase in SRA in tissues during infusion, was estimated as described by Garlick *et al.* (1973). Equation 2 was used to estimate the minimum k_s substituting S_{pmax} for S_i and estimating λ_i with λ_p.

Statistics

Within a tissue, for each variable, a t statistic was calculated comparing values from olacebo- and bST-treated steers. The average value for each tissue across treatment was compared using one-way analysis of variance with tissue as the main effect. Tissue means were compared using Fisher's least significant difference (LSD).

RESULTS

Tissue protein and nucleic acid content

Tissue concentrations of protein, RNA and DNA were not changed with bST treatment except for increased ($P < 0·05$) DNA concentration in the gastrocnemius (Table 2).

Table 2. *Protein, RNA and DNA concentrations (mg/g wet weight) and protein:nucleic acid ratios in tissues of beef steers injected with placebo or bovine somatotropin (bST)*

Variable	Treatment	Liver	Biceps femoris	Longissimus dorsi	Semitendinosus	Gastrocnemius	Triceps brachii	Duodenum	Jejunum	Ileum
Protein	bST	180·2	186·4	192·8	205·4	187·2	187·8	124·5	114·5	101·1
	Placebo	179·2	196·3	199·3	225·1	189·9	193·6	120·1	107·7	105·6
	SE	4·0	3·3	7·0	10·9	5·1	5·8	4·3	3·3	3·6
	Tissue mean	179·7^{c}	191·3^{b}	196·1^{b}	215·3^{a}	188·5^{b,c}	190·7^{b,c}	122·3^{d}	111·1^{d,e}	103·5^{e}
RNA	bST	4·29	1·82	1·09	1·10	1·10	2·08	4·12	3·18	3·89
	Placebo	3·81	1·52	0·93	0·88	0·90	1·76	4·05	3·10	3·81
	SE	0·27	0·39	0·06	0·10	0·10	0·22	0·19	0·19	0·17
	Tissue mean	4·05^{a}	1·67^{c}	1·01^{d}	0·99^{d}	1·00^{d}	1·92^{c}	4·09^{a}	3·14^{b}	3·85^{a}
DNA	bST	1·52	0·210	0·182	0·187	0·215*	0·233	4·91	6·45	5·46
	Placebo	1·37	0·189	0·183	0·197	0·184	0·221	4·90	6·16	5·66
	SE	0·11	0·017	0·011	0·012	0·007	0·012	0·51	0·25	0·36
	Tissue mean	1·44^{d}	0·200^{e}	0·182^{e}	0·192^{e}	0·200^{e}	0·227^{e}	4·91^{c}	6·31^{a}	5·56^{b}
Protein:DNA	bST	120·2	906	1084	1114	874*	817	27·0	17·8	18·9
	Placebo	136·8	1077	1109	1158	1038	884	24·6	17·6	19·0
	SE	12·2	86	95	77	49	55	2·3	0·8	1·4
	Tissue mean	128·5^{e}	992^{b,c}	1097^{a,b}	1136^{a}	956^{c,d}	850^{d}	25·8^{e}	17·7^{e}	19·0^{e}
Protein:RNA	bST	42·2	126	180	197	181	91·8	30·2	36·8	26·1
	Placebo	48·5	152	217	259	214	121·8	30·0	34·7	28·0
	SE	3·0	26	14	23	19	15·0	1·2	1·9	1·3
	Tissue mean	45·3^{d}	139^{b}	198^{a}	228^{a}	197^{a}	106·8^{c}	30·1^{d}	35·7^{d}	27·1^{d}
RNA:DNA	bST	2·84	9·29	6·04*	5·81	5·19	9·09	0·90	0·50	0·72
	Placebo	2·88	8·41	5·11	4·57	4·91	7·88	0·83	0·51	0·68
	SE	0·25	2·49	0·28	0·41	0·55	0·97	0·08	0·04	0·04
	Tissue mean	2·86^{c}	8·85^{a}	5·58^{b}	5·19^{b}	5·05^{b}	8·48^{a}	0·86^{d}	0·50^{d}	0·70^{d}

^{a,b,c,d,e} Mean values in horizontal rows (tissue mean across treatment) with different superscript letters differed significantly: $P < 0·05$.
Treatment means within each tissue differed significantly: * $P < 0·05$.

Table 3. *Tissue weights and total protein and nucleic acid content of tissues from steers injected with placebo or bovine somatotropin (bST)*

Variable	Treatment	Liver	Biceps femoris	Longissimus dorsi	Semitendinosus	Gastrocnemius	Triceps brachii	Small intestine†
Tissue wt (g)	bST	4643*	4168	3966*	1379	1205	1878	4214*
	Placebo	4364	3970	3586	1301	1209	1775	3688
	SE	84	127	100	53	48	43	138
Tissue wt (g/kg empty body-wt)	bST	17·0	15·3	14·5	5·0	4·4	6·9	15·4*
	Placebo	16·8	15·2	13·8	5·0	4·6	6·8	14·1
	SE	0·5	0·4	0·3	0·2	0·1	0·1	0·4
Total protein (g)	bST	836	777	765	285	225	354	478
	Placebo	782	781	716	293	229	344	417
	SE	24	33	36	22	10	17	24
Total RNA (g)	bST	19·9	7·7	4·3*	1·5	1·3	3·9	16·9
	Placebo	16·6	6·2	3·3	1·2	1·1	3·1	14·6
	SE	1·2	1·8	0·2	0·2	0·1	0·4	1·1
Total DNA (g)	bST	7·1	0·88	0·72	0·26	0·26*	0·44	21·9
	Placebo	6·0	0·75	0·65	0·26	0·22	0·39	19·6
	SE	0·6	0·08	0·04	0·02	0·01	0·02	1·6

Treatment means within each tissue differed significantly: * $P < 0·05$.

† An average value for protein, RNA and DNA concentration across the three sections of the small intestine was used to calculate total tissue content.

Protein:nucleic acid and nucleic acid ratios were relatively insensitive to bST treatment (Table 2). Protein:DNA decreased ($P < 0.05$) in gastrocnemius muscle, and RNA:DNA increased ($P < 0.05$) in longissimus dorsi in bST-treated steers.

While treatment with bST had only minor impact within tissues, there were striking differences in comparisons across tissue types across treatments (Table 2). Protein concentration was higher ($P < 0.05$) in muscle and liver than in sections of the small intestine and there were differences among muscles. RNA concentration was higher ($P < 0.05$) in liver and sections of the small intestine than in muscle, and DNA concentration was highest in the small intestine, intermediate in liver and lowest in muscle ($P < 0.05$). Both RNA and DNA concentrations differed ($P < 0.05$) across sections of the small intestine. As a consequence, the protein:DNA and RNA:DNA ratios were highest in muscles, intermediate in liver and lowest in small intestine ($P < 0.05$). Protein:RNA ratios in liver and small intestine were similar and were lower than muscle ratios ($P < 0.05$).

The bST increased ($P < 0.05$) tissue weight of liver, longissimus dorsi, and small intestine (Table 3). Empty BW (EBW) increased from 260·6 kg for placebo-treated steers to 273·0 kg for bST-treated steers (SE 4·0, $P = 0.06$). Carcass weight also increased ($P < 0.05$, SE 2·7) and was 175·9 and 184·8 kg for placebo- and bST-injected steers respectively. Expressed as a percentage of EBW, the small intestine increased ($P < 0.05$) with bST treatment (Table 3). Total tissue protein was not altered by treatment with bST. Total RNA increased ($P < 0.05$) in longissimus dorsi and total DNA increased ($P < 0.05$) in gastrocnemius muscle in response to bST (Table 3).

Tissue free leucine and SRA of free and bound leucine in tissues

For all muscles except biceps femoris, the tissue free leucine concentration decreased ($P < 0.05$) in bST-treated steers (Table 4). There was a trend for a decrease in the biceps femoris. Treatment with bST did not alter leucine concentration or the protein-bound leucine:tissue free leucine ratio in liver or small intestine. The protein-bound leucine:tissue free leucine ratio increased ($P < 0.05$) in longissimus dorsi and gastrocnemius muscles from bST-treated steers, and tended to be higher in other muscles. Across treatments, tissue free leucine concentration was higher ($P < 0.05$) in small intestine than in liver or muscle tissue. Protein-bound leucine relative to free leucine was highest in muscle, intermediate in liver and lowest in small intestine ($P < 0.05$).

The relative SRA of free leucine (tissue free SRA:plasma SRA) was not altered by treatment and ranged from 0·21 in ileal tissue to 0·56 in several muscles (Table 4). The relative SRA of protein-bound leucine in individual tissues compared with the liver increased ($P < 0.05$) with bST treatment in all muscle tissues (Table 4). Values ranged from 0·07–0·19 in muscle tissues to 2·0 or more in tissues from the small intestine.

Range of tissue fractional synthesis and absolute synthesis

The k_{smax} value was calculated for the soluble fraction of muscles and was not substantially different from that for the insoluble fraction (Table 5). Means for the k_{smax} (%/d) in the soluble fraction of muscles for bST and placebo treatments respectively were: biceps femoris 2·0, 1·8 (SE 0·2); longissimus dorsi 2·4, 2·2 (SE 0·1); semintendinosus 1·8, 1·6 (SE 0·1); gastrocnemius 2·6, 2·2 (SE 0·2); triceps brachii 2·7, 2·4 (SE 0·2). All subsequent calculations on muscle used k_s values from the insoluble muscle fraction. The proportion of muscle protein in the two fractions, averaged across all muscles, was 79% in the insoluble and 21% in the soluble fraction. Approximately 6% of total protein is probably stromal protein (Helander, 1957) which would precipitate with the myofibrillar fraction.

The k_{smin} and k_{smax} were calculated for the insoluble fraction of muscle by use of equation 2 and expressed as %/d (Table 5). The bST increased ($P < 0.05$) k_{smin} in all muscle tissues

Table 4. *Tissue free leucine concentration, relationship of tissue protein-bound leucine and tissue free leucine, and relative specific radioactivities (SRA) of free and protein-bound leucine in tissues from steers injected with placebo or bovine somatotropin (bST)*†

Variable	Treatment‡	Liver	Biceps femoris	Longissimus dorsi	Semitendinosus	Gastrocnemius	Triceps brachii	Duodenum	Ileum	
									Mean	SE
Tissue free leucine (nmol/g)	bST	267	97	101*	81*	86*	91*	792	619	260
	Placebo	297	126	141	130	127	138	526	568	233
	SE	20	14	10	12	10	13	262	—	—
	Tissue mean	282[a]	111[a]	121[a]	106[a]	107[a]	114[a]	659[b]	591[b]	—
Protein-bound leucine: tissue free leucine	bST	414	865	780*	1083	1005*	1023	171	195	42
	Placebo	373	704	609	765	701	736	156	151	37
	SE	25	97	52	108	86	106	38	—	—
	Tissue mean	394[a]	784[b, f]	695[d, e, f]	924[b]	853[b, e]	879[b]	164[c]	170[c]	—
Tissue free leucine SRA: plasma leucine SRA	bST	0·33	0·56	0·51	0·52	0·53	0·56	0·22	0·21	0·04
	Placebo	0·39	0·52	0·49	0·49	0·53	0·53	0·25	0·25	0·03
	SE	0·04	0·04	0·02	0·03	0·03	0·03	0·03	—	—
	Tissue mean	0·36[a]	0·54[b]	0·50[b]	0·50[b]	0·53[b]	0·54[b]	0·23[c]	0·24[c]	—
Tissue-bound leucine SRA: liver-bound leucine SRA	bST	—	0·15*	0·16*	0·11*	0·17*	0·19**	2·7	2·0	0·1
	Placebo	—	0·09	0·11	0·07	0·12	0·13	2·2	1·6	0·1
	SE	—	0·01	0·01	0·01	0·01	0·01	0·3	—	—

[a,b,c,d,e,f] Mean values in horizotal rows (tissue mean across treatment) with different superscript letters differed significantly: $P < 0·05$.

Treatment means within each tissue differed significantly: $*P < 0·05$, $**P < 0·01$.

† Plasma leucine SRA (disintegrations/min (dpm) per nmol) averaged 23·0 and 24·0 (SE 1·6) for bST and placebo treatment respectively. Liver-bound leucine SRA (dpm/nmol) averaged 0·44 and 0·51 (SE 0·05) for bST and placebo treatment respectively.

‡ n 5 per treatment except for ileum with n 4 for bST and n 5 for placebo treatment.

Table 5. *Range of the fractional rate of tissue protein synthesis* (k_s) *in steers injected with placebo or bovine somatotropin (bST)*†

Tissue	Minimum k_s (%/d)			Maximum k_s (%/d)		
	bST	Placebo	SE‡	bST	Placebo	SE‡
Liver	8·1	8·7	0·8	27·2	24·4	4·5
Biceps femoris	1·2**	0·6	0·1	2·3*	1·6	0·2
Longissimus dorsi	1·2*	0·9	0·1	2·4	2·1	0·2
Semitendinosus	0·9	0·7	0·1	1·9	1·5	0·1
Gastrocnemius	1·3*	1·0	0·08	2·6*	2·0	0·2
Triceps brachii	1·5*	1·1	0·08	2·8**	2·2	0·09
Duodenum	21·0	18·5	1·4	109·0	88·0	14
Ileum						
Mean	14·5	13·7		72·8	62·4	
SE	1·1	1·0		8·8	7·9	

Treatment means within each tissue differed significantly: * $P < 0·05$, ** $P < 0·01$.
† For muscle, the values are for the insoluble fraction.
‡ n 5 per treatment except for ileum with n 4 for bST, n 5 for placebo.

except semitendinosus, which tended to be higher. The bST increased ($P < 0·05$) the k_{smax} in biceps femoris, gastrocnemius and triceps brachii. Treatment did not alter k_{smin} or k_{smax} in liver or small intestine.

Absolute rates of tissue protein synthesis (g/d) increased ($P < 0·05$) with bST treatment in biceps femoris, gastrocnemius and triceps brachii muscles whether calculated from k_{smin} or k_{smax} estimates (Table 6). Protein synthesized by longissimus dorsi and small intestine increased ($P < 0·05$) with bST when calculated from k_{smin} or k_{smax} only, respectively. In all tissues, protein synthesized (g/unit RNA or DNA) was not altered by treatment with bST for either method of calculation (Table 6). Treatment with bST increased ($P < 0·05$) the amount of protein synthesized per unit EBW in biceps femoris and triceps brachii with both methods of calculation, in longissimus dorsi using k_{smin} and in gastrocnemius using k_{smax}.

Protein synthesized per unit DNA was similar across tissues except for liver, using the k_{smax} estimate, or small intestine, using the k_{smin} estimate (Table 6), despite the variation in DNA content of the tissues studied (Table 2). In contrast, protein synthesized per unit RNA varied ($P < 0·05$) among tissues and was highest in the small intestine, intermediate in liver, and lowest in muscle tissues. Overall, muscle tissues had lower RNA concentration (Table 2), more total protein per unit RNA and synthesized less protein per unit RNA (Table 6) than liver or small intestine tissues.

DISCUSSION

Tissue protein and nucleic acids

Measurements of tissue protein and nucleic acids were made to suggest cellular mechanisms responsible for changes in FSR of tissue proteins, both in response to bST and in comparisons across tissue types. Both protein and DNA concentrations were lower than those reported by DiMarco *et al.* (1987) for liver and small intestine of cattle. Protein: DNA ratios among muscles did not reflect the divergence previously reported (DiMarco *et al.* 1987) and were similar to those reported by Lipsey *et al.* (1978) for semitendinosus, but higher than those reported by Eversole *et al.* (1981) for semitendinosus and Trenkle *et al.* (1978) for longissimus muscles of cattle.

Table 6. *Range of tissue protein synthesis (g/d) and in relation to tissue DNA and RNA, and empty body-weight (EBW) in steers injected with placebo or bovine somatotropin (bST)*

Variable	Type of estimate	Treatment	Liver	Biceps femoris	Longissimus dorsi	Semitendinosus	Gastrocnemius	Triceps brachii	Small intestine†
Protein synthesis (g/d)	Min	bST	68·4	9·0**	9·6*	2·6	3·0*	5·2*	87·3
		Placebo	68·3	4·4	6·8	1·9	2·3	3·9	67·0
		SE	7·6	0·9	0·8	0·3	0·2	0·3	6·5
	Max	bST	228	17·8*	18·8	5·4	5·9*	9·7**	446*
		Placebo	191	12·7	14·8	4·3	4·6	7·7	305
		SE	37	1·5	1·8	0·6	0·3	0·4	39
Protein synthesis (g/g DNA)	Min	bST	9·7	10·4	13·7	10·0	11·7	12·0	4·0
		Placebo	12·0	6·5	10·6	7·7	10·5	10·0	3·5
		SE	1·4	1·3	1·7	1·4	1·1	0·7	0·2
		Tissue mean	$10·9^{a, b, c}$	$8·5^{c}$	$12·1^{a}$	$8·9^{b, c}$	$11·1^{a, b}$	$11·0^{a, b, c}$	$3·7^{d}$
	Max	bST	33·4	21·0	26·9	21·1	23·0	22·5	20·7
		Placebo	34·3	17·3	23·1	17·2	20·7	19·6	16·5
		SE	7·4	2·4	3·5	2·2	1·6	1·4	2·8
		Tissue mean	$33·9^{a}$	$19·2^{b}$	$25·0^{b}$	$19·2^{b}$	$21·8^{b}$	$21·1^{b}$	$18·6^{b}$
Protein synthesis (g/g RNA)	Min	bST	3·4	1·5	2·3	1·8	2·4	1·4	5·1
		Placebo	4·1	0·9	2·1	1·7	2·2	1·4	4·7
		SE	0·3	0·4	0·3	0·2	0·3	0·2	0·3
		Tissue mean	$3·8^{a}$	$1·2^{d}$	$2·2^{c}$	$1·7^{c, d}$	$2·3^{c}$	$1·4^{d}$	$4·9^{b}$
	Max	bST	11·6	2·93	4·50	3·68	4·75	2·54	26·4
		Placebo	11·3	2·47	4·51	3·78	4·28	2·72	22·4
		SE	1·9	0·59	0·59	0·37	0·56	0·36	3·8
		Tissue mean	$11·5^{a}$	$2·70^{c}$	$4·50^{c}$	$3·73^{c}$	$4·51^{c}$	$2·63^{c}$	$24·4^{b}$
Protein synthesis (g/kg EBW‡)	Min	bST	0·25	0·033*	0·035*	0·009	0·011	0·019*	0·32
		Placebo	0·26	0·017	0·026	0·007	0·009	0·015	0·26
		SE	0·03	0·003	0·003	0·001	0·001	0·001	0·02
		Tissue mean	$0·26^{a}$	$0·025^{c}$	$0·030^{c}$	$0·008^{c}$	$0·010^{c}$	$0·017^{c}$	$0·29^{b}$
	Max	bST	0·84	0·065*	0·069	0·020	0·022*	0·035**	1·63
		Placebo	0·74	0·049	0·057	0·016	0·017	0·030	1·18
		SE	0·14	0·005	0·006	0·002	0·001	0·001	0·16
		Tissue mean	$0·79^{a}$	$0·057^{c}$	$0·063^{c}$	$0·018^{c}$	$0·020^{c}$	$0·032^{c}$	$1·41^{b}$

Min, minimum; Max, maximum.
Treatment means within each tissue differed significantly: * $P < 0·05$, ** $P < 0·01$.
[a, b, c, d, e] Mean values in horizontal rows (tissue mean across treatment) with different superscript letters differed significantly: $P < 0·05$.
† Fractional rates of synthesis, DNA and RNA concentration were averaged across the three sections of the small intestine to derive an average value for small intestine.
‡ Total body protein synthesis g/kg EBW, ranged from 5·17 to 6·30 for bST-injected and from 4·86 to 5·92 for placebo-injected steers.

Compositional changes in response to bST were variable and occurred only in some muscle tissues (Table 2). The changes observed reflect a decrease in the DNA unit size (protein:DNA; Waterlow *et al.* 1978) in the gastrocnemius and an increase in the capacity for protein synthesis in the longissimus dorsi (RNA:DNA).

Although the DNA unit size decreased in the gastrocnemius, the activity of DNA (g protein synthesized/d per g DNA) was unchanged in any tissue (Table 6). A trend toward an increase was apparent in all tissues except liver. Across tissues the DNA concentration appeared to be related directly to FSR, whereas the DNA unit size was inversely related (Table 2). A similar relationship between FSR and DNA unit size was reported in muscle from rats (Waterlow *et al.* 1978).

There were no changes in RNA activity (g protein synthesized/d per g RNA) in any tissue in response to bST (Table 6). Thus the effect of bST to increase protein synthesis is probably initiated at the transcriptional level. A similar effect on RNA concentration and activity was observed by Pell & Bates (1987) in lambs receiving somatotropin injections. The changes observed in the concentration of RNA and in protein:RNA are in contrast to those observed in muscle of rats as they mature (Waterlow *et al.* 1978; Lewis *et al.* 1984). Rather, the capacity for protein synthesis declines with ageing whereas, in response to bST, the cellular changes observed showed increased capacity.

Kinetic measurements

Although the free leucine concentration decreased in most muscles in response to bST, this was not reflected in a treatment effect on tissue free leucine SRA (from values in Table 4). However, bST proportionately increased the quantity of leucine in the protein-bound pool compared with the tissue free pool. The tissue free leucine relative SRA (tissue free leucine SRA:plasma SRA) was highest in muscles, intermediate in liver and lowest in intestine. This is similar to that previously observed for muscle and liver in rats (Garlick *et al.* 1973), pigs (Simon *et al.* 1982, 1983) and lambs (Davis *et al.* 1981). In pigs (Simon *et al.* 1978, 1982, 1983) the relative SRA of intestinal tissues was more similar to that of liver. In all tissues the free leucine SRA is lower than in plasma due to dilution with amino acids from protein degradation. This source increases with increased fractional rate of protein turnover in a tissue in conjunction with the size of the free amino acid pool. In addition, SRA in the small intestine and to a lesser degree in the liver reflect dilution due to exogenous amino acids (Waterlow *et al.* 1978). Per unit weight, the tissue free leucine pool was highest in the small intestine where the protein-bound:free leucine ratio was lowest (Table 4).

The relative SRA of bound leucine (tissue-bound leucine SRA:liver-bound leucine SRA, Table 4) showed a similar pattern in muscle and intestinal tissue to that observed by Simon *et al.* (1983). The treatment response in muscle tissue suggests a greater stimulation by bST in this tissue relative to liver. An increase in tissue weight as a percentage of EBW in the intestine (Table 3) and amount of protein synthesis per unit EBW in some muscles (Table 6) support this concept also. This is predominantly with regard to synthesis of liver tissue protein only because total protein synthesis (tissue+export) was not measured, although probably some of the labelled protein was destined for export.

The FSR in specific tissues is presented as a range because of uncertainty in the SRA of the true precursor pool (leucyl tRNA). The fact that the SRA of both plasma and tissue free leucine was not changed by treatment with bST supports the assumption that the SRA of leucyl tRNA maintained a similar relationship to the $k_{s\min}$ and $k_{s\max}$ precursor estimates respectively. Consideration of precursor SRA to estimate tissue protein synthesis and existence of intracellular compartments have been discussed (for example, Fern & Garlick, 1973; Airhart *et al.* 1974; Khairallah *et al.* 1977; Everett *et al.* 1981). Because of the complexity of this issue, it would be misleading to present a single estimate only.

The range in k_s (Table 5) is greater for liver and small intestine than for muscle tissues due to the smaller free leucine relative SRA in these tissues (Table 4). From the k_{smin} and k_{smax} values, estimates of the half-life of tissue proteins range from 2·5–8·6 d in liver, 25–115 d in muscle and 0·6–5·1 d in tissues of the intestine. Regardless of method, the general interpretation is that exogenous bST increases k_s in muscle tissues. The increase ranged from 14 to 44% for k_{smax} and from 29 to 100% for k_{smin}. Pell & Bates (1987) used a flooding-dose technique and reported an increase in FSR of non-collagen protein in biceps femoris (30%) and semitendinosus (6%, not significant) muscles from lambs in response to bST. Nnanyelugo & Chatterjee (1985) observed an increase in FSR in gastrocnemius muscle when ST was given to restricted-fed rats and compared with restricted-fed controls. Millward *et al.* (1976) suggested that the primary regulation of protein content in skeletal muscle was through alterations in protein synthesis.

Although FSR was not affected in intestinal tissue, the absolute amount of protein synthesized per d increased (Table 6). Thus, liver is the only tissue measured where neither FSR nor absolute rate of protein synthesis increased in response to bST. This conclusion is based predominantly on protein retained in the tissue and does not include export proteins.

Our values for FSR (Table 5) are similar to those reported for growing heifers (Lobley *et al.* 1980) and mature lambs (Bryant & Smith, 1982) and about half of those reported for muscle of younger lambs (Davis *et al.* 1981; Pell & Bates, 1987) and pigs (Garlick *et al.* 1976; Simon *et al.* 1982). The FSR in muscle from preruminant lambs was 22%/d (Attaix *et al.* 1988). The FSR in muscle of young rats (Garlick *et al.* 1973; Lewis *et al.* 1984) was four to seven times higher than that reported for cattle. FSR in liver and intestine varied less among species than that in muscle.

We chose to sample a variety of muscles to determine whether there was variation in muscle response. Variation in FSR among muscles was reported in sheep (Bryant & Smith, 1982; Hunter *et al.* 1987) as was variation in response to bST (Pell & Bates, 1987). The present study does not give strong support to a differential protein synthetic response to bST among muscle tissues (Tables 5 and 6) except for the semitendinosus. Lack of response in semitendinosus muscle is in agreement with findings obtained in sheep in response to bST (Pell & Bates, 1987). Goldberg (1967) reported increased protein synthesis in red muscle fibres compared with white muscle fibres. The semitendinosus probably had the highest percentage of α_w fibres of the muscles sampled (Suzuki *et al.* 1976).

The relative rate of protein turnover in different body tissues and the subsequent impact on whole-animal protein turnover can be expressed in various ways. For example, liver, small intestine, biceps femoris and longissimus dorsi all make a similar contribution to EBW (Table 3), while the amount of protein synthesized per unit EBW is greatest in small intestine, intermediate in liver and lowest in the muscle tissues (Table 6). Based on an estimate for total muscle mass (Table 7), the minimum contribution of muscle to whole-body protein synthesis was 14–18% and the maximum contribution was 26–29%. These maximum estimates for muscle (26–29%) and for liver (13%) are slightly higher than those obtained by Lobley *et al.* (1980) in cattle, but similar to estimates in young lambs obtained with the flooding-dose technique (Attaix *et al.* 1988). The high contribution of tissues other than muscle to total protein synthesis emphasizes the need to consider regulation of protein turnover in non-muscle tissues as well as muscle tissues when studying regulation of protein turnover during growth.

Findings reported in the present paper, demonstrating an increase in FSR (muscle) or absolute synthesis rate (intestine), support previous indirect measurement of an increase in whole-body protein synthesis in response to exogenous bST. Both the secretion rate and metabolic clearance rate of ST decline with age (weight) in growing cattle (Trenkle and

Table 7. *Tissue protein synthesis as a percentage of whole-body protein synthesis, calculated from both minimum and maximum fractional rates of tissue protein synthesis* (k_s), *in steers injected with placebo or bovine somatotropin (bST)*†

	Minimum k_s			Maximum k_s		
Tissue	bST	Placebo	SE	bST	Placebo	SE
Liver	4·8	5·4	0·5	13·0	12·6	2·1
Muscle‡	18·2*	14·3	0·9	29·4	25·7	1·6
Small intestine	6·2	5·3	0·4	25·8	20·5	2·8
Total	29·2	25·0	—	68·2	58·8	—

Treatment means within each tissue differed significantly: * $P < 0{\cdot}05$.

† Minimum whole-body protein synthesis (g/d) in these steers was calculated from:

$$\frac{(\text{leucine irreversible loss} - \text{leucine oxidation, mmol/min}) \times 1440 \text{ min/d} \times 131 \text{ mg leucine/mmol}}{66 \text{ mg leucine/g protein}},$$

see Eisemann *et al.* (1989). Maximum whole-body protein synthesis was estimated from the minimal value by assuming the constant-infusion method underestimated leucine flux by 18% (Reeds *et al.* 1980).

‡ Calculated from average minimum k_s or maximum k_s values and average protein concentration in the five muscles sampled. Muscle weight was estimated as 40% of empty body-weight (Lobley *et al.* 1980).

Topel, 1978). The FSR in muscle tissue (Waterlow *et al.* 1978; Lewis *et al.* 1984) decreases with age to a greater extent than in liver (Waterlow *et al.* 1978; Goldspink & Kelly, 1984) or intestinal (Goldspink *et al.* 1984) tissues in the rat. It is possible that a greater response in FSR in muscle than the other two tissues in response to exogenous ST would occur both due to a greater decline in FSR in this tissue or a selective response to bST, or both. Considering FSR and absolute synthesis, the liver is the only tissue that did not increase in response to bST, and yet the weight of the liver increased. This may reflect both less stimulation of protein synthesis by bST and a change in degradation of protein, and suggests that response in the liver may differ from that in muscle and small intestine.

The authors would like to express appreciation to Dr R. F. Olsen and The Upjohn Company for donating the somatotropin used in this study. They thank J. Grinsted, J. Haley and the Animal Operations Staff for care and feeding of the steers; and L. Colbert, T. Elsasser, C. Felber, D. Hucht, A. Kozak, B. Lee, M. Pello, J. Ward, B. Phillips, E. Williams and J. Wray for technical assistance.

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Printed in Great Britain