

The effect of a cold environment on protein and energy metabolism in calves

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Eleven Holstein bull calves 35 d of age were assigned to one of three treatment groups: (1) W72, warm environment (20°), 72 g feed/kg body weight (BW)^{0.75} per d, (2) C72, cold environment (–5°), 72 g feed/kg BW^{0.75} per d, or (3) C90, cold environment (–5°), 90 g feed/kg BW^{0.75} per d. Fractional synthesis rates (FSR) of protein in the rumen wall, rumen papillae, omasum, duodenum, kidney, liver, heart, *longissimus dorsi*, *biceps femoris* and skin were determined following a continuous infusion of [³H]phenylalanine. Phenylalanine flux was elevated in both groups of cold-adapted calves. FSR of protein in the two muscles and skin were reduced along with N retention in the calves in the C72 group compared with the other two groups. Muscle protein degradation, estimated from urinary N²-methylhistidine excretion, tended to be elevated in both groups of cold-adapted calves. Reduced protein synthesis and increased protein degradation in the C72 group contributed to reduced muscle protein gain. It appears that when feed intake is limited in cold-adapted animals, muscle and skin have a lower priority for nutrients than other organs and tissues, resulting in reduced protein synthesis. It seems unlikely that thermogenesis due to enhanced protein synthesis contributed to the increased heat production in the cold.

Cold environment: Protein synthesis: Protein metabolism: Energy metabolism: Calves

In regions where temperatures fall far below freezing in the winter months, animals may experience significant energy losses due to the cold environment. Cold-induced thermogenesis is a homeostatic response permitting animals to increase heat production using substrates mobilized from body tissues or arising from dietary metabolizable energy. Since net protein deposition is positively related to the supply of metabolizable energy (Reeds & Fuller, 1983), it is likely that metabolizable energy available for protein deposition will be reduced by the requirements for thermogenesis. However, for animals with unlimited access to feed, increased intake often occurs during chronic cold exposure and this may compensate partially for the increased energy requirement. Protein turnover is a substrate cycle which accounts for perhaps 20% of thermoneutral heat production (Newsholme, 1987). One might predict that increased rates of protein turnover would contribute to cold-induced thermogenesis. Therefore, protein metabolism in cold-adapted animals would be expected to be influenced by the requirement for thermogenesis as well as the dietary energy level.

Relatively few studies have examined the effect of environmental temperature on the rate of protein turnover, particularly in domestic species. Aoyagi *et al.* (1988) found that in *ad lib.*-fed chicks exposed to 22° whole-body protein synthesis and degradation were increased compared with those exposed to 30°. Lindsay *et al.* (1988) showed that tyrosine catabolic rate was elevated in young restricted-fed pigs acclimated to 10° compared with others

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exposed to 35°, although environment did not influence rates of whole-body or muscle protein synthesis. Thompson *et al.* (1987) showed that urinary excretion of N⁷-methylhistidine was 35% higher in restricted-fed calves acutely exposed to -10° than in calves at 18°, suggesting increased myofibrillar proteolysis in the cold. The results cited previously are in some cases contradictory, perhaps due to the variety of temperature ranges, species and feeding regimens employed.

We hypothesized that increased rates of tissue protein turnover may contribute to heat production in cold-adapted calves and that feed intake may affect this response. We also hypothesized that responses may be tissue-specific since their functions and priority for available nutrients vary widely. The objectives of the present study were to examine the effect of cold-adaptation and two levels of feed intake on the whole-body phenylalanine flux and the fractional synthesis rate (FSR) of protein in different tissues in growing calves, as well as to estimate muscle protein degradation.

MATERIALS AND METHODS

Animals and management

Twelve Holstein bull calves 35 d of age housed in individual metabolism crates in temperature-controlled chambers were randomly assigned to one of three treatment groups: (1) W72, warm environment (20°), 72 g feed/kg body weight (BW)^{0.75} per d, (2) C72, cold environment (-5°), 72 g feed/kg BW^{0.75} per d, or (3) C90, cold environment (-5°), 90 g feed/kg BW^{0.75} per d. Feed was weighed on an as-fed basis. One calf in the C90 group developed a severe infection and was removed from the experiment. The lower level of feed intake was designed to meet National Research Council (1978) requirements at thermoneutrality while the higher intake of the C90 group was designed to approximate the usual response in *ad lib.* intake during adaptation to a cold environment (Fuller, 1965). Calves were adapted to treatments for 21 d during which time a pelleted lucerne (*Medicago sativa*)-barley ration containing 876 g dry matter (DM)/kg, 169 g crude protein (N × 6.25; CP)/kg DM and 16.3 MJ gross energy/kg DM was fed every 2 h from an automatic feeder in order to achieve a steady-state in nutrient absorption from the small intestine. The weight of any unconsumed feed was recorded each morning. Calves were weighed weekly and the daily feed allotment adjusted accordingly.

Balance trial

Starting at 49 d of age, total collection of urine and faeces was undertaken for seven consecutive days. Bacterial growth in the urine was inhibited by 100 ml 6 M-HCl, which also kept the final pH of the urine below 2, in order to minimize ammonia losses. A 20 ml portion of urine was taken daily for N⁷-methylhistidine determination. A pooled sample of urine or faeces comprised 10% of daily samples. CP in urine, faeces and feed was determined by the Kjeldahl method (Association of Official Analytical Chemists, 1984).

During the balance trial, heat production of each calf was measured once for an 8 h period at 52, 53 or 54 d of age by indirect calorimetry in an open-circuit system as described by Young *et al.* (1975). Calves were adapted to wearing a box-like hood and during the measurement of O₂ consumption feed was given every 2 h through a port in the hood. O₂ concentration in the incoming and outgoing air was continuously monitored by a dual-channel paramagnetic analyser (Servomex Model OA184; Taylor Servomex, Crowborough, UK) and the respiratory gas exchange system was calibrated according to the procedure of Young *et al.* (1984). Metabolic rate was calculated using McLean's (1972) equation.

Continuous infusion procedure

At 56 d of age each calf was fitted with two catheters, a 0.5 m infusion catheter in the right jugular vein and a 0.1 m sampling catheter in the left jugular vein. At 57 d of age each calf received a continuous infusion of L-[ring-2,6-³H]phenylalanine (Amersham Corp., Oakville, Canada) in sterile physiological saline (9 g NaCl and 10 mmol phenylalanine/l) at a rate of 30 ml/h (approximately 325 μ Ci/h) for 8 h via a peristaltic pump (Pharmacia Peristaltic Pump P-3 with 2.1 mm i.d. silicon tubing; Pharmacia Fine Chemicals, Uppsala, Sweden). Labelled phenylalanine was administered via a continuous infusion because a single flooding dose would have been prohibitively expensive for animals of the size used in the present experiment.

Tissue and blood sampling

Blood samples were collected in heparinized test-tubes (140 USP units heparin sodium; Allen and Hanburys, Toronto, Canada) before and every 20 min during the infusion and placed on ice. Plasma was removed by centrifugation (570 g for 15 min at 4°) within 2 h of sampling and stored at -70° until analysed.

At the conclusion of the infusion each calf was anaesthetized with thiopentone sodium (Intraval sodium, 0.38 ml/kg body-weight; M.T.C. Pharmaceuticals, Mississauga, Canada) and killed by exsanguination. Approximately 5 g tissue from the rumen wall, rumen papillae, omasal leaf, duodenum, kidney, liver, heart, *longissimus dorsi*, *biceps femoris* and perineal skin were immediately removed, rinsed with cold sterile saline if necessary, and placed on ice. The *longissimus dorsi* and *biceps femoris* muscles were selected because they are large and accessible for obtaining samples quickly from approximately the same area in different animals. In both muscles the α W-fibre type accounted for just over 50% of the total fibres with the remaining percentage divided approximately equally between the α R- and β R-fibre types (Johnston *et al.* 1981). Samples were weighed, minced and homogenized in 10 ml ice-cold 0.3 M-HClO₄ with a Polytron homogenizer (Brinkman Instruments, Rexdale, Canada). The homogenizer blades were rinsed with an additional 5 ml (10 ml for skin) of ice-cold 0.3 M-HClO₄. The homogenate was centrifuged at 2300 g to separate the protein pellet from the acid supernatant fraction. The protein pellet was washed and re-centrifuged twice with 10 ml ice-cold 0.3 M-HClO₄ to remove any free amino acids and lyophilized. Supernatant fractions, lyophilized pellets, and infusate portions were frozen at -50° until analysed. The supernatant fraction was assumed to represent the tissue intracellular phenylalanine fraction and the pellet to represent the tissue protein-bound phenylalanine fraction. Total weights of tissues and organs were determined following their complete dissection.

Analytical techniques

All chemicals were purchased from Sigma Chemical Co. (St Louis, USA) unless otherwise noted.

Specific radioactivity of phenylalanine in tissues and plasma. To determine the specific radioactivity of phenylalanine it was first converted by enzymic decarboxylation to β -phenylethylamine. A modification of the method of Suzuki & Yagi (1976), as outlined by McAllister (1987), was used.

For analysis of the protein-bound fraction, approximately 25 mg of the lyophilized protein pellet was hydrolysed in 3 ml 6 M-HCl for 24 h at 110°, dried in a vacuum centrifuge (Savant Speed-vac Concentrator Model SVC-200H; Savant Instruments Inc., Farmingdale, USA), and resuspended in 2 ml 0.5 M-sodium citrate. Portions of plasma (2.5 ml) were deproteinized by addition of 0.7 ml ice-cold 1.5 M-HClO₄ and centrifugation (3000 g at 4° for 15 min). Following neutralization of 2 ml deproteinized plasma with 0.6 ml saturated tripotassium citrate, samples were centrifuged again. This neutralized deproteinized plasma

was used for isolation of β -phenylethylamine. Supernatant fraction samples (2 ml) were also neutralized by addition of 0.5 ml saturated tripotassium citrate and treated in the same way as deproteinized neutralized plasma.

Radioactivity of β -phenylethylamine in the final extract was determined using liquid-scintillation spectrometry, and its concentration was analysed using high-performance liquid chromatography (HPLC) according to the method outlined by McAllister (1987). Ethanolamine (Fisher Scientific Co., Fair Lawn, USA) was used as an internal standard, and both standards and samples were derivatized with *o*-phthalaldehyde according to the method of Jones & Gilligan (1983).

Determination of free and protein-bound phenylalanine in tissues. The concentration of free phenylalanine in the intracellular fraction or bound in the protein fraction was determined by HPLC analysis. For free phenylalanine the analysis was done following neutralization of 0.5 ml of the tissue-homogenate supernatant fraction with 0.5 ml saturated potassium borate. For protein-bound phenylalanine 20 mg of the lyophilized protein pellet was hydrolysed in 3 ml 6 M-HCl. Samples were processed as described previously for β -phenylethylamine. These values were used for calculating values for R, protein-bound:intracellular free phenylalanine concentrations, as described by Garlick *et al.* (1973).

Determination of urinary N⁷-methylhistidine. Urine was analysed for N⁷-methylhistidine as described by Nakamura & Pisano (1976) and Wassner *et al.* (1980) with the following modifications. To 0.2 ml urine or standards (0.5 mM-N⁷-methylhistidine) 0.2 ml internal standard (0.5 mM-histidinol) was added. Samples were deproteinized with 0.2 ml 3.0 M-HClO₄ and centrifuged at 2300 g at 4° for 15 min. A 0.1 ml portion of deproteinized urine was mixed with 0.4 ml deionized water, 0.04 ml 1.5 M-NaOH, and 0.4 ml 0.2 M-Na₂B₄O₇ (pH 9.0). As they were vortexed, samples were derivatized with 0.25 ml of fluorescamine solution (160 mg fluorescamine/100 ml acetonitrile). After standing for a few seconds to consume excess fluorescamine, 0.4 ml 2.0 M-HCl was added to samples. Tubes were mixed, capped, and incubated in a 90° water-bath for 45 min. Following two extractions with 1.5 ml diethyl ether (Fisher Scientific Co., Fair Lawn, USA), samples were eluted from a 3 μ m reverse-phase C₁₈ column (Supelco, Inc., Bellefonte, USA) using a binary gradient; solvent A was 8 mM-ammonium acetate buffer (pH 5.0) and solvent B was acetonitrile. The gradient rose from 0.25 to 0.60 acetonitrile over 8.5 min at solvent flow-rate of 1.1 ml/min.

Protein content of tissue. Protein content of the freeze-dried pellets was determined according to the method of Hartree (1972) with bovine serum albumin as the standard. The protein content of the freeze-dried pellet was multiplied by the DM content in the fresh sample and by the weight of the fresh sample relative to that of the organ or tissue in order to determine the protein mass of individual organs and tissues.

Calculations

Plasma phenylalanine flux. The specific radioactivity (disintegrations/min (dpm) per nmol) of plasma phenylalanine was plotted *v.* sampling time for each animal, and the rise to plateau specific activity was fitted to a single exponential function using a non-linear iterative procedure (SAS Institute Inc., 1985). The equation used was:

$$S_p = S_p \max (1 - e^{-\lambda_p t}), \quad (1)$$

where S_p is the plasma phenylalanine specific radioactivity at any time, $S_p \max$ is the plateau specific radioactivity, λ_p is the rate-constant (/d), and t is time (d). Phenylalanine flux (F ; mmol/d) was calculated using $S_p \max$ from the formula:

$$F = I/S_p \max, \quad (2)$$

where I is the isotope infusion rate (dpm/d).

FSR of protein in tissues. The FSR (%/d) of protein in tissues was calculated as described by Garlick *et al.* (1973). Their assumptions for determining the value of the rate-constant describing the rise to plateau of intracellular free phenylalanine in individual tissues were also used.

In calculating the FSR of protein in tissues and organs, the intracellular free phenylalanine pool was assumed to represent the true precursor pool, phenylalanyl-tRNA, so calculated FSR are maximum estimates of the true rates (Baracos *et al.* 1991).

Absolute synthesis rate (ASR) of protein in tissues. The ASR of protein (g/d) was calculated as the product of protein content (g) and the FSR of protein in each tissue (%/d). In addition, the amount of skeletal muscle in the body was assumed to be 0.365 of BW from studies of calf body composition by Ugarte *et al.* (1974). For each calf, the product of the average protein content and the FSR of protein in the *longissimus dorsi* and *biceps femoris* muscles was used as an estimate of the ASR of protein in skeletal muscle.

Statistics

All results were analysed by one-way analysis of variance (ANOVA) and the unequal replication version of the Student–Newman–Keuls procedure for multiple comparison of means as described by Steele & Torrie (1980) using SPSS-X (SPSS Inc., 1988). Although individual standard errors are given for means, tests of significance were based on the pooled variance with 8 df from the one-way ANOVA. Linear and non-linear regressions were performed using SAS (SAS Institute Inc., 1985).

RESULTS

Calf feed intake, weight gain, heat production, and N balance

The average daily feed intake of the calves in the C90 group was about 1.20 times that of the other two groups (Table 1), and very few feed refusals were observed. Compared with the W72 group the average daily gain was reduced in the C72 group ($P = 0.025$) but not in the C90 group. Retarded body weight gain in the cold led to a significant reduction in the efficiency of use of feed for gain (Table 1; $P = 0.018$). Although heat production tended to be elevated in the cold there were no significant differences between treatments. N intake and faecal N excretion followed the same pattern as feed intake between the three treatment groups (Table 1; $P = 0.004$ and $P = 0.012$ respectively). However, urinary N excretion was elevated in the C72 group but not in the C90 group compared with the W72 group ($P = 0.024$), with the result that N retention was depressed in the C72 group compared with the other two groups ($P = 0.040$), but in the C90 group it was maintained at the same level as the W72 group. Urinary N⁷-methylhistidine excretion, an estimate of myofibrillar protein degradation, tended to be higher in both groups of cold-adapted calves (Table 1; $P = 0.098$).

Phenylalanine flux

All calves were infused with [³H]phenylalanine for 8 h and plasma phenylalanine specific radioactivity at each sampling time was pooled by group (Fig. 1). There was no effect of environment on the rate constant λ_p , with values (/d) of 43 (SE 0.9), 55 (SE 8.7), and 63 (SE 15.1) for the W72, C72, and C90 groups respectively ($P > 0.05$). Plateau plasma phenylalanine specific radioactivity was significantly higher in the W72 group compared with both groups in the cold ($P < 0.05$). In addition, the daily phenylalanine flux was elevated in both groups in the cold ($P < 0.01$), with means of 2.48 (SE 0.05), 2.82 (SE 0.11), and 3.02 (SE 0.10) mmol/d per kg BW for the W72, C72, and C90 groups respectively.

Table 1. Average body weight (BW)*, average daily intake*, average daily gain*, gain:feed (% efficiency)*, heat production†, N balance‡ and urinary N⁷-methylhistidine excretion‡ of calves adapted to two environmental temperatures

(Mean values with their standard errors)

Treatment group.....	W72		C72		C90	
	Mean	SE	Mean	SE	Mean	SE
n.....	4		4		3	
Environmental temperature (°)	20.3		-3.3		-3.3	
Body weight (kg)	68.5	3.4	61.3	3.3	72.3	5.9
Average daily intake (kg DM/d)	1.23 ^a	0.02	1.21 ^a	0.05	1.49 ^b	0.08
Average daily gain (kg/d)	0.61 ^a	0.04	0.26 ^b	0.10	0.45 ^{a,b}	0.05
Gain:feed (kg gain/kg DM)	0.497 ^a	0.035	0.215 ^b	0.079	0.302 ^b	0.026
Heat production (kJ/d per kg BW)	205.2	18.6	254.7	14.0	241.5	31.5
N balance (g N/d)						
Intake N	37.4 ^a	1.0	36.3 ^a	1.1	47.5 ^b	3.1
Faecal N	10.9 ^a	0.5	10.5 ^a	0.4	16.9 ^b	2.5
Urine N	8.5 ^a	0.5	16.2 ^b	2.4	12.7 ^{a,b}	1.2
Retained N	18.0 ^a	0.8	9.6 ^b	1.7	17.9 ^a	4.1
Urinary N ⁷ -methylhistidine excretion (μmol/d per kg BW)	1.90 ^c	0.13	2.57 ^d	0.29	2.50 ^d	0.15

DM, dry matter; W72, warm environment (20°), 72 g feed/kg BW^{0.75} per d; C72, cold environment (-5°), 72 g feed/kg BW^{0.75} per d; C90, cold environment (-5°), 90 g feed/kg BW^{0.75} per d (for details, see p. 128).

* Measured from 35 to 56 d of age.

† Measured at 52, 53, or 54 d of age.

‡ Measured from 49 to 56 d of age.

^{a,b,c,d} Means in the same row with different superscript letters were significantly different: ^{a,b} $P < 0.05$, ^{c,d} $P < 0.10$.

Fractional and absolute synthesis rates of protein in tissues

FSR of protein in muscles, skin, and kidney are shown in Fig. 2(a and b). FSR of protein in both skeletal muscles in the C72 group was about 50% of values for the other two groups ($P = 0.020$ and $P = 0.001$ for the *longissimus dorsi* and *biceps femoris* muscles respectively), while in the C90 group FSR of protein in both muscles were maintained at the same level as the W72 group. A fall in the FSR of protein in the kidney ($P = 0.015$) and the skin ($P = 0.126$) was also observed. The FSR (%/d) of protein in liver (30.3 (SE 1.3)), heart (10.3 (SE 3.6)), rumen wall (45.9 (SE 14.7)), rumen papillae (60.9 (SE 20.7)), omasum (68.0 (SE 5.7)), and duodenum (112 (SE 25.6)) were not significantly influenced by environmental treatment ($P > 0.05$).

ASR of protein in the two individual skeletal muscles and in the total skeletal musculature were depressed in the C72 group compared with the W72 group ($P < 0.03$), but not in the C90 group (Table 2), and a similar trend was seen in the skin ($P = 0.064$). ASR of protein in gastrointestinal tissues were highly variable and not affected by treatment ($P > 0.05$).

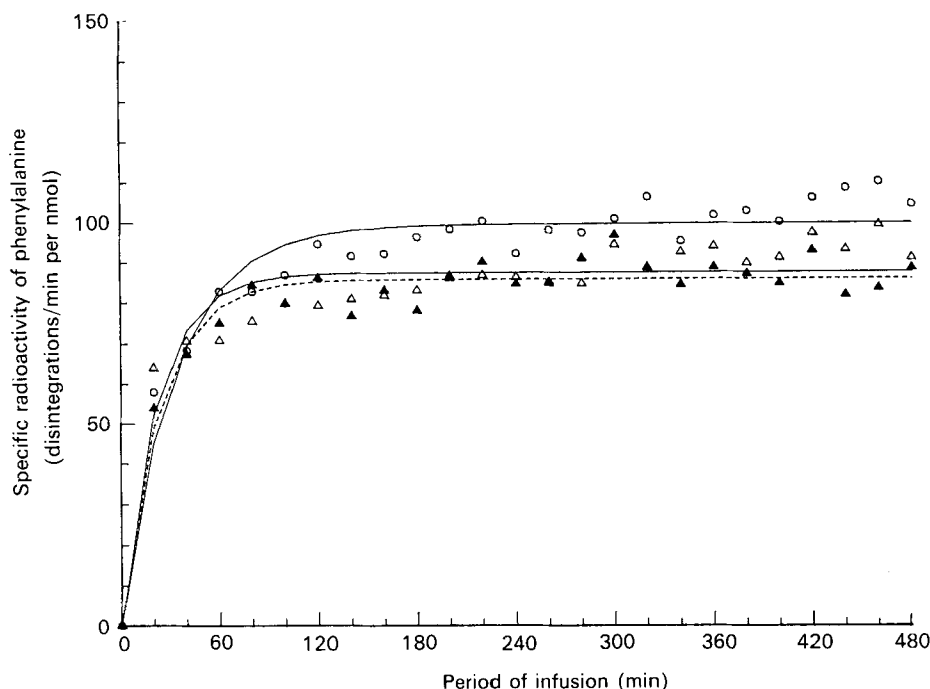


Fig. 1. The rise in plasma phenylalanine specific radioactivity during an 8 h infusion of L-[ring-2,6- ^3H]phenylalanine into calves in the W72 (○-○), C72 (△-△), and C90 (▲-▲) treatment groups. Points are means for three to four animals per group. W72, warm environment (20°), 72 g feed/kg body-weight ($\text{BW}^{0.75}$) per d; C72, cold environment (-5°), 72 g feed/kg $\text{BW}^{0.75}$ per d; C90, cold environment (-5°), 90 g feed/kg $\text{BW}^{0.75}$ per d. For details of treatments, see p. 128.

Muscle protein turnover

There was a significant positive relationship between protein intake (g N/d) and rate of protein synthesis (g N/d) in both muscle and skin in cold-adapted calves (Fig. 3; $P < 0.01$).

DISCUSSION

Methodological considerations

Use of a continuous infusion of a radioactively labelled amino acid to measure the FSR of protein in tissues has advantages as well as limitations which have been discussed in detail by several authors (Garlick *et al.* 1973; Waterlow *et al.* 1978; Loblely *et al.* 1980; Schaefer *et al.* 1986; Baracos *et al.* 1991). Briefly, calculation of the FSR of protein requires knowledge of the specific radioactivity (SRA) of the tracer amino acid in the precursor pool for protein synthesis, the amino acyl-tRNA. Usually, analytical difficulties preclude direct determination of the amino acyl-tRNA SRA, so either the intracellular or plasma free amino acid SRA is assumed to represent the SRA of the true precursor pool. Use of the former gives a maximum estimate of FSR due to dilution of the label by proteolysis (Rannels *et al.* 1982), while use of the latter gives a minimum estimate (Baracos *et al.* 1991).

Determination of FSR of protein can be influenced by other factors including intracellular recycling of label during the infusion when tissue protein turnover is rapid (Waterlow *et al.* 1978), or dilution of the intracellular tracer with unlabelled amino acid released by proteolysis following termination of the infusion. In experiments with small

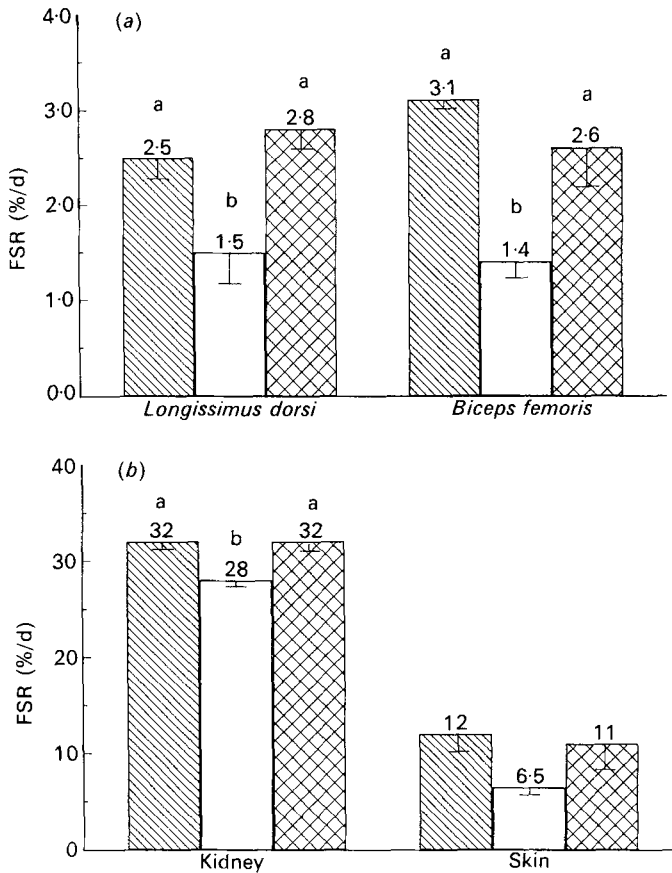


Fig. 2. The fractional synthesis rate (FSR; %/d) of protein in (a) muscle (b) kidney and skin from calves in the W72 (▨), C72 (□) and C90 (▩), treatment groups. Values are means for three to four animals per group with their standard errors represented by vertical bars. ^{a, b} Means in the same tissue with different superscript letters were significantly different ($P < 0.02$). W72, warm environment (20°), 72 g feed/kg body-weight ($BW^{0.75}$) per d; C72, cold environment (-5°), 72 g feed/kg $BW^{0.75}$ per d; C90, cold environment (-5°), 90 g feed/kg $BW^{0.75}$ per d. For details of treatments, see p. 128.

lambs, Attaix & Arnal (1987) used a flooding dose of [3 H]valine and a short labelling period to minimize the difference between intracellular and plasma SRA, as well as label recycling. Although their method is especially useful for measuring the FSR of protein in gastrointestinal tissues, the cost of label for larger ruminants is prohibitively expensive. Therefore, the continuous infusion of a tracer dose is the method of choice for estimating the FSR of protein in tissues of larger animals.

Animal performance and N balance

Depressed growth in both groups of calves in the cold (Table 1) is similar to findings reported by Ames *et al.* (1980) for lambs and feedlot steers. Although increased feed intake permitted the average daily gain of the C90 group to be intermediate to the other two groups, feed conversion efficiency was reduced. Pigs at 10° fed *ad lib.* showed similar reductions in efficiency (Fuller, 1965).

Metabolizable energy was probably diverted away from tissue growth toward heat production in the cold-adapted calves in the present experiment, as their heat production

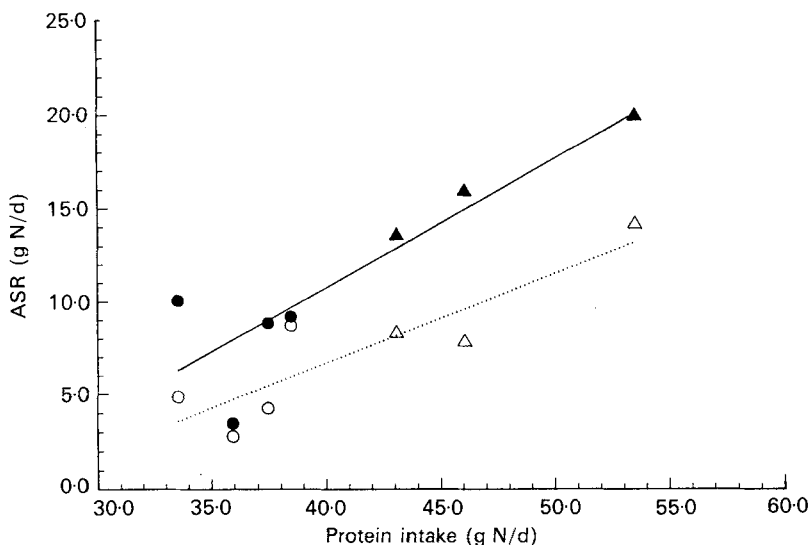


Fig. 3. The relationship between the daily intake of nitrogen (g N/d) and absolute synthesis rates (ASR) of protein in whole-body muscle and skin (g N/d) in cold-adapted calves. Points are individual values for calves in the C72 (muscle ●), skin (○)) and C90 (muscle ▲, skin △)) treatment groups. For muscle the equation of the line of best fit is: $y = 0.687$ (SE 0.158) - 16.8 (SE 6.58)x, where y is the absolute synthesis rate of protein and x is protein intake; n 7, P < 0.01, r² 0.79. For skin the equation of the line of best fit is: $y = 0.479$ (SE 0.113) - 12.5 (SE 4.71)x, where y is the absolute synthesis rate of protein and x is protein intake; n 7, P < 0.01, r² 0.78. C72, C90, cold environment (-5°), 72 and 90 g feed/kg body-weight^{0.75} per d respectively. For details of treatments, see p. 128.

Table 2. Absolute synthesis rate (ASR) of protein* (g/d) in tissues from calves adapted to two environmental temperatures (Mean values with their standard errors)

Treatment group....	W72		C72		C90	
	Mean	SE	Mean	SE	Mean	SE
n....	4		4		3	
<i>Longissimus dorsi</i>	1.96 ^a	0.34	0.80 ^b	0.20	2.38 ^a	0.55
<i>Biceps femoris</i>	2.97 ^a	0.18	1.14 ^b	0.21	2.67 ^a	0.48
All muscle†	96.5 ^a	8.4	49.2 ^b	9.3	102.3 ^a	11.5
Skin	75.0 ^c	13.6	32.1 ^d	7.8	62.6 ^c	12.6
Kidney	3.94	0.54	4.92	0.40	5.61	0.68
Liver	50.0	2.8	54.3	7.1	41.0	13.1
Heart	3.62	0.31	4.33	0.37	5.50	0.93
Rumen	61.4	16.9	67.8	4.2	87.1	22.4
Omasum	17.4	2.7	15.2	3.2	22.4	6.7
Intestine	291.2	143.1	173.4	43.9	317.7	207.8

* Calculated as described on p. 132; fractional synthesis rate of protein calculated assuming intracellular phenylalanine is the precursor pool.

† Whole-body content of muscle estimated as 0.365 of body weight (BW) (Ugarte *et al.* 1974); the fractional synthesis rate of muscle protein was the average of the two muscles sampled, assuming intracellular phenylalanine as the precursor pool.

^{a,b,c,d} Means in the same row with different superscript letters were significantly different: ^{a,b} P < 0.05, ^{c,d} P < 0.10.

W72, warm environment (20°), 72 g feed/kg BW^{0.75} per d; C72, cold environment (-5°), 72 g feed/kg BW^{0.75} per d; C90, cold environment (-5°), 90 g feed/kg BW^{0.75} per d (for details, see p. 128).

was 20% higher than that of calves in the warm environment. This increase was not significant but was similar in magnitude to the 8% increase in heat production reported for dairy-type calves adapted to 5° compared with 15° (Webster *et al.* 1978).

Reduced whole-body N retention in the C72 group compared with the W72 group suggests that more amino acids were oxidized as an energy source rather than used for protein synthesis (Lindsay *et al.* 1988). This is supported by the significant increase in urinary N excretion in the C72 group but not in the C90 group compared with the W72 group.

Phenylalanine flux

It was not possible to determine whether the elevation in phenylalanine flux in both groups of cold-adapted calves was due to an increased rate of whole-body protein synthesis or amino acid oxidation. Since protein synthesis in a variety of tissues was either unchanged or decreased in the cold-adapted calves (see below), it is unlikely that a greater proportion of the phenylalanine flux was used for protein synthesis. On the other hand, increased heat production in the cold-adapted calves could result from accelerated oxidation of energy substrates, including amino acids. Higher phenylalanine oxidation could also account for lower mean values of S_p max in the C72 and C90 groups. This hypothesis is supported by results demonstrating an elevated tyrosine catabolic rate in pigs acclimated to 10° compared with 35° (Lindsay *et al.* 1988).

FSR and ASR of protein in tissues

Major organs such as the kidney, liver and heart exhibited FSR of protein approximately threefold those reported for 373 kg steers by McBride *et al.* (1989) (see p. 132 and Fig. 2a and b); discrepancies may be attributed to differences in age between experimental animals. However, rates for muscle, skin and gastrointestinal tissues were similar to those reported for 10.5–11-month-old heifers by Lobley *et al.* (1980).

The reduced FSR of protein in skeletal muscle from calves in the C72 group agrees with findings of McAllister (1987) that the FSR of protein in *soleus* muscle was decreased in *ad lib.*-fed rats acclimated to 4°. Environmental temperature did not affect the FSR of protein in muscle from pigs fed at either a low or high level of intake and adapted to temperatures of either 10° or 35° (Lindsay *et al.* 1988). However, those results were confounded by the fact that feed intake per unit body weight was actually higher for both groups of animals at 10° than for those at 35°. Thus, feed intake may have been adequate to supply amino acid requirements for protein synthesis, even in pigs on the low level of intake at 10°. In the present experiment the FSR of protein in skin tended to mimic the pattern observed in muscle, but difficulties in sampling and homogenizing the skin elevated variability.

Depressed FSR of protein in muscle and skin along with reduced N retention in the C72 group suggest that feed intake was inadequate in this group to support lean tissue growth at the same rate as in the other two groups. The fact that a reduction in protein synthesis was observed only in muscle and skin, rather than a general decrease in all tissues, implies that those two tissues have a lower priority for limited nutrients than others (Buttery & Bryan, 1986). Allison & Wannemacher (1965) contended that during conditions of nutritional stress skin could serve as a significant labile protein reserve along with muscle, resulting in diminished protein synthesis. This concept is supported by results showing that protein synthesis in both skin and muscle was reduced in rats following a 2 d fast (Preedy *et al.* 1983). In addition, protein synthesis in both skin and muscle from the hindlimb was reduced in lactating dairy goats in negative N balance compared with their dry counterparts in positive N balance (Baracos *et al.* 1991). Therefore, skin protein may be mobilized along with muscle protein in order to meet amino acid requirements in situations

where feed intake is inadequate. Further studies on the effect of feed intake and cold-adaptation on amino acid release from skin are necessary to confirm this hypothesis.

A lack of response in liver protein synthesis to cold-adaptation in calves is supported by similar results in chicks fed *ad lib.* at 22° compared with 30° (Aoyagi *et al.* 1988) and in rats fed *ad lib.* at 5° compared with 25° (McAllister, 1987). In the present experiment the FSR of protein in the heart was also unchanged after acclimation to a cold environment, although McAllister (1987) observed that protein synthesis in the heart was elevated in rats exposed to 5° for 20 d compared with 25°. The decreased FSR of kidney protein (Fig. 2(b)) has not previously been reported in large animals exposed to cold environments.

None of the gastrointestinal tissues exhibited a response in FSR of protein to cold-adaptation, although the large variability in FSR may have masked any treatment effects. Some alteration might have been expected since results showing a faster rate of digesta passage through the gastrointestinal tract of cold-adapted sheep (Westra & Christopherson, 1976) suggest that increased enterocyte sloughing and, thus, increased enterocyte protein synthesis might occur as a result of cold-adaptation.

ASR of protein (Table 2) were mainly influenced by changes in FSR as protein mass of tissues did not vary with treatment. However, unlike the FSR, there was no significant effect of temperature on the ASR of protein in kidney.

Since none of the tissues studied exhibited an increase in the FSR or ASR of protein as a result of cold-adaptation, it is unlikely that significant changes in protein synthesis contributed to cold-induced thermogenesis in the present experiment.

Muscle protein turnover

It appears that the effect of acclimation to a cold environment on protein synthesis in muscle can be overcome by higher feed intake. Since the same diet was fed to both groups of calves in the cold, protein intake increased along with feed intake, as did the ASR of protein in muscle and skin in the cold-adapted calves (Fig. 3). The FSR of protein in the hindlimb increased in warm-acclimated lambs fed from 0.5 to 1.5 times maintenance (Garlick & Lobley, 1987), suggesting that muscle is sensitive to increments in nutrient intake. As discussed previously, skin protein synthesis may also be sensitive to nutrient intake.

Muscle protein degradation can be estimated from urinary N⁷-methylhistidine excretion subsequent to myofibrillar protein breakdown. Harris & Milne (1981) validated this method for use in cattle by showing that an intravenously injected dose of N⁷-[¹⁴CH₃]methylhistidine was rapidly and quantitatively recovered in urine. Although not all N⁷-methylhistidine comes from muscle, Nishizawa *et al.* (1979) calculated that it contributed more than 93% of the N⁷-methylhistidine contained in bovine tissues. Assuming that the mean muscle protein content of N⁷-methylhistidine in cattle is 3.5 μmol/g muscle protein (Nishizawa *et al.* 1979), muscle protein degradation was estimated to be 0.54 (SE 0.04), 0.73 (SE 0.08), and 0.72 (SE 0.04) g/d per kg BW for the W72, C72, and C90 groups respectively. Muscle protein degradation tended to be higher in both groups of calves in the cold compared with those in the warm ($P = 0.098$). These findings are supported by reports by Thompson *et al.* (1987) that urinary N⁷-methylhistidine excretion increased in restricted-fed calves acutely exposed to -10° compared with 18°, and by Brown *et al.* (1984) that muscle protein degradation was elevated in *ad lib.*-fed rats exposed to 4°. Elevated protein degradation in the cold would likely make amino acid-carbon skeletons available for oxidation and gluconeogenesis, as discussed previously.

Depressed muscle protein synthesis (expressed per kg BW) combined with a trend towards accelerated muscle protein degradation resulted in reduced muscle protein gain in the C72 group compared with the other two groups (0.86 (SE 0.10), 0.08 (SE 0.08) and 0.70

(SE 0.15) g/d per kg BW for the W72, C72 and C90 groups respectively ($P = 0.002$). Muscle protein deposition efficiency, indicated by the ratio protein gain:protein synthesized (Reeds *et al.* 1980), was 0.614, 0.091 and 0.495 in the W72, C72 and C90 groups respectively. Apparently, protein deposition in the C72 group was less efficient than in the other two groups.

Conclusion

Muscle and skin demonstrated marked reductions in protein synthesis following cold-adaptation. Substrate availability seems to play a major role in the response to a cold environment, as additional feed intake in the cold largely prevented the observed effects. Regardless of level of feed intake, phenylalanine flux was elevated in cold-adapted calves. Increased rates of protein degradation in cold-adapted calves probably make amino acids available for gluconeogenesis and oxidation and may also contribute to thermogenesis. However, elevated FSR of protein did not contribute to cold-induced thermogenesis under the conditions in the present study.

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