

## The effects of breed and level of nutrition on whole-body and muscle protein metabolism in pure-bred Aberdeen Angus and Charolais beef steers

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Eighteen pure-bred steers (live weight 350 kg) from each of two breeds, Aberdeen Angus (AA) and Charolais (CH), were split into three equal groups (six animals each) and offered three planes of nutrition during a 20-week period. The same ration formulation was offered to all animals with amounts adjusted at 3-week intervals to give predicted average weight gains of either 1.0 kg/d (M/M group) or 1.4 kg/d (H/H group). The remaining group (M/H) were offered the same amount of ration as the M/M group until 10 weeks before slaughter when the ration was increased to H. Data on animal performance, carcass characteristics and fibre-type composition in skeletal muscle are presented elsewhere (Maltin *et al.* 2000; Sinclair *et al.* 2000). On three occasions (17, 10 and 2 weeks before slaughter) the animals were transferred to metabolism stalls for 1 week, during which total urine collection for quantification of N<sup>7</sup>-methylhistidine (N<sup>7</sup>-MeH) elimination was performed for 4 d. On the last day, animals were infused for 11 h with [<sup>2</sup>H<sub>5</sub>]phenylalanine with frequent blood sampling (to allow determination of whole-body phenylalanine flux) followed by biopsies from *m. longissimus lumborum* and *m. vastus lateralis* to determine the fractional synthesis rate of mixed muscle protein. For both breeds, the absolute amount of N<sup>7</sup>-MeH eliminated increased with animal age or weight ( $P < 0.001$ ) and was significantly greater for CH steers, at all intake comparisons, than for AA ( $P < 0.001$ ). Estimates of fractional muscle breakdown rate (FBR; calculated from N<sup>7</sup>-MeH elimination and based on skeletal muscle as a fixed fraction of live weight) showed an age (or weight) decline for M/M and H/H groups of both breeds ( $P < 0.001$ ). FBR was greater for the H/H group ( $P = 0.044$ ). The M/H group also showed a lower FBR for the first two measurement periods (both at M intake) but increased when intake was raised to H. When allowance was made for differences in lean content (calculated from fat scores and eye muscle area in carcasses at the end of period 3), there were significant differences in muscle FBR with intake ( $P = 0.012$ ) but not between breed. Whole-body protein flux (WBPF; g/d) based on plasma phenylalanine kinetics increased with age or weight ( $P < 0.001$ ) and was similar between breeds. The WBPF was lower for M/M compared with H/H ( $P < 0.001$ ) based on either total or per kg live weight<sup>0.75</sup>. Muscle protein fractional synthesis rate (FSR) declined with age for both breeds and tended to be higher at H/H compared with M intakes (intake  $\times$  period effects,  $P < 0.05$ ). Changing intake from M to H caused a significant increase ( $P < 0.001$ ) in FSR. The FSR values for AA were significantly greater than for CH at comparable ages ( $P = 0.044$ ). Although FSR and FBR responded to nutrition, these changes in protein metabolism were not reflected in differences in meat eating quality (Sinclair *et al.* 2000).

**Protein turnover: N<sup>7</sup>-methylhistidine: Stable isotopes: Cattle**

**Abbreviations:** AA, Aberdeen Angus; CH, Charolais; FBR, fractional breakdown rate; FSR, fractional synthesis rate; IRL, irreversible loss rate; LL, *m. longissimus lumborum*; MeH, methylhistidine; VL, *m. vastus lateralis*; WBPF, whole-body protein flux.

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Protein gain, both within individual tissues and across the whole animal, is the difference between the opposing mechanisms of protein synthesis and degradation. In terms of improved animal growth, any strategy which increases this difference is advantageous and this had led to interest in procedures which reduce the rate of tissue breakdown, such as use of pharmacological agents (e.g. Dawson *et al.* 1991; Hayden *et al.* 1992; Boisclair *et al.* 1994) or genetic selection (e.g. McCarthy *et al.* 1983). Although lowered protein degradation is attractive in terms of energy saving, there may be accompanying disadvantages. For example, if aspects of meat quality (e.g. tenderness) are related to proteolytic activity present in muscle tissue at the time of slaughter then it may be advantageous to promote conditions of elevated proteolysis *in vivo* just prior to slaughter. The simplest, and most acceptable, way to achieve greater rates of proteolysis *in vivo* is by increasing intake, which has been shown to elevate both muscle protein synthesis and protein breakdown across a range of species (e.g. sheep, Lobley *et al.* 1992; cattle, Boisclair *et al.* 1993; pigs, Seve *et al.* 1993). Husbandry strategies, based on lower resource inputs but with a higher plane of nutrition in the period prior to slaughter, may yield meat of similar eating quality to that obtained from animals maintained on higher intakes and faster rates of live-weight gain throughout the rearing period. The effectiveness and timing of such strategies may differ between large- and small-framed animals and those considered 'early' as opposed to 'late' maturing (McCarthy *et al.* 1983).

A study was therefore instigated to examine the influence of nutritional and genetic factors on final meat quality in cattle. The model contained three breeds; pure-bred Aberdeen Angus (AA), Charolais (CH) and Holstein steers, with groups offered intakes to either match (0.8–1.0 kg live-weight gain/d) or surpass (1.4 kg/d) the UK national herd averages for growth during the 20 weeks prior to slaughter. The intakes of one group from each breed were adjusted to increase predicted growth rates from 1.0 to 1.4 kg/d in the 10 weeks preceding slaughter. The consequences for carcass composition, meat quality, muscle fibre-type size and chemical composition are described in companion papers (Maltin *et al.* 2000; Sinclair *et al.* 2000). The present paper details the response in whole-body protein flux (WBPF) and muscle protein turnover to intake, plus the effects of age and breed on these variables. These findings are then briefly compared with the responses in eating quality of the meat obtained by the different nutritional strategies. Due to cost constraints, the protein metabolism measurements were restricted to the AA and CH breeds.

## Materials and methods

### *Main experimental details*

The animals and diets used, plus the main experimental details have been described elsewhere (Sinclair *et al.* 2000). Briefly, eighteen (plus two spare) AA and eighteen (plus two spare) CH beef steers were subdivided into three groups of six, matched for similar average weight for each breed. A similar group of Holstein steers were also examined but, for these animals, kinetic measurements of the type

described in this study were not performed. The AA and CH steers were allocated to one of three dietary intakes for 20 weeks: (1) M/M, to yield predicted average growth rates of 1.0 kg/d; (2) H/H, for predicted live-weight gains of 1.4 kg/d; (3) M/H, which entailed M for 10 weeks followed by H for 10 weeks. The treatments were staggered to start at weekly intervals over 6 weeks so that one animal from each group was allocated to the study every 7 d. The pelleted ration was based on nutritionally-improved straw–barley–high-protein soyabean–molassed sugar-beet pulp (10:50:15:25, by weight) and contained 18.8 g N and 12.5 MJ metabolisable energy per kg DM. In addition, 1 kg straw was provided daily. During the growth phases, aspects of protein metabolism were studied at three intervals, 17, 10 and 2 weeks before slaughter. Thus, for the M/H group, the second examination was during the week before the switch from M to H intake. All animals were slaughtered and meat quality characteristics and muscle histochemical features examined (Maltin *et al.* 2000; Sinclair *et al.* 2000).

For the protein metabolism measurements, one animal from each group (six animals, one each of groups M/M, H/H and M/H, from each breed) was transferred each week (on day 0) for 6 weeks from the byre to the metabolism room where they were tethered in individual raised stalls and their pelleted ration supplied in paper bags (each containing 500 g) placed along the length of 24 h continuous belt feeders. Feed was supplied in the bagged batches to avoid the over-frequent provision of small quantities of pellets from the continuous feeders as that was found to cause behavioural responses. All animals had previously been trained to these conditions for at least one 3 d period. On day 3 in the stalls, animals had two temporary polyvinyl-chloride catheters (0.8 mm inner diameter × 1.2 mm outer diameter; Critchley Electrical Products, Auburn, NSW, Australia) inserted into an external jugular vein. One catheter (for infusion) was inserted 45 cm, close to the heart, and the other was inserted above this (for sampling) a distance of 25 cm. At the same time, samples of tissue (approximately 300–500 mg) from the *m. longissimus lumborum* (LL) and *m. vastus lateralis* (VL) were removed by scalpel excision under local anaesthesia. These provided samples for background protein-bound and free phenylalanine abundance, as did a plasma sample taken on day 5. Throughout the study, within muscles and between sides all biopsies were taken within 12 cm of each other.

On day 6, animals were infused for 11 h (at 40 g/h) with a solution of 24 mM-[<sup>2</sup>H<sub>5</sub>]phenylalanine (Mass Trace, Woburn, MA, USA), prepared in sterile 0.15 M-NaCl. The start of infusion for the six animals was staggered, by means of domestic time clocks, at hourly intervals between 00.00 and 05.00 hours. Blood samples (6 ml) were taken into 0.2 ml heparin (50 IU/ml) at hourly intervals from 07.00 hours (occasionally 06.00 hours) for all animals. After 10 h of infusion biopsies were again taken from the same muscles (from the opposite side but of similar position to the 'background' sample), following which another blood sample was taken (at approximately 11 h of infusion). For the two subsequent periods of measurement, the sides for jugular catheterisation, plus pre-infusion and infusion biopsies were alternated.

For the 96 h between days 3 and 6 in the stalls, total urine collections were made through a grid in the centre of the stall and collected by gravity into 4 M-H<sub>2</sub>SO<sub>4</sub>, sufficient to maintain a pH < 3. Urine collections were well-mixed and weighed daily and subsamples (2 × 20 ml) stored at -20°. On day 7 animals were returned to individual pens in the byre.

#### Analytical procedures

Plasma was prepared immediately after collection by centrifugation of blood at 1000 g for 15 min and then stored at -20° until further analysis. The biopsies were immediately rinsed in ice-cold 0.15 M-NaCl to remove adherent blood, lightly blotted dry, any superficial fat discarded and then the muscles frozen in liquid N. Samples were stored at -20° until further processing.

Preparations of plasma free and muscle free and protein-bound [<sup>2</sup>H<sub>5</sub>]phenylalanine were made as described previously (Connell *et al.* 1997). The enrichment determinations were also as reported by Connell *et al.* (1997), except for muscle-homogenate free [<sup>2</sup>H<sub>5</sub>]phenylalanine where approximately 60 mg frozen muscle were homogenised, on ice, in 3 ml 70 g sulfosalicylic acid/l and the supernatant desalted on Dowex-50 (H<sup>+</sup> form, 100–200 mesh, X8) with 2 M-NH<sub>4</sub>OH. After freeze-drying, the residue was dissolved in 400 μl 0.1 M-HCl, transferred to a v-vial and dried at 90°C under a stream of N<sub>2</sub>. Then 100 μl acetyl chloride–iso-butanol (1:9, v/v) was added and the mixture heated at 90°C for 20 min. The solution was cooled, transferred to a hydrolysis tube, dried under a stream of N<sub>2</sub> at 40°C and 1 ml chloroform–pentafluorobenzoyl chloride (1:0.005, v/v) mixture added, followed by 1 ml saturated NaHCO<sub>3</sub> solution. The total mixture was shaken vigorously and centrifuged (1000 g for 3 min). The upper aqueous layer was removed and discarded and then a wash cycle of addition of 1 ml water, centrifugation and aqueous discard was repeated three times. The organic layer was transferred to a v-vial, dried under a stream of N<sub>2</sub> at 40°C, then 50 μl methanol added, followed by careful mixing and redrying. Finally, 45 μl ethyl acetate was added, the mixture shaken well before analysis of the iso-butylpentafluoro-benzoyl phenylalanine determined by GC–MS in electron impact mode with m/z ions 148.1 and 153.1 monitored.

The concentration of N<sup>τ</sup>-methylhistidine (MeH) in urine was quantified by an isotope dilution technique. Briefly, 0.075 g urine were added to 0.5 g 20 μM-N<sup>τ</sup>-[3-<sup>13</sup>C]methylhistidine standard solution (99 atom %; Mass Trace), this gave a mixture approximately equimolar for N<sup>τ</sup>-MeH from urine and the internal standard. Samples were then processed and analysed as described previously (Thompson *et al.* 1996). Six urine samples (three each from AA and CH) were also tested for the presence of N<sup>τ</sup>-acetyl-MeH, balanine (β-alanyl-N<sup>τ</sup>-MeH) and protein-bound N<sup>τ</sup>-MeH. A mixed urine–internal standard sample was portioned into three, one was measured as described earlier while the other two were hydrolysed at 110°C in a final concentration of 6 M-HCl for either 1 h or 18 h prior to analysis. The presence of peptide- or protein-bound N<sup>τ</sup>-MeH would be indicated by an increase in concentration. The mean values relative to the unhydrolysed sample were 1.009 and 1.000 (SED 0.0033;

$P=0.039$ ) for the 1 h and 18 h hydrolysis respectively. Despite the finding that mild hydrolysis increased free N<sup>τ</sup>-MeH, probably from cleavage of N<sup>τ</sup>-acetyl-MeH, the effect was less than 1 % of the total, approximately equivalent to the variance associated with quantification by the internal standard procedure. Subsequent analyses were performed, therefore, on untreated urine. Creatinine measurements were also performed on each urine subsample by a Technicon Instruments Co. Ltd (1965) procedure based on the Jaffe reaction.

The amounts of total and protein-bound N<sup>τ</sup>-MeH in muscle were also determined by an isotope dilution procedure. Mixed, minced muscle samples from twenty animals (six AA and CH, eight Holstein, selected from all intakes) were first determined for total N using a macro N analyser (Fosse Heraeus Macro N; Fosse Heraeus, York, UK). For total N<sup>τ</sup>-MeH, 1 g minced muscle was hydrolysed in 200 ml 5.6 M-HCl under reflux at 110°C for 18 h. The acid was removed under reduced pressure and the residue dissolved in 250 g 0.1 M-HCl; approximately 8 ml of this was clarified by centrifugation and 5 g supernatant mixed with 10 nmol [3-<sup>13</sup>C]N<sup>τ</sup>-MeH. For protein-bound N<sup>τ</sup>-MeH, 1 g muscle was accurately weighed and homogenised in 4 ml cold acetone–water (7:3, v/v). The pellet weight was measured after 2 × 2.5 ml washes with 70 g sulfosalicylic acid/l and a known portion (equivalent to approximately 80 % of the original muscle weight) removed, hydrolysed and treated exactly the same as for total muscle. The clarified supernatants were then desalted and the N<sup>τ</sup>-MeH eluted on Dowex-50 (H<sup>+</sup>) resin using pyridine elution prior to preparation of the heptafluorobutyl derivative and measurement by GC–MS (Thompson *et al.* 1996). The combined free, balanine- and N-acetyl-bound N<sup>τ</sup>-MeH was determined as the difference between total and protein-bound N<sup>τ</sup>-MeH.

#### Calculations

Phenylalanine irreversible loss rate (ILR, mmol/h) was calculated from the standard formula:

$$\left( \frac{\text{atom \% excess infusate}}{\text{atom \% excess plasma free phenylalanine}} - 1 \right) \times \text{infusion rate of } [^2\text{H}_5]\text{phenylalanine (mmol/h),}$$

with all values over the last 4 h of infusion (from 7 to 11 h) taken to provide the mean denominator. These values were converted to WBPF (g/d):

$$\frac{\text{ILR}}{1000} \times 24 \times \frac{165}{0.035},$$

where 0.035 is the fraction (w/w) of phenylalanine (molecular mass 165) in body protein.

Because the infusion starts were staggered automatically during the night, it was not possible to construct full curves for the initial rate of change of phenylalanine enrichment with time, but such data were obtained for approximately 20 % of the infusions, i.e. those which started at 04.00 and 05.00 hours. Analysis of these yielded an average value for the rate constant  $\theta = 26.3 \text{ d}^{-1}$  and this value was used for all animals to define the area under the curve based on

integration of the first order rate equation:

$$S_t = S_{\max} (1 - e^{-\theta t}),$$

where  $S$  is the mean plasma free [ $^2\text{H}_5$ ]phenylalanine enrichment between 7 to 11 h ( $S_{\max}$ ) and where  $t$  (d) is the time of biopsy.

The fractional rate of muscle protein synthesis (FSR; %/d) was calculated:

$$\frac{S_B}{(\text{average precursor area})} \times \frac{1}{t_b},$$

where  $S_B$  is the enrichment of protein-bound phenylalanine at  $t_b$ , the time (d) after the start of the infusion at which the biopsy was taken. The denominator was calculated either from the average area under the plasma free phenylalanine enrichment curve (obtained as described earlier) to give  $k_{sp}$ , or from this value times the ratio muscle (averaged from the LL and VL homogenates): plasma free [ $^2\text{H}_5$ ]phenylalanine enrichment for the average of the last 3 h of infusion. This yielded a value,  $k_{sh}$ , based on the homogenate ('intracellular') muscle pool.

#### Statistical analysis

Repeated measures analyses were performed for each variable to test for compound symmetry (Box, 1950). In all cases, except one, departures from compound symmetry were not significant at  $P < 0.05$  so a split plot ANOVA was considered suitable where animals were treated as blocks, intake as whole plot, period as sub-plot and with treatment as breed  $\times$  intake  $\times$  period. Linear and quadratic effects were also tested. The one variable that showed a significant effect (by repeated measures analysis) was live weight; this was due to substitution of three animals during the study. For other comparisons, e.g. effect of hydrolysis procedures on  $N^T$ -MeH values, one way ANOVA with animals as a block factor was employed.

## Results

A number of technical difficulties were encountered that reduced the amount of data obtained. Animal tolerance to the experimental conditions was variable. Three animals

(one AA and two CH) did not accept satisfactorily the experimental conditions in the metabolism stalls and were replaced. These inclusions disturbed the selections of the initial groups based on similar mean live weights. Whilst the majority of the steers consumed the ration offered, unseasonably hot weather during the first and second measurement periods caused some difficulties for CH animals offered the high ration. Four measurements were excluded over these two periods for four individual animals, because less than 90% of feed was consumed. There were also a number of technical failures (mainly chewed or disconnected infusion lines). In total, 80% of the planned observations were made.

#### $N^T$ -methylhistidine content of muscle

Values for protein-bound  $N^T$ -MeH (780  $\mu\text{mol/kg}$  wet weight muscle) were substantially greater than the widely adopted value of 551  $\mu\text{mol/kg}$  muscle obtained previously by conventional amino acid analysis (Nishizawa *et al.* 1979) (Table 1). Known quantities of  $N^T$ -MeH added to the hydrolysate were recovered at 103% by the GC-MS methodology. The internal standard (isotope dilution) approach requires only the assumption that natural abundance and isotopically-labelled  $N^T$ -MeH follow similar physical and chemical routes during the quantification procedure and, in theory, is the most accurate and precise approach available. The values obtained were also slightly greater than those reported by Jones *et al.* (1985), based on HPLC analyses of a range of prime beef cuts (topside, chuck, skin, silverside, leg, brisket). Those samples had a wide range of fat (1.4–24.9%) and total N (28.3–41.0 g N/kg fresh weight) with a mean  $N^T$ -MeH of 611 (SE 14)  $\mu\text{mol/kg}$  ( $n$  36; range 437–760  $\mu\text{mol/kg}$ ). These values increased to 709 (SE 1.4)  $\mu\text{mol/kg}$  ( $n$  24; ranges across cuts 657–781  $\mu\text{mol/kg}$ ) when the contribution of fat and collagen to muscle weight was removed. In that study, higher  $N^T$ -MeH contents were observed for flank muscle compared with the prime cuts (+33%).

#### Urinary $N^T$ -methylhistidine elimination

$N^T$ -MeH elimination was determined for each day of collection and these results were pooled for each experimental

**Table 1.** Total and protein-bound  $N^T$ -methylhistidine ( $N^T$ -MeH)\* in skeletal muscle of Charolais, Aberdeen Angus and Holstein steers fed on three levels of dietary intake†  
(Mean values with their standard errors)

	Charolais‡ ( $n$ 6)		Aberdeen Angus‡ ( $n$ 6)		Holstein§ ( $n$ 8)	
	Mean	SE	Mean	SE	Mean	SE
$N^T$ -MeH ( $\mu\text{mol/kg}$ wet weight muscle)						
Total	946	25.0	930	27.4	930	17.3
Protein-bound	785	9.7	774	34.7	779	21.1
Non-protein bound	161	29.6	155	38.5	151	21.1

\* Measured as described on p. 277.

† M/M intake predicted live-weight gain of 1.0 kg/d; H/H intake predicted live-weight gain of 1.4 kg/d; M/H entailed M intake for 10 weeks followed by H for 10 weeks (diets were fed for 20 weeks before slaughter). For details of diets see Sinclair *et al.* (2000).

‡ Two animals from each of M/M, M/H and H/H diet groups quantified. There were no significant differences between breed or intake.

§ Four animals from each of M/M and H/H diet groups quantified. There were no significant differences between intakes.

|| Obtained as the difference between total and protein-bound  $N^T$ -MeH.



period (4 d) to give the mean values analysed statistically (Table 2). For only four collections (all CH) are data not reported, due to intakes being less than 90 % of that offered. In another nine cases (out of 104 possible measurements) incomplete urine collections were obtained on some days. In these circumstances, values were adjusted by multiplying the  $N^T$ -MeH:creatinine ratio by the average total creatinine elimination on the other three days of collection.

Total  $N^T$ -MeH in the urine was significantly greater ( $P < 0.001$ ) in the CH compared with AA steers (Table 2). This effect was still present ( $P = 0.001$ ) when the data were adjusted for differences in live weight. For both breeds  $N^T$ -MeH elimination on both a total ( $P < 0.001$ ) and per kg live weight basis ( $P = 0.001$ ) increased with level of intake. Furthermore, for M/H animals values were equivalent to those of the M/M group during measurement period 2 but were elevated to those of group H/H during period 3, in line with the increase in intake. Over the time course of the study, total  $N^T$ -MeH elimination increased ( $P < 0.001$ ) as the animals became both older and heavier. A significant decrease ( $P < 0.001$ ) was also observed with age (or weight) for values expressed per kg live weight.

#### Estimated muscle fractional breakdown rates

If the isotope dilution values for protein-bound  $N^T$ -MeH and the body composition data for cattle reported by Nishizawa *et al.* (1979) are combined, then estimated fractional breakdown rates (FBR) of protein can be calculated. These data have similar statistics to  $N^T$ -MeH elimination/kg per d and yield FBR values of between 0.62 and 0.97 %/d. Again there was a decline with age (or weight) and higher values at greater intakes, with the decline for group M/H arrested or reversed as the intake was increased from M to H (Table 2). Such calculations are compromised by the estimation of

muscle mass, plus the assumption that body composition is similar for all the animals, regardless of age, nutritional history and breed. This assumption is palpably false, but only for period 3 data can a more realistic calculation be attempted. Based on the carcass compositions determined at slaughter, 2 weeks after the last kinetic analyses, lean (muscle) mass for each animal can be predicted from regression equations (D. Horner, personal communication), using fat class and eye muscle area ( $\text{cm}^2$ , at the 10th rib). These equations yield the revised data shown in Table 3 and show clear breed differences ( $P < 0.001$ ) for both the lean percentage and total lean in the carcass. The carcass lean for the CH steers (35–36 % of live weight) is similar to the value reported by Nishizawa *et al.* (1979), but the AA had a lower proportion ( $P < 0.001$ ) of body weight as lean mass (32–33 %). In consequence, the revised FBR values for period 3 are now higher ( $P < 0.001$ ) than calculated previously (compare Tables 2 and 3) and the breed differences disappear. In contrast, the intake effects remain, with both breeds showing increased breakdown between the M/M and H/H (including M/H) intakes.

#### Whole-body phenylalanine and protein flux

For comparative purposes the phenylalanine ILR data have been scaled to equivalent WBPF (the original ILR data as  $\text{mmol/h}$  for example can be obtained by division of the values by 113.1) (Table 4). Total WBPF increased with period ( $P < 0.001$ ) as the animals became heavier and received greater absolute intakes. There was no difference between breeds but WBPF was significantly increased ( $P = 0.001$ ) with intake. There was also a large increase ( $P < 0.001$ ) for the M/H group after the transition from M to H intake.

When the data were scaled to metabolic body size (kg live

**Table 2.** Urinary elimination of  $N^T$ -methylhistidine ( $N^T$ -MeH) and calculated muscle fractional breakdown rate (FBR) over three periods for growing Aberdeen Angus and Charolais steers fed either M/M, H/H or M/H levels of intake\*  
(Mean values for six animals per group)

Intake ...	Aberdeen Angus						Charolais						Significance effects (ANOVA)†					
	Aberdeen Angus			Charolais			Breed		Intake		Period		Breed		Intake		Period	
	M/M	M/H	H/H	M/M	M/H	H/H	SED	P	SED	P	SED	P	SED	P	SED	P	SED	P
<b><math>N^T</math>-MeH elimination (mmol/d)</b>																		
Period 1	0.838	0.908	1.047	0.996	1.134	1.239	0.041	< 0.001	0.051	< 0.001	0.025	< 0.001						
Period 2	0.815	0.963	1.142	1.136	1.112	1.359												
Period 3‡	0.898	1.135	1.234	1.213	1.323	1.367												
<b><math>N^T</math>-MeH elimination (<math>\mu\text{mol/kg per d}</math>)</b>																		
Period 1	2.083	2.077	2.298	2.154	2.466	2.656	0.067	0.001	0.082	0.001	0.049	< 0.001						
Period 2	1.826	1.933	2.264	2.117	2.116	2.415												
Period 3‡	1.804	1.980	2.175	2.146	2.244	2.267												
<b>FBR (%/d)§</b>																		
Period 1	0.742	0.740	0.818	0.767	0.878	0.946	0.024	0.001	0.029	0.002	0.018	< 0.001						
Period 2	0.651	0.688	0.807	0.754	0.753	0.860												
Period 3‡	0.643	0.705	0.705	0.765	0.799	0.807												

\* M/M intake predicted live-weight gain of 1.0 kg/d; H/H intake predicted live-weight gain of 1.4 kg/d; M/H entailed M intake for 10 weeks followed by H for 10 weeks (diets were fed for 20 weeks before slaughter). For details of diets see Sinclair *et al.* (2000).

† ANOVA with animals as blocks and tested for breed  $\times$  intake  $\times$  period effects, including linear trends. There were three missing observations from a theoretical total of 108 with a maximum of 57 residual degrees of freedom. There were no interactions between breed, period and intake.

‡ At the end of period 2 the M/H group were switched from M to H intake.

§ Calculated from  $N^T$ -MeH elimination data and assuming skeletal muscle is 0.36 of live weight (Nishizawa *et al.* 1979) and contains 780  $\mu\text{mol}$  protein-bound  $N^T$ -MeH per kg (see Table 1).

**Table 3.** Predicted lean mass and muscle fractional breakdown rate (FBR), two weeks prior to slaughter, for growing Aberdeen Angus and Charolais steers fed either M/M, H/H or M/H levels of intake\*  
(Means values for six animals per group)

Intake ...							Significance effects (ANOVA)†		
	Aberdeen Angus			Charolais			<i>P</i>		
	M/M	M/H	H/H	M/M	M/H	H/H	SED	Breed	Intake
Lean in carcass (%)‡	60.4	61.1	60.7	65.3	65.4	65.5	0.59§	<0.001	NS
Lean in carcass (kg)	164	193	190	207	216	219	6.5§	<0.001	0.03
N <sup>15</sup> -MeH elimination (µmol/d per kg lean body mass)	6.86	7.35	8.15	7.01	7.66	7.53	0.282	NS	0.012
FBR (%/d)	0.880	0.942	1.045	0.899	0.981	0.965	0.0295	NS	0.012
Lean tissue (% live weight)	32.2	33.0	32.9	35.3	36.0	35.1	0.67§	<0.001	NS

\* M/M intake predicted live-weight gain of 1.0 kg/d; H/H intake predicted live-weight gain of 1.4 kg/d; M/H entailed M intake for 10 weeks followed by H for 10 weeks (diets were fed for 20 weeks before slaughter). For details of diets see Sinclair *et al.* (2000).

† Two-way ANOVA with a maximum of 28 residual degrees of freedom (two missing points).

‡ Calculated from equation: lean % = A + 0.116 × eye muscle area (cm<sup>2</sup>, at 10th rib); where A is 59.227, 56.788 and 51.655 for fat classes 2, 4L and 5L respectively. Values of A for fat classes 3 and 4H were set, by interpolation, as 58.008 and 54.222 respectively.

§ For breed effects.

|| For intake effects.

weight<sup>0.75</sup>) a significant breed effect ( $P < 0.001$ ) became apparent, with lower values for the CH steers during periods 2 and 3. The increases observed for total WBPF with age (or weight) became declines ( $P < 0.001$ ) for the M/M and H/H groups when expressed on a kg<sup>0.75</sup> basis. This decline was reversed for the M/H group when switched from M to H intakes. The overall significant effect of intake was maintained ( $P = 0.001$ ).

#### Muscle protein synthesis rates

Free phenylalanine enrichments from muscle homogenates were lower ( $P < 0.001$ ) than from plasma, due to intracellular dilution by unlabelled amino acid released from muscle protein breakdown. In consequence, precursor

areas were lower and calculated FSR values higher ( $P < 0.001$ ). Regardless of the precursor selected there were two notable features. First, the values for the AA were greater than for CH by, on average, 11.5% ( $k_{sp}$ ,  $P = 0.067$ ) and 13.8% ( $k_{sh}$ ,  $P = 0.044$ ). Second, FSR across both breeds was greater in VL compared to LL ( $P < 0.001$ ; +16.0%,  $k_{sp}$ ; +17.3%,  $k_{sh}$ ).

For both muscles, but particularly LL, there were also significant intake × period interactions (e.g.  $k_{sp}$  LL,  $P < 0.001$ ;  $k_{sh}$  LL,  $P = 0.003$ ;  $k_{sp}$  VL,  $P = 0.054$ ) with a strong linear trend ( $P < 0.009$  or better for the three comparisons mentioned). This can be seen clearly in Table 5 where  $k_{sp}$  and  $k_{sh}$  for the combined muscle data (i.e. average of LL and VL) showed a decline between periods 1 to 3 for the M/M and H/H groups. In contrast there was an increase for the M/H

**Table 4.** Live weights and whole-body protein flux (WBPF) over three periods for growing Aberdeen Angus and Charolais steers fed either M/M, H/H or M/H levels of intake\*  
(Mean values for six animals per group)

Intake ...							Significance effects (ANOVA)†									
	Aberdeen Angus			Charolais			Breed		Intake		Period		Intake × period		Period × breed	
	M/M	M/H	H/H	M/M	M/H	H/H	SED	<i>P</i>	SED	<i>P</i>	SED	<i>P</i>	SED	<i>P</i>	SED	<i>P</i>
Live weight (kg)																
Period 1	399	437	456	460	460	463	11.8	0.002	14.4	0.049	4.2	<0.001	15.6	0.053	12.7	0.038
Period 2	451	498	508	540	524	549										
Period 3	499	573	569	575	590	594										
WBPF (g/d)‡																
Period 1	1972	1974	2334	2199	2027	2238	6.90	NS	84.5	0.001	53.3	<0.001	113.2	<0.001	92.4	0.029
Period 2	1927	2016	2457	1971	2032	2348										
Period 3	2079	2813	2534	2000	2390	2358										
WBPF (g/kg live weight <sup>0.75</sup> /d)																
Period 1	22.0	20.6	23.8	22.0	20.3	22.3	0.43	<0.001	0.53	0.001	0.48	<0.001	0.86	<0.001	0.70	0.050
Period 2	19.7	19.1	22.9	17.6	18.6	21.0										
Period 3	19.8	24.0	22.7	17.1	20.0	19.4										

\* M/M intake predicted live-weight gain of 1.0 kg/d; H/H intake predicted live-weight gain of 1.4 kg/d; M/H entailed M intake for 10 weeks followed by H for 10 weeks (diets were fed for 20 weeks before slaughter). For details of diets see Sinclair *et al.* (2000).

† ANOVA with animals as blocks and tested for breed × intake × period effects, including linear trends. There were twenty-two missing observations from a theoretical total of 108 with a maximum of 38 residual degrees of freedom.

‡ Calculated from phenylalanine flux (mol/h) × 24 × 165/0.035 where 165 is molecular mass of phenylalanine and 0.035 is the fraction of body protein as phenylalanine.

**Table 5.** Combined fractional rates of protein synthesis (FSR; %/d) from *m. longissimus lumborum* and *m. vastus lateralis* based either on plasma ( $k_{sp}$ ) or (mean) muscle homogenate ( $k_{sh}$ ) free [ $^2H_5$ ]phenylalanine enrichments as precursor for growing Aberdeen Angus and Charolais steers fed either M/M, H/H or M/H levels of intake\*  
(Mean values for six animals per group)

Intake ...							Significance effects (ANOVA)†				
	Aberdeen Angus			Charolais			Breed		Intake × period		
	M/M	M/H	H/H	M/M	M/H	H/H	SED	P	SED	P	
FSR ( $k_{sp}$ )											
Period 1	1.35	0.97	1.47	1.34	1.17	1.27	0.070	0.067	0.122	0.006	(linear 0.001)
Period 2	1.34	1.31	1.20	1.07	0.96	1.02					
Period 3	1.29	1.46	1.19	1.04	1.36	1.14					
FSR ( $k_{sh}$ )											
Period 1	2.21	1.55	2.58	2.05	2.04	1.90	0.122	0.044	0.215	0.024	(linear 0.006)
Period 2	2.16	2.20	2.03	1.79	1.59	1.72					
Period 3	1.88	2.31	2.07	1.65	2.18	1.76					

\* M/M intake predicted live-weight gain of 1.0 kg/d; H/H intake predicted live-weight gain of 1.4 kg/d; M/H entailed M intake for 10 weeks followed by H for 10 weeks (diets were fed for 20 weeks before slaughter). For details of diets see Sinclair *et al.* (2000).

† ANOVA with animals as blocks and tested for breed × intake × period effects, including linear trends. There were twenty-four missing observations from a theoretical total of 108, with a maximum of 36 residual degrees of freedom.

group for both breeds between periods 2 and 3, i.e. the transition from M to H intake. Indeed, during period 2 for M/M and M/H groups, when both were receiving the same intakes, the FSR data were similar. The decline in FSR for the M/M group between periods 2 and 3 amplifies the effect of the M/H group which exhibited increased protein synthesis ( $P < 0.05$ ) during period 3 when live-weight gain was augmented compared with period 2 (1.25 v. 1.02 kg/d; Sinclair *et al.* 2000).

## Discussion

### Whole-body protein flux

With longitudinal studies on growing animals, protein metabolic measurements reflect two contradictory events. First, as the lean body mass of the animal increases so does the absolute rate of protein turnover (for both protein synthesis and breakdown; Attaix *et al.* 1988). Second, this is accompanied by lower activity per unit mass (either on a metabolic body weight or lean tissue basis; Attaix *et al.* 1988). This is most clearly seen in young animals (Lobley, 1993) but an age-linked decline in WBPF, expressed per  $kg^{0.75}$ , was also observed in the current study. Part of the decrease may be associated with lean tissue mass (which dominates protein turnover) contributing a smaller proportion of live weight as the animals fatten. Care has to be exerted, therefore, when interpreting intake-related events in the current study as the animals (both within- and between-breeds) may be of dissimilar weight, due to previous dietary history, and different physiological (developmental) age, although of similar chronological age. Even with these provisos, however, it is clear that the transition for the M/H group from M to H intake markedly increased the absolute phenylalanine flux and reversed the age (or weight) decline observed when the data were considered on a metabolic body weight basis. This is in agreement with many other studies, which have demonstrated that whole-body amino acid (protein) flux is nutrient sensitive (e.g. Lobley *et al.* 1987; Harris *et al.* 1992).

### Muscle protein metabolism

*N<sup>7</sup>-methylhistidine elimination.* The use of  $N^7$ -MeH elimination has been a popular method to estimate muscle protein breakdown and, indirectly, protein synthesis if protein gain is also determined. Although criticised on several theoretical grounds (Rennie & Millward, 1983), the simple, non-invasive technique has been widely used, particularly for cattle (e.g. Harris & Milne, 1981; McCarthy *et al.* 1983; van Eenaeme *et al.* 1998). The adoption of isotope dilution greatly improved the precision and the major analytical (as opposed to biological) uncertainty then became the accuracy of the urine collection. The current FBR are below other estimates in the literature, which themselves encompass a wide range (1.39–2.44 %/d; Harris & Milne, 1981; McCarthy *et al.* 1983; Gompath & Kitts, 1984; Hayden *et al.* 1992; Rathmacher *et al.* 1992; Morgan *et al.* 1993), but these earlier data were based on lower estimates of the protein-bound  $N^7$ -MeH in bovine skeletal muscle. The estimate from isotope dilution has increased that value by 42% and therefore the earlier FBR need to be adjusted downwards by a similar proportion.

In the current study, and based on simple assumptions of body weight composition, there was an apparent decline (within M and H intakes) with age and/or weight on the estimated FBR. Nutrition had a greater influence on FBR than did development or maturity status, however, as exemplified by the values obtained for animals switched from M/H. This estimated increase in protein muscle breakdown at higher intakes is in agreement with more direct kinetic observations across a range of farm species (e.g. Lobley *et al.* 1992; Boisclair *et al.* 1993; Seve *et al.* 1993; Thomson *et al.* 1997), although a reduction in degradation at intakes between 0.6 and 1.8 × maintenance has been reported in lambs (McDonagh *et al.* 1999). In cattle,  $N^7$ -MeH elimination has also been shown to be sensitive to total metabolisable energy intake (Smith *et al.* 1992) but, contrariwise, may also be augmented on low energy regimens (Ndibualonji *et al.* 1995). Improved gain is not invariably linked with increased  $N^7$ -MeH output, however, because

bulls have lower rates of elimination than steers despite superior growth performance (Hayden *et al.* 1992).

Breed differences in estimated rates of muscle protein breakdown have been studied previously by McCarthy *et al.* (1983) who examined large-frame (CH, Holstein, Angus cross) and small-frame (polled Hereford) cattle but failed to find any differences in the estimated fractional rate, although larger total outputs occurred due to body weight differences between the breeds. In contrast, the current study yielded an apparent higher FBR for CH than AA, when compared per unit body weight. When corrected for estimated muscle mass at slaughter, however, the FBR were similar between breeds at comparable intakes. Thus, while nutrition does affect muscle protein degradation, the influence of genotype appears to be rather limited.

*Synthesis.* The problems discussed for WBPF apply equally to measurements of tissue protein synthesis. For example, during period 1 values for muscle protein synthesis in AA steers were higher for the H/H compared with M/M group. This is in accord with other observations in various farm species that showed muscle protein metabolism is sensitive to intake (e.g. Oddy *et al.* 1987; Dawson *et al.* 1991; Lobley *et al.* 1992; Boisclair *et al.* 1993; Seve *et al.* 1993). It should be noted that similar comparisons for CH steers were compromised by variable intakes for the H/H group during period 1. By the period 3 such comparisons are compromised by weight differences between the groups, with further complications between development and intake. For these reasons, it is more appropriate to examine trends within each group.

With time (age and weight) for M/M and H/H intakes of both breeds, muscle FSR declined, following the trend for WBPF when expressed on a metabolic mass basis. Again this agrees with data obtained either within studies, mainly on rodents (e.g. Lewis *et al.* 1984), or from comparisons between species (see Lobley, 1993). What was clear, however, was that the switch to H intake for the M/H group reversed the decline in FSR and, in most cases, yielded a value greater than that for the H/H group at any of the measurement periods. These higher FSR may be partly due to differences in 'physiological' age (i.e. lower weight and stage of maturity) but also because the most rapid live-weight gains overall occurred between periods 2 and 3 for the M/H group (Sinclair *et al.* 2000).

The decision to opt for biopsy of two muscles, rather than just one, was based on two rationales. First, to determine if the direction of any effect was common to different major muscle groupings. This was the case. Second, to see if a 'common' value could be applied for comparative purposes, e.g. the contribution of muscle to WBPF or the relationship with  $N^7$ -MeH elimination. The significant difference between VL and LL synthesis rates contrasts with previous observations in cattle (Dawson *et al.* 1991) and sheep (Lobley *et al.* 1990) but not with other ovine studies (Wester *et al.* 1998). Such differences may relate to differences in fibre type proportions, which have been shown in rodents to influence protein metabolism in muscles (Garlick *et al.* 1989). The differences between VL and LL were numerically small, however, and for comparative purposes the average value has been taken as representative of skeletal muscle as a whole, although this

assumption requires further testing. The contribution of muscle protein synthesis to WBPF can be assessed if  $k_{sp}$  values are adopted because both calculations involve a common precursor (plasma free phenylalanine). For the period 3 measurements skeletal muscle of the AA made a significantly lower contribution to WBPF than for the CH (15.9 v. 20.6%; SED 1.89,  $P=0.023$ ). These contributions cover the range reported for other species (Lobley *et al.* 1980) and the differences may again reflect the stage of maturity of the two breeds at slaughter. As muscle gain and mass reach asymptotes then the proportional contribution to whole-body protein metabolism would be expected to decline.

#### *Comparison of tissue protein turnover approaches*

Few studies have attempted to measure muscle protein synthesis and breakdown simultaneously in farm species. The closest approach involves the arterio-venous technique (e.g. Lobley *et al.* 1992; Boisclair *et al.* 1993), but this is confounded by the vascular drainage of other tissues (skin, bone, fat). Even the current study is compromised. For example, uncertainties arise as to how much  $N^7$ -MeH arises from skeletal muscle and the assumption that two small biopsy samples are representative of the total musculature may not be valid. Nonetheless, the differences between estimated FBR (Table 3) and calculated FSR (Table 5) would indicate expected fractional rates of gain of 0.14–0.52 %/d based on  $k_{sp}$  and 0.75–1.37 %/d based on  $k_{sh}$ , for period 3 measurements. Values for fractional rates of gain based on either live-weight changes or increase in daily creatinine elimination (to allow for changes in body muscle:fat composition) ranged from 0.2 to 0.3 %/d (data not shown). Such comparisons would indicate a closer (but not perfect) match if synthesis rates based on  $k_{sp}$  were used. Other data, both from sheep (Lobley *et al.* 1992) and human subjects (Ljungquist *et al.* 1997) have shown, however, the precursor free pools for muscle protein synthesis from leucine and phenylalanine to be intracellular. If the same findings can be extrapolated to cattle, the current analysis suggests that use of  $N^7$ -MeH leads to a marked underestimate of muscle mixed protein breakdown. These apparent contradictions need to be resolved by further analyses, based on rates of synthesis for the total musculature and for different muscle fractions (e.g. sarcoplasmic and myofibrillar).

#### *Relationship between protein turnover and meat quality*

Although the various nutritional treatments did induce changes in both protein synthesis and degradation these differences did not correlate with sensory variables of meat quality within each breed. Treatments M/M, M/H and H/H yielded meat of similar tenderness, flavour and overall acceptability (Sinclair *et al.* 2000). Thus, provided adequate nutrition with associated rates of gain was supplied, meat of consistent quality was obtained. Even though there were differences between breeds, with meat from CH having lower ratings for sensory variables than AA (Sinclair *et al.* 2000), these occurred under situations where muscle FBR was similar between the two breeds, data again not supportive



of a link between muscle proteolysis *in vivo* and final eating quality.

These findings contrast with other studies that have reported significant relationships between the calpain system (a contributor to muscle proteolysis) and objective measures of meat quality (myofibril fragmentation index, shear force). These have usually involved experimental extremes (sub- to supra-maintenance intake, plus inclusion of clenbuterol or insulin-like growth factor treatment; Thomson *et al.* 1997; McDonagh *et al.* 1999). Such designs increase the chances of revealing correlations, either real or apparent, but the results of the current study suggest that more subtle interactions may operate.

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