METABOLISM IN COMPENSATORY GROWTH. III. THE UREA, GLUCOSE AND CO_2 ENTRY RATES IN ANIMAL UNDERGOING COMPENSATORY GROWTH

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ABSTRAK

MAHYUDDIN, PRAPTI dan E. TELENI. 1996. Metabolisme dalam pertumbuhan kompensatori. III. Laju pemasukan urea, glukosa dan CO_2 pada ternak yang mengalami pertumbuhan kompensatori. *Jurñal Ilmu Ternak dan Veteriner* 2(1).

Pengukuran laju pemasukan glukosa (GER), urea (UER) dan CO_2 (CO_2ER) dilakukan pada 4 titik kurva pertumbuhan, yaitu: sebelum pembatasan pemberian pakan (P1), 8 minggu setelah pembatasan pemberian pakan (P2), 3 minggu (P3) dan 15 minggu (P4) setelah ternak diberi pakan kembali secara *ad libitum*. Enam belas ekor domba jantan Merino lepas sapih yang dipakai dalam percobaan ini diberi pelet *lucern* (*Medicago sativa*) secara *ad libitum* selama 3 minggu; kemudian domba-domba ini dibagi ke dalam 2 kelompok, masing-masing 8 ekor. Kelompok I terus diberi pakan secara *ad libitum* dan Kelompok II diberi pelet *lucern* sebanyak 1/2 kebutuhan dasarnya selama 8 minggu dan kemudian diberi pakan secara *ad libitum* sampai akhir percobaan. Selama diberi pakan terbatas (P2), UER, kadar urea urin dan urea yang ditransfer dari darah ke rumen 74% lebih rendah pada Kelompok II daripada Kelompok I disebabkan turunnya konsumsi nitrogen. Pada P2, GER dan CO_2ER naik secara mencolok yang menunjukkan perubahan ke arah anabolik. Ini diikuti oleh kenaikan glukosa yang diambil dan dioksidasi oleh otot kaki. Pentingnya glukosa dalam metabolisme otot selama pertumbuhan kompensatori terlihat dengan naiknya secara dramatis kecepatan oksidasi glukosa per unit bobot otot. Kelihatannya prioritas penggunaan glukosa yang diambil oleh otot selama pertumbuhan kompensatori adalah untuk oksidasi menjadi CO_2 dan laktat.

Kata kunci: Laju pemasukan glukosa, laju pemasukan CO_2, urea darah

ABSTRACT

MAHYUDDIN, PRAPTI and E. TELENI. 1996. Metabolism in compensatory growth. III. The urea, glucose and CO_2 entry rates in animal undergoing compensatory growth. *Jurnal Ilmu Ternak dan Veteriner* 2 (1).

Glucose (GER), Urea (UER) and CO_2 (CO_2 ER) entry rates were studied at four points in the growth curve viz: before feed restriction (P1) after 8 weeks of feed restriction (P2), after 3 weeks (P3) and 15 weeks (P4) following resumption of *ad libitum* feeding. Sixteen Merino wethers were used and offerred pelleted lucerne (*Medicago sativa*) *ad libitum* for 3 weeks; then they were divided into 2 groups of eight. Group I continued to be fed *ad libitum* and Group II was fed pelleted lucerne at half maintenance level for 8 weeks and then fed *ad libitum* until the end of experiment. During feed restriction (P2), UER, urinary urea and urea transferred from the blood to the gut were 74% lower in group II than those in group I due to the reduction of N intake. At P2 GER and CO_2ER were also lower (53% and 56%, respectively) because of the reduction of available glucose precursor and metabolic rate. Similarly AV concentration difference of glucose, glucose taken up by the hind-limb muscle and the percentage of glucose taken up by muscle that was oxidised were reduced by 52%, 86% and 48%, respectively. When animals resumed *ad libitum* feeding, the components of urea entry rate (except plasma urea concentration), GER and CO_2ER were markedly increased indicating a switch to the anabolic mode, followed by increased glucose taken up and oxidised by the hind-limb muscle. The significance of glucose in muscle metabolism during compensatory growth was shown in the dramatic increase in the actual rate of glucose oxidation per unit muscle weight. It appears that the priority of usage of glucose taken up by muscle during compensatory growth is for oxidation to both CO_2 and lactate.

Key words: Glucose entry rate, CO_2 entry rate, plasma urea

INTRODUCTION

Lambs undergoing compensatory growth were estimated to utilise metabolisable energy (ME) for growth more efficiently (29%) than lambs growing normally (MAHYUDDIN, 1995). A possible reason for this might be explained by the observation by a number of studies (FOOT and TULLOH, 1977; LEDGER and SAYERS, 1977; GRAHAM and SEARL, 1979; MURRAY and SLEZACEK, 1980) that animals tended to lower maintenance energy requirements when they have been either loosing or maintaining live weight for a period of

time. Another possible reason which explain the increase in efficiency of nutrient utilisation by animals in compensatory growth is the hormonal/enzyme milleu which is stimulated by resumption of *ad libitum* feeding after a period of restricted feeding. The sudden increased influx of nutrients into body pools at the resumption of *ad libitum* feeding, could have a "rebound" effect on the hormonal/enzyme millieu. This millieu might be sustained for a period of time, thus facilitating the phenomenon of compensatory growth.

The efficiency of ME for growth is influenced by the balance between amino acids and energy-yielding substrate such as glucose and long-chain fatty acids. If amino acids are available in the blood circulation in large amounts when glucose and fatty acids concentrations are low, amino acids would not be utilised completely for protein synthesis. Instead, they would either be oxidised or used for glucose synthesis in the liver (LINDSAY, 1980). During starvation, most amino acids in the circulation are derived from protein degraded in muscle tissues. The gluconeogenic amino acids such as alanine and glutamine are converted to glucose in the liver while branch-chain amino acids (BCAA) might be oxidised in the muscle (LINDSAY, 1982). In well fed animals, THORNTON and TUME (1984) suggested that glucose supply both from absorption and gluconeogenesis (propionate and amino acids) could contribute to the increased rate of fat deposition as the result of the probable increased supply of NADPH and glycerol phosphate.

Reports on the pool metabolites of animals undergoing compensatory growth has not been available. The experiment below was one of a series of experiment (MAHYUDDIN and TELENI, 1995) undertaken to describle metabolic changes at selected point in the growth curve of these animals. This experiment aimed to study the urea, glucose and CO_2 entry rate in lambs undergoing compensatory growth.

MATERIALS AND METHODS

Animals and feeding

Sixteen Merino wethers, used in this study were fed pelleted lucerne (*Medicago sativa*) *ad libitum* in individual pens, approximately three weeks before experimentation. These animals were randomly divided into two groups of eight. Group I (mean liveweight: 28.4 ± 0.82 kg) continued to be fed *ad libitum* the pelleted lucerne diet, while Group II (mean liveweight: 28.6 + 1.5 kg) was fed the pelleted lucerne at half the estimated maintenance energy level (MAFF, 1975) for eight weeks. At the end of eight weeks, Group II was fed *ad libitum* the same diet until the experiment was terminated.

Four animals in each group were selected at random for metabolism studied which consists of a 7-day preliminary period, a 7-day intake/digestibility measurement (see Fig. 1, MAHYUDDIN and TELENI, 1995) and a 4-day entry rate measurements. The 4-day measurement includes urea entry rate (UER), blood flow, glucose entry rate (GER) and CO_2 entry rate (CO_2ER). During metabolism studies, the animals were kept in individual cages, fed through continuous feeders, with drinking water being available at all times. At the end of each metabolism study period, the animals were returned to their respective pens.

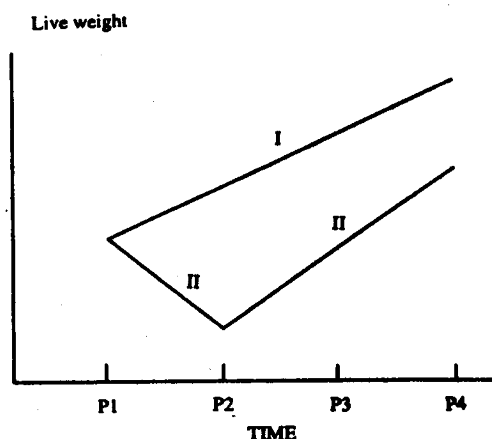

Figure 1. Growth curve of sheep as described in MAHYUDDIN and TELENI. 1995. The measurement periods of the four metabolism study are indicated by:

P1 : Eleven days immediately before animals in Group II were subjected to restricted feeding

P2 : Eleven days immediately after 8 weeks of restricted feeding of animals in Group II

P3 : Eleven days immediately after 3 weeks of resumption of *ad libitum* feeding by animals in Group II

P4 : Eleven days immediately after 15 weeks of resumption of *ad libitum* feeding by animals in Group II

Statistical analysis

A Split-plot analysis was done with contrast of individual treatments (period) and interactions (group x period) were made; the significance of these effects being tested using two-tailed t-test (see MAHYUDDIN and TELENI, 1995).

Catheterisation

The hind-limb muscle preparation used for muscle metabolism studies was similar to the hind-limb model described by TELENI and ANNISON (1986). Animals were catheterised one week before each experimental period. Polyethylene catheters (1.0 mm ID x 1.5 mm OD and 0.86 mm ID x 1.27 mm OD) (dural Plastic and Engineering, NSW) filled with sterile heparinised saline (250 IU/ml) were surgically inserted into an external jugular vein and into the femoral artery (under local anaesthesia) respectively (TELENI and ANNISON, 1986).

Blood collection

During the measurement of AV concentration difference of metaboliter, arterial and venous blood were withdrawn simultaneously using continuous sampling technique described below (see blood flow measurement). Blood samples (10 ml) were collected into tubes placed in an ice bath. Where the continuous sampling technique was not used, *e.g.* during urea and CO_2 entry rate determination, blood was collected in discrete samples with minimal disturbance to the animals.

Pairs of arterial and venous blood samples for blood gas analysis were withdrawn anaerobically into 2 ml heparinised syringes (10 IU heparine/ml blood) and kept on ice until analysed. Other pairs of arterial and venous blood (5 ml) were withdrawn into 5 ml syringes to the exclusion of any air and dispensed into respective McCartney bottles contained 1 ml of carbon dioxide for specific radioactivity (SRA) determination. The McCartney bottles contained 1 ml of 0.5 M H_2SO_4 and a glass tube insert containing 1 ml of 1 M CO_2 free NaOH. Blood samples were dispensed into bottles by piercing their air-tight lids with 21 G needles attached to 5 ml syringes and injecting blood to mix with the H_2SO_4 + Cu_2SO_4 solution.

Blood flow measurement

Blood flow was determined using tritiated water (TOH) as described by ODDY *et al.* (1981) and TELENI and ANNISON (1986). Ten ml of TOH (925 kBq/ml) was mixed continuously in a vial using a magnetic stirrer. In the first minute 1 ml (925 kBq/ml) was infused into the jugular vein. The remainder of the TOH was infused continuously at an exponentially decreasing concentration by adding a TOH diluent (9.25 kBq/ml) into the vial at the rate of 1 ml/min for 59 minutes. This procedure allowed the TOH to equilibrate with muscular water. During the TOH infusion, arterial and venous blood samples were drawn continuously using a peristaltic pump at the rate of 1 ml/min. At the same time heparin (500 IU/ml) was infused into the samples (0.04 ml/min) at point near the exit of the catheter from the relevant blood vessel, to avoid blood clotting. Blood was collected over 10 minutes intervals into 10 ml tubes which were kept in an ice bath.

A sample of 0.5 ml blood from each tube was deproteinised by adding 1.5 ml of 0.5 M perchloric acids and centrifuged at 3,000 rpm for 20 minutes. One ml of the supernatant was added to 10 ml of scintillation Cocktail II and counted for tritium in a liquid scintilation counter. Blood flow across the hind-limb was calculated using the equation of KETY and SCHMID (1945):

$$MBF = [Cv\,(eq).S]/[O(Ca\text{-}Cv)dt] \times 100$$

where MBF is muscle blood flow (ml/min/100 g muscle);

Ca is the TOH concentration of arterial blood during approach to equilibrium;

Cv is the TOH concentration of venous blood to equilibrium;

Cv (eq) is the concentration of TOH ml of venous at equilibrium;

S is the partition coefficient which is the ratio of tissue TOH concentration per g to venous TOH concentration per g at equilibrium = 0.855 (ODDY *et al.*, 1981).

Biokinetics of metabolites

1. Urea entry rate

Urea entry rate was estimated by infusing [^{14}C] urea continuously into a jugular vein for 9 h at the constant rate of 3.43 kBq/min. Arterial blood samples were collected at half-hourly intervals in the last three hours of infusion. Estimation of urea entry rate was always carried out before other ^{14}C l labelled metabolites were infused to avoid contamination of blood plasma with ^{14}C.

Urea entry rate was calculated as:

$$UER\ (mg/ml) = \frac{IR}{SRA}$$

where IR is the infusion rate (Bq/min);

SRA is the mean plateau specific radioactivity of plasma urea (Bq/mg);

2. Glucose entry rate

Glucose entry rate (GER) was estimated by infusing [U-^{14}C] glucose continuously into the jugular vein for

9 h at a constant rate of 4.80 kBq/min. Blood samples were collected during the last 3 h of [U-^{14}C] glucose infusion. In some cases blood samples for GER determination were derived from blood samples collected over 1 h at 10 minute intervals during blood flow estimation. It was assumed that glucose SRA was at a plateau by 6 h of continuous [U-^{14}C] glucose infusion (TELENI, 1984).

a) Calculation of glucose metabolism in whole body and hind-limb muscle

The calculation of parameters of glucose biokinetics are based on those of TELENI (1984) and ODDY *et al.* (1985).

Whole body

Glucose entry rate (GER) and CO_2 entry rate (CO_2 ER) were calculated on the basis similar to urea entry rate.

$$CO_2 \text{ from glucose } (CO_2gl,\%) = \frac{CO_2 \text{ SRA}}{\text{SRA.gl}} \times 100$$

where CO_2SRA is the mean specific radioactivity at "plateau" or ^{14}C in arterial blood (Bq/mgC) during infusion of labelled glucose.

$$\text{Glucose oxidation rate (GOR,mgC/min)} = \frac{CO_2\text{,gl}}{100} \times CO_2\text{,ER}$$

$$\text{Glucose oxidised (GOX,\%)} = \frac{\text{GOR}}{\text{GER}} \times 100$$

Hind-limb muscle

$$\text{Glucose uptake (GU,mg/min/kg)} = \frac{\text{BF} \times (\text{A.gl} - \text{V.gl})}{100}$$

where BF is blood flow to the hind-limb muscle (ml/min/kg);
A.gl is arterial concentration of glucose (mg/100 ml);
V.gl is venous concentration of glucose (mg/100 ml).
CO_2 output (CO_2,out,mgC/min/kg) = BF x (V.CO_2 – A.CO_2) x 0.012
where V.CO2 is venous concentration of CO_2 (mM);
A.CO_2 is arterial concentration of CO_2 (mM).

$$^{14}CO_2 \text{ production (Bq/ml)} = \frac{12\,(\text{A.}CO_2 \times CO_2\text{, SRA}) - (\text{V.}CO_2 \times \text{V.}CO_2\text{, SRA})}{1000}$$

$$^{14}\text{C-Glucose uptake (14C.GU, Bq/ml)} = \frac{40\,(\text{A.gl} - \text{V.gl}) \times \text{SRA.gl}}{1000}$$

$$\text{Glucose oxidised (MOX,\%)} = \frac{^{14}\text{CO2, prod}}{^{14}\text{C.GU}} \times 100$$

$$CO_2 \text{ from glucose} = \frac{\text{MOR}}{CO_2\text{, out}} \times 100$$

where MOR is muscle glucose oxidation rate.

CO_2 entry rate

Entry rate of CO_2 was estimated using $NaH^{14}CO_3$ infused continuously into jugular vein for at least 12 h at a constant of 4.63 kBq/min. It was considered important to infused $NaH^{14}CO_3$ for a longer period before blood sampling because the equilibrium of CO_2 in the body pools is considered to take more than 8 h (see BERGMAN and HOGUE, 1967).

Radioisotopes

The isotope used were D-[U^{14}C] glucose, [^{14}C] urea and Sodium (^{14}C) Bicarbonate ($NaH^{14}CO_3$). Infusates were prepared by diluting isotopes to appropriate concentrations with a sterile physiological saline. Infusates were analysed for radioactivity before and after infusion into the animals to check for any loss of activity. The D-[^{14}C] glucose infusate was made by diluting the isotope to give a concentration of 12.95 kBq/ml. Glucose carrier (less than 0.06% of expected glucose entry rate in sheep fed at maintenance level) was added to the infusate at the rate of 205.7 mg D-glucose carrier per litre infusate + 1 ml sterile alcohol before being dispensed into a polyethylene bottle and stored at -20°C.

The $NaH^{14}CO_3$ infusate was made by diluting the stock to give a concentration of 16.65 kBq/ml. Sodium bicarbonate was also added as a carrier to the infusate at the rate of 1.68 g/l (less than 0.06% of expected CO_2 entry rate in sheep fed maintenance energy level). Preparation of the infusate was carried out immediately before each infusion to avoid possible loss of radioactivity during storage. Furthermore, 2-3 drops of 1 M NaOH were added to each infusate to maintain the solution slightly alkaline.

[^{14}C] urea was diluted, in a similar manner, to give a concentration of 11.56 kBq/ml.

Metabolites

1. Urea

Plasma and urinary urea were determined using a Technicon Autoanalyser (Technicon Method No SE 40001FD4). When necessary, urine sample were appropriately diluted with distilled water before analysis.

Plasma samples were deproteinised (1:1) with Somogyi reagent (SOMOGYI, 1945) and centrifuged at 3000 rpm for 20 minutes. One ml of supernatant was mixed with 10 ml of Cocktail II and counted in the liquid Scintillation Counter. Radioactivity in the supernatant was assumed to be due to ^{14}C urea (COCIMANO and LENG, 1967). For this reason urea entry rate was always determined first in an animal where there was negligible ^{14}C contamination in blood plasma. The remaining supernatant was used for urea concentration determination.

2. Glucose

Two ml of plasma was diluted with 4 ml of sterile distilled water and mixed with 2 ml Somogyi reagent then centrifuged for 20 minutes at 3,000 rpm. Four ml of the supernatant was used for radioactivity determination, the remaining aliquot was used for determination of glucose using the Technicon Autoanalyser technique.

The determination of glucose SRA was carried out using glucose pentaacetate derivative method of JONES (1965).

3. Blood CO_2

Blood CO_2 concentration was calculated using the equation of Henderson-Hasselbach : Total CO_2 (mM) = antilog (pH 6.12) x 0.444 pCO_2 + 0.314 pCO_2

4. Blood oxygen

Blood O_2 was calculated from measured pCO_2, pH and Hb using the equation:

O_2 (mM) = 0.394 O_2 (vol %)

O_2 (vol %) = [Hb (g/100 ml) x O_2 saturation % x 1.34]/100,

where O_2 saturation % at 37°C and pH 7.4 = $(pO_2/34.1)^{2.68}/[1 + (pO_2/34.1)^{2.68}]$ after correction of Bohr effect, i.e. log pO_2 = - 0.48 per 0.1 pH unit difference from 7.40.

5. Blood CO_2 SRA

Blood CO_2 SRA was determined by the gravimetric method of LENG and LEONARD (1965). The $BaCO_3$ formed, was solubilised using TRIS-EDTA buffer as described by HINKS *et al.* (1966).

6. Lactate, free fatty acids (FFA), insulin and growth hormone

Lactate was analysed by the method of HOHORST (1965).

Free fatty acids were determined in 1 ml plasma using a modified version of the "Dole" method published and by KELLY (1965). Palmitic acid (0.05-0.20 µmole/ml) was used as standard.

Plasma insulin was determined using the radioimmunoassay technique described by ROSSELIN *et al.* (1966). Ovine insulin and purified ovine serum were used as a standard. The ^{125}I labelled insulin was iodinated, according to the Chloramine-T method of HUNTER and GREENWOOD (1962) and purified by gel filtration (JORGENSEN and LARSEN, 1972). The sensitivity of assay was 2.4 mU/l. The sample was analysed for radioactivity using a Packard Auto-Gamma Scintillation Spectrometer (Model 5210).

The concentration of growth hormone was measured by the talc radioimmunoassay originally described by WALLACE and BASSETT (1970). The assay had a sensitivity of 1.0 µg/l.

RESULTS AND DISCUSSION

Urea biokinetics

Data on urea biokinetics are presented in Table 1. Plasma urea concentration was not affected by underfeeding nor resumption of *ad libitum* feeding. However, during underfeeding (P2), urea entry rate, urinary urea excretion rate and the rate of urea transferred from the blood to the gut (sheep in Group II) were approximately 74% lower than those in Group I. These values were significantly increased in Group II at P3, and the incremental increases (P2 to P3) were higher than those in Group I. At P4 the mean value of each of the parameters above were not statistically different between groups. Pooling data from all animals for regressions of plasma urea entry rate on plasma urea concentration or on N intake, showed significant relationships of $r = 0.42$ ($P < 0.05$) and $r = 0.53$ ($P < 0.05$), respectively (see Fig. 2).

In the present study, there was a tendency that plasma urea concentration was increased (although not statistically significant) at 3 weeks resumption of *ad*

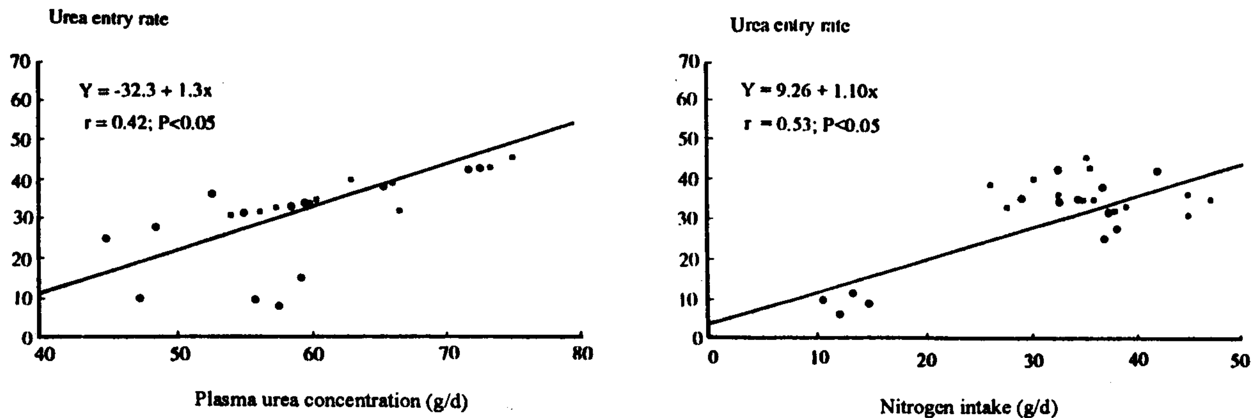

Figure 2. The relationship between urea entry rate and plasma urea concentration N intake derived from pooled data on animals in normal (*) and interrupted growth (?)

libitum feeding. ELLENBERGER *et al.* (1989) observed a decline in blood urea nitrogen (BUN) in steers in early realimentation (0-14 days) following feed restriction, but BUN was increased again afterward to a level similar to that in control animals. It was suggested that the decline of BUN (despite the protein intake was increased) is an indication of the efficient use of nutrient and the high demand for increasing visceral growth (see STANGASSINGER and GIESECKE, 1986) during compensatory growth. It seems that 2 weeks of realimentation is a period where nutrient is most efficiently used; since at 3 weeks of resumption of *ad libitum* feeding there was a tendency of plasma urea to increase in this experiment.

The increase in plasma urea was followed by increased urea entry rate (UER) or urea production which is a reflection of amino acid catabolism. However, estimation of UER gives too high values for the rate of amino acid catabolism since part of urea is formed from ammonia which is absorbed from the digestive tract. The increase in protein intake during the period of resumption of *ad libitum* feeding apparently was also followed by increased urinary urea suggesting that there was protein wastage. This is probably true since plasma concentrations in all period of this experiment were higher than 10 mg/100 ml which according to PRESTON *et al.* (1965) is an indication of protein wastage.

During feed restriction, there was a reduction of amino acid catabolism resulting from reduced N intake indicated by a decline in urea entry rate (UER) and urinary urea. This inturn decreased the rate of urea transferred to the gut (Table 1).

If it is assumed that the degradation of 100 g protein could potentially produce 55 g glucose and 35 g urea (KREBS, 1964) and that the urea excreted in the urine of sheep in this study came from catabolised body protein, the maximum amount of glucose derived from amino acids can be estimated (Table 4). It can be seen from the table that during 3 weeks of feed resumption, the contribution of amino acid to glucose production increased remarkably although it subsequently declined.

Table 1. Urea biokinetics (g/d) in sheep in normal (Group I) and interrupted (Group II) growth over different periods (P)

	Group	Period P1	P2	P3	P4
Plasma urea (mg/dl)	I	61.3	60.2	62.1	63.9
	II	56.8	54.6	63.0	59.9
Urea entry rate (g/d)	I	48.5	51.1	51.8	54.1
	II	47.2	13.4*	52.6**	49.8
Urinary urea (g/d)	I	24.5	27.9	30.2	30.8
	II	25.8	7.3*	27.2**	26.1
Urea transferred to gut#	I	24.0	23.3	21.9	22.3
	II	21.4	6.2*	25.3**	23.9

P1 is the period when both Groups I and II were on *ad libitum* feeding
P2 is the period when Group II was on restricted feeding
P3 and P4 are periods of 3 and 15 weeks, respectively, of resumption of *ad libitum* feeding by Group II
\# Estimated by substracting urinary urea excreted from urea entry rate
* Significant between Group I and II at P2 ($P < 0.05$)
** Significant P2-P3 increment between Group I and II ($P < 0.05$)

Perhaps this suggests that the amino acid was not efficiently utilised for protein deposition particularly during the first 3 weeks of feed resumption. This could be due to the imbalance between the available energy-yielding substrates and amino acids in the body.

Glucose metabolism

Data on glucose biokinetics and CO_2 entry rate are summarised in Table 2. Neither the plasma glucose concentrations, GER expressed per unit liveweight nor the proportion of CO_2 entry derived from glucose oxidation was significantly different between sheep in Group I and those in Group II over the different periods. A similar result was observed for the percentage of glucose oxidised except that at P4, a higher percentage of glucose was oxidised in Group II than in Group I. The GER and CO_2 entry rates were higher in Group I than in Group II at P2 due to reduction in entry rates of approximately 53% and 56%, respectively.

In the hind-limb muscles, the different periods of growth did not affect blood flow nor the percentage of glucose extracted from the circulation. The glucose AV concentration differences across the hind-limb muscles were significantly differently between Group I and II only at P2 where the mean value for Group I was approximately 52% greater than that in Group II. The mean glucose taken up by the hind-limb muscle was higher in Group I than that in Group II by approximately 86%. The rate of increase of glucose uptake in the period P2 to P3, however, was significantly greater in Group II than in Group I. Group II sustained an increased uptake of glucose so that at P4, glucose uptake by this group, was approximately 48% higher than that in Group I. The mean percentage of glucose uptake that was oxidised at P4 was higher in Group I than in Group II by approximately 30%. No significant different between the two groups was detected at P2 or in the period from P2 to P3.

The reduction of glucose and CO_2 entry rates in the underfed animals were expected due to the reduction of available glucose precursors and the reduction in metabolic rate. When animals resumed *ad libitum* feeding, the glucose and CO_2 entry rates were increased markedly, indicating a switch to the anabolic mode by these animals. Such a switch was evident in the observed increase in glucose uptake by the hind-limb muscles of sheep in Group II when *ad libitum* feeding was resumed (Table 2). The increased GER which would have resulted in increased secretion of insulin was the most probable cause of the increased glucose uptake by muscles (McDOWELL, 1983; ODDY *et al.*, 1987). In the current study the increase in plasma insulin concentration in animals in Group II during resumption of *ad libitum* feeding, was not statistically significant because of a high variation between animals (Table 3). No significant difference in plasma growth hormone concentration was

Table 2. Glucose biokinetics and CO_2 entry rate in sheep in normal (Group I) and interrupted growth (Group II) over different periods (P)

	Group	Period[a]			
		P1	P2	P3	P4
Whole body:					
Plasma glucose (mM)	I	3.5	3.4	3.4	3.4
	II	3.3	3.0	3.4	3.6
Glucose entry rate (mmole/h)	I	29.4	30.0	24.0	29.8
	II	29.3	14.2*	21.2**	33.4
Glucose entry rate (mmole/h/kg LW)	I	1.13	0.88	0.73	0.76
	II	1.09	0.68	0.81	0.94
Glucose oxidised (%)	I	20.2	22.2	31.9	30.7
	II	25.2	17.6	36.4	41.0***
CO_2 entry rate (mmole/h)	I	813	644	718	650
	II	809	283*	638**	880
CO_2 from glucose (%)	I	4.4	6.0	6.5	9.0
	II	5.6	5.6	7.2	9.4
Hind-limb muscle:					
Blood flow (ml/min/kg muscle)	I	104.8	108.3	117.8	100
	II	133.8	87.8	105.8	121.3
Glucose AV conc difference (μM)	I	158	160	130	109
	II	136	87.8*	105.8	121.3
Glucose extraction (%)	I	4.5	4.7	3.9	3.2
	II	4.1	3.7	3.2	3.7
Glucose uptake (μmole/min/kg muscle)	I	16.4	17.1	15.1	10.7
	II	18.2	9.2*	11.5**	5.8***
Glucose oxidised (%)	I	11.4	15.6	20.3	32.1
	II	17.6	20.2	24.7	24.6
Glucose glycolysed (%)#	I	NA	22.6	32.2	19.8
	II	NA	26.9	39.0	23.7
Maximum glucose oxidised (%)##	I	59.3	62.1	66.4	NA
	II	56.3	50.0	59.6	NA
CO_2 from glucose (%)	I	5.5	7.6	8.3	9.1
	II	4.3	5.3*	6.4	7.6

[a]see Table 1 for P1, P2, P3 and P4
by Group II
NA = not available
\# Assumming that 1 mole glucose produces 2 moles of lactate
\#\# Assumming that complete oxidation of 1 mole glucose requires 6 moles of oxygen
* Significant between Group I and II at P2 ($P < 0.05$)
** Significant P2-P3 increment between Group I and II ($P < 0.05$)
*** Significant between Group I and II at P4 ($P<0.05$)

also observed (Table 3). BLUM *et al.* (1985), ELLENBERGER *et al.* (1989) HAYDEN *et al.* (1993) and HENRICK *et al.* (1994), however, observed an elevation and reduction of growth hormone and insulin, respectively in steers during feed restriction (maintenance level) and a reduction and elevation of growth hormone and insulin, respectively during resumption of *ad libitum* feeding.

The rate of glucose oxidation in the whole body paralleled the rate of glucose oxidation by the hind-limb muscles (Table 2) suggesting the significance of this tissue in total body metabolism. Although the percentage contribution of glucose to CO_2 production in hind-limb muscle did not change significantly during compensatory growth (Table 2); the actual rate of oxidation of glucose per unit muscle weight was dramatically increased (Table 5), thus indicating the significance of glucose in muscle metabolism during compensatory growth.

If the amount of glucose oxidised to CO_2 and that apparently glycolysed to lactate are accounted for and if it is assumed that an insignificant amount of glucose is converted to amino acids such as alanine (MALLETE *et al.*, 1969; FELIG *et al.*, 1970), it might be assumed that the balance of glucose taken up by muscle would probably be assimilated into glycogen. If these assumptions were accepted, it can be suggested that during compensatory growth, the rate of storage of glycogen in hind-limb muscle will probably be not significantly in the first three weeks of *ad libitum* feeding (see Table 6). The priority of usage of glucose taken up by muscle during this period appears to be oxidation to both CO_2 and lactate.

Table 3. The mean arterial plasma concentrations of lactate, free fatty acids, growth hormone and insulin, the ratio of growth hormone: insulin and the arteriovenous (AV) concentration differences of lactate, free fatty acids and oxygen in sheep in normal (Group I) and interrupted (Group II) growth over different periods (P)

	Group	Period[a] P1	P2	P3	P4
Whole body:					
Arterial concentration:					
Lactate (mM)	I	0.76	1.07	0.59	1.12
	II	0.60	1.03	0.61	1.2
Free fatty acids (µM)	I	498	691	1080	753
	II	516	561	1355	676
Growth hormone (µg/L)	I	3.0	2.5	4.6	4.2
	II	4.7	5.8	8.8	2.6
GH : insulin	I	0.18	0.09	0.19	0.13
	II	0.18	0.33	0.37	0.11
Hind-limb muscle:					
AV concentration difference:					
Lactate (µM)	I	0.17	-0.07	-0.08	-0.04
	II	0.13	-0.06	-0.11	-0.06
Free fatty acids (µM)	I	90	44	260	-260
	II	90	29	845	-15
Oxygen (mM)	I	1.53	1.54	1.14	NA
	II	1.48	1.30	1.09	NA

[a] see Table 1, for P1, P2, P3 and P4

Relationship between GER and protein intake

When data of GER and protein intake (see MAHYUDDIN and TELENI, 1995) were pooled, there was a significant correlation ($r = 0.47$, $P < 0.05$ see Figure 3) between the two parameters. Increasing protein content in the diet was observed to increase GER (FORD and

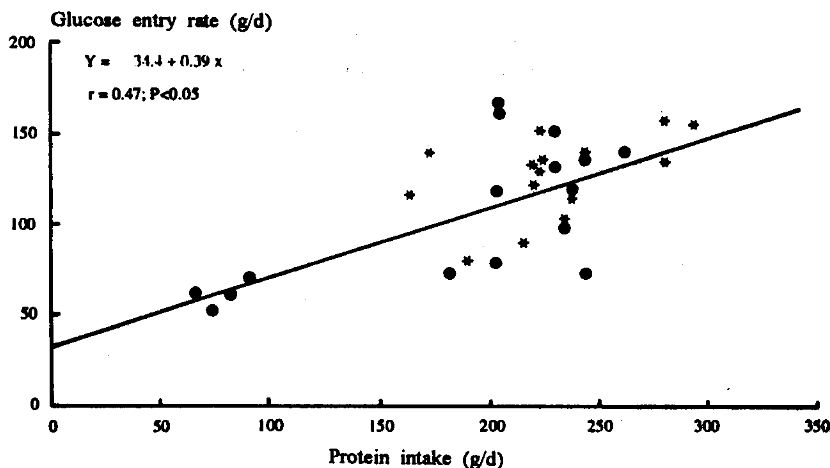

Figure 3. The relationship between glucose entry rate and protein intake derived from pooled data on animals in normal (*) and interrupted growth (?)

Table 4. The maximum amount of glucose that can be derived from amino acids as calculated from urinary urea excretion by sheep in normal (Group I) and compensatory (Group II) growth over different periods (P)

	Group	Periods[a]			
		P1	P2	P3	P4
Glucose from amino acids (mmole/h)	I	8.8	10.3	10.8	11.3
	II	9.5	2.5	9.7	95
Contribution to glucose entry rate (%)	I	30	34	45	38
	II	32	18	46	28

[a] see Table 1, for P1, P2, P3 and P4

Table 5. The rate of glucose oxidation in the whole body and hind-limb muscles in normal (Group I) and compensatory (Group II) growth over different periods

	Group	Period[a]			
		P1	P2	P3	P4
Whole body					
Glucose oxidation rate (mmole/h)	I	1.9	2.7	3.1	3.4
	II	3.2	1.9	2.8	3.9
Hind-limb muscle					
Glucose oxidation rate (umole/h/kg muscle)	I	5.9	6.7	7.6	9.3
	II	7.4	2.5	7.8	13.7

[a] see Table 1, for P1, P2, P3 and P4

Table 6. Estimated percentage and rate of glucose (taken up by hind-limb muscle) converted to glycogen in normal (Group I) and compensatory (Group II) growth

	Group	Period[a]		
		P2	P3	P4
Glucose to glycogen (%)	I	61.6	47.5	48.1
	II	52.9	36.3	51.7
Glucose to glycogen (rate) (umole/m/kg/muscle)	I	10.6	7.2	5.1
	II	4.9	4.2	8.2

[a] see Table 1, for P1, P2, P3 and P4
* Calculated by substraction from glucose taken up by muscle the amount of glucose oxidised to CO_2 and that apparently converted to lactate

REILLEY, 1969). It has been suggested (LINDSAY, 1970) that such a relationship between GER and protein intake might be a reflection of a relationship that normally exists between protein and ME intake. It is probably true in this experiment since lucerne is highly degradable in the rumen (COELHO DA SILVA *et al.*, 1972; DE BOER *et al.*, 1987; KUSWANDI, 1988) a relatively low rate of absorption of amino acids from the small intestine is likely to occur.

CONCLUSION

In ruminants, restricted feeding results in reduction of urea, glucose and CO_2 entry rate because of reduced N intake, available glucose precursor and metabolic rate. The reduction of urea entry rate was reflected in the lower concentration of urinary urea and urea transferred from blood to the gut. Whereas the reduction of glucose and CO_2 entry was reflected in the lower AV concentration of glucose, glucose taken up by the hind-limb muscle and the percentage of glucose taken up by muscle that was oxidised.

Animals on *ad libitum* feeding, following restricted feeding show a switch to the anabolic mode indicated by increased urea, glucose and CO_2 entry rates. This was followed by the increased in glucose taken up and that was oxidised by the hind-limb muscle. This period of compensatory growth was also demonstrated by the dramatic increase in the actual rate of glucose oxidation per unit muscle weight.

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