Influence of hepatic ammonia removal on ureagenesis, amino acid utilization and energy metabolism in the ovine liver

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The mass transfers of O2, glucose, NH3, urea and amino acids across the portal-drained viscerae (PDV) and the liver were quantified, by arterio–venous techniques, during the last 4 h of a 100 h infusion of 0 (basal), 150 or 400 μmol NH4HCO3/min into the mesenteric vein of three sheep given 800 g grass pellets/d and arranged in a 3×3 Latin-square design. Urea irreversible loss rate (ILR) was also determined by continuous infusion of [14C]urea over the last 52 h of each experimental period. PDV and liver movements of glucose, O2 and amino acids were unaltered by NH4HCO3 administration, although there was an increase in PDV absorption of non-essential amino acids (P=0.037) and a trend for higher liver O2 consumption and portal appearance of total amino acid-N, glucogenic and non-essential amino acids at the highest level of infusion. PDV extraction of urea-N (P=0.015) and liver removal of NH3 (P<0.001), release of urea-N (P=0.002) and urea ILR (P=0.001) were all increased by NH4HCO3 infusion. Hepatic urea-N release (γ) and NH3 extraction (x) were linearly related (R2=0.89), with the slope of the regression different from unity, both for estimations based on liver mass transfers (1.16; SE=0.144; Pγ,x=0.31) and [14C]urea (0.97; SE=0.123; Pγ,x=0.84). The study indicates that a sustained 1.5 or 2.4-fold increase in the basal NH3 supply to the liver did not impair glucose or amino acid supply to non-splanchnic tissues; nor were additional N inputs to the ornithine cycle necessary to convert excess NH3 to urea. Half of the extra NH3 removed by the liver was, apparently, utilized by perportal glutamate dehydrogenase and aspartate aminotransferase for sequential glutamate and aspartate synthesis and converted to urea as the 2-amino moiety of aspartate.

Sheep: Liver: Ammonia: Ureagenesis

NH3 of both endogenous and gastrointestinal origin is normally removed completely by the liver and converted to urea. Hepatic ureagenesis depends, however, on the coordinated supply of N to the ornithine cycle from two different precursors, mitochondrial NH3 and cytosolic aspartate. Blood free amino acids are, together with NH3, the only N-substrates extracted by the liver in amounts sufficient to maintain the rates of ureagenesis observed in ruminants in vivo (Huntington, 1989; Reynolds et al. 1991; Lobley et al. 1995). If amino acids were the predominant N-donors to aspartate via transamination reactions with glutamate, the immediate aspartate-N precursor, then the ratio NH3 removal: urea-N production across the liver should be 0·5 or even lower because NH3 derived from the 5-amido group of glutamine can also contribute N to the mitochondrial synthesis of carbamoyl phosphate (Nissim et al. 1992). Such ratios, on both an absolute and incremental basis, have been observed in several studies with ruminants in vivo (see reviews by Reynolds, 1992; Parker et al. 1995; Lescoat et al. 1996), leading to the hypothesis that, to detoxify NH3, the liver would require an equal N input from amino acids. This would penalize net protein availability to the animal. Other data, however, yield values greater than 0.5 (see Huntington, 1986; Seal & Reynolds, 1993; Parker et al. 1995), suggesting that blood NH3 can provide N to both urea-N precursors. Indeed, the equimolar conversion of NH3 to urea-N has been firmly established by isotopic studies in vitro (Luo et al. 1995; Brosnan et al. 1996). Although this efficient conversion has not always been observed in response to increased hepatic NH3 extraction in vivo (Huntington, 1986; Reynolds et al. 1991; Goetsch et al. 1996), this may relate to experimental conditions in which both diet

Abbreviations: GDH, glutamate dehydrogenase; GIT, gastrointestinal tract; ILR, irreversible loss rate; PDV, portal-drained viscerae; RMS, residual mean square.

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quality and/or quantity were altered. Thus, factors other than portal NH$_3$ flow that might also stimulate ureagenesis from amino acids, were changed simultaneously.

Ureagenesis can influence both liver energy expenditure and gluconeogenesis. The theoretical energy costs of urea synthesis have been exceeded in many studies in vivo (e.g. Reynolds et al. 1991; Lobley et al. 1995), but the data are confounded by the number of factors altered by the nutritional treatments imposed. Similarly, evidence for interaction between NH$_3$-stimulated ureagenesis and hepatic glucose production comes primarily from studies in vitro (Weekes et al. 1978; Martinrequero et al. 1993), where non-physiological concentrations and balances of substrates are often employed.

The objective of the present experiment was to investigate the response of hepatic urea-N production and amino acid production to a constant input of 50 mmol NaCl/min plus one of: (1) no NH$_3$-HCO$_3$; (2) NH$_3$-HCO$_3$ at 400 mmol/min; (3) NH$_3$-HCO$_3$ at 150 mmol/min; (4) NH$_3$-HCO$_3$ at 40 mmol/min. The collection lines were allowed to pass through ice-cold water to reduce both risk of blood clotting and enzyme activities in the blood samples. The blood samples were collected directly into 10 ml syringes stored in ice-cold water. An additional mixed blood sample (10–15 ml) was collected during each experimental period for determination of blood and plasma DM. Three urine samples were also collected during the last 4 h of the experimental period. An additional urine sample was collected 18 h before the [14C]urea infusion for determination of background radioactivity.

**Materials and methods**

**Animals**

Three Suffolk cross-bred wethers (35–40 kg body weight), surgically prepared with indwelling catheters in the posterior aorta, portal, hepatic and mesenteric veins (Lobley et al. 1995), were placed in metabolism cages under continuous feeding. The specific radioactivity of urea was determined on 4 g of blood and plasma DM. Three urine samples were collected during each experimental period for determination of in vivo urea acidification and amino acid metabolism.

**Blood analysis**

Blood samples in each syringe were carefully mixed and analysed for blood p(O$_2$), p(CO$_2$), pH, bicarbonate and haemoglobin concentration immediately after collection using a Blood Gas Analyser (Acid Base Laboratory ABL3, Radiometer, Copenhagen, Denmark). The packed cell volume was determined by the microhaematocrit method. One portion (0.5 g) of blood was deproteinized with 5 g 120 g/l TCA and processed for gravimetric determination of p-amino hippuric acid as previously described (Lobley et al. 1995). Then 4 ml blood was centrifuged at 1200 g for 10 min at 4°C and the plasma (approximately 2.5 ml) collected. Two portions (0.5 ml) of plasma were used for the enzymic determination of NH$_3$ (Mondzak et al. 1965) and glucose concentration (Bergmeyer, 1985) by automated procedures (Kone Autoanalyzer, Espoo, Finland). Two additional portions of 1.2 and 0.9 ml plasma were stored at −20°C until analysis for urea and amino acid concentration respectively.

The four arterial, portal vein and hepatic vein plasma samples from each experimental period were pooled in proportion to the plasma flow (pooled sample weight, approximately 0.5 g), and processed for amino acid analysis in physiological fluids with an Alpha Plus Amino Acid Analyser (Pharmacia-LKB Biochrom Ltd, Cambridge, Cambs, UK) as described by Lobley et al. (1995). One portion of 0.3 g plasma was enriched with 0.3 g of a solution of 5 mmol-[15N$_2$]urea (99.7 atom %), carefully mixed, deproteinized with 0.1 ml sulfoalicylic acid (480 g/l) and centrifuged at 7000 g for 5 min. From the supernatant fraction, the N-methyl-N- (tertiary butyldimethylsilyl) trifluoroacetamide derivative of urea was prepared as described by Calder & Smith (1980). Plasma urea enrichment was determined by electron impact GC–mass spectrometry analysis on a VG Trio-1 mass spectrometer (VG Masslab, Manchester, UK) coupled to a Hewlett Packard 5890 GC (VG Organic, Manchester, UK; Calder & Smith, 1980). The fragment ions at m/z 231 and 233 were monitored under selective ion recording conditions.

**Urine analysis**

The specific radioactivity of urea was determined on 4 g urine acidified with 1 g 250 g/l TCA. Scintillation liquid (10 ml) (Ultima Gold, Camberra Packard Ltd, Pangbourne, Berkshire, UK) was added to 0.2 g acidified urine and the $^{14}$C radioactivity was measured by liquid scintillation
Calculations

O₂ concentration in blood (mmol/l) was calculated as

\[ H \times 1.34 \times S/22.4 \]

where \( H \) is the haemoglobin content of blood (g/l), 1.34 is the maximum O₂ transport capacity of the haemoglobin (ml O₂/g haemoglobin), 22.4 is the gas constant (ml O₂/mmol O₂) and \( S \) is the O₂ saturation of the haemoglobin estimated from the equation developed by Margaria (1963):

\[
S = \frac{[(1 + k_1 \times cp(O_2))k_1 \times cp(O_2)]^3}{k_2 - 1} + \frac{k_1 - 1}{[(1 + k_1 \times cp(O_2))k_1 \times cp(O_2)]^4} + k_2 - 1.
\]

In this equation, the value of \( p(O_2) \) has been corrected \((cp(O_2))\) to allow for the effects of \( p(CO_2) \) and blood pH on \( S \) according to the formula proposed by Kelman (1966):

\[
cp(O_2) = p(O_2) \times 10^{0.4 \times (pH - 7.4) + 0.06 \times (\log 40 - \log p(CO_2))},
\]

and \( k_1 (0.005491) \) and \( k_2 (1042) \) were estimated by fitting the equation to the data obtained for the O₂ saturation of sheep haemoglobin at different O₂ tensions reported by Bartels & Harms (1959).

Blood flows (F, g/min) were calculated as:

\[
F_p = I/(C_p - C_a) \quad \text{and} \quad F_h = I/(C_h - C_a),
\]

where \( F_p \) and \( F_h \) are the blood flows in the portal and hepatic veins respectively, \( I \) is the infusion rate of p-amino hippuric acid (μmol/min) and \( C_p \), \( C_h \) and \( C_a \) are the concentrations of p-amino hippuric acid (μmol/g) in posterior aorta, portal vein and hepatic vein respectively.

Plasma flows and whole-blood water flows (g/min) were calculated as:

\[
F \times (1 - PCV) \quad \text{and} \quad F \times (1 - BDM),
\]

respectively, where \( F \) is the relevant blood flow and PCV and BDM are the corresponding packed cell volume and blood DM content of the sample respectively.

Mass transfers of metabolites and O₂ across the portal-drained viscera (PDV) and the liver (μmol/min or mmol/min) were calculated as:

\[
F_p \times (C_p - C_a) \quad \text{and} \quad (F_h C_h) - \left( F_p C_p \right) - (F_h - F_p) \times C_a,
\]

where \( F_p \) and \( F_h \) are the blood (for O₂ and NH₃), plasma (for amino acids and glucose) or whole-blood water (for urea) flows in the portal vein and hepatic vein (g/min) respectively, and \( C_p \), \( C_h \) and \( C_a \) are the concentrations of O₂ in blood (μmol/g) and metabolites in plasma (amino acids, NH₃ and glucose; nmol/g) or plasma water fraction (urea, estimated as plasma urea concentration : plasma water fraction; μmol/g) in posterior aorta, portal vein and hepatic vein respectively. Urea transfers were calculated as whole-blood water transfers under the assumption that plasma and blood water fractions have equal urea concentrations (Milano, 1997). NH₃ concentrations in blood and plasma were assumed to be equal (Milano, 1997) and thus plasma concentration and blood flows quantified to yield transfers across the PDV and the liver. Virtually no glucose is transported within the erythrocytes in sheep (Arai et al. 1995) and only for glycine and threonine is plasma transfer across the PDV significantly smaller than blood movements (Lobley et al. 1996a). Therefore, glucose and amino acid movements across the PDV and the liver were estimated as plasma transfers.

The irreversible loss rate (ILR, μmol/min) of urea was calculated as:

\[
\frac{I}{Sr},
\]

where \( I \) is the infusion rate (kBq/min) of \([^{14}C]\)urea and \( Sr \) is the specific radioactivity of \([^{14}C]\)urea (kBq/μmol) in urine.

Statistical analysis

The data were initially analysed by ANOVA for the effects of treatment (rate of infusion of NH₄HCO₃), block (animals) and period, with two residual degrees of freedom. In the case of portal vein NH₃ concentration and urea and NH₃ transfers across the PDV and the liver, the mean squares for the effect of period and block were equal to, or lower than, that of the residual and never significant. For these data, the period and block sum of squares were therefore included in the residual sum of squares and the data re-analysed for the effect of treatment alone, with 6 d.f. for the residual mean square (RMS). The relationships between hepatic NH₃ removal, hepatic urea-N release and urea-N IRL were studied by regression analysis.

Results

Animals and catheter patency

All animals completed the experiment and the catheters maintained their full patency (i.e. they allowed blood collection as well as infusion of solutions) during the experiment, with the exception of one mesenteric catheter which, after the first experimental period, was suitable only for infusion.

Blood variables (Table 1)

The 4 d infusion of NH₄HCO₃ had no measurable effect on blood pH, \( p(CO_2) \), HCO₃⁻ or haemoglobin concentration and therefore the acid–base status of the sheep remained unaltered during the experiment.

Plasma ammonia, urea and amino acid concentrations (Table 1)

An apparent small reduction of 14 μmol/l was detected during treatment C150 in the average arterial concentration of NH₃ (\( P < 0.05 \)) which otherwise lay between 60 and 75 μmol/l, within the ranges normally reported for sheep (e.g. Orzechowski et al. 1988; Lobley et al. 1995, 1996b). No changes were observed in NH₃ concentration in the hepatic vein, while that in the portal vein increased (\( P < 0.003 \)) by 191 (C150) and 400 (C400) μmol/l during the NH₄HCO₃ administration. The increases in portal NH₃
concentration were larger than would be expected (i.e. 125 and 290 μmol/l for C150 and C400 respectively) on the basis of the portal blood flow, basal portal NH₃ concentration and the infusion rates of NH₄HCO₃.

The arterial concentrations of urea increased by 163 and 365 mmol/l after the 4 d infusion of NH₄HCO₃ at 150 and 400 μmol/min (P < 0.05), with parallel responses in the portal and hepatic venous concentrations.

With the exception of leucine, which increased (P < 0.05) from 157 μmol/l in C0 to 186 μmol/l in C400, no changes were detected in the arterial concentration of amino acids as a result of the infusion of NH₄HCO₃ (results not shown). Regardless of the treatment, plasma concentrations of several amino acids (e.g. phenylalanine, P < 0.05; leucine, P < 0.01; isoleucine, P < 0.05; results not shown) in the first experimental period were lower than in subsequent periods.

### Blood flow, gas exchange and glucose transfers (Table 2)

The blood flows in the portal and hepatic veins were not

### Table 1. Arterial blood variables and plasma ammonia and urea concentrations in sheep infused with 0 (C0), 150 (C150) or 400 (C400) μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d

(Means values for three sheep with the standard error of the difference between means)

<table>
<thead>
<tr>
<th>Arterial blood variables</th>
<th>C0</th>
<th>C150</th>
<th>C400</th>
<th>SED</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.416</td>
<td>7.453</td>
<td>7.436</td>
<td>0.029</td>
<td>0.55</td>
</tr>
<tr>
<td>Blood p(CO₂) (mmHg)</td>
<td>35.0</td>
<td>35.03</td>
<td>33.32</td>
<td>0.134</td>
<td>0.80</td>
</tr>
<tr>
<td>Blood HCO₃⁻ (mmol/l)</td>
<td>22.24</td>
<td>24.35</td>
<td>22.18</td>
<td>0.273</td>
<td>0.55</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>103.6</td>
<td>101.6</td>
<td>102.6</td>
<td>1.62</td>
<td>0.57</td>
</tr>
<tr>
<td>NH₃ (μmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>63</td>
<td>49</td>
<td>74</td>
<td>2.8</td>
<td>0.024</td>
</tr>
<tr>
<td>Portal vein</td>
<td>343</td>
<td>534</td>
<td>743</td>
<td>68.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>30</td>
<td>43</td>
<td>45</td>
<td>13.6</td>
<td>0.563</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>4.36</td>
<td>5.99</td>
<td>8.01</td>
<td>0.406</td>
<td>0.024</td>
</tr>
<tr>
<td>Portal vein</td>
<td>4.23</td>
<td>5.80</td>
<td>7.84</td>
<td>0.406</td>
<td>0.025</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>4.50</td>
<td>6.19</td>
<td>8.32</td>
<td>0.423</td>
<td>0.024</td>
</tr>
</tbody>
</table>

† The data were analysed by ANOVA, with 2 or 6 (portal vein) d.f. for the error term. t (0, 6) = 2.45.

* For details of procedures, see pp. 308–309.

### Table 2. Blood flow and net mass transfer of oxygen, glucose, ammonia, urea and amino acid-nitrogen across the portal-drained viscera (PDV) and the liver and urea irreversible loss rate (ILR) in sheep infused with 0 (C0), 150 (C150) or 400 (C400) μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d

(Means values for three sheep with the standard error of the difference between means)

<table>
<thead>
<tr>
<th>Blood flow (g/min)</th>
<th>C0</th>
<th>C150</th>
<th>C400</th>
<th>SED</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic artery</td>
<td>104</td>
<td>66</td>
<td>82</td>
<td>52</td>
<td>0.78</td>
</tr>
<tr>
<td>Portal vein</td>
<td>1268</td>
<td>1218</td>
<td>1380</td>
<td>248</td>
<td>0.82</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>1372</td>
<td>1284</td>
<td>1463</td>
<td>197</td>
<td>0.71</td>
</tr>
<tr>
<td>PDV (μmol/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂§</td>
<td>-1593</td>
<td>-1647</td>
<td>-1843</td>
<td>105.0</td>
<td>0.24</td>
</tr>
<tr>
<td>Glucose§</td>
<td>-32</td>
<td>-39</td>
<td>-88</td>
<td>37.0</td>
<td>0.43</td>
</tr>
<tr>
<td>NH₃</td>
<td>344</td>
<td>589</td>
<td>908</td>
<td>39.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea-N</td>
<td>-322</td>
<td>-430</td>
<td>-610</td>
<td>24.6</td>
<td>0.015</td>
</tr>
<tr>
<td>Amino acid-N§</td>
<td>316</td>
<td>346</td>
<td>522</td>
<td>46.4</td>
<td>0.080</td>
</tr>
<tr>
<td>Liver (μmol/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂§</td>
<td>-1610</td>
<td>-1600</td>
<td>-2003</td>
<td>174.0</td>
<td>0.13</td>
</tr>
<tr>
<td>Glucose§</td>
<td>315</td>
<td>325</td>
<td>352</td>
<td>10.6</td>
<td>0.13</td>
</tr>
<tr>
<td>NH₃</td>
<td>-391</td>
<td>-600</td>
<td>-954</td>
<td>46.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea-N</td>
<td>710</td>
<td>912</td>
<td>1356</td>
<td>105.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Amino acid-N§</td>
<td>-263</td>
<td>-331</td>
<td>-377</td>
<td>197.9</td>
<td>0.86</td>
</tr>
<tr>
<td>Urea IRL (μmol urea-N/min)</td>
<td>774</td>
<td>1012</td>
<td>1336</td>
<td>79.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

† The data were analysed by ANOVA with 2 or 6 d.f. for the error term; t (0, 5, 2) = 4.30; t (0, 5, 6) = 2.45.

§ § 2 d.f.

†† Does not include valine and proline.
altered by NH₄HCO₃ infusion. The small contribution of the hepatic artery to the liver blood flow (6 %) was also unaltered. The liver and the PDV each accounted for 0.5 of the O₂ consumption measured across the splanchnic bed (3-20 mmol/min) in C0. Based on the traditional estimation of 0.66 mol O₂/mol urea (i.e. 4 ATP/mol urea), urea synthesis would account for 0.15 of liver energy expenditure under basal dietary conditions. The O₂ consumption by both tissues showed an upward trend during the infusion of 400 μmol NH₄HCO₃/min, but only 0.54 of the observed increase in liver O₂ consumption could be theoretically accounted for by the additional urea synthesised. Basal glucose uptake by the PDV was, at 32 μmol/min, 0.10 of that released by the liver, with neither altered by the administration of NH₄HCO₃.

**Ammonia and urea transfers (Table 2)**

Dietary N intake was 16.4 g/d, equivalent to 815 μmol N/min under continuous feeding conditions. Although urea transfer to the gastrointestinal tract (GIT) remained constant at 0.45–0.47 of liver production across all three treatments, the absolute transfer increased with both levels of NH₄HCO₃ infusion (P < 0.02). This endogenous urea-N recycling to the GIT was equivalent to 0.40–0.75 of ingested N.

Basal NH₃ appearance in the portal vein (μmol/min; i.e. net NH₃ appearance corrected for the infusion rate of NH₄HCO₃) amounted to 0.42 of dietary N intake in C0, increased by 95 and 164 μmol/min in C150 and C400 respectively (P < 0.02), and showed a linear correlation with urea-N transfer to the GIT (x, μmol/min; y = 188.5 (SE 43.12) + 0.53 (SE 0.091) x; P < 0.001; RMS 1324.8; R² 0.83).

Lever extraction of NH₃ was augmented (P < 0.001) in response to the NH₃ load and was 1.02–1.14 higher than NH₃ appearance across the PDV, indicating that the liver also removed NH₃ released from non-splanchnic tissues. Urea-N production by the liver was 1.81 times the basal NH₃ uptake for treatment C0 and rose significantly by 202 and 646 μmol/min following the infusion of 150 and 400 μmol NH₄HCO₃/min. Similar trends were observed for urea IRL (μmol/min), which showed good concordance with hepatic urea production (x, μmol/min; y = 321.4 (SE 160.38) + 0.734 (SE 0.155) x; P = 0.002; Pᵇ[b] = 0.13; RMS 18137.77; R² 0.76). A strong linear relationship was observed between urea-N release (y, μmol/min) and NH₃ extraction (x, μmol/min) across the liver, with the slope of the regression equation not different from unity, regardless of whether estimations were based on GC–mass spectrometry analysis (y = 241.3 (SE 100.05) + 1.16 (SE 0.144) x; P < 0.001; Pᵇ[b] = 0.31; RMS 10626.8; R² 0.89; Fig. 1) or [¹⁴C]urea IRL (y = 409.5 (SE 84.60) + 0.97 (SE 0.123) x; P < 0.001; Pᵇ[b] = 0.84; RMS 7597.6; R² 0.89).

**Amino acid transfers (Table 3)**

For all treatments there was net absorption of amino acids across the PDV and net removal by the liver, with the exception of glutamate, citrulline and ornithine, which exhibited net release by the liver (results not shown). Initial calculations also showed a net hepatic release of valine; later studies revealed that a non-identified peak co-chromatographed with valine and interfered with the determination of plasma valine concentrations. Valine data were, therefore, not included in the estimation of net PDV, hepatic and total splanchnic transfers of total, essential and branched-chain amino acids.

Under basal dietary conditions, net amino acid-N absorption by the GIT (316 μmol/min) accounted for 0.38 of the dietary N intake (or 0.28 of dietary N plus urea-N inputs to the GIT). The movement of amino acids across the PDV remained unaltered by the infusion of either level of NH₄HCO₃ (P > 0.05), although portal appearances of arginine (P < 0.03) and non-essential amino acids (P < 0.04) increased while those of total amino acid-N (see Table 2), threonine, asparagine, glutamate, glutamine and glycine also tended to be greater (P < 0.1; results not shown) at the highest level of infusion. Net absorption of amino acid-N and urea-N removal by the GIT were highly correlated (R² 0.84; Fig. 2).

Hepatic extraction of total, essential, non-essential, branched-chain, glucogenic or individual amino acids was unaltered by the treatments. Hepatic extraction of total amino acid-N accounted, on average, for 0.82 of the amino acid-N appearance across the PDV. The hepatic fractional removal (hepatic removal:PDV appearance) varied markedly among individual amino acids, however, being close to, or higher than, unity for tyrosine, phenylalanine, histidine, threonine and the glucogenic serine, alanine, glycine and glutamine (the latter removed at a rate seven times higher than the PDV appearance), intermediate (0.5–0.7) for methionine and lysine and lowest (< 0.2) for leucine and isoleucine (results not shown).

**Liver nitrogen balance**

The net balance of N across the liver (estimated as: NH₃ removal + free amino acid-N removal – urea-N release) was not affected by the NH₄HCO₃ infusion and averaged –21 (SEM 21.8) μmol/min.
Table 3. Net mass transfer of amino acids across the portal-drained viscera (PDV), the liver and the splanchnic bed (μmol/min) in sheep infused with 0 (C0), 150 (C150) or 400 (C400) μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d† (Mean values for three sheep with the standard error of the difference between means)

<table>
<thead>
<tr>
<th></th>
<th>TA</th>
<th>EA</th>
<th>BA</th>
<th>NA</th>
<th>GA</th>
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TA, total amino acids (Asp, Asn, Glu, Gln, Ser, Gly, Ala, Tyr, Arg, Thr, Met, Ile, Leu, Phe, Lys, His, Cit, Cmr); EA, essential amino acids (Thr, Met, Ile, Leu, Phe, Lys, His); BA, branched chain amino acids (Leu, Ile); NA, non-essential amino acids (Asp, Asn, Glu, Gln, Ser, Gly, Ala, Tyr, Arg); GA, glucogenic amino acids (Ser, Gln, Gly, Ala).

* For details of procedures, see pp. 308–309.
† Positive and negative values indicate net production and net extraction of the metabolite by the relevant organ respectively.
‡ The data were analysed by ANOVA, with 2 d.f. for the error term; t (0.05, 2) = 4.30.

Discussion

The present study was designed to address the question of whether increasing the portal supply of NH₃ to the liver could alter the availability of energy and amino acids to non-splanchnic tissues, thus compromising protein and energy deposition in growing male lambs. Data from previous studies in vivo and in vitro indicated that both phenomena might indeed arise under conditions of high input of NH₃ to the liver, but the information available was controversial on both a qualitative and a quantitative basis because NH₃ was not the only variable modified by the treatments (Orzechowski et al. 1988; Reynolds et al. 1991; Lobley et al. 1995; Luo et al. 1995). In most cases either nutrient supply or acid–base status had also been altered and, thus, the interpretation of the results was confounded. In order to minimize these problems an experimental design was adopted in which only one variable, the NH₃ supply to the liver, was modified by direct infusion of two levels of NH₄HCO₃ into the mesenteric vein.

Liver oxygen consumption and gluconeogenesis

There is little disagreement, at present, that stimulation of ureagenesis increases liver O₂ consumption (e.g. Reynolds et al. 1991); the resultant elevated energy expenditure will reduce whole-body energy deposition. Yet doubts still exist about the actual magnitude of this effect. The measured increase in liver O₂ consumption during the administration of NH₃⁺ into the mesenteric vein has usually ranged from 1.5 to 5.5 mol O₂ per additional mol of urea synthesized (i.e. 2–8-fold greater than theoretical estimates based on 4 ATP or 0.66 mol O₂ per mol urea synthesized; Milano, 1994; Lobley et al. 1995, 1996b). With the exception of determinations carried out in fasted sheep infused with NH₄HCO₃ (Milano, 1994), the differences with pre-infusion levels never achieved statistical significance.
This experiment is, unfortunately, no exception in that the largest average increase in live O₂ consumption (393 μmol/min or 1·2 μmol O₂ per additional μmol urea synthesized, for C400) was 1·85 times higher than the expected theoretical maximum but the variance was, again, large (SED 174 μmol O₂/min, P = 0·13). If energy expenditure elicited by NH₃-stimulated ureagenesis exceeds theoretical values then other reactions (e.g. transport of substrates across subcellular membranes, intrahepatic cycling of glutamine) must be also enhanced. Lately, however, it has been pointed out that if the traditional P:O of 3·1 were replaced by other less efficient ratios (e.g. 2·1 or even 1·5:1; see Lobley, 1994), then much of the discrepancy between theoretical considerations and empirical evidence would disappear.

Studies in isolated sheep hepatocytes have revealed that gluconeogenesis from propionate could be depressed by 20–40% at NH₃Cl concentrations of 500–660 μmol/l (Weekes et al. 1978; Luo et al. 1995). When this was tested in vivo, however, the results were contradictory. Liver glucose output was either depressed by 48% (Orzechowski et al. 1988) or unaltered (Barej et al. 1987) during short-term infusions (120 min) of NH₃Cl into the mesenteric vein of sheep at rates sufficient to achieve portal concentrations of 800–850 μmol/l and exceed the capacity of the liver for NH₃ removal (1·5 μmol/min per g wet tissue). In the present experiment, hepatic glucose production remained unaffected after a 4 d administration of NH₄HCO₃ and at similar portal NH₃ flows and concentrations (i.e. 740 μmol/l in C400) to the previous studies. This suggests that the high portal NH₃ load that may occur in ruminants, e.g. those given rations with a high non-protein-N content or fresh forage rich in rapidly degradable protein, does not impair liver glucose supply for extra-hepatic tissue metabolism.

Ammonia, urea and amino acid transfers

Experimental evidence obtained from studies of splanchnic transfer of N-compounds over the last decade (see reviews of Reynolds, 1992; Parker et al. 1995; Lescoat et al. 1996), indicated that, on an incremental basis, removal of NH₃ by the liver is associated with a larger than equimolar release of urea-N; the slope of the regression of urea-N release v. NH₃ uptake across studies ranged between 1·6 and 1·9. Free amino acids were the only N-compounds available to the liver in sufficient amounts to provide the additional N. This led to the hypothesis that amino acid availability for hepatic synthesis of export proteins, or for non-splanchnic tissue utilization, would be progressively reduced as the rate of NH₃ removal by the liver increased (Reynolds, 1992; Parker et al. 1995).

The hypothesis gained further support from long-term studies in sheep. When the basal rate of hepatic NH₃ removal was increased by continuous administration of NH₄Cl into the mesenteric vein for 5 d, not only was the additional urea-N released from the liver 2-fold greater than the additional NH₃ removed but whole-body leucine oxidation increased significantly by 18% (Lobley et al. 1995). Slight decreases in plasma pH (0·08 units) and plasma HCO₃⁻ concentration (5 mmol/l) were also reported during the NH₄Cl infusions. Studies in human subjects (Reaich et al. 1992) and rodents (May et al. 1992) have demonstrated that acidosis elicited by the administration of NH₄Cl can stimulate protein breakdown and amino acid oxidation, leaving a surplus of amino acid-N available for urea synthesis. Thus, it was unclear whether the higher leucine oxidation and the additional N appearing in urea observed by Lobley et al. (1995) were consequences of the higher NH₃ removal or of the concomitant mild acidosis.

In a subsequent study, in which acidosis was avoided by a continuous 4 d infusion of NH₄HCO₃ into the mesenteric vein, the incremental values for urea-N release: NH₃ removal across the liver of growing lambs were 1·12 and 1·17 after hepatic NH₃ uptake was increased by 208 or 325 μmol/min respectively (Lobley et al. 1996b). The nutritional conditions involved were, however, substantially different, with a higher feed intake (2·0 v. 1·2 times energy maintenance) compared with the initial experiment of Lobley et al. (1995). This increased markedly the amino acid-N absorbed by the animals (1155 v. 372 μmol/min) and reduced the ratio NH₄⁻: amino acid-N appearing in the portal vein from 1·3–1·9 to 0·5–0·8, lower than expected for diets with high non-protein-N content (1·1–1·5; Maltby et al. 1991). In addition, it is well established that the activity of urea cycle enzymes is enhanced in response to high-protein diets, primarily as a consequence of changes in enzyme mass (Morris, 1992). There was concern, therefore, that these changes may have altered the hepatic capacity to handle excess NH₃, as shown in the perfused rat liver, where the ability to form urea from 600 μM-NH₄Cl increased with the amount of casein in the diet (Saheki, 1972).

Thus, the present experiment was designed to meet two fundamental criteria. First, the acid–base status of the animals remained unchanged during the administration of NH₄Cl and, in this respect, the choice of the bicarbonate salt seemed appropriate because it had been used without obvious alteration of the acid–base status in two experiments at a rate similar to that of C150 (Milano, 1994; Lobley et al. 1996b). At higher doses, however, NaHCO₃ had been shown to cause acute metabolic alkalosis in rats (Boon et al. 1994) and dogs (Rodriguez et al. 1989). This raised concerns about the possible long-term increase in plasma HCO₃⁻ concentration with the highest level of NH₄HCO₃ infusion (C400). Nevertheless, the acid–base status of the sheep remained within the normal range throughout the experiment.

Second, amino acid-N appearance across the PDV had to match that reported by Lobley et al. (1995) (i.e. 406 v. 372 μmol/min) but at comparatively higher rates of hepatic NH₃ removal (900 v. 600 μmol NH₃/min). It was, therefore, critical that the additional source of N would not alter the net amino acid supply to the lambs. It was expected that some of the urea synthesized by the liver, as a result of the infusion of NH₃HCO₃, would be transferred to the GIT. This could increase net microbial protein yield if the basal (C0) rumen degradable N supply was below that required to sustain optimum microbial protein yield in vivo (29–32 g N/kg organic matter truly digested in the rumen: Agricultural and Food Research Council, 1993). Any additional amino acid absorption could result in increased urea synthesis and tend to lower the apparent efficiency of conversion of NH₃ to urea. The marked trend towards higher values of PDV...
amino acid-N absorption in C400 and the linear correlation between amino acid-N and urea-N transfers across the PDV suggest that the assumption of unchanged amino acid supply did not hold for the highest level of infusion.

Nevertheless, the slope of the regression of hepatic urea-N release (1.16; SE 0.14) or [14C]urea-N IRL (0.97; SE 0.12) on hepatic NH₃ removal, together with the steady hepatic amino acid extraction observed under these experimental conditions support earlier observations in vivo in steer (Maltby et al., 1991) and sheep (Lobley et al., 1996b) and contradict the concept that equal inputs of N from sources exogenous or endogenous to the liver, namely amino acids, are necessary to maintain NH₃-stimulated ureagenesis. Studies in vitro, where [15N]urea was synthesized from [15NH₄]Cl, the only exogenous N source (Luo et al., 1995; Brosnan et al., 1996), have provided evidence that NH₃-N can enter the ornithine cycle by two separate routes: synthesis of carbamoyl phosphate, through a reaction catalysed by carbamoyl phosphate synthase I, and reductive amination of 2-oxoglutarate followed by transamination to aspartate, a pathway controlled by glutamate dehydrogenase (GDH) and aspartate aminotransferase. If the additional NH₃ supply to the liver in the current experiment was converted to urea-N without extra amino acid-N inputs to the ornithine cycle, then the GDH–aspartate aminotransferase pathway must have had the capacity to utilize half the extra NH₃ removed. For C400, for example, this required an additional flux of 281 μmol N/min to glutamate and aspartate synthesis, which represents an 8-fold increase in the net flux through GDH (calculated as: liver NH₃ removal – (0.5 × urea-N release)), from an estimated basal rate (C0) of 36 μmol N/min. The capacity of liver GDH to utilize excess NH₃ to synthesize glutamate is, therefore, central to the question of detoxifying NH₃ with or without alteration in the partition of amino acid-N flow towards ureagenesis.

The current study indicates that the capacity of GDH to meet such demands is either inherent or can be adapted when portal NH₃ input to the liver is augmented and sustained for 4 d at nearly 3-fold that in basal conditions. Distinction between these mechanisms seems of less practical significance for long-term than for short-term (i.e. 2–3 h) increases in NH₃ supply to the liver. The former (i.e. intrinsically high liver GDH activity) would be of importance to allow removal of acute excesses of NH₃ without penalizing amino acid availability to the animal. Confirmation of equal partition of the additional NH₃ inputs to the liver between the GDH–aspartate aminotransferase and carbamoyl phosphate synthase I pathways requires further experimentation, however. First, it is not certain that the conclusions of the current, and previous, experiments in growing lambs and steers can be extrapolated to other diets (e.g. fresh grass or legumes), feeding routines (e.g. discontinuous feeding) and physiological conditions (e.g. pregnancy, lactation) where different balances of hormones and nutrients (e.g. propionate) may occur. Second, the deviation from unity of the slope of the regression of urea-N release v. NH₃ removal across the liver (i.e. 0.16) might still represent a minor, but not negligible, stimulation of amino acid-N utilization for ureagenesis. With a 300 μmol/min increase in hepatic NH₃ extraction, for example, the deviation of the ratio from unity observed in the current study would amount to 0.96 g N/d. Moreover, enhancement of amino acid oxidation during sustained increase of NH₃ inputs to the liver could be entirely unrelated to limitations in N flux through GDH, because amino acids could also be used as energy substrates to meet the increased demands of hepatic ureagenesis. In C400, for example, with an increase in liver O₂ consumption of 393 μmol/min, an extra 84 μmol amino acid-N per min might have been oxidized to provide the necessary energy to sustain ureagenesis (i.e. 176 J/min, based on 448 kJ/mol O₂ and 24 MJ/kg protein or 2.1 kJ/mol protein N). The numerical agreement between this value and the additional amino acid-N flow to urea in C400 as measured by GC–mass spectrometry (83 μmol/min, estimated from additional urea-N release – additional NH₃ removed across the liver) is remarkable, but probably coincidental.

Acknowledgements

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