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Ovine Hepatic Metabolism 1. The Effect of Portal Amino Acid Concentration on the Metabolism of Glucose and Urea

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Abstract: The study was designed to address the hepatic response (urea and glucose production, glycogen synthesis, and oxygen consumption) to physiological changes in Amino Acid (AA) concentrations. To elucidate this objective, Four South African Mutton Merino Wethers weighing 23-33 Kg were used and the caudate lobe of the liver of mean weight 18.70±1.5 g was perfused with modified Krebs-Henseleit medium. Mixtures of amino acids representing Low Nitrogen (LN), High Nitrogen (HN = 3 X LN) and LN or HN plus either extra alanine, lysine, or glutamine, were perfused at a flow rate ranging from 65-100 ml/min. The data indicates an increase in hepatic urea production and a 20-240% decrease in glucose production in response to high amino acid mixture. The inhibition of glucose production was inhibited by 22% for the HN infusion and further 20% by added alanine, lysine or glutamine. However, the addition of amino acid did not affect urea production and the oxygen consumption.

Key words: Amino acids, liver, perfusion, sheep

INTRODUCTION

The liver perfusion model has a great advantage over isolated and cultured hepatocytes techniques, as the hepatic architecture, polarity and the integrity of the cytoskeleton is maintained[1]. Ruminants absorb a large proportion of dietary nitrogen across the Portal-Drained Viscera (PDV) as ammonia nitrogen, which is detoxified by conversion to urea in the liver. In our earlier reports, an improvement of the liver perfusion model was adapted for sheep[2]. As previously reported the perfusion of a physiological mixture of amino acids increased urea production by more than 170% [2], while glucose output did not increase when either glutamine or alanine were added. Under conditions of chronic supply, the liver removes most amino acids in excess of its net anabolic needs. While glucose production was stimulated in response to individual amino acids, glucose production decreased as a result of infusion of an amino acid mixture. Such an interrelationship between ureagenesis and gluconeogenesis warranted a further investigation to fully understand the mechanisms involved. We therefore tested, in the perfused liver, the response of different amino acid concentrations on the metabolism of glucose and urea.

MATERIALS AND METHODS

Animals and diet: Four South African Mutton Merinowethers weighing 23 - 33 kg (live weight) were used in this experiment. The sheep were fed a Lucerne-mixed diet (*Medicago sativa*) and tef (*Eragrotis tef*) diet mixed 1:1, which was supplied in equal meals of 600 g.

Experimental procedure: Each sheep was anaesthetised using 6% sodium pentobarbitone intravenously at a dose rate of 15 mg Kg^{-1}. The abdominal organs were exposed from the right side via a paracostal incision (3cm caudal to the last rib). The detailed experimental procedures and surgical protocol of Ali *et al.*[2] were followed.

Perfusion system and treatments: After placing a portal catheter[2], the isolated lobes were perfused in a non-recirculating mode with Krebs-Henseleit buffer containing NH_4Cl (0.3 mM), lactate (2.1 mM), pyruvate (0.3 mM) and propionate (0.5 mM). Solutions containing a mixture of amino acids at LN or HN were infused continuously into the portal cannula using a precision micropump.

The LN mixture reflected the concentrations of amino acids in the portal venous blood of sheep on a poor quality protein diet. Both LN and HN were also infused with an additional amino acid, either Ala, Lys or Gln at a final (HN= 3LN), LN or HN + Ala (0.2 mM), LN or HN + Lys (0.2 mM), or LN or HN + Gln (0.2 mM). The final concentrations of added amino acid contributed in the LN and HN mixtures to the perfusion buffer are shown in Table 1.

Effluent perfusates were collected at a regular interval throughout the perfusion. The concentrations of amino

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Table 1: The final concentrations of amino acids in the Low nitrogen (LN) and high nitrogen (HN) perfusates

Amino acid	LN µm	HN µm
Alanine	102.7	308.1
Arginine	80.8	80.8
Aspartic acid	8.4	25.2
Cysteine	25	75
Glutamic acid	42.8	128.4
Glutamine	92.38	277.14
Glycine	177.4	532.2
Histidine	18.8	56.4
Isoleucine	48.9	146.7
Leucine	52.2	156.6
Lysine	23.6	70.8
Methionine	27	81
Phenylalanine	18.8	56.4
Proline	16.15	48.45
Serine	44.4	133.2
Threonine	49	147
Tyrosine	41.2	123.6
Valine	122.7	368.1
Tryptophan	20	60

acids, urea, ammonia and glucose were determined in these samples. To monitor the liver viability, electron microscopy was performed according to the method adopted by Florman *et al.*,[3].

Chemical analysis: Using diagnostic kits (Boehringer Mannheim, Germany), the concentrations of urea and glucose were measured using the methods described by Fawcet and Scott[4] and Werner *et al.*,[5], respectively. The liver glycogen was measured using an enzymatic hydrolysis with amyloglucosidase.

Statistical analysis: All studies used 3 separate perfusions. Results are expressed as means±SEM. The data was analysed using ANOVA, followed by the Student's t-test for mean separation. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Perfusion data: The basic parameters for the liver perfusion are presented in Table 2. To ensure that the preparation was viable, the liver was studied microscopically. In addition, viability tests as outlined by Ali *et al.*,[2], were performed to assure the suitability of liver for accurate physiological measurements.

Figure 1 shows the effect of different concentrations of AA on the glucose and urea outputs from the perfused lobe. The highest glucose release (900 nmol g^{-1} min^{-1}) from the liver was obtained when the physiologically balanced LN mixture was perfused. This output was significantly higher than any of the other values. The addition of either one of the three amino acid significantly reduced this output (e.g. from 903.14±89.5 to 639.83±55.10 nmole g^{-1} min^{-1} for glutamine). Furthermore, increasing the total amino acid output three fold also significantly

Table 2:Basic Perfusion data of sheep

Parameter	Mean±SEM
Mass of caudate lobe (g)	18.76±1.50
O_2 supply (μmol g^{-1} min^{-1})	4.29±0.11
O_2 consumption (μmol g^{-1} min^{-1})	2.02±0.12

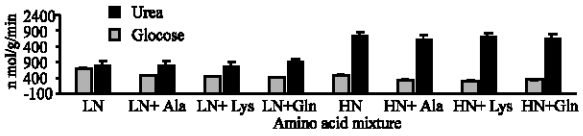

Fig. 1: Effect of various levels of amino acids on hepatic output of glucose and urea

Table 3: The effect of amino acids on liver glycogen synthesis during the perfusion of a sheep liver lobe

Treatment	Glycogen ((g 100g)$^{-1}$)
Without amino acids	
Before perfusion	1.76 ± 0.28*
After perfusion	0.63 ± 0.34¥
With amino acids	
Before perfusion	2.71 ± 1.02
After perfusion	2.43 ± 0.98

* Significantly different from after perfusion (None) ($p < 0.05$)
¥ Significantly different from all treatments

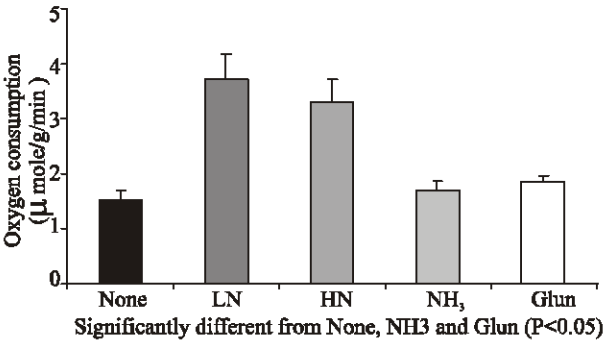

Fig. 2: Oxygen consumption by the liver lobe of sheep in response to changing nitrogen supply

reduced the glucose output by the same amount. Additional single AA loads on top of the HN mixture further significantly reduced the glucose output. The urea output, unlike that of glucose, doubled when the AA mixture was changed from LN to HN. Further addition of single amino acids to either the LN or the HN mixtures failed to elicit any significant response in glucose production.

The glycogen concentrations in the lobe, both pre- and post-perfusion, are given in Table 3. It is clear that at least some of the additional amino acids metabolised by the liver were indeed converted to glucose, but were stored as glycogen rather than being released as free glucose.

The oxygen consumption by the perfused lobe is illustrated in Fig. 2. These data suggest a significant

increase in metabolic cost associated with the increased gluconeogenic and ornithine cycle activities resulting from the provision of suitable substrates. However, additional amino acids above normal portal concentrations did not lead to any further energy demand. Similarly, the addition of propionate or NH_4Cl to the buffer showed no increase of O_2 consumption.

DISCUSSION

The functional and structural integrity of the perfused liver were assessed by different parameters including gross appearance, perfusion flow rate, pH, K^+ efflux, O_2 consumption, gluconeogenesis, ureagenesis and the enzymatic activity of the perfusate. Of these, oxygen supply plays a determining role.

The composition of perfusion media might play a pivotal role in the study of glucose metabolism in the isolated perfused rat liver[6]. The contribution of amino acids to glucose synthesis is highly variable ranging from 2-40% and the hepatic conversion of gluconeogenic AA into glucose is regulated to a great extent by the availability[7] of the amino acids. Glutamine is the primary amino acid utilized for urea synthesis, regardless of whether it is supplemented as the sole amino acid, or in the presence of a physiologic mixture of amino acids[8].

The release of hepatic urea in response to a high amino acid mixture differed to that due to amino acid imbalance (1800 nmole g^{-1} min^{-1} in HN and 1000 nmole g^{-1} min^{-1}). This finding is in agreement with the decrease in urea production in the rat liver (Yassuda et al., 2003). In sheep, the increase in urea formation in the presence of amino acids can be explained by the preferential utilization of the amide nitrogen of glutamine[9]. In the rat, the infusion of Alanine (gluconeogenic) increased urea production in rat, however lysine (ketogenic) showed no effect[10].

The present study indicated that hepatic glucose production was decreased by 20-40% (900 mmole/g/min in high amino acid to 500 mmole/g/min) in LN. This effect may be due to an increase in glycogen synthesis. The most significant reduction was seen when the liver was exposed to HN plus alanine, lysine or glutamine. Similar findings were obtained in ewes, where net hepatic glucose release increased with increased amino acid uptake[11]. In contrast, urea release was doubled in response to these treatments. However, the infusion of glutamine or alanine alone increases glucose production by approximately 400%, while the increase in urea production was insignificant[2]. Shattuck *et al.*,[12] indicated that the infusion of amino acid solutions caused an increase in glucose concentration was also found in the rat liver.

Gluconeogenesis is a continual process that is of great importance in ruminants where carbohydrates are fermented to Volatile Fatty Acids (VFA) in the rumen. The gluconegenicity of amino acid alanine and glutamine was very low in hepatocytes from fed sheep, but was significantly increased in hepatocytes from starved animals. Alanine makes a substantial contribution to ureagenesis, whereas the conversion to glucose was very low[13].

It should be noted that the addition of alanine or glutamine on top of LN or HN did not significantly affect urea release. The carbon deficit cannot be explained by the release of amino acids such as glutamic acid. It is possible that there was an increase in oxidative and non-oxidative (i.e. glycogen synthesis) glucose disposal as a result of increased amino acids load, thereby leading to a decrease of glucose release as a result of HN treatment.

The perfusion model technique could assist in identifying amino acid(s) acting as anabolic signals and it is vital to identify those contributing to urea synthesis and their fate. Our results indicate the importance of the non-recirculating perfusion model in the assessment of amino acid status on hepatic metabolism.

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