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Amino Acids

Extracellular amino acid levels in the human liver during transplantation: a microdialysis study from donor to recipient

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Summary. Using microdialysis, we have monitored extracellular levels of amino acids and related amines in the human liver at three stages of the transplantation procedure: donor retrieval, back table preparation and during 48 h post-implantation. By comparing the ratio of mean levels at the donor and back table stages, with the ratio between early (2–6 h) and late (43–48 h) post-reperfusion, these amines were classified into one of three groups. In one group, back table levels were markedly higher than during the donor stage, with levels declining over time post-reperfusion. A second group had much lower levels in the back table than during the donor phase, and post-reperfusion levels were either stable or increased over time. Concentrations of amino acids in the final group remained relatively constant at all stages. This study illustrates the value of microdialysis in providing organ-specific metabolic data that may indicate specific mechanisms of poor graft function.

Keywords: Liver – Transplantation – Microdialysis – Amino acids – Ethanolamine – Putrescine

Introduction

Orthotopic liver transplantation (OLT) is often the only form of therapy available for end-stage liver disease. As demand on liver transplant programmes grows, there is a need to increase the donor pool, and this has led to the use of more marginal donor grafts, with a consequent increased risk of subsequent graft dysfunction and graft loss. A need for better predictive factors or biomarkers to assess the viability of marginal donor organs has led to the use of microdialysis as a monitoring tool during the post-transplantation period (Nowak et al., 2002; Silva et al., 2005) in order to gain insight into the biochemical changes occurring in the graft at this time.

Microdialysis (Plock and Kloft, 2005) allows the harvesting of low molecular weight substances from the extracellular fluid of the tissue or organ in which the microdialysis catheter is implanted, for subsequent quantitation by various analytical platforms, and has been used for many years as an investigational technique in neuro-physiology and pharmacology (Bourne, 2003), and more recently in the clinical setting to investigate ischaemia and reperfusion in a variety of conditions (Ungerstedt and Rostami, 2004).

While ischaemia/reperfusion injury is the major cause of early liver dysfunction that may be experienced in the recipient following OLT (Carini and Albano, 2003; Casillas-Ramirez et al., 2006), other factors arising during donor retrieval and cold preservation may also contribute. Indeed, it can be argued that if biomarkers in microdialysates are to direct clinical intervention, then their measurement before the graft is implanted is essential.

We have therefore carried out a study in which microdialysis was employed to monitor the liver graft from donor retrieval through to 48 h post-reperfusion, focusing on glucose metabolism (Silva et al., 2006b) and urea cycle intermediates (Silva et al., 2006a), as these are two key aspects of normal liver function that may be disrupted in a marginal organ. These findings have then been related to clinical outcome in order to identify possible biochemical factors that may be predictive of subsequent graft dysfunction (Silva et al., 2006a, b).

As the liver is the major site of nitrogen and, specifically, amino acid metabolism in the body, we also examined the hypothesis that other aspects of nitrogen metabolism may be disrupted during liver transplantation. We therefore adopted a more global approach to studying extracellular levels of amino acids and related amines

rather than focusing on particular pathway intermediates. There were no cases of primary non-function of grafts within this patient group, so the data presented may be seen to represent a 'normal' metabolic response to the insults the organ underwent during this procedure, and may give insight into how the liver adapts to these. However, six of the livers were considered to show features of initial poor function (IPF) on the basis of 24 h post-operative plasma levels of aspartate aminotransferase (AST) being in excess of 2000 IU/L (Strasberg et al., 1994). Consequently, extracellular levels of amino acids and amines during the donor and back table phases of the procedure in this subset of patients were also compared to the remaining patients with 24 h post-operative AST levels of less than 2000 IU/L, to see if there were any differences that might have been indicative of subsequent IPF.

Materials and methods

Patients

This study was approved by the South Birmingham Research Ethics Committee. Fifteen consecutive, consented adult patients undergoing elective OLT were recruited to the study. The median age of these 15 recipients was 53 (range; 20–68) and there were 11 males. Median donor age was 50 (range; 16–66) and the cause of brain death was an intra-cranial bleed in 14 donors, with 12 of them being spontaneous and 2 post traumatic. One donor was found to be brain dead following carbon monoxide poisoning. The aetiology of liver disease in the recipients and further clinical details, are described elsewhere (Silva et al., 2006b).

There were no primary non-function of grafts within this patient group but, as described above, six of the livers were considered to show features of IPF.

Microdialysis

The microdialysis catheter used was a CMA 61 Liver MD Catheter (CMA Microdialysis, Stockholm, Sweden); diameter 0.6 mm, active membrane length 10 mm, molecular weight cut-off 20,000. The catheter was inserted into the liver at the level of the falciform ligament as previously described (Silva et al., 2006b), and was then connected to a microinfusion pump (CMA 106, CMA Microdialysis, Stockholm, Sweden) placed in a sterile glove, and continuously perfused with an isotonic sterile solution (contents sodium chloride 147 mmol/l; potassium chloride 4 mmol/l; calcium chloride 2.3 mmol/l; osmolality 290 mOsm/kg; pH 6; T1 perfusion fluid; CMA Microdialysis, Stockholm, Sweden), initially at 15 µl/min for 6 min, a flush sequence to remove air from within the system, and thereafter at 0.3 µl/min.

During the course of these investigations, 3 microdialysis catheters were used for each of the 15 recipient patients. The first of these was implanted in the donor graft at the beginning of the retrieval process. After changing the first flush sequence sample at 15–20 min, hourly samples of perfusate were then collected into sterile microvials until shortly before the liver was explanted from the donor (this averaged 3–4 samples per patient). In order not to compromise sterility, it was not possible to continue microdialysis monitoring after cross clamping of the donor's aorta, and during the subsequent stages of donor graft retrieval. After bleeding out of the donor, the graft was perfused with cold (4 °C) preservation fluids. The protocol followed used 1 l of University of Wisconsin (UW) solution via the portal vein, and 3 l of Marshall's hypertonic citrate solution via the aorta in the hepatic arteries. After explantation, the liver was then vacuum packed in ice and transported to the Queen Elizabeth Hospital, Birmingham.

During preparation of the liver for implantation at the back table procedure, which entailed the removal of fascia and fat attached to the blood vessels and bile ducts, a second microdialysis catheter was inserted into the graft. The catheter was inserted into the same track created by the first microdialysis catheter. This was easily done since the first catheter, minus its connecting tubes which were cut off when the graft was vacuum packed following the retrieval process, remained in the graft during transport. The first flush sequence sample was again changed after 15–20 min and hourly samples of perfusate were then collected, again averaging 3–4 samples per patient. During the back table procedure, the graft was completely immersed in ice slush (frozen 0.9% saline) throughout. The back table procedure takes 1–2 h, following which the graft remains in ice at 4 °C until the recipient is ready to have the graft implanted. This second catheter was removed just before the graft was transferred into the recipient for implantation. The third microdialysis catheter was implanted into the graft at the end of the recipient operation, following reperfusion of the graft. The third catheter too was inserted into the same tract which could be easily identified following reperfusion. After again collecting a flush sequence sample at 15–20 min, serial hourly dialysate samples were then obtained over the next 48 h, after which the catheter was removed. As with our previous study (Silva et al., 2005), these samples were analysed for a number of different constituents. As previous experience has indicated that temporal amino acid changes resolve only slowly during the post-transplant period, and in view of the extended analytical time per sample required for a full amino acid profile, only one sample from the middle of each of the following time windows was analysed for each patient: (hours, following transplantation: 2–6, 7–12, 13–18, 19–24, 25–30, 31–36, 37–42, 43–48). However, amino acid analysis was performed on all samples collected during the graft procurement and back table stages since the speed of temporal changes was unknown.

Amino acid analysis

Using HPLC with fluorimetric detection of o-phthaldialdehyde derivatives, extracellular concentrations of 24 amino acids and related amines were determined. Of the amino acids, only histidine could not be reliably quantitated as this appeared in the tail of the much larger threonine peak.

The system consisted of a Chromspher ODS column (5 µm, 150 × 3 mm, with guard column; Varian Chrompack, Walton-on-Thames, UK) with a CMA 280 fluorescence detector (maximum excitation, 340–360 nm; maximum emission, 495 nm: CMA Microdialysis Ltd, Stockholm, Sweden). The derivatisation reagent was prepared by mixing 975 µl of incomplete o-phthaldialdehyde reagent solution (OPA, 1 mg/ml in potassium borate buffer, pH 10.4; Sigma, Poole, UK) with 25 µl of 10% (v/v) mercaptopropionic acid in methanol. Derivatisation of dialysis samples and standards, as well as sample injection, were carried out with a CMA 200 refrigerated autosampler (CMA Microdialysis Ltd, Stockholm, Sweden). The mobile phase gradient consisted of 100 mM sodium acetate buffer, pH 6.9, with a deionised water/acetonitrile/methanol mixture (1/3/6 v/v) increasing linearly from 8 to 48% (v/v) over 46 min. This was delivered using a PM-80 twin-reciprocating pump with LC-26A vacuum degasser (BAS Technicol, Congleton, UK). Data were collected and analysed using EZChrom software (Aston Scientific, Stoke Mandeville, UK) after calibration with a range of standard amino acid solutions (10–40 µM). Dialysate levels were not corrected for recovery.

Statistical analysis

Comparison between mean dialysate levels in the donor retrieval and back table stages was by two-way Student's paired *t*-test; significance at $p < 0.05$. Temporal changes in dialysate levels during the 48 h post-implantation period were assessed by 1-way ANOVA with post-hoc comparison by Dunnett's test to the 2–6 h value when significance ($p < 0.05$) was attained.

Results

Donor retrieval

Although, on average, we obtained 3–4 samples during each retrieval process, the levels of all amino acids remained relatively constant throughout. Microdialysis was terminated before aortic cross-clamping, when blood supply to the organ was stopped, and before sterile perfusion with preservation media. Mean levels of each of the amino acids investigated during this part of the transplantation procedure are shown in Table 1.

Back table procedure

As above, extracellular levels of all amino acids remained relatively constant throughout the back table procedure period, so mean levels are again presented (Table 1). With the exception of alanine, aspartate, β-alanine, glutamine and serine, there were statistically significant differences in mean levels between the donor and back table phases for all amino acids and amines. For ethanolamine, GABA, glutamate, glycine, putrescine and taurine, extracellular levels were significantly greater during the back table phase. However, for the remainder of the amino acids studied, donor levels were significantly greater than during the back table procedure (Table 1).

Table 1. Comparison of mean dialysate levels of amino acids and related amines obtained during the donor retrieval and back table phases of liver transplantation

Amino acid	Abbreviation	Donor (μM) mean ± SEM	Back table (μM) mean ± SEM	Paired *t*-test
Alanine	ALA	196.6 ± 12.1	177.5 ± 15.4	ns
Arginine	ARG	9.6 ± 1.3	1.9 ± 0.3	***
Aspartate	ASP	36.4 ± 4.5	32.1 ± 3.2	ns
β-alanine	BALA	8.9 ± 0.7	10.8 ± 1.1	ns
Citrulline	CIT	9.8 ± 1.1	2.0 ± 0.2	***
Ethanolamine	ETH	23.0 ± 2.6	67.5 ± 6.3	***
γ-aminobutyric acid	GABA	7.3 ± 0.9	12.1 ± 1.2	**
Glutamate	GLU	134.1 ± 12.9	299.4 ± 19.3	***
Glutamine	GLN	189.0 ± 11.1	190.8 ± 11.9	ns
Glycine	GLY	214.8 ± 16.3	398.3 ± 19.2	***
Isoleucine	ILEU	23.3 ± 2.3	5.8 ± 0.7	***
Leucine	LEU	60.7 ± 5.1	14.9 ± 1.5	***
Lysine	LYS	55.6 ± 5.3	13.7 ± 1.9	***
Methionine	MET	10.3 ± 1.1	2.7 ± 0.6	***
Ornithine	ORN	34.2 ± 3.4	13.2 ± 2.6	***
Phenylalanine	PHE	25.1 ± 1.6	5.5 ± 0.5	***
Putrescine	PUT	3.0 ± 0.4	6.8 ± 1.1	*
Serine	SER	53.7 ± 6.2	58.4 ± 5.9	ns
Taurine	TAU	41.2 ± 3.6	85.4 ± 6.5	***
Threonine	THR	36.7 ± 3.0	23.7 ± 3.0	***
Tryptophan	TRY	6.1 ± 0.5	1.7 ± 0.2	***
Tyrosine	TYR	17.1 ± 1.7	4.0 ± 0.5	***
Valine	VAL	61.2 ± 4.5	16.1 ± 2.5	***

Values (μM) are the mean (±SEM) of all samples obtained during each of the two phases ($n = 15$). Comparison between the donor retrieval and back table phases was by two-way Student's paired *t*-test ($^{*}\ p < 0.05$; $^{**}\ p < 0.005$; $^{***}\ p < 0.001$; *ns* not significant)

Post-implantation

Most of the amino acids reported here were being investigated for the first time in the post-liver transplant situation. Extracellular levels of β-alanine, leucine, methionine, ornithine, serine, threonine, tryptophan, tyrosine and valine did not vary significantly throughout the 48 h post-reperfusion period (data not shown). Three amines (aspartate, ethanolamine and putrescine) showed a progressive decline throughout the monitoring period (Fig. 1), although the time at which this decline became statistically significant varied. Extracellular levels had reduced significantly by 13 h post-implantation for ethanolamine, 31 h for putrescine, and 43 h for aspartate. Finally, extracellular levels of three amino acids (isoleucine, lysine and phenylalanine) progressively increased over the first 24 h post-implantation before reaching a plateau level thereafter (Fig. 2).

We have previously reported post-implantation extracellular levels of a further 8 amino acids (alanine, arginine, citrulline, GABA, glutamate, glutamine, glycine and taurine) in a separate cohort of patients (Silva et al., 2005). In the present study, the overall temporal profiles of these 8 amino acids remain as we reported previously, as shown in Fig. 3. As before, citrulline and glycine showed no significant changes over the 48 h monitoring period (data not shown). Although alanine declined slowly throughout this period, this also did not reach statistical significance in this study (data not shown). For glutamine, the modest increase seen in both studies over the first 12 h post-reperfusion reached statistical significance on this occasion (Fig. 3), after which levels remained relatively constant throughout the remainder of the monitoring period. The progressive increase in extracellular arginine, the progressive and gradual decrease in both glutamate and GABA, and the more immediate decline of extracellular taurine levels (Fig. 3) all confirmed our previous findings (Silva et al., 2005).

The release or uptake of each individual amine or amino acid was assessed by comparing the pre-transplant (the ratio of donor to back table levels) and the post-transplant situations (the ratio of early to late post-reperfusion phases) and the results of this are shown in Fig. 4. This approach appears to divide the amino acids and amines into one of three groups.

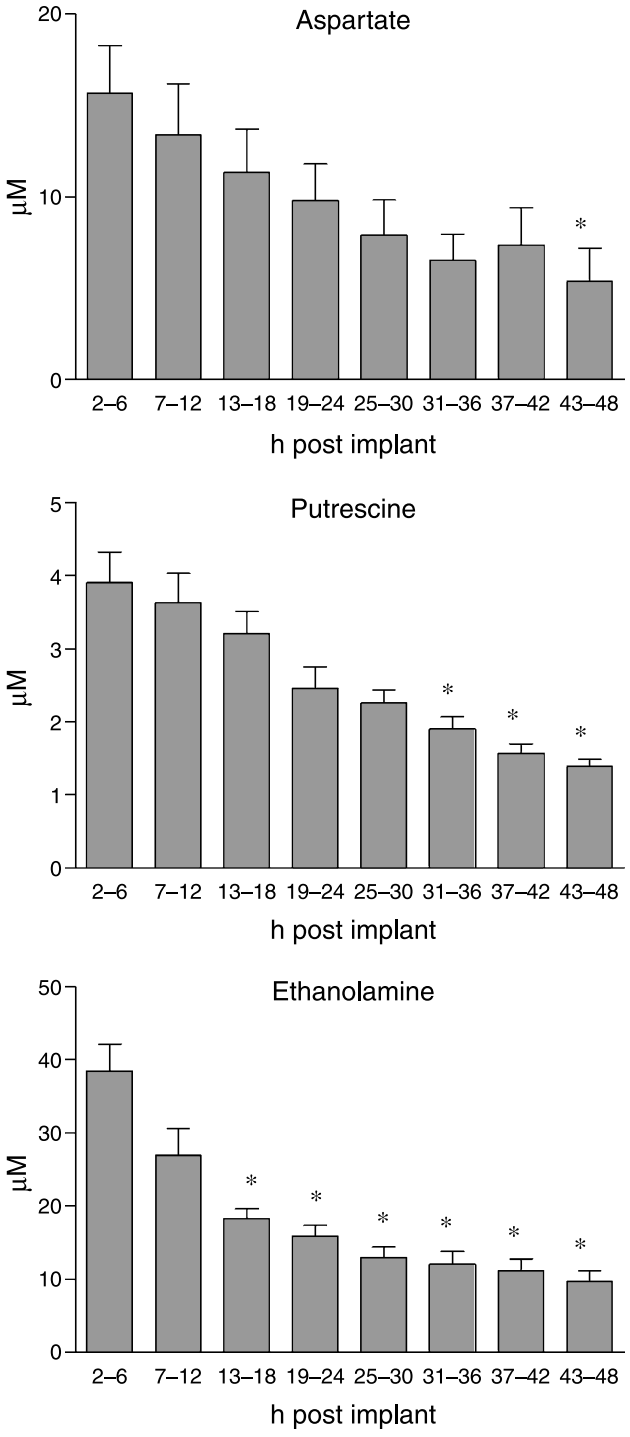

Fig. 1. Dialysate levels (μM, mean ± SEM) of aspartate, putrescine, and ethanolamine at selected time intervals during the 48 h after liver transplantation. $^{*}p < 0.05$, in comparison with value at 2–6 h (1-way ANOVA with post-hoc Dunnett's test)

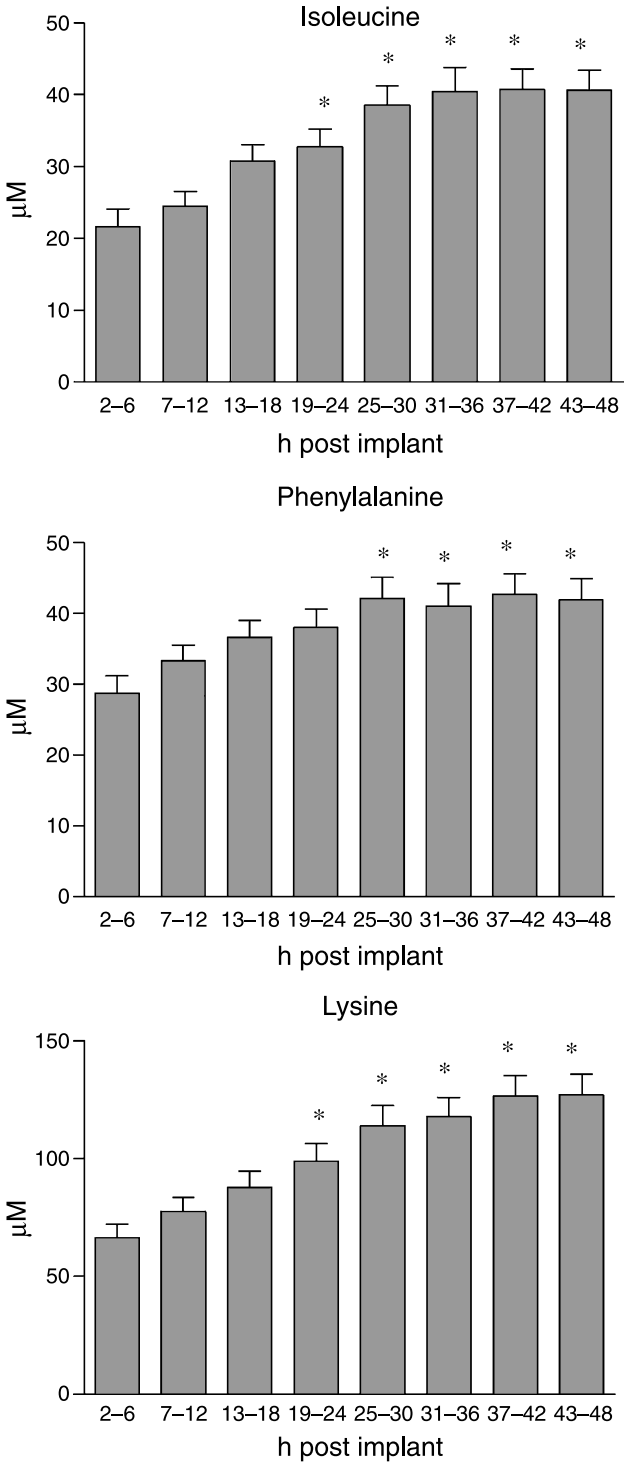

Fig. 2. Dialysate levels (μM, mean ± SEM) of isoleucine, phenylalanine and lysine at selected time intervals during the 48 h after liver transplantation. $^{*}p < 0.05$, in comparison with value at 2–6 h (1-way ANOVA with post-hoc Dunnett's test)

In the first group (Fig. 4, Group A), containing aspartate, ethanolamine, GABA, glutamate, putrescine and taurine, extracellular levels immediately post reperfusion are high, although below the mean levels seen during the back table procedure. Thereafter, there is a progressive decline, although the rate of this decline varies between amino

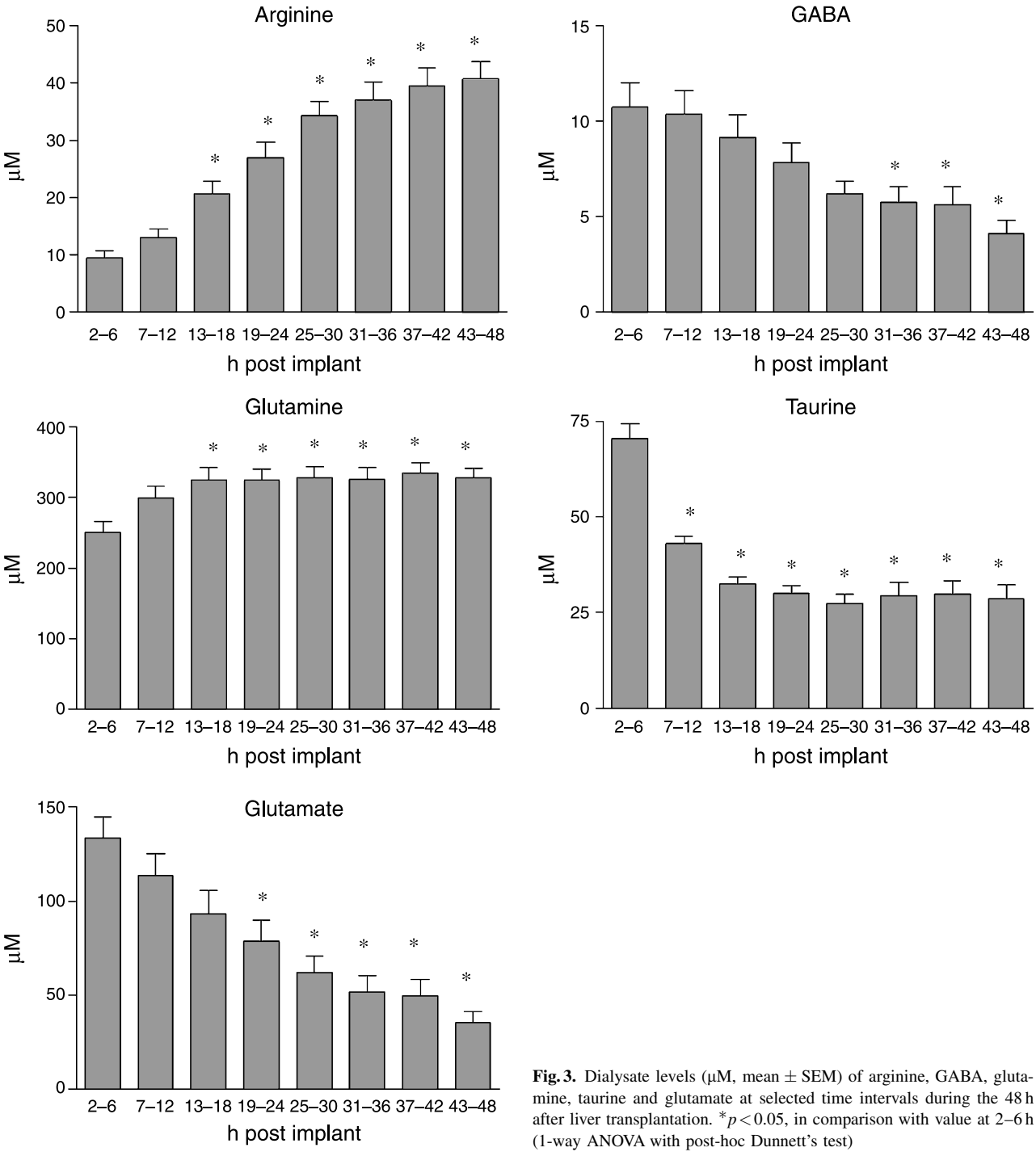

Fig. 3. Dialysate levels (μM, mean ± SEM) of arginine, GABA, glutamine, taurine and glutamate at selected time intervals during the 48 h after liver transplantation. $^{*}p < 0.05$, in comparison with value at 2–6 h (1-way ANOVA with post-hoc Dunnett's test)

acids. With the exception of aspartate, this group of amines also shows significantly higher extracellular levels in the back table phase as opposed to the donor retrieval phase.

In contrast, in the group which contains alanine, β-alanine, glutamine, glycine, serine and threonine (Fig. 4, Group B), there was little variation (less than 2-fold) either between the donor and back table phases, or between the early (2–6 h) and late (43–48 h) post-reperfusion phases.

The final, and largest group of amino acids (Fig. 4, Group C), comprising arginine, citrulline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, tryptophan, tyrosine and valine are all present in the extracellular fluid in the donor organ at least three times the concentration found in the back table phase. After transplantation and reperfusion, the extracellular levels of this group either remained essentially unchanged (e.g. citrulline, leu-

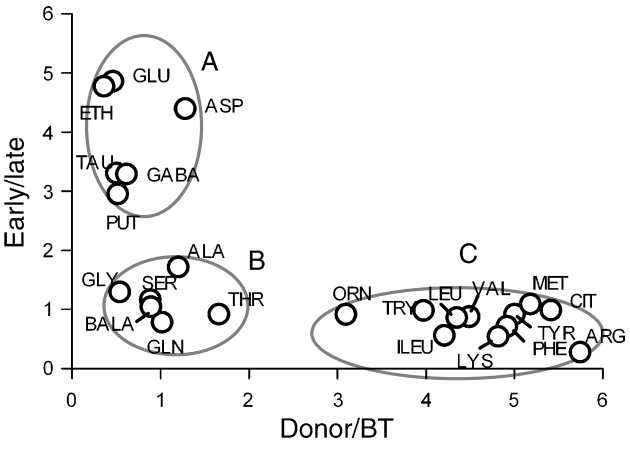

Fig. 4. A comparison of the extracellular/intracellular translocation of individual amino acids and related amines at various stages of the liver transplantation procedure. The x-axis represents the ratio of mean dialysate concentration (μM) of each compound between the donor retrieval phase and the back table phase, prior to transplantation. The y-axis represents the ratio of mean dialysate concentration (μM) of each compound between the early (2–6 h) and late (43–48 h) phases, post-transplantation. The ovals (labeled A, B and C) indicate possible sub-groupings of the amino acids and related amines. Abbreviations of the individual compounds are as listed in Table 1

cine, methionine, ornithine, tryptophan, tyrosine and valine), increased slightly, albeit significantly (e.g. lysine, isoleucine and phenylalanine (Fig. 2)) or, in the case of arginine, increased markedly and progressively (Fig. 3). Mean concentration values in the 2–6 h post-reperfusion samples were (μM, mean ± SEM), arginine, 9.5 ± 1.2; citrulline, 19.9 ± 1.8; isoleucine, 21.7 ± 2.5; leucine, 73.3 ± 7.0; lysine, 66.7 ± 5.6; methionine, 43.0 ± 19.3; ornithine, 49.4 ± 3.9; phenylalanine, 28.8 ± 2.4; tryptophan, 8.7 ± 0.7; tyrosine 31.7 ± 3.5; and valine 77.7 ± 4.7.

Amino acid levels and initial poor function of the graft

As described above, 6 of the 15 patients with 24 h post-operative AST levels of above 2000 IU/L were identified as demonstrating IPF of the graft. Comparing amino acid levels during the donor and back table phases of the procedure to those obtained in the remaining 9 patients where 24 h AST levels were below 2000 IU/L (non-IPF) revealed significantly increased aspartate levels in the donor liver in the IPF group (μM, mean ± SEM, 47.7 ± 7.5 (IPF); 28.8 ± 4.5 (non-IPF), Student's *t*-test, $p < 0.05$). During the back table procedure, extracellular levels of β-alanine (μM, mean ± SEM, 16.0 ± 1.4 (IPF); 7.9 ± 0.8 (non-IPF), GABA (μM, mean ± SEM, 15.1 ± 2.4 (IPF); 9.6 ± 1.1 (non-IPF), glutamine (μM, mean ± SEM, 230.9 ± 29.6 (IPF); 168.8 ± 5.2 (non-IPF), and threonine (μM, mean ± SEM, 33.4 ± 7.6 (IPF); 19.0 ± 1.5 (non-IPF), were all significantly greater in the IPF group (Student's *t*-test, $p < 0.05$).

Discussion

In interpreting this data, it is important to recognise that the technique of microdialysis samples from the interstitial fluid of the liver tissue, i.e. that compartment where cells signal and communicate with one another, and this has been shown to be an appropriate means of monitoring tissue metabolism in the transplanted liver (Nowak et al., 2002). Temporal changes in amino acid concentration within the extracellular fluid may therefore reflect efflux from, or uptake into, the intracellular compartment, and these processes may be either passive or active. In addition, during times when circulatory supply to the graft is intact, blood may also influence levels of amino acids in the extracellular compartment. Interruption or changes in the activity of the large family of amino acid transporters (Broer, 2002) across the liver cell or plasma membranes will certainly influence extracellular levels.

During the course of the liver transplantation procedure, the graft undergoes a succession of challenging insults and each of these may affect translocation of amino acids within these tissue compartments. The samples obtained during the graft procurement phase were from the in-situ organ, perfused by blood, in brainstem-dead but heart-beating donors.

Microdialysis during the back table procedure monitors the effects of both hypothermia and the presence of UW and Marshall's solutions, the compositions of which are designed to protect the graft against cell swelling, the effects of cold ischaemia during storage and transport, and the subsequent rewarming ischaemia/reperfusion injury after re-implantation. Thus, UW solution contains a number of cell impermeable agents, notably lactobionic acid to prevent cell swelling during storage, as well as glutathione to augment antioxidant capacity, and adenosine to stimulate ATP generation and preserve energy metabolism, during reperfusion (Southard and Belzer, 1995).

As shown in Fig. 4, the amines investigated in this study could be broadly classified into one of three groups on the basis of their compartmental translocation during the various phases of this study, and this will be used as a basis for interpretation of this data, although exceptions will be highlighted.

For ethanolamine, GABA, glutamate, putrescine and taurine (Group A), extracellular levels were significantly greater during the cold ischaemia of the back table procedure than during donor retrieval. Indeed, this same

group of amino acids also showed decreasing extracellular levels from a high initial level following restoration of blood supply in the recipient and the associated warm ischaemia and reperfusion injury. As mentioned above, these changes could simply signify passive release following the death of some cells as a result of the metabolic stresses involved, or they could represent active processes in order to signal a protective or restorative response. The individual variation in the kinetics of the decrease of this group of compounds following implantation suggests that at least some of the changes are actively regulated.

Some of these post-reperfusion findings confirmed and extended our previous observations (Silva et al., 2005). GABA has been shown to act as a transmitter in the liver (Erdo and Wolff, 1990) and may be involved in the regulation of hepatic regeneration (Erlitzki et al., 2000). Glutamate is also involved in hepatic cellular signaling (Gill et al., 2000; Gill and Pulido, 2001), while the role of taurine as an osmoregulatory agent within the liver is well established (Warskulat et al., 1997; Wettstein et al., 2000).

Increases in extracellular ethanolamine in neuronal tissue have been reported previously in stressful situations such as hypoglycaemia, hypoxia and epilepsy (Buratta et al., 1998). Parallel increases of taurine and ethanolamine have also been observed in the extracellular fluid of neural tissue and CSF under hypo-osmolar conditions (Lehmann et al., 1991), and a possible osmoregulatory role in the liver is consistent with the similar temporal profile of these 2 amines post-reperfusion seen here. Another possible mechanism by which ethanolamine release may represent an active process is by virtue of it being a by-product of the enzymatic hydrolysis of N-acylethanolamines, a group of compounds that have been shown to possess anti-inflammatory, immunosuppressive and anti-oxidant properties (Tsuboi et al., 2005) and that have been shown to accumulate after cerebral ischaemia (Berger et al., 2004). Finally, ethanolamine enhances hepatic regenerative processes (Mimuro et al., 2002).

The apparent release of putrescine, a polyamine produced by the decarboxylation of the urea cycle intermediate, ornithine, may be related to its ability to protect liver cells by reducing lipid peroxidation (Nagoshi et al., 1994), or to reverse liver necrosis and hepatocyte apoptosis (Tzirogiannis et al., 2004).

Two other amino acids are worthy of mention at this point. As seen in Fig. 1, aspartate also declined from a high initial level after reperfusion although this decrease did not reach significance for 43 h. However, in contrast to the other amino acids in this sub-group, there was no difference between donor and back table levels. In contrast, extracellular glycine levels remained relatively constant post-reperfusion, but back table levels were almost double those found during donor retrieval, which again may be associated with a protective role as glycine has been shown to protect hepatocytes against hypoxia (Carini et al., 1997), as well as minimizing reperfusion injury in perfused rat livers (Zhong et al., 1996). Furthermore, increasing plasma glycine by intravenous infusion of this amino acid improved survival in a rat model of liver transplantation (Schemmer et al., 1999).

In the second sub-group of amino acids (alanine, β-alanine, glutamine, glycine, serine and threonine (Group B)), changes in extracellular level were less marked although still statistically significant on occasions. Glycine has been considered above, and although threonine values did not vary post-reperfusion, donor levels were almost twice as high as those in the back table. Glutamine levels did not vary between the donor and back table situations, but increased significantly over the first 12 h of the post-reperfusion period, before then stabilising. This may reflect activity in glutamate/glutamine cycling in response to the initial elevated levels of glutamate following reperfusion (Watford, 2000). However, for alanine, β-alanine and serine, extracellular levels did not vary significantly at any stage of the liver transplantation procedure.

The final, and largest, group of amino acids (Group C) was characterised by markedly higher extracellular levels during donor retrieval than during the back table procedure. This may indicate increased uptake of these primary amines during the back table procedure, perhaps to accelerate protein synthesis, or it may be a consequence of the lack of blood supply to the graft at this stage. Indeed, once the graft has been implanted and reperfused in the recipient, extracellular levels return to the donor retrieval situation or above, in some cases almost immediately, and in others more slowly and progressively. This sub-group contains all of the essential amino acids with the exception of threonine although, as stated above, threonine levels are significantly higher in the donor retrieval situation, but not to the same degree.

No members of Group C exhibited the declining post-reperfusion levels seen with those amines in Group A that may be associated with an active signaling function. Extracellular levels for most of this group remained relatively constant during the 48 h post-reperfusion period, which argues against the view that some of the elevated levels described previously arise simply from hepatocyte damage following probe implantation. Isoleucine, lysine and phenylalanine increased progressively over the first 24 h post-reperfusion before then stabilizing and this may

signify increased availability in order to restore or accelerate protein synthesis. Indeed, acute phase protein synthesis requires a high proportion of aromatic amino acids such as phenylalanine, and this may result in a disproportionate breakdown of skeletal muscle in order to provide it (Reeds et al., 1994). Furthermore, there is evidence of an increased requirement for branched-chain amino acids for protein synthesis following OLT (Luzi et al., 1997; Mager et al., 2006).

In agreement with our previous findings (Silva et al., 2005), a marked and progressive increase in the concentration of extracellular arginine following transplantation was observed. Also included in Group C are citrulline and ornithine which, along with arginine, are metabolically closely related, being part of the urea cycle, the formation of polyamines including putrescine as discussed above, and in nitric oxide synthesis, and these metabolic relationships have been discussed and investigated further elsewhere (Silva et al., 2006a).

Finally, all of the amine changes discussed so far apply to the complete cohort of 15 patients as there were no cases of primary non-function of grafts. However, there was plasma-based evidence of IPF in 6 of the implanted grafts, and analysis of the pre-transplant data revealed some differences between these grafts and those that performed well from the outset.

In the donor retrieval situation, extracellular aspartate levels were significantly higher in those grafts that subsequently exhibited IPF. During back table microdialysis, elevated extracellular levels of β-alanine, GABA, glutamine and threonine were seen in the IPF grafts. β-Alanine has been shown to be a GABA uptake inhibitor (Ishiwari et al., 2004), a possible explanation for the concurrent increase in these two amines, and may also act as a protective agent against hypoxic liver injury (Vairetti et al., 2002).

In conclusion, this study illustrates the value of microdialysis in providing organ-specific metabolic data, and has revealed some interesting and consistent patterns of amino acid translocation during the metabolic challenges suffered by the liver during the various phases of liver transplantation. Although no data are available from this study on grafts that suffered PNF, we are currently extending this work into animal models, under more controlled conditions, to further explore the metabolic changes identified in the pre-transplant grafts that subsequently resulted in IPF.

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References

Berger C, Schmid PC, Schabitz WR, Wolf M, Schwab S, Schmid HHO (2004) Massive accumulation of N-acylethanolamines after stroke. Cell signalling in acute cerebral ischemia? J Neurochem 88: 1159–1167

Bourne JA (2003) Intracerebral microdialysis: 30 years as a tool for the neuroscientist. Clin Exp Pharmacol Physiol 30: 16–24

Broer S (2002) Adaptation of plasma membrane amino acid transport mechanisms to physiological demands. Pflugers Arch Eur J Physiol 444: 457–466

Buratta S, Hamberger A, Ryberg H, Nystrom B, Sandberg M, Mozzi R (1998) Effects of serine and ethanolamine administration on phospholipid-related compounds and neurotransmitter amino acids in the rabbit hippocampus. J Neurochem 71: 2145–2150

Carini R, Albano E (2003) Recent insights on the mechanisms of liver preconditioning. Gastroenterology 125: 1480–1491

Carini R, Bellomo G, De Cesaris MG, Albano E (1997) Glycine protects against hepatocyte killing by KCN or hypoxia by preventing Na^+ intracellular overload in the rat. Hepatology 26: 107–112

Casillas-Ramirez A, Mosbah IB, Ramalho F, Rosello-Catafau J, Peralta C (2006) Past and future approaches to ischemia-reperfusion lesion associated with liver transplantation. Life Sci 79: 1881–1894

Erdo SL, Wolff JR (1990) γ-Aminobutyric acid outside the mammalian brain. J Neurochem 54: 363–372

Erlitzki R, Gong Y, Zhang M, Minuk G (2000) Identification of γ-aminobutyric acid receptor subunit types in human and rat liver. Am J Physiol Gastrointest Liver Physiol 279: G733–G739

Gill SS, Mueller RW, McGuire PF, Pulido OM (2000) Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity. Toxicol Pathol 28: 277–284

Gill SS, Pulido OM (2001) Glutamate receptors in peripheral tissues: current knowledge, future research, and implications for toxicology. Toxicol Pathol 29: 208–223

Ishiwari K, Mingote S, Correa M, Trevitt JT, Carlson BB, Salamone JD (2004) The GABA uptake inhibitor β-alanine reduces pilocarpine-induced tremor and increases extracellular GABA in substantia nigra pars reticulata as measured by microdialysis. J Neurosci Methods 140: 39–46

Lehmann A, Carlstrom C, Nagelhus EA, Ottersen OP (1991) Elevation of taurine in hippocampal extracellular fluid and cerebrospinal-fluid of acutely hypoosmotic rats – contribution by influx from blood. J Neurochem 56: 690–697

Luzi L, Perseghin G, Regalia E, Sereni LP, Battezatti A, Baratti D, Bianchi E, Terruzzi I, Hilden H, Groop LC, Pulvirenti A, Taskinen M-R, Gennari L, Mazzaferro V (1997) Metabolic effects of liver transplantation in cirrhotic patients. J Clin Invest 99: 692–700

Mager DR, Wykes LJ, Roberts EA, Ball RO, Pencharz PB (2006) Effect of orthotopic liver tranplantation (OLT) on branched-chain amino acid requirement. Pediatr Res 59: 829–834

Mimuro A, Aoki T, Tsuchida A, Miyashita T, Koyanagi Y, Enosawa S (2002) Effect of ethanolamine on liver regeneration after 90% hepatectomy in rats. Transplant Proc 34: 2664–2667

Nagoshi S, Ohta Y, Matsui A, Fujiwara K (1994) Protective action of putrescine against rat-liver injury. Scand J Gastroenterol 29: 166–171

Nowak G, Ungerstedt J, Wernerman J, Ungerstedt U, Ericzon B-G (2002) Clinical experience in continuous graft monitoring with microdialysis early after liver transplantation'. Br J Surg 89: 1169–1175

Plock N, Kloft C (2005) Microdialysis – theoretical background and recent implementation in applied life-sciences. Eur J Pharmaceut Sci 25: 1–24

Reeds PJ, Fjeld CR, Jahoor F (1994) Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J Nutr 124: 906–910

Schemmer P, Bradford BU, Rose ML, Bunzendahl H, Raleigh JA, Lemasters JJ, Thurman RG (1999) Intravenous glycine improves survival in rat liver transplantation. Am J Physiol 276: G924–G932

Silva MA, Mirza DF, Buckels JAC, Bramhall SR, Mayer D, Wigmore SJ, Murphy N, Richards DA (2006a) Arginine and urea metabolism in the liver graft; a study using microdialysis in human orthotopic transplantation. Transplantation 82: 1304–1311

Silva MA, Murphy N, Richards DA, Wigmore SJ, Bramhall SR, Buckels JAC, Adams DH, Mirza DF (2006b) Interstitial lactic acidosis in the graft during organ harvest, cold storage and reperfusion of human liver allografts predicts subsequent ischemia reperfusion injury. Transplantation 82: 227–233

Silva MA, Richards DA, Bramhall SR, Adams DH, Mirza DF, Murphy N (2005) A study of the metabolites of ischemia-reperfusion injury and selected amino acids in the liver using microdialysis during transplantation. Transplantation 79: 828–835

Southard JH, Belzer FO (1995) Organ preservation. Annu Rev Med 46: 235–247

Strasberg SM, Howard TK, Molmenti EP, Hertl M (1994) Selecting the donor liver – risk-factors for poor function after orthotopic liver-transplantation. Hepatology 20: 829–838

Tsuboi K, Sun Y-X, Okamoto Y, Araki N, Tonai T, Ueda N (2005) Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. J Biol Chem 280: 11082–11092

Tzirogiannis KN, Panoutsopoulos GI, Demonakou MD, Papadimas GK, Kondyli VG, Kourentzi KT, Hereti RI, Mykoniatis MG (2004) The hepatoprotective effect of putrescine against cadmium-induced acute liver injury. Arch Toxicol 78: 321–329

Ungerstedt U, Rostami E (2004) Microdialysis in neurointensive care. Curr Pharmaceut Des 10: 2145–2152

Vairetti M, Carini R, De Cesaris MG, Splendore R, Richelmi P, Berte F, Albano E (2002) Beta-alanine protection against hypoxic liver injury in the rat. Biochim Biophys Acta 1587: 83–91

Warskulat U, Wettstein M, Haussinger D (1997) Osmoregulated taurine transport in H411E hepatoma cells and perfused rat liver. Biochem J 321: 683–690

Watford M (2000) Glutamine and glutamate metabolism across the liver sinusoid. J Nutr 130: 983S–987S

Wettstein M, Peters-Regehr T, Kubitz R, Fischer R, Holneicher C, Monnighoff I, Haussinger D (2000) Release of osmolytes induced by phagocytosis and hormones in rat liver. Am J Physiol: Gastrointest Liver Physiol 278: G227–G233

Zhong Z, Jones S, Thurman RG (1996) Glycine minimizes reperfusion injury in a low-flow, reflow liver perfusion model in the rat. Am J Physiol 270: G332–G338

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