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Abstract

Arginine is an important substrate in health and disease. It is a commonly held view that arginase-1 release from injured erythrocytes and hepatocytes leads to arginine breakdown. However, the true relationship between plasma arginase-1 concentration and activity has remained unaddressed. Blood was sampled from patients undergoing liver resection, a known cause of hepatocyte injury and arginase-1 release, to determine arginase-1, arginine and ornithine plasma levels. Arginase activity was assessed *in vitro* by measuring changes in arginine and ornithine plasma levels during incubation of plasma and whole blood samples at 37ºC. Arginase-1 plasma levels increased 8 to 10-fold during liver resection, while arginine and ornithine levels remained unchanged. In accordance with these *in vivo* findings, arginine and ornithine levels remained unchanged in plasma incubated at 37˚C irrespective of arginase-1 concentration. In contrast, arginine plasma levels in whole blood decreased significantly during incubation, with ornithine increasing stoichiometrically. These changes were irrespective of arginase-1 plasma levels and were explained by arginase activity, present in intact erythrocytes. Next, plasma samples with 1000-fold normal arginase-1 concentrations were obtained from patients undergoing cadaveric liver transplantation. Here, a significant decrease of arginine plasma levels occurred *in vivo* and *in vitro*. In contrast with commonly held views, moderately increased arginase-1 plasma levels do not affect plasma arginine. Very high plasma arginase-1 levels are required to induce potential clinical relevant effects.

Key words: arginase-1; arginine; cell injury

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Introduction

The amino acid arginine is an important substrate for protein synthesis and for the production of agmatine and creatine (1). It is best known however as the precursor for the imunoregulatory and vasoactive molecule nitric oxide (NO), although the conversion to NO represents only 1% of arginine plasma flux (2). Quantitatively, arginine plasma flux is determined by arginine intake, endogenous arginine synthesis and protein breakdown on one side and protein synthesis and arginine catabolism by the enzyme arginase on the other side (3). Arginase converts arginine to urea and ornithine and arginase activity accounts for 10 % of total plasma arginine turnover (4). Arginase exists in 2 isoforms with only 58% sequence identity (5). Arginase-1 is a cytosolic protein, predominantly found in the liver and to a lesser extent in erythrocytes (6). Arginase-2 is located in mitochondria and more ubiquitously present (5), amongst others in the kidneys and the spleen but not in mature erythrocytes (6, 7). Hepatic arginase-1 activity serves urea synthesis and nitrogen homeostasis. In the liver ornithine is recycled to citrulline and back to arginine. Due to compartmentalization of this urea cycle, plasma arginine is not a substrate for hepatic arginase-1 (8, 9). Ornithine generated outside the urea cycle can be converted to proline, an important constituent of collagen, and to the polyamines, which are important for cell proliferation (1). Changes in protein turnover, arginine intake or arginase activity can affect arginine plasma levels (3). Arginine deficiency may result in microcirculatory disturbances, pulmonary and systemic hypertension (10, 11), disturbed collagen synthesis and wound healing (12), impaired immune function (13, 14) and in onset of postoperative infections (15).

Injury to arginase-1 expressing cells such as hepatocytes and erythrocytes leads to arginase-1 release into the circulation and increased arginase-1 plasma levels (10,

11, 16-19). It is generally believed that such an increase leads to plasma arginine breakdown compromising arginine availability, potentially leading to microcirculatory dysfunction (10, 11) and immune suppression (19, 20). Alternatively, regarding its effect on cell proliferation, arginine depletion by exogenous arginase may become a promising anti-cancer treatment (21). The true relation between the concentration of arginase-1 in plasma and its actual activity however, has never been verified.

Aim of this study was to investigate the effects of arginase-1 release on arginine plasma levels, using liver surgery (resection and transplantation) as a model of hepatocyte injury and arginase-1 release and to establish the potential significance of circulating arginase-1 for arginine metabolism.

Methods

Patients

Patients undergoing liver resection for secondary malignancies in an otherwise normal liver (n=16, Table 1) were studied. A routinely placed peripheral arterial catheter was used for blood sampling. Informed consent was obtained from every participant and the research protocol was approved by the Institutional Medical Ethical Committee.

Arginase-1 protein release and indices of in vivo arginase enzyme activity during liver resection

Arginase-1 release and its effect on arginine and ornithine levels were studied *in vivo* in 10 patients undergoing hepatectomy with intermittent hepatic inflow occlusion (Pringle manoeuvre) (22). Arterial blood was sampled pre-operatively, before liver transection, before and after each event of an intermittent Pringle manoeuvre (2 x 15 minutes ischemia and 5 minutes reperfusion) and 90 minutes postoperatively. Blood was collected in pre-chilled heparinized vacuum tubes (Becton, Dickinson & Co., Franklin Lakes, NJ), immediately placed on ice and processed as described below.

In vitro arginase enzyme activity in whole blood and plasma after liver manipulation

To study the effect of plasma arginase activity, separate from other processes regulating arginine levels *in vivo*, plasma samples with varying arginase-1 levels were incubated. In addition arginase activity was assessed in corresponding whole blood samples. Arterial blood was obtained from 6 patients undergoing liver resection, pre-operatively and after liver manipulation (before liver transection), when

based upon prior experience peak arginase-1 concentrations were already expected (23).

Pre-incubation blood processing

Blood was collected in two 4 mL heparin tubes (BD). One tube was centrifuged (4,000 x G, room temperature, 5 min) to obtain plasma. The plasma was removed and transferred to clean tubes for incubation. The whole blood samples were incubated without any further processing.

Incubation

Incubations were performed immediately after sampling. Plasma and whole blood samples were divided over three aliquots per fraction that were incubated for 0, 20 or 40 minutes at 37°C. Thereafter, samples were placed on ice and processed immediately as described below. Arginase-1, arginine, ornithine and other amino acid concentrations were measured as described below. Arginase activity was expressed as the increment of ornithine concentration per minute. Since kinetic assays like these do not discriminate between the activities of different iso-enzymes the term arginase activity will be used without further specification when referring to the data of this assay.

Arginase-1 protein release and plasma arginase enzyme activity after liver transplantation

Roth et al. described a decrease of plasma arginine levels from ±100 µmol/l to ±4 µmol/l within 30 minutes following cadaveric liver transplantation (16), which was ascribed to arginase release from the graft and overwhelming plasma arginase activity. Ethical permission was granted to include 4 patients undergoing cadaveric liver transplantation at the University Hospital, Leuven, Belgium (Table 2) as optimal

positive control to the present study. Arterial blood was drawn in heparin tubes (BD) pre-operatively, at the end of the anhepatic phase and 3, 20 and 60 minutes following reperfusion of the graft. Whole blood was centrifuged at 4,000 x G for 5 minutes. Argininase-1, arginine, ornithine and other amino acid concentrations were measured in all samples. Plasma obtained three minutes following reperfusion was incubated at 37°C for 25-40 (mean 30) minutes to assess plasma arginase activity as described above.

Relation between arginase activity and arginase-1 concentration in plasma

Plasma arginase activity at physiological arginine concentration

Aliquots of a plasma sample from one liver transplant recipient, obtained immediately following reperfusion (arginase-1 concentration 25 µg/mL) were merged with aliquots of a plasma sample obtained from a healthy subject (arginase-1 concentration below detection limit (10 ng/ml). By this means an arginase-1 dilution curve ranging from 25 µg/ml to 195 ng/ml was created. Before merging, phosphate buffered saline (PBS, pH 7.4) containing 85 mmol/l arginine was added to the “healthy” plasma aliquots. The volume of arginine-enriched PBS added to each aliquot was adjusted so that the final arginine concentration after merging equalled 85 µmol/l in each sample. Accordingly, plasma samples were created containing varying arginase-1 and equal arginine concentrations, the maximum concentration of PBS in these samples was <0.1%. All samples were immediately incubated at 37ºC for 0, 5 or 30 minutes.

Plasma arginase activity at above-Km arginine concentration

In a similar fashion as described above, plasma aliquots containing various concentrations of arginase-1 and 20 mmol/l arginine were created. However, in this case arginine was directly dissolved in the “healthy” plasma aliquots to avoid dilution

of the plasma with PBS. Eventually plasma samples were created containing 15,000, 1,500 or 150 ng/ml arginase-1 with 20 mmol/l arginine. These samples were immediately incubated at 37ºC for 0, 5 or 30 minutes.

Arginase-1 concentration in erythrocytes

Blood was obtained from five healthy volunteers in vacuum tubes containing EDTA (Becton, Dickinson & Co.). To remove plasma and mononuclear cells a previously described protocol was applied using a separation fluid (Lymphoprep 1.077, Axis-Shield, PoC AS, Oslo, Norway) (24). One ml of packed erythrocytes was added to 74 ml distilled water and gently shaken for 10 min to induce 100% haemolysis (25). The homogenate was centrifuged (4,000 rpm, RT, 15 min) and the supernatant was assayed for arginase-1.

Post-incubation sample processing and laboratory analysis

Whole blood samples were centrifuged (4,000 rpm, 4°C, 5 min) to obtain plasma. One hundred µL plasma was deproteinized with 8 mg sulphosalicylic acid and stored at -80°C, the remainder was stored untreated (-80°C). Amino acid levels were analyzed in SSA deproteinized samples by high performance liquid chromatography as described before (26). Arginase-1 concentrations were measured in untreated samples using enzyme-linked immunosorbent assay (ELISA) (27) (kindly provided by Hycult biotechnology, Uden, the Netherlands). The detection limit of this assay is 10 ng/mL arginase-1.

Statistics

Changes in amino acid and arginase-1 concentrations *in vivo* and *in vitro* were tested using a paired t-test or 1-way ANOVA for repeated measures when more than two serial observations were available. Statistical calculations were made using Prism 4.0

for Windows (GraphPad Software Inc. San Diego, CA). Results are expressed as mean (SEM), patient characteristics as median (range). A p-value <0.05 was considered to indicate statistical significance.

Results

Effects of liver manipulation and warm ischemia

As expected, mean (SEM) arginase-1 plasma levels increased significantly during liver manipulation (8-fold), before hepatic inflow occlusion (Fig 1a). Despite this, plasma concentrations of arginine and ornithine remained unchanged during the same period (Fig 1b). During inflow occlusion no further significant changes of arginase-1 plasma levels were observed (Fig 1a). At the same time plasma levels of arginine *(and most other amino acids, data not shown)* increased. Ornithine concentrations remained unchanged (Fig 1b). Ninety minutes postoperatively, mean arginase-1 plasma concentration was declined to 17% of the last measured intraoperative value. From this, an arginase-1 plasma half life of less than 1 hour was calculated.

In vitro arginase activity

Blood was sampled from 6 patients undergoing liver resection pre-operatively and after liver manipulation. Pre-operative arterial arginase-1 plasma levels were 18.1 (8.0) ng/ml). In line with abovementioned data, arginase-1 plasma levels increased significantly during liver manipulation (10-fold to 184 (54) ng/mL), without affecting arginine and ornithine levels *in vivo* (p=0.72 and p=0.16 respectively) (Fig 2).

Plasma

Incubation of pre-operatively collected plasma samples for 40 minutes at 37°C did not lead to changes in arginine and ornithine concentration (p=0.26; Fig 2a). In accordance with *in vivo* findings, no significant changes in arginine and ornithine concentrations were observed during incubation of plasma samples containing a 10-

fold increased arginase-1 concentration (p=0.33; Fig 2b). Plasma concentrations of other amino acids were not affected by *in vitro* incubation (Table 3).

Whole blood

Arginase-1 plasma levels in incubated whole blood remained stable (data not shown), ruling out a potential increase of arginase activity due to haemolysis. After 40 minutes of incubation a significant decrease of arginine concentrations with a concomitant increase of ornithine concentrations was found in plasma of all whole blood samples, irrespective of the amount of arginase-1 in the plasma (Fig 2c,d). These highly significant changes were not found for other amino acids (Table 4).

Plasma arginase activity after liver transplantation

In vivo arginase-1 levels and indices of arginase activity

After reperfusion, plasma arginase-1 levels increased steeply to 1000-fold normal values (Fig 3a). In addition we observed a rapid decline of plasma arginine levels with a concomitant increase in the plasma levels of ornithine (Fig 3a). A one-phase exponential decay curve fitted along the mean arginase-1 concentrations 3, 20 and 60 minutes post reperfusion, resulted in a calculated plasma half life of approximately 40 minutes. Within 3 minutes following reperfusion *in vivo* arginine plasma levels were relatively stabilized (Fig 3b). Plasma concentrations of other amino acids that depend on the liver for their plasma clearance such as alanine and methionine also declined following reperfusion of the liver, although not as steeply as arginine concentrations (Fig 3b).

In vitro arginase activity after liver transplantation

Incubation of plasma sampled 3 minutes following liver transplantation (arginase-1 13.2 (4.2 µg/ml) at 37ºC for 30 minutes resulted in a significant decrease of plasma arginine concentration (from 61.1 (7.4) to 28.4 (6.7) µmol/l). This was accompanied by a similar increase of ornithine concentration (from 125.2 (6.6) to 163.7 (10.4) µmol/l) (p=0.002) (Fig 3c). Other amino acids remained unaffected (Table 3).

Relation between arginase enzyme activity and arginase-1 protein concentration in plasma

Plasma arginase activity at physiological arginine concentration

Plasma samples containing different arginase-1 levels were incubated for 5 and 30 minutes at 37ºC in the presence of 85 µmol/l arginine. At this arginine concentration the relation between arginase-1 concentration and arginase activity appeared to be non-linear, with a lower specific activity (activity per unit of enzyme) at higher arginase-1 concentrations. Below an arginase-1 concentration of 1.6 µg/ml however, there was in fact a linear relationship between arginase-1 concentration and arginase activity (r^2=0.99) (Figure 4a). After 30 minutes of incubation with an initial arginine concentration of 85 µmol/l, arginine plasma levels decreased dependent on the arginase-1 level. At arginase-1 levels below 2 µg/ml and an initial arginine concentration of 85 µmol/l, arginine levels decreased less than 10% within 30 minutes (Figure 4b).

Plasma arginase activity at above-Km arginine concentration

Plasma with varying amounts of arginase-1 was incubated in the presence of 20 mmol/l arginine, which is well above the Km for arginase-1. This resulted in an

enduring linear ornithine formation. (Figure 4c), showing that the intrinsic activity of the enzyme in isolated plasma was preserved during *in vitro* incubation.

Arginase-1 concentration in erythrocytes

Erythrocyte arginase-1 concentration, measured by ELISA was 17.0 (1.1) µg per ml red blood cells.

Discussion

Increased plasma arginase-1 activity is frequently named as a cause of low arginine plasma levels in patients with hepatocellular or erythrocyte injury (10, 11, 16, 17, 20, 28, 29). This could be clinically relevant since low arginine levels may induce immunological and microcirculatory dysfunction (14, 30, 31). The results from the present study however, show that a potentially relevant effect of plasma arginase-1 on arginine metabolism only occurs when very high arginase-1 concentrations are reached. We were able to study the relation between plasma arginase-1 levels and plasma arginase activity by the application of a recently developed ELISA (27) that allows exact quantification of the arginase-1 concentration in plasma.

Arginase-1 was released from the liver into the plasma in patients undergoing liver resection, leading to an 8-fold increase of circulating arginase-1, without affecting arginine and ornithine plasma levels. These data show no evidence for increased arginase activity despite increased arginase-1 plasma levels. From these data alone however, it cannot be ruled out that actual arginase activity was compensated by other mechanisms regulating plasma arginine and ornithine levels. Plasma arginine levels are regulated *in vivo* by protein breakdown and endogenous arginine synthesis. The latter occurs in the kidney where citrulline, derived from intestinal glutamine metabolism becomes converted to arginine (9, 32). We have demonstrated recently that endogenous arginine synthesis remains unchanged during liver surgery (32). Quantitatively, arginine clearance is primarily determined by protein synthesis. This is illustrated by the increased plasma levels of arginine and most other amino acids during hepatic inflow occlusion, reflecting abolishment of hepatic protein synthesis. Ornithine concentrations remained unchanged, which is in line with the absence of physiological hepatic ornithine uptake (9).

To study plasma arginase activity isolated from in vivo regulatory mechanisms, plasma and whole blood samples were incubated in vitro. In agreement with the *in vivo* data no significant changes in arginine and ornithine concentrations were found during incubation of plasma samples with a mean arginase-1 concentration of 185 ng/ml. To explore whether plasma arginase activity was dependent on factors present in whole blood but not in plasma, whole blood samples were incubated at 37°C for 40 minutes. A significant increase of ornithine was found, accompanied by a similar decrease of arginine. No changes were found in the plasma concentrations of other amino acids such as glutamate, an ornithine precursor or citrulline, an arginine product. This strongly suggests that the observed changes actually specifically reflect arginase activity. Whole blood arginase activity however was not related to the arginase-1 plasma concentration, which underlines that plasma arginase-1 does not affect plasma arginine levels. More likely whole blood arginase activity occurs in the cellular fraction (erythrocytes and/or leucocytes).

Several studies have reported arginase activity in plasma in clinical settings, which seemingly conflicts with our data. Roth and co-workers showed that liver transplantation leads to an immediate decrease of plasma arginine to virtually zero with a stoichiometric increase of plasma ornithine, which is highly indicative for arginase activity (11, 16). We were able to reproduce these data, although the decrease of arginine levels was not as outspoken as described by Roth et al. Moreover plasma concentrations of other amino acids such as methionine and alanine decreased as well, reflecting restoration of liver function. Nonetheless, the specific steep decline of arginine in the first three minutes following reperfusion and the simultaneous increase of ornithine levels is in fact highly indicative for arginase activity. Arginase-1 plasma levels were 100-fold higher after liver transplantation than

during liver resection and 1000-fold higher than normal reference values. *In vitro* assessment of plasma obtained 3 minutes following transplantation revealed specific and stoichiometric changes in arginine and ornithine concentration that were related to arginase-1 concentration. Arginase activity per µg arginase-1 (specific activity) appeared to decline at increasing arginase-1 concentrations. This is probably due to the high initial reaction velocity at high arginase-1 concentrations, leading to rapid decline of arginine concentration and arginase activity. In samples with an arginase-1 concentration <1.5 µg/ml arginine depletion did not occur. At these low enzyme concentrations there was a linear relation between plasma arginase-1 concentration and arginase activity. Moreover incubation of plasma samples with 20 mmol/l arginine (above Km) showed that the intrinsic activity of the enzyme was preserved during incubation. Theoretically, the activity of arginase-1 at low enzyme and/or substrate concentrations may be limited by endogenous arginase inhibition. The most potent endogenous arginase inhibitor known is N-ω-hydroxyarginine (NOHA), an intermediate of NO synthesis. However, regarding the high NOHA concentrations required to cause arginase inhibition (IC_{50}≈400µM) (33) it appears unlikely that any of the presently known arginase inhibitors affected the present results.

Due to limited availability of methods to quantify arginase (-1 and -2) protein content in biological samples, various semiquantitative arginase activity assays, relying on supraphysiological pH and arginine concentrations have been developed (20, 34). The wide-spread use of such biochemical assays has led to the interchangeable use of the terms arginase activity and arginase concentration. The *in vitro* experiments in the current study were designed to study arginase activity under physiological conditions rather than to quantify plasma levels of arginase. The data show that detection of arginase activity in a plasma sample under optimal biochemical

conditions does not necessarily imply that arginase is active in plasma under physiological conditions.

Currently, there is growing interest in the role of arginine and arginase in haemolytic anaemia (10, 34). In a recent publication it was reported that haemolytic patients with sickle cell anaemia had reduced arginine levels and a 5-fold increase in plasma arginase activity, measured by a semiquantitative assay (10). In the same study it was found that erythrocyte arginase activity was similarly increased. Another recent study reported a 14-fold increase in erythrocyte arginase activity in sickle cell disease, measured semiquantitatively (34). In the light of our current data, which show that a considerable amount of plasma arginine catabolism is performed by arginase-1 within the intact erythrocyte, it can be speculated that increased arginase activity in the blood of sickle cell patients should be ascribed to increased arginase activity in the cellular fraction rather than to increased arginase levels in plasma.

Finally, it has been suggested that increased circulating arginase concentrations disturb arginine metabolism following blood transfusion due to haemolysis of stored erythrocytes (20). We found that normal erythrocytes contain 17 μg arginase-1 per ml cells. Therefore one unit of red blood cell concentrate (300 ml) contains 5.1 mg arginase-1. Considering a distribution volume of 3 L (plasma volume) for these erythrocytes after transfusion, one unit of packed red blood cells can increase arginase-1 plasma levels with 1.7 μg/mL in the extreme situation of 100% haemolysis of the transfused erythrocytes. This theoretical example shows that plasma arginase-1 activity during blood transfusion will not likely affect arginine metabolism substantially. This assumption is further confirmed by clinical observations in patients receiving massive blood transfusion during thoracoabdominal aorta surgery, showing

moderate changes in plasma arginase-1 concentrations but no changes in plasma arginine concentrations (Hanssen et al., *unpublished data*).

In conclusion, increased plasma levels of arginase-1 occurring during liver resection do not lead to arginine breakdown. The threshold beyond which the plasma level of arginase-1 significantly affects plasma arginine concentration is probably rarely reached in clinical practice, with the exceptions of liver transplantation.

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FIGURE LEGENDS

Figure 1. Arterial arginase-1 **(a)** and arginine and ornithine **(b)** concentrations were measured in 10 patients undergoing partial hepatectomy with intermittent Pringle manoeuvre (15' clamping, 5' reperfusion; clamping (C)/unclamping (U) indicated by arrows). Arginase-1 levels increased from the start of the procedure ($p=0.0005$), without affecting arginine levels ($p=0.43$). A significant increase of arginine levels was observed during hepatic pedicle clamping ($p<0.0001$), most likely due to abolishment of hepatic amino acid clearance. Data are mean (SEM).

Figure 2. Blood was drawn from 6 patients undergoing liver resection immediately before surgery **(a,c)** and after liver manipulation **(b,d)**. Mean (SEM) preoperative arginase-1 plasma concentration was 18.2 (8.0) ng/mL, which increased to 184 (54) ng/mL following liver manipulation (10-fold increase, $p=0.028$), without affecting arginine and ornithine levels *in vivo* ($p=0.72$ and $p=0.16$, respectively). Plasma, whole blood and erythrocytes suspended in PBS + 80 µmol/l arginine obtained before and during surgery were incubated for 0, 20 and 40 minutes at 37°C. No significant changes in arginine and ornithine concentration were found in plasma **(a,b)** irrespective of the amount of arginase-1 in the samples. Arginine concentrations in whole blood **(c,d)** decreased significantly. Decreasing arginine levels were accompanied by stoichiometric increases of ornithine levels in all cases, indicating arginase activity. The amount of extracellular arginase-1 in the incubated whole blood samples did not influence the amount of ornithine formed. * $p<0.005$

Figure 3. a. Arterial arginase-1, arginine and ornithine concentrations were measured in 4 patients undergoing cadaveric liver transplantation. Preoperative

arginase-1 levels were elevated above reference values, reflecting underlying liver disease eventually necessitating liver transplantation. Immediately following reperfusion a large increase of plasma arginase-1 levels occurred ($p=0.008$). This was accompanied by a sharp decrease of arginine ($p<0.0001$) and concomitant increase of ornithine levels ($p=0.0003$). **b.** The decline in plasma arginine levels was most outspoken in the first three minutes, where after arginine levels remained relatively stable.

c. Incubation of plasma samples drawn 3 minutes following reperfusion of the liver graft (containing a mean (SEM) arginase-1 concentration of 13 (4.2) µg/mL) led to a significant decrease of arginine levels with a concomitant increase of ornithine levels ($p=0.002$), proving plasma arginase activity.

Figure 4. Plasma samples with varying arginase-1 concentrations were created by mixing plasma from a liver transplant recipient ([arginase-1] 25 µg/mL) and from a healthy volunteer ([arginase-1] below 10 ng/mL). Starting arginine levels were adjusted by adding arginine and samples were incubated at 37ºC. **a.** Plasma samples were incubated for 5 minutes in the presence of 85 µmol/l arginine, which approximates the normal arginine concentration in human plasma. The data show that arginase activity was related to arginase-1 concentration; however the formation of ornithine at the highest arginase-1 concentrations was rapidly limited by decreasing substrate availability. The dashed line indicates the theoretical relation between arginase-1 concentration and arginase activity at a constant arginine concentration of 85 µmol/l. **b.** Plasma samples with varying concentrations of arginase-1 were incubated in the presence of 85 µmol/l arginine for 20 minutes. During this period arginine plasma levels decreased dependent on the arginase-1

plasma level. At arginase-1 levels below 2,000 ng/ml, the decrease of arginine levels in 20 minutes was below 10%. Below an arginine plasma level of 50 µmol/l, arginase activity rapidly becomes limited by substrate availability. Lx indicates mean arginase-1 levels reached during liver resection, LTx indicates mean arginase-1 levels reached after liver transplantation. **c.** Plasma samples with varying concentrations of arginase-1 were incubated in the presence of 20 mmol/l arginine, which maintained substrate availability above Km values during the incubation. In this experiment the ornithine formation rate remained unchanged showing that the intrinsic activity of the enzyme was maintained during the incubations.

Table 1. patient characteristics – liver resection

	In vivo study (n=10)	*In vitro* study (n=6)
Surgical trauma	Liver resection with intermittent Pringle manoeuvre	Liver mobilization/manipulation prior to liver resection
Gender (M/F)	5/5	3/3
Age (years)	50 (33-75)	54 (36-74)
Colorectal liver metastases/Other liver metastases	8/2	6/0
AST (IU/L)	23 (7-37)	34 (8-59)
LDH (IU/L)	360 (295-490)	387 (351-422)
Creatinine (μmol/l)	103 (67-118)	93 (56-106)

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Table 2. patient characteristics – liver transplantation

	M/F	Age	indication	Cold ischemia	AST Pre	AST 3' rep	LDH Pre	LDH 3' rep	Creat Pre	Creat 3' rep
1	F	23	Acute Budd-Chiari	10:51	1956	1786	2417	4680	410	318
2	M	55	Alcoholic cirrhosis + HCC	8:57	40	479	274	1544	62	61
3	M	61	Alcoholic cirrhosis	11:26	71	458	572	1582	62	68
4	M	73	Hepatitis C cirrhosis + HCC	12:29	68	290	329	855	21	98

Gender, Age (years), Indication for liver transplantation, Cold ischemia time (hours:min) and AST (IU/L), LDH (IU/L) and creatinine (µmol/l) levels preoperative and 3 minutes after reperfusion. HCC=hepatocellular carcinoma

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Table 3. Changes in plasma amino acid concentrations during incubation of plasma

	concentration (µmol/l)	absolute increase (µmol/l)		relative increase (%)	
			p-value		p-value
Glutamate	114 (9.3)	2.3 (2.9)	0.451	3.0 (3.1)	0.350
Asparagine	68 (11.7)	4.3 (2.2)	0.083	4.6 (2.8)	0.130
Serine	137 (14.3)	2.6 (2.7)	0.355	1.3 (1.8)	0.481
Glutamine	626 (52.9)	11.6 (12.5)	0.372	1.7 (2.3)	0.481
Histidine	188 (105.1)	0.6 (5.3)	0.915	-5.7 (9.8)	0.573
Glycine	329 (40.3)	9.8 (8.7)	0.287	8.8 (12.5)	0.496
Threonine	155 (18.9)	0.5 (4.4)	0.911	-1.3 (2.8)	0.653
Citrulline	40 (2.7)	0.7 (1.0)	0.526	1.3 (2.3)	0.582
Alanine	404 (38.5)	12.8 (8.3)	0.148	3.2 (2.2)	0.187
Taurine	61 (7.5)	2.1 (1.2)	0.113	10.2 (8.2)	0.237
α-amino butyric acid	17 (1.3)	0.7 (0.4)	0.136	3.7 (2.3)	0.140
Tyrosine	84 (15.6)	6.6 (5.4)	0.246	5.1 (4.4)	0.272
Valine	156 (12.0)	7.5 (5.1)	0.173	3.2 (2.7)	0.273
Methionine	38 (11.1)	1.5 (0.8)	0.098	2.8 (2.3)	0.265
Isoleucine	52 (3.5)	2.3 (1.5)	0.157	3.5 (2.6)	0.207
Phenylalanine	68 (11.5	2.0 (1.2)	0.122	2.2 (1.7)	0.213
Tryptophane	51 (7.5)	1.3 (1.3)	0.370	3.7 (3.1)	0.251
Leucine	101 (8.5)	4.3 (2.2)	0.075	3.2 (2.0)	0.134
Lysine	212 (19.0)	8.0 (5.8)	0.193	2.8 (2.6)	0.313
Σ AA	3073 (291.4)	90.3 (61.7)	0.171	2.3 (2.0)	0.283
Σ Arginine & Ornithine	161 (10.0)	4.8 (3.7)	0.219	1.7 (2.1)	0.424

Pooled data of all plasma incubation experiments. Plasma was obtained from patients undergoing liver resection before liver manipulation (n=6), after liver manipulation (n=6) and after liver transplantation (n=4). Arterial concentrations before incubation are given as well as absolute (µmol/l) and relative (%) increases at the end of the incubation (mean 37.5 minutes). Results were similar for all three experiments except for changes in arginine and ornithine concentrations in plasma obtained after liver transplantation. Changes in arginine and ornithine concentrations during the various experiments are presented in Figures 2 and 3c. The sum of arginine and ornithine concentrations remained unchanged during all experiments. Data are presented as mean (SEM), absolute and relative changes were tested vs. zero by a one-sample t-test. Σ AA = sum of all amino acids, Σ Arginine & Ornithine = sum of arginine and ornithine concentrations.

Table 4. Changes in plasma amino acid concentrations during incubation of whole blood

	concentration (µmol/l)	absolute increase (µmol/l)		relative increase (%)	
			p-value		p-value
Glutamate	109 (6.8)	9.6 (4.7)	0.078	8.3 (4.0)	0.077
Asparagine	43 (2.0)	3.8 (1.7)	0.066	7.7 (3.8)	0.079
Serine	118 (10.7)	3.6 (5.3)	0.514	1.7 (4.4)	0.711
Glutamine	527 (9.3)	3.1 (23.6)	0.898	0.5 (4.5)	0.924
Histidine	61 (13.7)	6.3 (4.4)	0.196	6.6 (5.2)	0.247
Glycine	305 (53.0)	45.9 (41.8)	0.308	55.4 (41.3)	0.221
Threonine	117 (9.5)	4.1 (5.0)	0.433	2.0 (4.8)	0.692
Citrulline	39 (3.0)	1.0 (1.1)	0.394	2.2 (2.7)	0.437
Arginine	68 (3.1)	-18.3 (2.2)	<0.001	-26.7 (3.1)	<0.001
Alanine	321 (26.0)	28.4 (14.6)	0.094	9.3 (4.2)	0.062
Taurine	58 (5.3)	-1.4 (10.9)	0.903	3.2 (16.3)	0.850
α-amino butyric acid	14 (0.8)	1.3 (0.5)	0.049	8.4 (3.4)	0.043
Tyrosine	49 (2.9)	2.1 (1.7)	0.239	3.5 (3.3)	0.327
Valine	148 (11.0)	9.3 (5.3)	0.127	5.0 (3.2)	0.161
Methionine	16 (0.9)	0.5 (0.5)	0.351	2.3 (3.0)	0.473
Isoleucine	46 (3.6)	2.9 (1.6)	0.111	4.6 (3.3)	0.209
Phenylalanine	43 (2.6)	1.9 (1.3)	0.201	4.0 (3.0)	0.214
Tryptophane	34 (4.0)	2.1 (2.3)	0.391	7.8 (8.7)	0.401
Leucine	85 (7.4)	7.3 (2.9)	0.042	7.1 (2.9)	0.045
Ornithine	73 (5.9)	37.6 (7.5)	<0.001	45.1 (5.8)	<0.001
Lysine	167 (14.1)	19.0 (11.2)	0.132	10.0 (5.2)	0.094
Σ AA	2445 (111.6)	172.1 (105.1)	0.145	6.9 (4.3)	0.153
Σ Arginine & Ornithine	145 (8.5)	19.3 (7.4)	0.024	12.0 (4.1)	0.015

Pooled data of all whole blood incubation experiments. Whole blood was obtained from patient undergoing liver resection before liver manipulation (n=6) and after liver manipulation (n=6). Arterial concentrations before incubation are given as well as absolute (µmol/l) and relative (%) increases at the end of the incubation (mean 37.5 minutes). Results were similar for both experiments. Data are presented as mean (SEM), absolute and relative changes were tested vs. zero by a one-sample t-test. Σ AA = sum of all amino acids, Σ Arginine & Ornithine = sum of arginine and ornithine concentrations.

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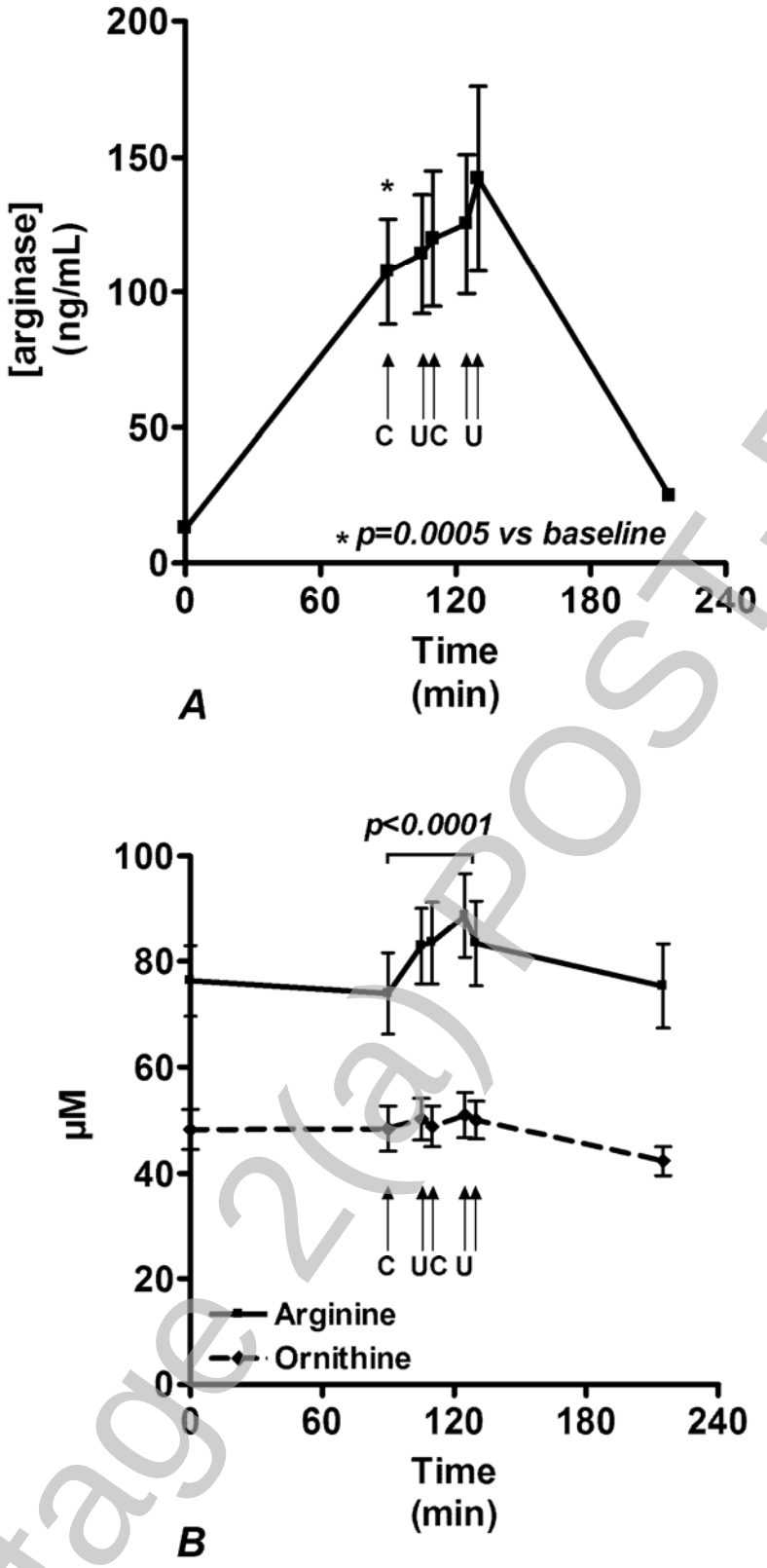

Figure 1

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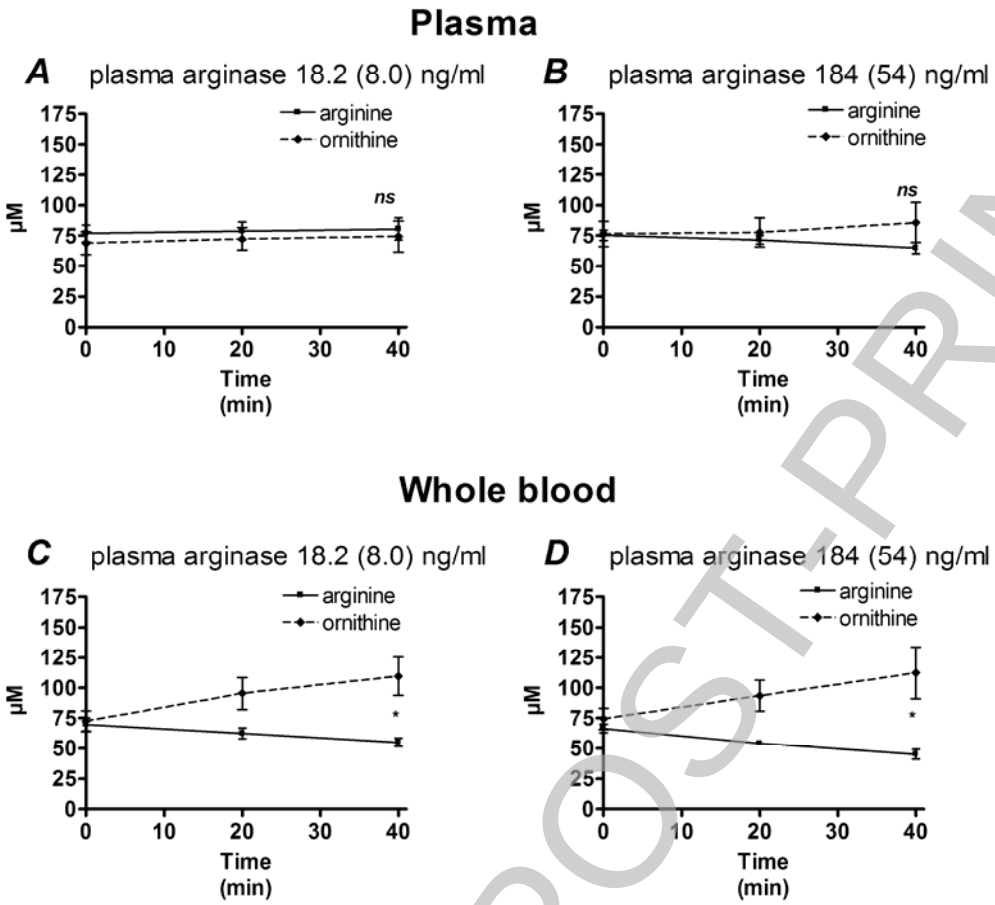

Figure 2

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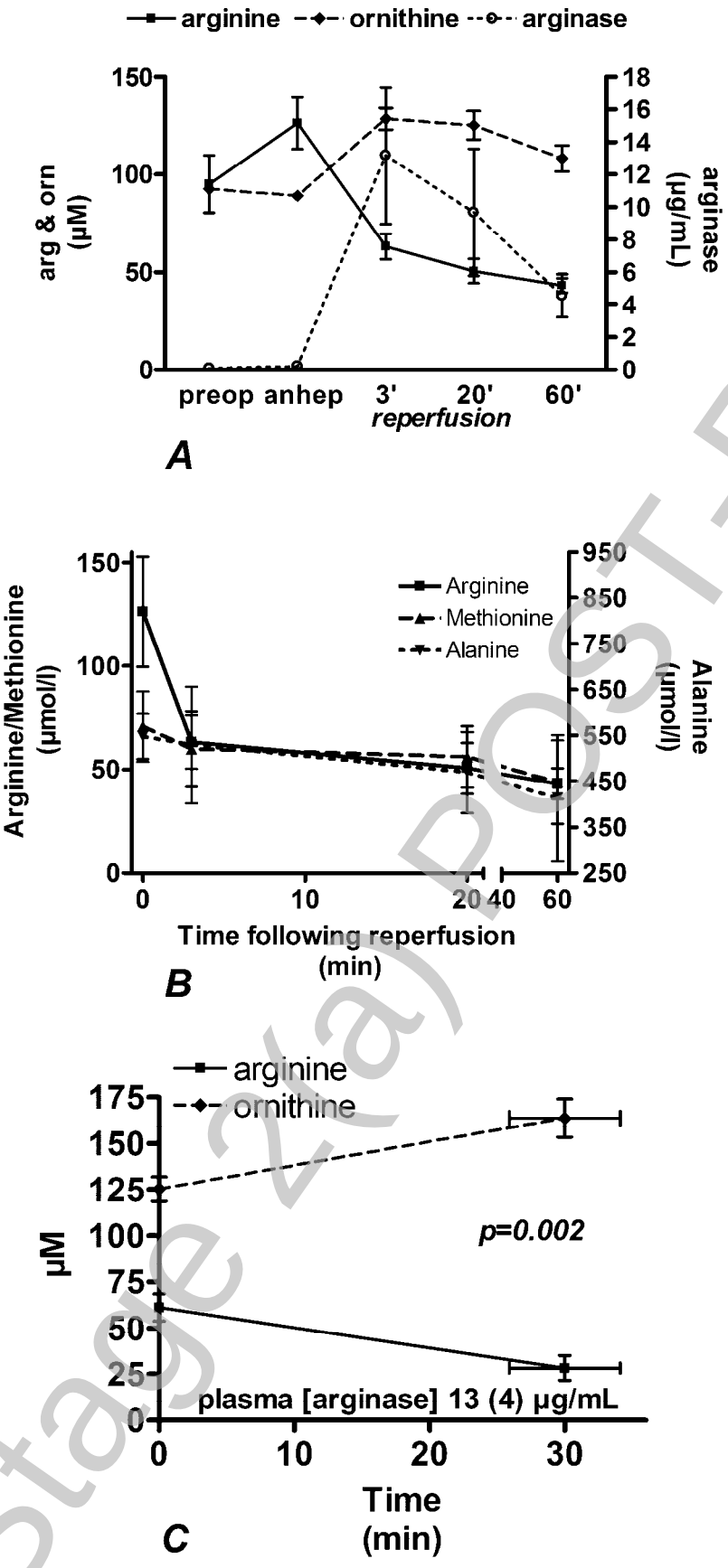

Figure 3

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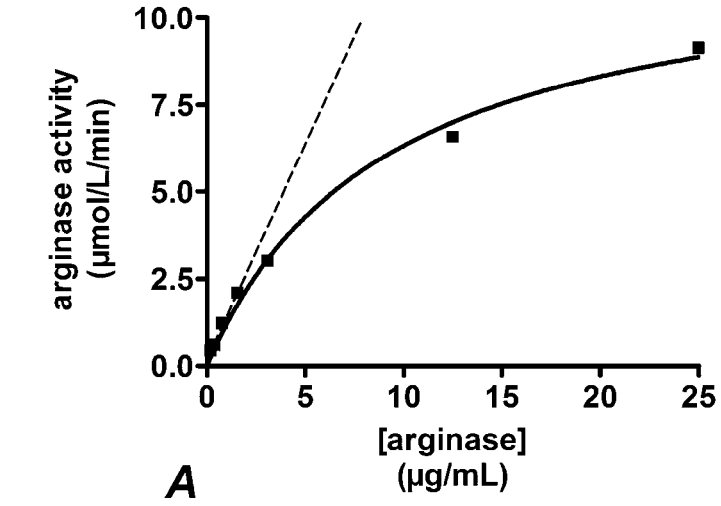

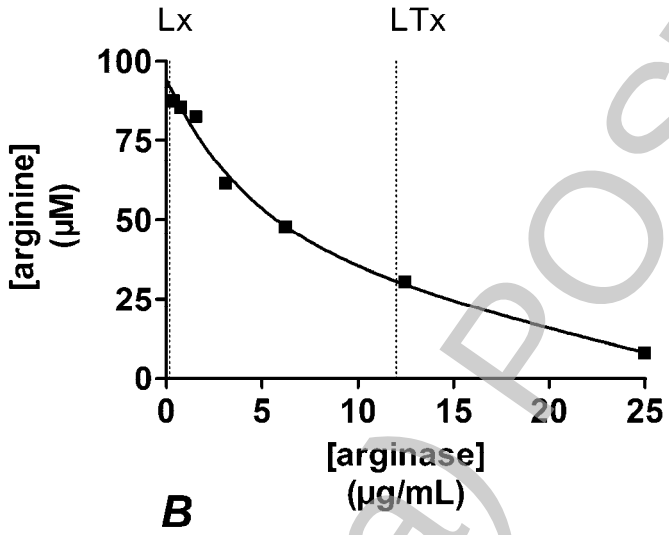

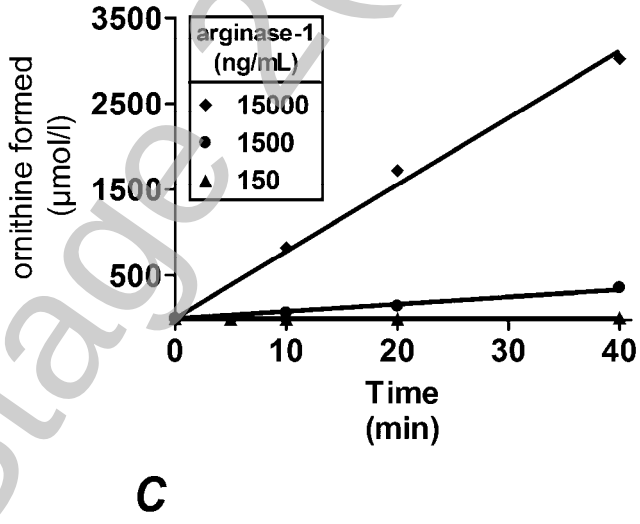

Figure 4