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Arginase Increases Total Pancreatic and Islet Blood Flow in Anaesthetized Mice

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Abstract

Background. Previous studies have demonstrated that the high basal pancreatic islet blood perfusion is crucially dependent on nitric oxide formation. Arginase can interfere with the formation of nitric oxide by limiting substrate availability. The aim of the present study was to evaluate the influence of arginase on islet blood perfusion in anasthetized mice.

Methods. The blood perfusion of the pancreatic islets was measured with a microsphere technique in anaesthetized NMRI mice after administration of arginase.

Results: Arginase administration increased both total pancreatic and islet blood flow to the same degree. Also adrenal blood flow was increased, whereas other organ blood flow values were unaffected.

Conclusion. Arginase induces a paradoxical increase in pancreatic and islet blood flow, the reasons for which are still unknown.

Introduction

Nitric oxide is formed from arginine through the actions of nitric oxide synthase and is the single most important general and local vasodilator in the body (1, 2). Administration of arginine to healthy volunteers induces formation of nitric oxide (NO) and decreases blood pressure (3), whereas inhibition of nitric oxide synthase increases blood pressure (4). Besides nitric oxide synthase also arginase, which catalyzes the final step in the urea cycle (5–7), competes for arginine.

Arginase is particularly prevalent in the liver, and is released after damage to hepatocytes, such as seen in ischemia/reperfusion injuries after liver transplantation (8, 9). Theoretically it could be assumed that this would impair the flow through vascular beds mainly regulated by NO through substrate depletion (10). However, administration of arginase to pigs (9) or rats (11, 12) did not affect blood perfusion in most vascular beds. Indeed, in one study a paradoxical increase in intestinal, ventricular, splenic and liver blood flow was observed (13).

Intraportal transplantation of islets in humans is usually associated with a transient increase in transaminase levels (14, 15), which may be due to a so called IBMIR reaction (instant blood mediated inflammatory reaction) caused by the introduction of foreign material into the blood stream of the portal vein (16, 17). This reaction is likely to induce cell death in both the implanted islets as well as in the surrounding hepatocytes, with subsequent local release of arginase. Since pancreatic islets are very dependent on normal NO production to maintain their

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Abbreviations: NO, nitric oxide

high blood perfusion, both in normal animals (18, 19) and after transplantation (20, 21) we deemed it of interest to examine if arginase affects the blood perfusion of endogenous islets.

Materials and methods

Animals

Adult, male NMRI-mice weighing approximately 30 g were purchased from Scan-Bur B&K (Sollentuna, Sweden). The animals had free access to tap water and pelleted food throughout the experiments. The experiments were approved by the animal ethics committee at Uppsala University, Uppsala, Sweden.

Surgical procedures

The animals were anaesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg body weight; Mebumal®; Apoteksbolaget, Umeå, Sweden). The animals were traceheostomized and placed on a thermal pad preset to maintain body temperature at 37.5–38.0 ºC. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and vein. The catheter in the femoral artery was used to continuously infuse Ringer solution (6 ml/kg body weight/h) throughout the experiments, and the aortic catheter was used to monitor mean arterial blood pressure by a transducer (PDCR 75/1; Druck Ltd., Groby, Leicestershire, UK).
The animals were then injected intravenously with 0.2 ml of either saline alone or 300 IU arginase (Sigma-Aldrich, Stockholm, Sweden) dissolved in saline immediately before injection. Blood flow measurements were made 20 min later.

Blood flow measurements

These measurements were performed according to a protocol previously described in detail (22, 23). Briefly, 20 min after administration of saline or arginase, 7 x 10^4 non-radioactive microspheres (E-Z Trac™; ITM Products, San Diego, CA, USA) with a mean diameter of 10 μm were injected during 5 sec via the catheter placed with its tip in the ascending aorta. Starting 5 sec before the microsphere injection, and continuing for a total of 60 sec, an arterial blood sample was collected from the catheter in the femoral artery at a rate of approximately 0.20 ml/min. The exact withdrawal rate was determined in each animal by weighing the sample. After obtaining the reference sample, another blood sample was secured for measurement of blood glucose and serum insulin concentrations. The animals were killed and the whole pancreas and adrenal glands as well as samples from the duodenum, colon and liver were removed, blotted and weighed. The tissue samples were then treated with a freeze-thawing technique to visualize the microspheres as previously de-

Table 1. Measurements were made in pentobarbital-anaesthetized male NMRI-mice 20 min after an intravenous injection of 0.2 ml saline or 300 IU arginase dissolved in 0.2 ml saline

Substance given No of animals	Saline 8	Arginase 9
Body weight (g)	33.6 ± 1.5	32.8 ± 0.8
Pancreas weight (mg)	323 ± 14	307 ± 17
B-glucose (mmol/l)		
0’	8.8 ±1.2	8.1 ± 0.5
20’	8.1 ± 1.1	8.6 ± 0.6
Serum insulin concentration (ng/ml)	2.87 ± 0.35	3.02 ± 0.40
Mean arterial blood pressure (mm Hg)	79 ± 3	99 ± 6*
Islet blood flow (% of pancreatic blood flow)	0.84 ± 0.16	1.23 ± 0.21
Duodenal blood flow (ml/min x g)	5.35 ± 0.86	7.61 ± 1.47
Colonic blood flow (ml/min x g)	2.16 ± 0.57	2.46 ± 1.08
Arterial liver flow (ml/min x g)	0.11 ± 0.03	0.34 ± 0.13

Values are means ± SEM. * denotes P=0.008 when compared to the saline-injected mice (Student’s unpaired *t*-test).

scribed (24). The blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$ where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of the reference sample (ml/min), N_{org} is number of microspheres present in the organ and N_{ref} is number of microspheres in the reference sample. A difference <10% in blood flow values between the adrenal glands was used to confirm adequate mixing of the spheres in the circulation.

Measurements of blood glucose and serum insulin concentrations

Arterial blood samples were obtained after securing the reference blood sample and later analyzed for blood glucose concentrations with a blood glucose meter (Medisense®; Svenska Medisense AB, Stockholm, Sweden) and serum insulin concentrations with ELISA (Mouse Insulin ELISA^{R}; Mercodia AB, Uppsala, Sweden).

Statistical calculations

All values are given as means ± SEM. Probabilities (P) of chance differences between the groups were calculated with Student’s two-tailed t-test or the Mann-Whitney rank sum test (SigmaStat^{R}; SSPD, Erfart, Germany).

Results

Arginase did not affect either blood glucose or serum insulin concentrations, but markedly increased mean arterial blood pressure (Table 1).

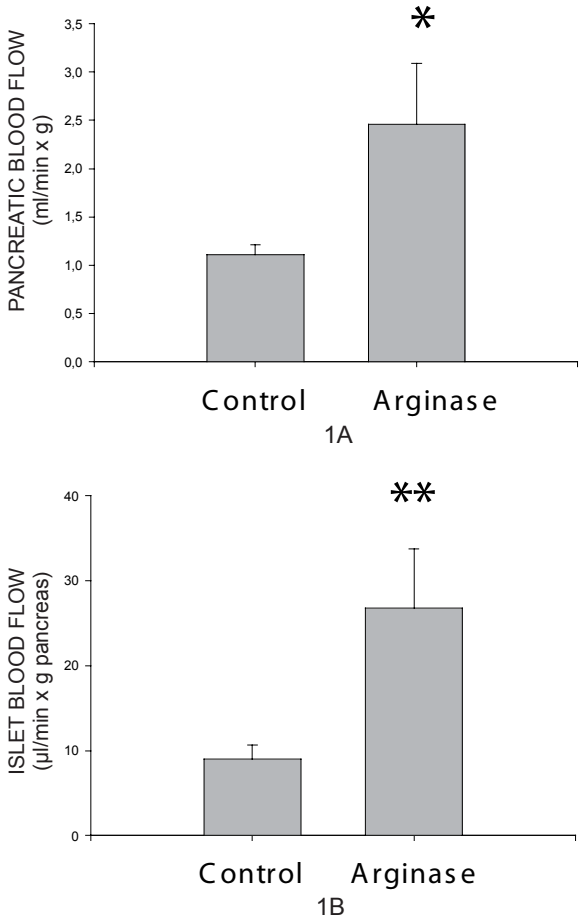

Figure 1. Total pancreatic blood flow (**A**) and islet blood flow (**B**) in anaesthetized NMRI mice 20 min after an intravenous injection of saline (control) or 300 IU arginase. Values are means ± SEM for 8–9 experiments. * denotes P=0.03 and ** P=0.008 when compared to the control animals (Mann-Whitney rank sum test).

Both total pancreatic (Figure 1A) and islet blood flow (Figure 1B) increased after arginase administration. The vascular conductance of the islets was increased (0.12 ± 0.02 vs. 0.35 ± 0.10 µl/min x g x mm Hg in controls and arginase-treated rats, respectively, P=0.034), and a similar trend, although not statistically significant, was seen in whole pancreatic blood flow (15.1 ± 1.3 vs. 29.3 ± 15.7 µl/min x g x mm Hg; P=0.072). The fractional islet blood flow, *i.e*. the fraction of total pancreatic islet blood flow diverted through the islets, did not change (Table 1). There were no effects on duodenal, colonic or arterial liver blood flow (Table 1). Adrenal blood flow was more than doubled after treatment with arginase (Figure 2).

Discussion

Arginase has a much wider distribution than other urea cycle enzymes, which suggests that it also possesses other important physiological functions, including regu-

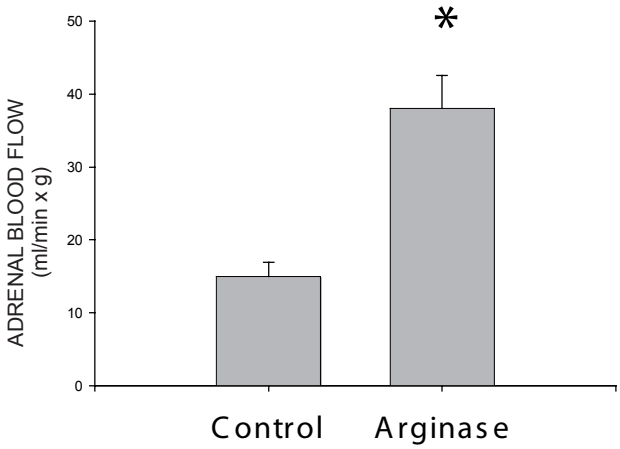

Figure 2. Adrenal blood flow in anaesthetized NMRI mice 20 min after an intravenous injection of saline (control) or 300 IU arginase. Values are means ± SEM for 8–9 experiments. * denotes P=0.045 when compared to the control animals (Mann-Whitney rank sum test).

lation of the synthesis of polyamines, proline, glutamate and NO (10, 25). Vascular tissues express arginase which metabolizes L-arginine to urea and thereby reduces bioavailability of arginine for nitric oxide formation (6). In line with this, inhibition of arginase in cultured endothelial cells stimulates NO synthesis (26, 27), whereas arginase over-expression inhibits NO synthesis (10). It has been suggested that arginase may modulate or down-regulate NOS activity by substrate competition also in macrophages (28) and T-lymphocytes (29). That arginase had some effect in the present study was clear from the increased mean arterial blood pressure, which presumably reflects a disturbance of NO production in some resistance vessels (6).

The islet blood perfusion is very high in comparison with that of the exocrine pancreatic parenchyma, and the blood flow to these two compartments is regulated independently from one another (30, 31). The islet blood flow is affected through a complex interplay between metabolic and nervous mediators as well as endothelium-derived substances, mainly nitric oxide (NO) (18, 19, 32). The latter substance has even been suggested to be permissive for the high basal islet blood perfusion (30). In a previous study we found that administration of arginine did not influence islet blood flow in itself, suggesting that substrate supply is not normally limiting for NO effects in the islets (33), which also confirms findings in other organs (11). In the present study, we used a dose of arginase which in other studies has decreased serum arginine concentrations (12, 13). In spite of this, we saw no major changes in organ blood flows, besides an increase in adrenal, total pancreatic and islet blood flow. This is unlikely to be due to the increased mean arterial blood pressure, since we have previously shown that this is well within the interval in which these blood flow values are autoregulated (34). That also islet blood flow increases, is surprising in view of the sensitivity of islets to NO (see above). The reasons for this arginase-induced increase are unknown, but it should be noted that it is intracellular, not extracellular, arginine concentrations which determine the rate of NO formation (35). Thus, it may well be that the intracellular stores of arginine in the

pancreas are sufficient to maintain an adequate NO-synthesis during the time span of the experiment. It should also be noted that a previous study in rats demonstrated an increase in intestinal, ventricular, splenic and liver blood flow after arginase administration (13). No mechanism for this increase could be discerned. We could not see any changes in intestinal blood flow in the present study, which presumably reflects species differences (see below).

The arginase content of the pancreas and pancreatic islets varies between species. Thus, in mice and rats the cytosolic isoform arginase I is preferentially expressed (36–38), whereas the mitochondrial arginase II is the major isoform found in human islets (38). There is no arginase II in rodent islets, whereas exocrine tissue contains weak arginase I and no arginase II (37). Arginase I is widely expressed in mouse tissues, especially in the upper gastrointestinal tract, and is seen also during embryonic development in pancreas and is much more pronounced in glandular cells in early development (39). Studies with Western blots demonstrated that rat islets and RINm5F cells express mainly arginase I (37). Interestingly, functional studies have shown that the level of islet arginase activity can regulate the rate of cytokine-induced NO generation (38). However, no other physiological role of arginase in the pancreas has been suggested, and it may well be that endogenous arginase plays a minor role in normal endocrine and exocrine pancreatic function. In view of this, it is unlikely that intra-islet arginase affects NO availability in the native pancreas.

An important consideration of the present findings is related to clinical islet transplantation. These are performed intra-portally, so that islets embolize in small portal tributaries within the liver (40, 41). This is associated with damage to surrounding hepatocytes, which are prone to release arginase when injured. This can affect regional blood circulation, *e.g*. after liver transplantation (9, 42, 43). The local concentrations of arginase are likely to be much higher around the embolized islets, but their effects are unknown. It is known that intraportally implanted islets revascularize within 3–5 days (44), *i.e*. in a time span when damaged hepatocytes are present. The present findings suggest that arginase release may not be disadvantageous for graft blood flow, even though this must be proven in a more adequate model.

The major finding in the present study is that arginase administration causes only minor effects on the blood flow of most organs studied, but, in view of the previously demonstrated sensitivity of islet blood flow to NO, paradoxically increased both total pancreatic and islet blood flow to the same degree. The reasons for this increase are unknown. From a practical point of view the hyperperfusion is an advantage, since release of arginase from damaged hepatocytes, which is likely to occur after intraportal implantation of islets, is unlikely to adversely affect the blood perfusion of the graft.

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References

1. Huang PL (2003) Endothelial nitric oxide synthase and endothelial dysfunction. Curr Hypertens Rep 5: 473–80.
2. Bruckdorfer R (2005) The basics about nitric oxide. Mol Aspects Med 26: 3–31.
3. Nakaki T, Hishikawa K, Suzuki H, Saruta T, Kato R (1990) L-arginine-induced hypotension. Lancet 336: 696.
4. Arnal JF, Dinh-Xuan AT, Pueyo M, Darblade B, Rami J (1999) Endothelium-derived nitric oxide and vascular physiology and pathology. Cell Mol Life Sci 55: 1078–87.
5. Jackson MJ, Beaudet AL, O'Brien WE (1986) Mammalian urea cycle enzymes. Annu Rev Genet 20: 431–64
6. Boucher JL, Moali C, Tenu JP (1999) Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. Cell Mol Life Sci 55: 1015–28.
7. Wu G, Morris SM, Jr. (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336 (Pt 1): 1–17.
8. Ashamiss F, Wierzbicki Z, Chrzanowska A, Scibior D, Pacholczyk M, Kosieradzki M, Lagiewska B, Porembska Z, Rowinski W (2004) Clinical significance of arginase after liver transplantation. Ann Transplant 9: 58–60.
9. Längle F, Roth E, Steininger R, Winkler S, Mühlbacher F (1995) Arginase release following liver reperfusion. Evidence of hemodynamic action of arginase infusions. Transplantation 59: 1542–9.
10. Li H, Meininger CJ, Hawker JR, Jr., Haynes TE, Kepka-Lenhart D, Mistry SK, Morris SM, Jr., Wu G (2001) Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells. Am J Physiol Endocrinol Metab 280: E75–82.
11. Prins HA, Houdijk AP, Wiezer MJ, Teerlink T, van Lambalgen AA, Thijs LG, van Leeuwen PA (2000) The effect of mild endotoxemia during low arginine plasma levels on organ blood flow in rats. Crit Care Med 28: 1991–7.
12. Nijveldt RJ, Prins HA, van Kemenade FJ, Teerlink T, van Lambalgen AA, Boelens PG, Rauwerda JA, van Leeuwen PA (2001) Low arginine plasma levels do not aggravate renal blood flow after experimental renal ischaemia/reperfusion. Eur J Vasc Endovasc Surg 22: 232–9.
13. Prins HA, Houdijk AP, van Lambalgen AA, Teerlink T, Meijer S, Thijs LG, van Leeuwen PA (1998) Paradoxical changes in organ blood flow after arginase infusion in the non-stressed rat. Shock 9: 422–7.
14. Barshes NR, Lee TC, Goodpastor SE, Balkrishnan R, Schock AP, Mote A, Brunicardi FC, Alejandro R, Ricordi C, Goss JA (2005) Transaminitis after pancreatic islet transplantation. J Am Coll Surg 200: 353–61.
15. Rafael E, Ryan EA, Paty BW, Oberholzer J, Imes S, Senior P, McDonald C, Lakey JR, Shapiro AM (2003) Changes in liver enzymes after clinical islet transplantation. Transplantation 76: 1280–4.
16. Bennet W, Groth CG, Larsson R, Nilsson B, Korsgren O (2000) Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. Ups J Med Sci 105: 125–33.
17. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, Bretzel RG, Elgue G, Larsson R, Nilsson B, Korsgren O (1999) Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? Diabetes 48: 1907–14.
18. Moldovan S, Livingston E, Zhang RS, Kleinman R, Guth P, Brunicardi FC (1996) Glucose-induced islet hyperemia is mediated by nitric oxide. Am J Surg 171: 16–20.

19. Svensson AM, Östenson CG, Sandler S, Efendic S, Jansson L (1994) Inhibition of nitric oxide synthase by N^G-nitro-L-arginine causes a preferential decrease in pancreatic islet blood flow in normal rats and spontaneously diabetic GK rats. Endocrinology 135: 849–53.
20. Jansson L, Bodin B, Carlsson P-O (2005) Changes in graft blood flow early after syngeneic rat pancreas-duodenum transplantation. Upsala J Med Sci 110 57–68.
21. Svensson AM, Sandler S, Jansson L (1995) The blood flow in pancreatico-duodenal grafts in rats: inhibition of nitric oxide synthase preferentially decreases islet blood flow. Eur J Pharmacol 275: 99–103.
22. Carlsson PO, Källskog Ö, Bodin B, Andersson A, Jansson L (2002) Multiple injections of coloured microspheres for islet blood flow measurements in anaesthetised rats: influence of microsphere size. Ups J Med Sci 107: 111–20.
23. Carlsson PO, Andersson A, Jansson L (1996) Pancreatic islet blood flow in normal and obese-hyperglycemic (ob/ob) mice. Am J Physiol 271: E990–5.
24. Jansson L, Hellerström C (1981) A rapid method of visualizing the pancreatic islets for studies of islet capillary blood flow using non-radioactive microspheres. Acta Physiol Scand 113: 371–4.
25. Kepka-Lenhart D, Mistry SK, Wu G, Morris SM, Jr. (2000) Arginase I: a limiting factor for nitric oxide and polyamine synthesis by activated macrophages? Am J Physiol Regul Integr Comp Physiol 279: R2237–42.
26. Buga GM, Singh R, Pervin S, Rogers NE, Schmitz DA, Jenkinson CP, Cederbaum SD, Ignarro LJ (1996) Arginase activity in endothelial cells: inhibition by N^G-hydroxy-L-arginine during high-output NO production. Am J Physiol 271: H1988–98.
27. Chicoine LG, Paffett ML, Young TL, Nelin LD (2004) Arginase inhibition increases nitric oxide production in bovine pulmonary arterial endothelial cells. Am J Physiol Lung Cell Mol Physiol 287: L60-8.
28. Wang WW, Jenkinson CP, Griscavage JM, Kern RM, Arabolos NS, Byrns RE, Cederbaum SD, Ignarro LJ (1995) Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. Biochem Biophys Res Commun 210: 1009–16.
29. Bansal V, Rodriguez P, Wu G, Eichler DC, Zabaleta J, Taheri F, Ochoa JB (2004) Citrulline can preserve proliferation and prevent the loss of CD3 zeta chain under conditions of low arginine. JPEN J Parenter Enteral Nutr 28: 423–30.
30. Brunicardi FC, Stagner J, Bonner-Weir S, Wayland H, Kleinman R, Livingston E, Guth P, Menger M, McCuskey R, Intaglietta M, Charles A, Ashley S, Cheung A, Ipp E, Gilman S, Howard T, Passaro E, Jr. (1996) Microcirculation of the islets of Langerhans. Long Beach Veterans Administration Regional Medical Education Center Symposium. Diabetes 45: 385–92.
31. Jansson L (1994) The regulation of pancreatic islet blood flow. Diabetes Metab Rev 10: 407–16.
32. Moncada S, Palmer RM, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43: 109–42.
33. Svensson AM, Jansson L (1996) L-arginine and pancreatic islet blood flow in anesthetized rats. Experientia 52: 239–41.
34. Iwase M, Sandler S, Carlsson PO, Hellerstrom C, Jansson L (2001) The pancreatic islets in spontaneously hypertensive rats: islet blood flow and insulin production. Eur J Endocrinol 144: 169–78.
35. Griffith OW, Park KH, Aisaka K, Gross SS; (1992); The role of plasma arginine in nitric oxide synthesis studied with arginase treated guinea pigs and rats. In Moncada S (ed); The biology of nitric oxide. 1. Physiological and clinical aspects.; Vol. Portland, OR, USA; Portland Press; 6–10.
36. Herzfeld A, Raper SM (1976) The heterogeneity of arginases in rat tissues. Biochem J 153: 469–78.
37. Yu H, Yoo PK, Aguirre CC, Tsoa RW, Kern RM, Grody WW, Cederbaum SD, Iyer RK (2003) Widespread expression of arginase I in mouse tissues. Biochemical and physiological implications. J Histochem Cytochem 51: 1151–60.
38. Stickings P, Mistry SK, Boucher JL, Morris SM, Cunningham JM (2002) Arginase expression and modulation of IL-1beta-induced nitric oxide generation in rat and human islets of Langerhans. Nitric Oxide 7: 289–96.
39. Yu H, Iyer RK, Yoo PK, Kern RM, Grody WW, Cederbaum SD (2002) Arginase expression in mouse embryonic development. Mech Dev 115: 151–5.
40. Ricordi C (2003) Islet transplantation: a brave new world. Diabetes 52: 1595–603.

41. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343: 230–8.
42. Roth E, Steininger R, Winkler S, Langle F, Grunberger T, Fugger R, Muhlbacher F (1994) L-Arginine deficiency after liver transplantation as an effect of arginase efflux from the graft. Influence on nitric oxide metabolism. Transplantation 57: 665–9.
43. Längle F, Steininger R, Waldmann E, Grünberger T, Benditte H, Mittlbock M, Soliman T, Schindl M, Windberger U, Mühlbacher F, Roth E (1997) Improvement of cardiac output and liver blood flow and reduction of pulmonary vascular resistance by intravenous infusion of L-arginine during the early reperfusion period in pig liver transplantation. Transplantation 63: 1225–33.
44. Andersson A, Korsgren O, Jansson L (1989) Intraportally transplanted pancreatic islets revascularized from hepatic arterial system. Diabetes 38 Suppl 1: 192–5.

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