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ORIGINAL ARTICLE

Dynamic changes in blood flow and oxygen consumption in the portal-drained viscera of growing pigs receiving acute administration of L-arginine

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Abstract This study tested the hypothesis that an increase in arginine concentration in the portal vein may affect blood flow and oxygen consumption in the portal-drained viscera (PDV) of swine. Eight barrows (70 kg body weight) were surgically fitted with chronic catheters in the portal vein, ileal vein, and carotid artery. Thirteen days after the surgery, pigs that had been fasted for 12 h were randomly allocated to receive administration of either L-alanine (103 mg/kg body weight, isonitrogenous control) or L-arginine–HCl (61 mg/kg body weight) via the portal vein. Portal vein blood flow (PVBF) was measured with infusion of *p*-aminohippuric acid into the ileal vein, and blood samples were simultaneously obtained every 0.5 h for 4 h. Compared with the control, arginine infusion increased PVBF at 30–90 min after infusion but decreased PDV oxygen consumption at 60–150 min after infusion ($P < 0.05$). Plasma concentrations of glutamate at infusion times of 180–240 min and of arginine at infusion times of 60–240 min in arginine-infused pigs were higher than those for the control group ($P < 0.05$). Plasma concentrations of insulin and glucagon at the infusion times of 30–90 min were higher and of free fatty acids at the infusion times of 60–120 min were lower than those for the control pigs ($P < 0.05$). These results indicate that increasing arginine concentration in the portal vein enhances PDV blood flow, reduces PDV oxygen consumption, and beneficially alters the metabolic profile in swine, an established animal model for studying human nutrition and metabolism.

Keywords Arginine · Blood flow · Oxygen consumption · Pigs · Digestion

Abbreviations

NO	Nitric oxide
PAH	*p*-Aminohippuric acid
PDV	Portal-drained viscera
PVBF	Portal vein blood flow

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Introduction

Arginine may affect multiple metabolic pathways involving nutrient transport (Kim and Wu 2009), fatty acid and glucose syntheses (Fu et al. 2005; Jobgen et al. 2006), amino acid degradation (Blachier et al. 2011; Kim et al. 2007), and cellular redox state (Wu and Meininger 2009; Yin and Tan 2010). Arginine is the physiological precursor for the synthesis of nitric oxide (NO), which stimulates the oxidation of fatty acids and glucose in a cell-specific manner (Jobgen et al. 2006). Recent work has shown that dietary supplementation with arginine reduced plasma

levels of glucose, homocysteine, and asymmetric dimethylarginine (risk factors for the metabolic syndrome) (Marliss et al. 2006), while improving endothelium-dependent relaxation (an indicator of cardiovascular function), in both type I and type II models of diabetes mellitus (Fu et al. 2005; Kohli et al. 2004). Using ^{1}H nuclear magnetic resonance spectroscopy, He et al. (2009) have reported that dietary arginine supplementation alters the catabolism of fat and amino acids in the whole body, enhances protein synthesis in skeletal muscle, and modulates intestinal microbial metabolites.

The small intestine of pigs is crucial for the metabolism of amino acids (Burrin et al. 2000; Stoll et al. 1998; Wu et al. 2005) and nitrogen recycling (Bergen and Wu 2009). However, extensive investigations over the past two decades have indicated extensive catabolism of amino acids by the portal-drained viscera (PDV) of pigs and humans (Wu et al. 2005, 2009). Results from tracer studies with fed piglets indicate the preferential use of enteral amino acids rather than arterial amino acids for mucosal protein synthesis (Dudley et al. 1994; Stoll and Burrin 2006). At present, little is known about effects of arginine on nutrient metabolism in the PDV of mammals. We hypothesized that an increase in arginine concentration in the portal vein may affect blood flow, oxygen consumption, and circulating metabolites in the PDV. The present study tested this hypothesis using growing pigs [well-established animal models for studying human nutrition and metabolism (Tan et al. 2011)] and our new technique for implanting chronic portal vein and ileal mesenteric vein catheters in swine (Yin et al. 2010).

Materials and methods

Animals

Eight barrows (Duroc × Large White × Landrace) with an average initial BW of 70 kg were housed individually in stainless steel metabolism pens (0.8 × 1.8 m) in an environmentally controlled room with an average temperature of 25 °C, and had free access to drinking water and corn- and soybean meal-based diets (Yin et al. 1993). After a 1-week adjusting period, pigs were fasted for 24 h and surgically fitted with chronic catheters in the portal vein, ileal vein, and carotid artery, as described by Yen and Killefer (1987) with some modifications (Huang et al. 2003; Yin et al. 2010). Briefly, a 2-cm stainless steel tube (2.41-mm O.D. × 1.68-mm I.D.), which was made using adapter luerstus-15 g (VWR International Ltd., Mississauga, ON, Canada), was inserted into the top tip of a portal vein catheter (Micro-Renathane tubing, 2.41-mm O.D. × 1.68-mm I.D.; Braintree Scientific Inc., NY, USA). The portal vein was punctured with a No. 12 needle, and then the portal vein catheter was inserted, using an introducer, into the portal vein, 4–6 cm toward the liver. A catheter (Micro-Renathane tubing, 2.41-mm O.D. × 1.68-mm I.D.) was inserted into the carotid artery and toward the aorta for ~12 cm (Huang et al. 2003). Finally, a catheter (1.17-mm O.D. × 0.76-mm I.D., RenaPulse Tubing; Braintree Scientific Inc.) was inserted into the ileal vein for ~6 cm (Huang et al. 2003). After the surgery, pigs were returned to their metabolic crates for recovery. An antibiotic (penicillin) was administered intravenously to the animal during the first 5 days of the recovery period. The catheters were checked for potency by flushing and filling with heparinized saline solution daily during the 13-day recovery period. The experiment was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol, and approved by the Animal Care and Use Committee of The Chinese Academy of Sciences (Deng et al. 2009).

PAH and arginine infusion

After a 13-day period of recovery from the surgery, pigs that had been fasted for 12 h were infused with PAH for the measurement of the portal vein blood flow (PVBF), as described by Yen and Killefer (1987). Briefly, a concentrated PAH (Sigma, St Louis, MO, USA) solution was prepared with sterile physiological saline solution (9 g NaCl/L) and the pH was adjusted to 7.4 with 1 M NaOH, followed by filtration through a 0.2-μm filter. A priming dose of PAH was first administered into the ileal vein catheter at a rate of 3.82 ml/min for 5 min, and then followed by constant infusion (0.2 mg PAH/kg BW per min) at a rate of 1 ml/min for 4 h (Li et al. 2008).

Simultaneously, pigs were assigned randomly to receive intravenous administration of the same volume of either L-alanine (103 mg/kg BW, isonitrogenous control; Ajinomoto Inc, Tokyo, Japan) or L-arginine–HCl (61 mg/kg body wt; Ajinomoto Inc, Tokyo, Japan) solution via the portal vein. The period of administration of arginine or alanine lasted for 5 min. The dose of arginine was chosen to double arginine concentration in plasma at 60 min after its intravenous infusion (Wu et al. 2007b). The portal administration of arginine differs from its intravenous administration because ~6–8 % of arginine in the portal vein blood is taken up by the liver of pigs during the first pass (Wu et al. 2007a). All solutions were filtrated through 0.2-μm filters before intravenous administration.

Blood sample collection and analysis

Blood samples were collected, into heparinized tubes (BD Inc., Franklin Lakes, NJ, USA; with each tube providing 132 USP units of sodium heparin), from the portal vein and

carotid artery before arginine infusion and, thereafter, every 0.5 h for 4 h, for the determination of packed cell volume and partial pressure of oxygen (Huang et al. 2003). Additionally, some blood samples were centrifuged at 2,500×*g* at 4 °C for 15 min to obtain plasma, which was stored at −80 °C until analysis. Concentrations of PAH in plasma were measured as described by Harvey and Brothers (1962).

Plasma samples from carotid artery were used for analyzing metabolic profiles. Amino acids (only at infusion time of 0, 60, 120, 180, and 240 min) were analyzed by HPLC (Wu 1997). Authentic amino acid standards (Sigma Chemicals Inc, St. Louis, MO) were used to quantify amino acids in samples. Triglycerides, cholesterol, glucose, total protein, and urea were measured using Biochemical Analytical Instrument (Beckman CX4) and commercial kits (Sino-German Beijing Leadman Biotech Ltd., Beijing, China) (Tan et al. 2009). Tumor necrosis factor-α, insulin, growth hormone, insulin-like growth factor-I, leptin, and glucagon were determined by radioimmunoassays using kits from Tianjin Nine Tripods Biomedical Engineering Inc. (Tianjin, China). Free fatty acids were measured according to Yin et al. (2000) using an assay kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Calculations and statistical analysis

Portal vein blood flow was estimated using the PAH indicator dilution technique (Yen and Killefer 1987). Oxygen consumption by the PDV was calculated based on PVBF and concentration differences between portal vein and carotid arterial blood. Statistical analysis of the difference between treatment means was analyzed by the unpaired *t* test and the difference between after and before infusion means within the same treatment by ANOVA for repeated measurements using SPSS 13.0 (SPSS Inc., Chicago, IL). All data are given as mean ± SEM. *P* values <0.05 were taken to indicate significance.

Results

Portal vein blood flow and oxygen consumption by the PDV

Compared with alanine treatment, arginine infusion increased PVBF at 30, 60, and 90 min after infusion but decreased oxygen consumption by the PDV at 60, 90, 120, and 150 min after infusion ($P < 0.05$, Fig. 1). At 150 min after infusion, PVBF did not differ ($P > 0.05$) between control and arginine groups.

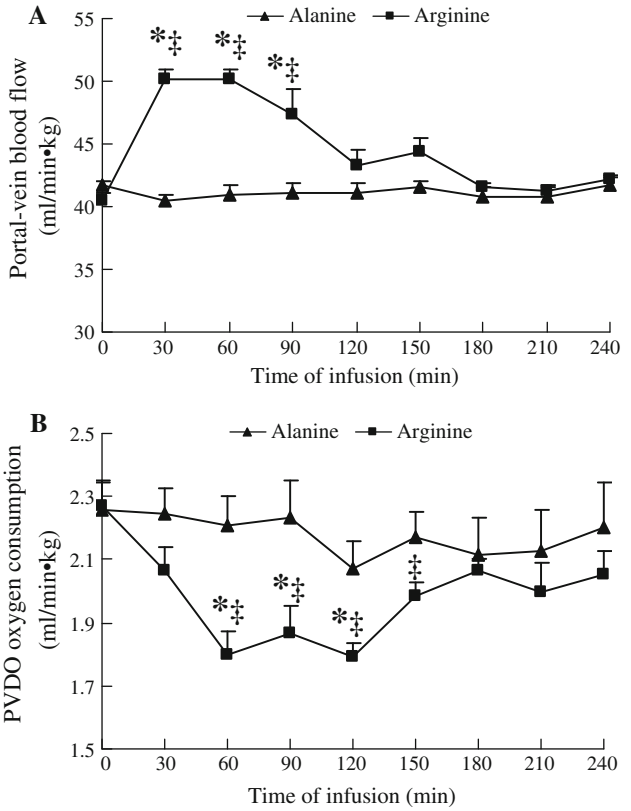

Fig. 1 Portal vein blood flow (PVBF) rates (**a**) and PDV oxygen consumption (**b**) after infusion of arginine or alanine via portal vein. Values are mean ± SEM, $n = 4$. *$P < 0.05$ versus alanine treatment on the same infusion time. ‡$P < 0.05$ versus before infusion within the same treatment. *PVDO* oxygen consumption by the portal-drained viscera

After arginine infusion, PVBF at the infusion times of 30, 60, and 90 min were higher ($P < 0.05$) but oxygen consumption by the PDV at 60, 90, 120, and 150 min were lower ($P < 0.05$) than the values for pre-infusion (Fig. 1). However, for pigs that were infused with alanine, there was no difference ($P > 0.05$) in PVBF or oxygen consumption by the PDV between the pre- and post-infusion periods (Fig. 1).

Plasma amino acids

Compared with the control, plasma concentrations of glutamate at the infusion times of 180 and 240 min (Fig. 2a) and of arginine at the infusion times of 60, 120, 180, and 240 min (Fig. 2e) were higher ($P < 0.05$) in arginine-infused pigs. However, arginine infusion decreased ($P < 0.05$) plasma concentrations of cystine at 60, 120, 180, and 240 min after infusion and of valine at 60 and 120 min after infusion compared with the control pigs (Fig. 2c, d).

Compared with pre- and post- infusion, plasma concentrations of cystine at the infusion times of 60, 120, 180, and 240 min were lower ($P < 0.05$) but plasma concentrations

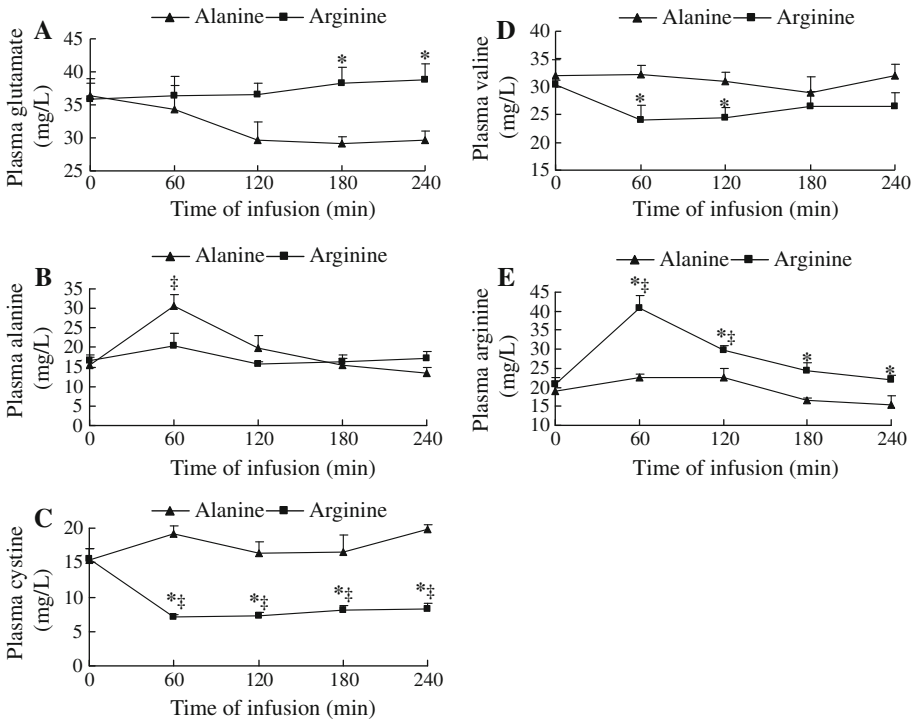

Fig. 2 Plasma concentrations of glutamate (**a**), alanine (**b**), cystine (**c**), valine (**d**), and arginine (**e**) in carotid artery after intravenous infusion of arginine or alanine via the portal vein. Values are mean ± SEM, $n = 4$. *$P < 0.05$ versus alanine treatment on the same infusion time. ‡$P < 0.05$ versus before infusion within the same treatment

of arginine at the infusion times of 60 and 120 min were higher ($P < 0.05$) than those at pre-infusion time. Arginine infusion did not affect ($P > 0.05$) concentrations of other amino acids in the plasma (data not shown). Concentrations of amino acids in the plasma except for alanine at the infusion time of 60 min (Fig. 2b) did not change ($P > 0.05$) in response to alanine infusion.

Plasma hormones and metabolites

After arginine infusion, plasma concentrations of insulin (Fig. 3a) and glucagon (Fig. 3c) at the infusion times of 30, 60, and 90 min were higher ($P < 0.05$) and FFA (Fig. 3d) at the infusion times of 60, 90, and 120 min were lower ($P < 0.05$) than the control pigs. Plasma insulin and glucagon reached maximal values at the infusion times of 30 and 60 min, respectively, but plasma FFA decreased ($P < 0.05$) to minimal values at the infusion time of 60 min (Fig. 3a, c, d). In comparison with values for the pre-infusion time, alanine or arginine infusion did not affect ($P > 0.05$) the concentrations of triglycerides, cholesterol, glucose, total protein, urea nitrogen, tumor necrosis factor-α, growth hormone, insulin-like growth factor-I, or leptin in any of the studied pigs (Fig. 3b, e; some data not shown).

Discussion

There is growing interest in the metabolism of amino acids by the intestine (Blachier et al. 2009; Boutry et al. 2011) and the PDV (Wu 2009). Using a new technique for implanting chronic portal vein and ileal mesenteric vein catheters in pigs (Yin et al. 2010), we determined the dynamic changes of blood flow, oxygen consumption, and plasma metabolites in pigs receiving acute intravenous administration of arginine. Results demonstrated for the first time that arginine infusion increased PVBF and decreased oxygen consumption by the PDV compared with the control group. Additionally, arginine infusion increased plasma concentrations of glutamate, arginine, insulin, and glucagon, while reducing plasma concentrations of cystine and valine. Because arginine is a basic acid, its HCl salt was used for intravenous infusion to avoid an acid–base imbalance (Satterfield et al. 2011, 2012). In a separate study, we found that an isomolar intravenous administration of NaCl (0.289 mmol/kg body weight) to the NaCl moiety in 61 L-arginine–HCl/kg body weight did not affect any of the blood parameters measured (B. Tan, G.Y. Wu, and Y.L. Yin; our unpublished data). Collectively, results of this work indicate that elevated levels of arginine in the circulation can affect the endocrine status and nutrient metabolism in the PDV.

A great deal of research has been conducted on the effects of dietary or intravenous administration of L-arginine on the circulatory system in animal models (Dallinger et al. 2003; Gannon et al. 1992; Garhöfer et al. 2005; Tangphao et al. 1999; Tay et al. 2002; Wu et al. 2009). Arginine decreases mean arterial pressure which indicates systemic vasodilatation (Dallinger et al. 2003) and increases retinal and choroidal blood flow (Garhöfer et al.

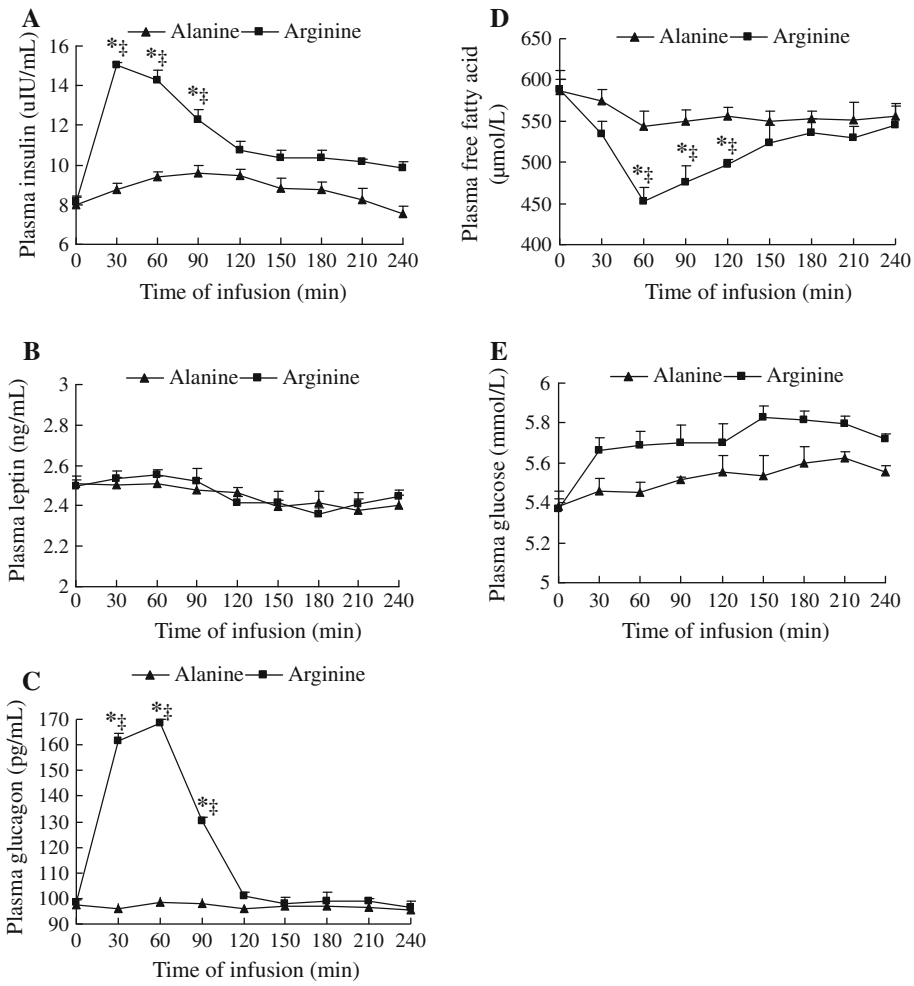

Fig. 3 Plasma concentrations of insulin (**a**), leptin (**b**), glucagon (**c**), free fatty acid (**d**), and glucose (**e**) in carotid artery after intravenous infusion of arginine or alanine via the portal vein. Values are mean ± SEM, $n = 4$. $^{*}P < 0.05$ versus the value for alanine treatment on the same infusion time. $^{‡}P < 0.05$ versus the value before infusion within the same treatment

2005). Ohta et al. (2007) demonstrated that infusion of L-arginine (150 mg/kg body weight) into rats enhanced blood flow in soleus muscle, skin, duodenum, heart, and brain compared with pre-infusion values. An important role of NO in the control of blood flow has been extensively studied (Baraona et al. 2002; Mattson et al. 1992; Tay et al. 2002). We did not measure NO production by the PDV or the whole body of pigs in the current study, but Poeze et al. (2011) recently reported that arginine supplementation increased systemic NO synthesis. Because L-arginine is the precursor of NO (Wu et al. 2009), the effects of arginine administration on PVBF are likely mediated, in part, by endogenous synthesis of NO (Wu and Meininger 2009). The increase in PVBF may augment the uptake of nutrients from the lumen of the small intestine into the portal vein and the removal of their products via the circulation. At the same time, a decrease in oxygen consumption by the PDV in response to arginine infusion suggests a role of elevated levels of NO in inhibiting the oxidation of energy substrates (primarily amino acids and glucose) by the small intestine. This would enhance the entry of dietary macronutrients into the systemic circulation and the efficiency of their utilization for lean tissue gains in the body (e.g., protein synthesis) (Tan et al. 2010; Yao et al. 2008).

Increases in concentrations of insulin and glucagon in the blood of arginine-treated pigs are in keeping with a known role of arginine in the physiology of the pancreas (Wu et al. 2009). Insulin may regulate blood flow and exert its potent vasodilator effects at physiologic concentrations (Bhanot and McNeill 1996; Tay et al. 2002). For example, Simonsen et al. (2002) reported that increased levels of insulin (during euglycemia) enhance PVBF, while decreasing oxygen consumption by 20 % and plasma FFA concentrations. Thus, impaired blood flow occurs in subjects with insulin resistance (Wu and Meininger 2009). Studies with the swine model are expected to provide therapeutic means for obese patients. In this regard, it is noteworthy that dietary arginine supplementation affected the serum metabolome in growing pigs (He et al. 2009). Consistent with this finding, dietary supplementation with arginine reduced plasma concentrations of urea in pigs (Kim and Wu 2004, 2009), while increasing plasma levels of arginine, ornithine, and creatine in pigs (Kim and Wu 2004; Wu and Morris 1998). Interestingly, changes in

plasma metabolites in response to acute arginine administration are not identical with those for long-term supplementation of arginine to pigs (He et al. 2009; Tan et al. 2009, 2011). For example, arginine infusion increased plasma glutamate levels (Fig. 2a) and did not reduce plasma concentrations of urea (data not shown), which could not be expected on the basis of current knowledge about the role for arginine in regulating nutrient utilization in pigs (Wu et al. 2007a). These different results may be explained by differences in the administered doses of arginine and the nutritional state of animals, as well as changes in gene expression brought about by long-term arginine supplementation.

As expected, an increase in circulating levels of arginine increased plasma concentrations of insulin (Fig. 3a) and glucagon (Fig. 3c) at the infusion times of 30, 60, and 90 min, which confirmed the previous reports for healthy pigs (Kim and Wu 2004; Yao et al. 2008) and humans (Eaton and Schade 1974; Floyd et al. 1966; Palmer et al. 1975), diabetic subjects (Palmer et al. 1976; Stingl et al. 2002), and obese subjects (Copinschi et al. 1967). Secretion of insulin and glucagon by specific cells in the pancreas is regulated usually in the opposite direction, but a striking exception is that insulin and glucagon secretion is stimulated by arginine or a protein meal (Muller et al. 1971). This is likely due to membrane depolarization in both pancreatic α- and β-cells that is coupled with the transport of arginine (a positively charged amino acid) (Newsholme et al. 2011). The fine balance between insulin and glucagon contributes the regulation of nutrient metabolism via pathways involving cAMP signaling (McKnight et al. 2010). Glucagon activates adenylyl cyclase to generate cAMP, which stimulates protein kinase A (PKA) (Mersmann and Smith 2005). PKA acts to phosphorylate many enzymes involved in fat and glucose metabolism (Jobgen et al. 2006). For example, PKA phosphorylates hormone-sensitive lipase, which hydrolyzes triacylglycerides to free fatty acids plus glycerol. Fatty acids are then oxidized in multiple tissues (including skeletal muscle, heart, adipose tissue, and liver) via the mitochondrial β-oxidation pathway (Jobgen et al. 2006). Insulin has opposing effects to glucagon, causing uptake of glucose and amino acids from the blood to various tissues and stimulation of glycogen, protein and lipid synthesis (Newsholme et al. 2011).

There are suggestions that arginine stimulates insulin release by modulating the insulinogenic signal evoked by glucose (Efendic et al. 1971). However, plasma glucose levels are relatively stable after arginine infusion in this study (Fig. 3e). This may be due to concurrent increases in both insulin and glucagon secretion in response to arginine administration. Glucagon stimulates conversion of amino acids to glucose in the liver, whereas insulin promotes the uptake of glucose by muscle for storage as glycogen. Because plasma glucose levels were not determined between infusion times of 0 and 30 min, we cannot rule out a possible increase in plasma glucose during this period. Notably, arginine has been reported to stimulate Ca^{2+} influx through voltage-sensitive Ca^{2+} channels (Smith et al. 1997). Whether this biochemical event may activate Ca^{2+}-dependent signaling pathways in porcine tissues (including the PDV, liver, skeletal muscle, and muscle) remains to be determined.

Insulin and glucagon secretion were closely related to plasma leptin, which was observed in both lean and obese healthy and insulin-resistant subjects and hyperinsulinemic hypothyroidism (Segal et al. 1996; Boden et al. 1996; Ahren and Larsson 1997; Kautzky-Willer et al. 1999). Stingl et al. (2002) reported that arginine infusion transiently decreased plasma concentrations of leptin both in insulin-deficient and hyperinsulinemic diabetic patients. Decrease of serum leptin concentration in pigs receiving long-term arginine supplementation was also observed in our previous study (Tan et al. 2011). In the current work, we found that the arginine-induced rise in insulin and glucagon secretion did not affect plasma leptin levels, arguing against a key role for insulin and glucagon in the regulation of leptin secretion. The plasma levels of leptin are highly correlated with adipose tissue mass (Friedman and Halaas 1998). Indeed, circulating levels of leptin do not increase after a meal (Maffei et al. 1995), as reported for short-term administration of insulin and glucagon (Pratley et al. 1996; Kauter et al. 2000) or acute arginine infusion (Fig. 3b).

Another novel observation of the present study is that plasma FFA concentrations decreased in response to arginine infusion (60, 90, and 120 min) (Fig. 3d), which followed the increases in glucagon and insulin. This is consistent with a role for these two hormones in regulating fat metabolism in liver (Witters and Trasko 1979), adipose tissue (Jensen et al. 1991), and the whole body (Carlson et al. 1993). In moderately obese men, insulin infusion lowered plasma FFA (Saad et al. 1998). The decrease in plasma FFA may be due to an increase in the uptake of FFA by skeletal muscle for oxidation (Tan et al. 2011). Arginine improves β-cell function (Fasching et al. 1994) and stimulates the β-oxidation of fatty acid, thereby decreasing plasma FFA and TG (Jobgen et al. 2006). Thus, enhancing the circulating levels of arginine within a physiological range may beneficially modulate the metabolic profile in mammals.

Effects of arginine administration on plasma concentrations of glutamate, cystine, and valine (Fig. 2) deserve comments. An effect of arginine on increasing glutamate levels in arterial blood appeared late in the infusion possibly because a gradual increase in ornithine production from arginine via arginase can drive glutamate formation via ornithine aminotransferase and pyrroline-5-carboxylate dehydrogenase in animals (Wu et al. 2011). In contrast, arginine infusion reduced concentrations of both cystine and

valine in the plasma of growing pigs (Fig. 2), suggesting a role for arginine in regulating the metabolism of these two amino acids. Elucidating the underlying mechanisms would require tracer studies of the synthesis and catabolism of cystine or the degradation of valine in the body. Such a complex experiment is beyond the scope of the present work but merits consideration in future investigation.

In conclusion, results of this study indicate that an increase in arginine concentration in the portal vein enhances blood flow, reduces oxygen consumption by the PDV, and alters circulating levels of metabolites. Additionally, arginine infusion simultaneously increased plasma concentrations of insulin and glucagon without affecting leptin levels. These results indicate that elevations of arginine in the plasma within a physiological range are effective in modulating PDV metabolism and metabolic profiles in swine and may have important implications for human health.

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Conflict of interest The authors declare that they have no conflict of interest.

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