Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

# Arginine Nutrition in Neonatal Pigs[1,2]

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ABSTRACT The concentration of arginine (an essential amino acid for neonates) in sow's milk is remarkably low, and thus endogenous synthesis of arginine plays a crucial role in maintaining arginine homeostasis in milk-fed piglets. Paradoxically, intestinal synthesis of citrulline from glutamine/glutamate and proline (the endogenous source of arginine) declines markedly in 7- to 21-d-old suckling pigs, compared with 1- to 3-d-old pigs. Therefore, plasma concentrations of arginine and its immediate precursors (ornithine and citrulline) decrease progressively by 20–41%, whereas plasma ammonia levels increase progressively by 18–46%, between d 3 and 14 of life. Dietary supplementation of 0.2 and 0.4% arginine to 7- to 21-d-old pigs (artificially reared on a milk feeding system) dose dependently enhances the plasma arginine concentration (30 and 61%), reduces the plasma ammonia level (20 and 35%), and increases weight gain (28 and 66%). These compelling metabolic and growth data demonstrate unequivocally that arginine is insufficient for supporting the maximal growth in milk-fed young pigs and that this arginine deficiency represents a major obstacle to realizing the growth potential in piglets. A low concentration of mitochondrial N-acetylglutamate (an activator of both pyrroline-5-carboxylate synthase and carbamoylphosphate synthase-I) is responsible for the striking decline in the intestinal synthesis of citrulline and arginine during the suckling period. Accordingly, oral administration of N-carbamoylglutamate [a metabolically stable analogue of N-acetylglutamate; 2 × 50 mg/(kg body wt · d)] enhances plasma arginine level (68%) and weight gain (61%) of 4- to 14-d-old sow-reared pigs. Thus, the metabolic activation of intestinal citrulline and arginine synthesis provides a novel, effective means to increase endogenous arginine provision and therefore piglet growth (a major goal of animal agriculture). Our findings not only generate new fundamental knowledge about amino acid utilization by neonatal pigs, but they also have important practical implications for improving the efficiency of pork production. J. Nutr. 134: 2783S–2790S, 2004.

KEY WORDS: • *amino acids* • *intestine* • *metabolism* • *milk* • *swine*

Arginine is an essential amino acid for the maximal growth of young mammals (1–3). It is the most abundant nitrogen carrier in tissue proteins (4) and is used by multiple pathways, including arginase, nitric oxide (NO)[4] synthase, arginine:glycine amidinotransferase, and arginyl-tRNA synthetase (5). Serving as a precursor for the synthesis of creatine, proline, glutamate, polyamines, and NO, arginine displays remarkable metabolic and regulatory versatility in cells. Notably, young mammals (including piglets) have a particularly high requirement of arginine for growth and metabolic function (1,2). An arginine deficiency (defined as insufficient arginine for supporting maximal growth or metabolic function in animals) may occur under various nutritional and clinical conditions. These conditions include a low supply of dietary arginine, reduced intestinal synthesis of citrulline, inherited deficiencies of arginine-synthetic enzymes, impaired intestinal transport of arginine, overexpression of intestinal arginase gene, and/or impaired conversion of citrulline into arginine in kidneys (1,6). Arginine deficiency causes growth retardation, intestinal and reproductive dysfunction, impaired immune and neurological development, cardiovascular and pulmonary abnormalities, impaired wound healing, hyperammonemia, and even death in animals (7–10). Because of the crucial metabolic roles of arginine, there was growing interest in its biochemistry, nutrition, and physiology in recent years (6,8). The major objective of this article is to review current knowledge about arginine nutrition in the neonatal pig, which has enormous agricultural importance worldwide (11) and is also an established animal model for studying human infant metabolism (9).

### *Submaximal growth of sow-reared piglets*

Although sow's milk was traditionally thought to provide adequate amino acids for supporting piglets' growth, recent

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[4] Abbreviations used: CPS-I, carbamoylphosphate synthase-I; NAG, N-acetylglutamate; NCG, N-carbamoylglutamate; NO, nitric oxide; P5C, pyrroline-5-carboxylate.

studies identified submaximal growth of sow-reared piglets (12,13). For example, artificial rearing data show that the biological potential for neonatal pig growth is ≥400 g/d (average from birth to 21 d of age), or ≥74% greater than that for sow-reared piglets (230 g/d) (13). Interestingly, suckling piglets exhibit submaximal growth from d 8 after birth (13). The metabolic basis for the submaximal growth of sow-reared piglets is unknown, but it may be due to inadequate intake of protein (or an essential amino acid) and/or energy. Because preweaning growth is a major determinant of neonatal survival and postweaning growth performance in pigs (11), improving the growth of suckling piglets will greatly enhance the efficiency of pork production.

### *Arginine deficiency in sow's milk and the crucial role of endogenous arginine synthesis in maintaining its homeostasis*

We and others demonstrated that arginine is deficient in sow's milk on the basis of *1*) the amino acid patterns in the milk and piglets; and *2*) the arginine supply from sow's milk vs. the estimated arginine requirement of piglets for growth and metabolic function (14–16). For example, our analysis of amino acids, using established HPLC methods (4,15), showed that the ratio of arginine/lysine on a gram basis was 0.35 ± 0.02 and 0.97 ± 0.05 (means ± SEM, $n = 10$) in sow's whole milk (d 7 of lactation) and 7-d-old pigs, respectively. This data suggests that a substantial amount of arginine is synthesized by the neonate to compensate for low levels of arginine in milk. Quantitatively, the relative contribution of milk vs. endogenous synthesis to meet arginine requirements by the suckling neonate can be estimated on the basis of arginine intake plus arginine accretion and catabolism in the body (17–20). Our calculations indicate that sow's milk provides ≤40% of arginine requirements by the 1-wk-old pig (**Table 1**). Thus, endogenous synthesis of arginine must play a crucial role in maintaining arginine homeostasis in sow-reared piglets (21), as reported for human infants (22) and neonatal mice (23). This conclusion is further supported by our finding that an inhibition of intestinal conversion of ornithine into pyrroline-5-carboxylate (P5C) for 12 h reduces plasma levels of ornithine, citrulline, and arginine by 59, 52, and 76%, respectively, in 4-d-old sow-reared pigs (21).

**TABLE 1**

*Relative contributions of milk vs. endogenous synthesis of arginine to arginine requirements by the 7-d-old (2.5 kg) sow-reared pig*

| | Amounts of arginine |
|---|---|
| | *g/d* |
| Arginine provision from sow's milk | ≤1.01 |
| Milk intake (0.78 *L/d;* 1.43 *g/L* of whole milk)[1] | 1.12 |
| Undigestible arginine in sow's milk (9.6%)[2] | 0.11 |
| Arginine requirements for growth and metabolic function | ≥2.7 |
| Body weight gain (200 *g/d;* 27.2 *g* progein)[3] | 1.88 |
| Arginine catabolism via arginase and NO synthase[4] | 0.65 |
| Arginine utilization for creatine synthesis[5] | 0.17 |
| Arginine provision from endogenous synthesis | ≥1.69 |

[1] Wu and Knabe (15).
[2] Mavromichalis et al. (17).
[3] Wu et al. (18).
[4] Murphy et al. (19).
[5] Calculated on the basis of urinary excretion of creatinine [0.38 mmol/(kg body wt · d)] (20).

### *Pathways of the endogenous arginine synthesis in neonatal pigs*

Although arginine is formed in the liver via the urea cycle, there is no net synthesis of arginine by this organ due to an exceedingly high activity of cytosolic arginase that rapidly hydrolyzes arginine (24). Studies over the past 30 y established that the citrulline released by absorptive epithelial cells (enterocytes) of the small intestine is almost the exclusive source of endogenous arginine in mammals (5,25,26). The kidneys are the major site for the conversion of intestine-derived citrulline into arginine via argininosuccinate synthase and lyase in adult animals, but this synthetic pathway occurs virtually in all cell types during the life cycle (5). Our published work showed that glutamine/glutamate and proline (abundant amino acids in sow's milk) are the precursors for citrulline and arginine synthesis in pig enterocytes (26,27). P5C is the common intermediate in the pathways of arginine synthesis from glutamine/glutamate and proline (**Fig. 1**). Both enzymological and metabolic evidence established that P5C synthase and N-acetylglutamate (NAG) synthase are 2 key regulatory enzymes in the intestinal synthesis of citrulline (25,28,29).

We found that in 1- to 7-d-old pigs most of the citrulline synthesized in enterocytes is converted locally to arginine because of high activities of argininosuccinate synthase and lyase (16,27). However, in older piglets (14- to 21-d old), enterocytes release most of the synthesized citrulline owing to limited conversion of citrulline into arginine because of a low argininosuccinate synthase activity (27). Thus, the small intestine shifts from the major site of net arginine synthesis in 1-wk-old pigs to the major site of net citrulline production in 2- to 3-wk-old pigs. In both newborn and suckling piglets, there is net synthesis of arginine by enterocytes because of the near absence of intestinal arginase (28). Citrulline released by the small intestine is not taken up by the liver and is used for arginine synthesis by extrahepatic cells and tissues (primarily the kidneys) (5,30). Likewise, the uptake of physiological concentrations of arginine by the liver is limited due to a low activity of the amino acid transport system $y^+$ in hepatocytes (31). Therefore, intestine-derived citrulline or arginine is equally effective as a source of arginine for the whole body.

### *Progressive decline in intestinal synthesis of citrulline and arginine in sow-reared piglets*

We were puzzled by one of our previous findings that intestinal synthesis of citrulline and arginine from glutamine and glutamate decreases by 70–73% in 7-d-old suckling pigs in comparison with newborn pigs and declines further in 14- to 21-d-old pigs (16,27) (**Fig. 2**). Similarly, rates of citrulline and arginine synthesis from proline in enterocytes are 75–88% lower in 7-d-old pigs, when compared with newborn piglets and remain at reduced levels in 14- to 21-d-old pigs (27). Because the ratios of small-intestinal weight (or mucosal protein weight) to body weight do not change substantially in newborn and suckling piglets (28–32 g small intestine per kg body wt from 1 to 21 d of age), intestinal synthesis of citrulline and arginine per kg body wt is also strikingly low in 7- to 21-d-old piglets compared with 1- to 3-d-old piglets (10). The metabolic basis for the progressive decrease in citrulline and arginine synthesis by enterocytes of 7- to 21-d-old pigs was not

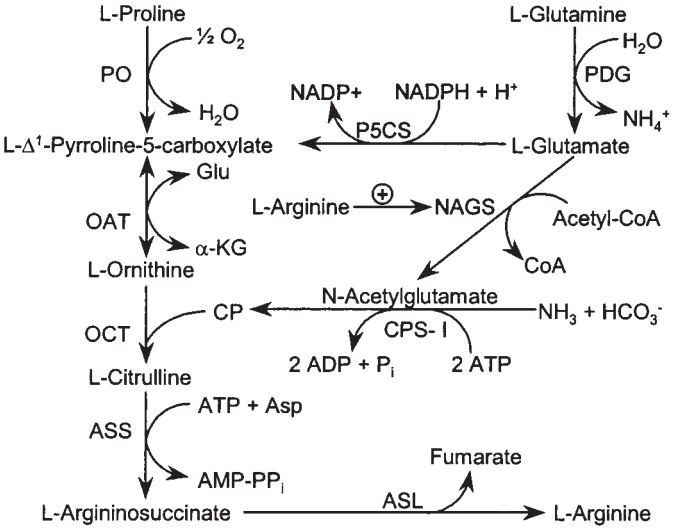


**FIGURE 1** Pathways of intestinal citrulline and arginine synthesis from glutamine and proline in pigs. ASL, argininosuccinate lyase (EC 4.3.2.1); Asp, aspartate; ASS, argininosuccinate synthase (EC 6.3.4.5); CP, carbamoylphosphate; CPS-I (EC 6.3.4.16); Glu, glutamate; α-KG, α-ketoglutarate; NAGS (EC 2.3.1.1); OAT, ornithine aminotransferase (EC 2.6.1.13); OCT, ornithine carbamoyltransferase (2.1.3.3); PDG, phosphate-dependent glutaminase (EC 3.5.1.2); P5CS (EC number not assigned); and PO, proline oxidase (EC number not assigned). All enzymes, except for cytosolic ASS and ASL, are located in mitochondria. Arginine and N-acetylglutamate are allosteric activators of NAGS and CPS-I, respectively.

previously identified but may result from decreases in cellular activities of both P5C synthase and NAG synthase. This intriguing finding also raised an important question of whether arginine is insufficient for supporting the metabolic needs of 7- to 21-d-old sow-reared piglets (16,21).

### *Arginine deficiency in sow-reared piglets*

Because of the practical limitation in conducting a nitrogen-balance study with sow-reared piglets, we decided to measure plasma biochemical variables (e.g., arginine, ammonia, and nitrite plus nitrate, stable oxidation products of NO) as indicators of arginine status in piglets (33). The rationale was as follows: *1*) arginine plays a crucial role in ammonia detoxification via the hepatic urea cycle and is the physiological substrate for NO synthesis (30); *2*) a decrease in plasma arginine concentrations and an increase in plasma ammonia levels are sensitive indicators of arginine deficiency in neonates, including piglets (2,9); and *3*) plasma concentrations of nitrite plus nitrate reflect the availability of arginine for supporting systemic NO synthesis in neonates (34).

Our results showed that plasma levels of most amino acids did not change in piglets during the suckling period (33), suggesting that mechanisms exist for maintaining their homeostasis. However, plasma concentrations of arginine and its immediate precursors (ornithine and citrulline) decreased progressively by 20–41%, with increasing postnatal age from 3 to 14 d (33). Intriguingly, a substantial decrease in arginine availability occurs around the time (d 8 of life) (33) when sow-reared piglets begin to exhibit submaximal growth (13). In addition, plasma concentrations of ammonia increased progressively by 18–46%, whereas those of nitrite plus nitrate decreased by 16–29%, in 7- to 14-d-old suckling pigs, compared with 1- to 3-d-old pigs. These metabolic data on impaired hepatic ureagenesis and reduced systemic NO synthesis suggest a hitherto unrecognized deficiency of arginine in 7- to 21-d-old sow-reared pigs. Although piglets continue to grow during the 21-d suckling period, this does not necessarily mean that arginine supply from milk plus endogenous synthesis meets arginine requirements for their maximal growth (a major goal of animal production) and optimal metabolic function, as exemplified by submaximal growth and impaired NO synthesis in arginine-deficient young rats (35).

### *Arginine deficiency as a growth-limiting factor in young pigs*

As noted above, arginine content is relatively low in sow's milk (14,15), as reported for the milk of humans and cows (14). There is experimental evidence supporting the notion that a reduced availability of arginine may limit the maximal growth of young pigs. For example, Leibholz (36) reported that in piglets weaned at 3 to 4 d of age supplementing 0.2 and 0.4% arginine to a dried milk diet containing 19.2% crude protein and 0.75% arginine (similar to the protein and arginine content in sow's milk) numerically increased weight gain by 43 and 93%, respectively, during d 7 to 14 of life (statistical analysis was not performed). Most recently, we found that dietary supplementation of 0.2 and 0.4% arginine (as 0.242 and 0.484% arginine-HCl) to 7- to 21-d-old pigs (artificially reared on a milk feeding system) dose dependently enhanced plasma arginine concentration (30 and 61%), reduced plasma ammonia level (20 and 35%), and increased weight gain (28 and 66%) (37). Dietary supplementation of 0.2 and 0.4% arginine resulted in elevated plasma levels of ornithine and citrulline but had no effect on plasma levels of lysine and histidine (37), suggesting the absence of amino acid antagonism under the experimental conditions.

Food intake was similar between the control and the arginine-supplemented piglets reared on the milk feeding system (37). We propose that dietary arginine supplementation enhanced the growth performance of milk-fed piglets through the following mechanisms: *1*) augmenting arginine availability for tissue protein synthesis; *2*) stimulating the arginine-dependent production of NO (a major vasodilator, a key regulator of immune response, and a versatile signaling molecule) by endothelial and endocrine cells as well as other cell types; *3*) promoting the NO-mediated secretion of insulin and somatotropin (anabolic hormones); *4*) improving tissue insulin

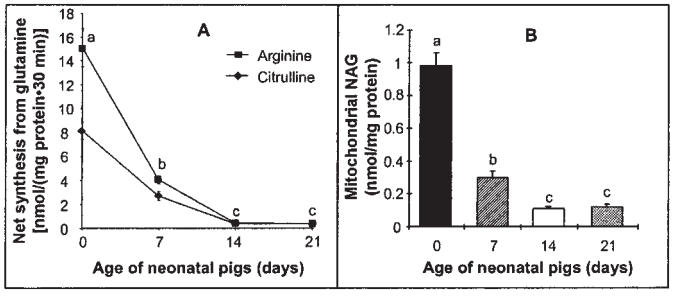


**FIGURE 2** Net synthesis of citrulline and arginine from glutamine (*panel A*) and postnatal changes in mitochondrial N-acetylglutamate (NAG) concentration (*panel B*) in jejunal enterocytes of sow-reared piglets. Data are means ± SEM, $n = 6$. Data for citrulline and arginine synthesis are adapted from Wu (27). NAG was measured using HPLC, as previously described (32). a–c: For both arginine and citrulline synthesis values and for NAG values, means sharing different letters are different ($P < 0.01$), as analyzed by one-way ANOVA (SAS Institute).

sensitivity; and 5) increasing polyamine synthesis in various tissues. Our finding that dietary supplementation of 0.2% arginine had no effect on plasma levels of insulin and somatotropin, and yet increased piglet weight gain (37) suggests that the growth-promoting effect of arginine did not result solely from an increase in the circulating levels of these 2 hormones. Collectively, our results indicate that *1*) the supply of arginine from the milk-based diet and endogenous synthesis is inadequate for supporting the maximal growth of 7- to 21-d-old pigs, *2*) increasing the arginine provision has a great potential to enhance neonatal pig growth, and *3*) an ideal dietary ratio of arginine/lysine is ≥55:100 to maximize weight gain in piglets <21 d of age. Thus, both metabolic and growth data provide direct, compelling evidence to substantiate an arginine deficiency in milk-fed young pigs.

### *Needs for metabolic activation of arginine synthesis in piglets*

Although dietary arginine supplementation to artificially reared piglets was shown to improve growth performance (36,37), it may not be cost effective or practical for family farms or corporate producers at present. This is because weaning at such an early age (d 4 or 7) is labor and resource intensive, and requires highly specialized and expensive facilities as well as costly diets and arginine. Thus, piglets are normally nursed by sows until 14 to 21 d of age in the current U.S. swine industry (11). In view of a reduced arginine supply from endogenous synthesis in suckling piglets and the great potential of arginine to enhance neonatal pig growth, it is of crucial importance to identify an effective means for improving arginine nutrition in preweaning piglets.

After several years' studies, we now recognize that there are practical difficulties in improving arginine provision in sow-reared piglets through either dietary arginine supplementation to sows or oral administration of arginine and citrulline to the neonates. This is primarily because of extensive arginine catabolism by the lactating mammary gland (38) and nutritional antagonism among basic and/or structurally related amino acids (31). For example, we found that supplementing 0.4% arginine (as 0.48% arginine-HCl) to the sow's conventional diet, containing 0.81% arginine (15) ($n = 6$ sows), between d 1 and 21 of lactation had no effect ($P > 0.05$) on total arginine content in milk, compared with isonitrogenous supplementation of 0.82% alanine (e.g., 1.45 ± 0.17 vs. 1.53 ± 0.19 g/L of whole milk; means ± SEM, $n = 6$ sows). Oral administration of arginine to sow-reared piglets (145 mg/kg body wt) 2 times/d enhanced plasma concentrations of arginine but reduced those of lysine and histidine (essential amino acids) compared with alanine administration (**Table 2**). Plasma concentrations of arginine were higher in sow-reared piglets receiving oral administration of citrulline twice daily than in isonitrogenous-control pigs (**Table 3**). This result supports the view that the sow-reared piglet has a high capacity for synthesizing arginine from citrulline (16) and that arginine deficiency in 7- to 21-d-old sow-reared piglets results from a limited supply of citrulline from the small intestine. An adverse effect of the citrulline administration at the chosen dose (121 mg/kg body wt) was a significant reduction in plasma concentrations of tryptophan, histidine, and lysine (Table 3), suggesting impaired intestinal absorption of these amino acids. Consistent with this notion, we found that in vitro transport of 0.5 mmol/L tryptophan, histidine, and lysine by incubated porcine enterocytes was 35, 28, and 32% lower, respectively, in the presence of 5 mmol/L citrulline, compared with its absence ($n = 6$). Thus, oral administration of relatively large doses of arginine or citrulline twice daily is unlikely to be beneficial for improving amino acid nutrition in sow-reared piglets. Interestingly, oral arginine administration at the chosen daily dose 6 times/d had no effect on plasma concentrations of arginine and, instead, enhanced plasma levels of cortisol (an indicator of stress) (Table 2). Cortisol is a potent

**TABLE 2**

*Enterocyte arginase activity as well as plasma levels of cortisol and amino acids in 14-d-old sow-reared piglets receiving oral administration of water, alanine, or arginine*[1]

| Treatment | Frequency of oral dosing | Enterocyte arginase activity[2] | Plasma cortisol | Plasma amino acids, *μmol/L* | | |
|---|---|---|---|---|---|---|
| | | | | Arginine | Lysine | Histidine |
| | *times/d* | | *nmol/L* | | | |
| Experiment 1 | | | | | | |
| None | 0 | 0.22 ± 0.03[a] | 75 ± 8.2[a] | 147 ± 10[b] | 241 ± 17[a] | 113 ± 9.7[a] |
| Water | 2 | 0.24 ± 0.04[a] | 78 ± 9.1[a] | 149 ± 12[b] | 232 ± 14[a] | 118 ± 13[a] |
| Alanine | 2 | 0.28 ± 0.03[a] | 86 ± 12[a] | 145 ± 8.2[b] | 237 ± 12[a] | 108 ± 7.3[a] |
| Arginine | 2 | 0.30 ± 0.04[a] | 83 ± 10[a] | 265 ± 14[a] | 170 ± 10[b] | 74 ± 5.5[b] |
| Experiment 2 | | | | | | |
| None | 0 | 0.26 ± 0.04[b] | 72 ± 7.4[b] | 152 ± 9.3[a] | 239 ± 13[a] | 106 ± 8.2[a] |
| Water | 6 | 2.83 ± 0.35[a] | 416 ± 46[a] | 114 ± 7.7[b] | 225 ± 12[a] | 114 ± 9.5[a] |
| Alanine | 6 | 2.57 ± 0.17[a] | 421 ± 53[a] | 116 ± 7.2[b] | 234 ± 11[a] | 101 ± 8.6[a] |
| Arginine | 6 | 2.94 ± 0.21[a] | 437 ± 58[a] | 122 ± 7.8[b] | 229 ± 14[a] | 97 ± 8.2[a] |

[1] Data are means ± SEM, $n = 10$. At 4 d of age, pigs received oral administration of arginine-HCl (145 mg/kg body wt), alanine (245.5 mg/kg body wt; isonitrogenous control), or water every 12 h (2 times/d) (Experiment 1), or of arginine-HCl (48.3 mg/kg body wt), alanine (81.8 mg/kg body wt), or water every 4 h (6 times/d) (Experiment 2). The amount of arginine (provided as arginine-HCl) represents 53% of estimated daily arginine intake by the piglet from milk (18). Some pigs did not receive oral administration of any solution and were not handled (the None group). At 14 d of age, 2 h after oral administration of water, alanine or arginine, jugular venous blood samples (3 mL) were obtained for determining plasma amino acids and cortisol, as previously described (5,8). Arginase activity was measured as described by Wu et al. (16). Data were analyzed by two-way ANOVA and the Student-Newman-Keul multiple comparison test (SAS, Cary, NC). a–c: Means without a common superscript letter within a column of each experiment differ ($P < 0.01$).

[2] Values are nmol/(mg protein · min).

**TABLE 3**

*Plasma concentrations of amino acids in 14-d-old sow-reared piglets receiving oral administration of alanine or citrulline*[1]

| Treatment | Plasma amino acid concentrations | | | | | |
|---|---|---|---|---|---|---|
| | Citrulline | Arginine | Tryptophan | Histidine | Lysine | Glutamine |
| | | | *μmol/L* | | | |
| Alanine | 76 ± 5.2 | 138 ± 10 | 45 ± 2.1 | 103 ± 6.6 | 237 ± 14 | 516 ± 19 |
| Citrulline | 148 ± 10* | 260 ± 18* | 36 ± 1.6* | 88 ± 5.0* | 194 ± 10* | 504 ± 23 |

[1] Data are means = SEM, $n = 10$. At 4 d of age, pigs received oral administration of citrulline (121 mg/kg body wt) or alanine (185 mg/kg body wt; isonitrogenous control) every 12 h (twice daily). At 14 d of age, 2 h after oral administration of alanine or citrulline, jugular venous blood samples (3 mL) were obtained for determining plasma amino acids, as previously described (5). Data were analyzed by unpaired *t* test (SAS Institute). * $P < 0.01$ vs. the alanine group.

inducer of arginase expression in enterocytes of 7- to 21-d-old pigs (39). Thus, plasma cortisol level and intestinal arginase activity were higher in sow-reared piglets receiving oral administration of water alone 6 times/d than in the control (unstressed) pigs that were not handled or gavaged (Table 2). This resulted in a lower level of plasma arginine in the stressed piglets (Table 2). Our finding may be explained by the behavioral nature of neonatal pigs, which exhibit significant stress in response to frequent handling and gavaging.

In view of the foregoing results, metabolic activation of intestinal citrulline and arginine synthesis may be an attractive approach to augment arginine provision in sow-reared piglets. However, only by elucidating the mechanism responsible for the marked decline in intestinal synthesis of citrulline and arginine (the major source of endogenous arginine) in sow-reared piglets, can we design a scientifically sound means to enhance arginine availability in neonates. This consideration prompted us to address the question of why intestinal citrulline synthesis is low in 7- to 21-d-old pigs, compared with newborn pigs.

### *A possible role for NAG in regulating intestinal synthesis of citrulline and arginine in neonatal pigs*

NAG synthase, which catalyzes the synthesis of NAG from glutamate and acetyl-CoA, is restricted to mitochondria of the liver and intestinal mucosa (30,40). NAG is an allosteric activator of carbamoylphosphate synthase-I (CPS-I) (5), which synthesizes mitochondrial carbamoyl-phosphate necessary for the conversion of ornithine into citrulline (Fig. 1).

Two lines of evidence from our work suggest that NAG may play an important role in regulating intestinal synthesis of citrulline and arginine in pigs. First, although ornithine aminotransferase and ornithine carbamoyltransferase are abundant in pig enterocytes, only ~35% of proline-derived P5C is converted into citrulline in enterocytes of 14-d-old pigs (41). This suggests a low concentration of mitochondrial carbamoylphosphate in enterocytes of 2- to 3-wk-old pigs. Second, mitochondrial NAG concentration [measured as described by Bush et al. (32)] was decreased progressively in enterocytes of 7- to 14-d-old pigs, compared with newborn pigs, as was intestinal synthesis of citrulline and arginine (Fig. 2). Further, we found that a reduced availability of mitochondrial NAG resulted from a marked postnatal decline ($P < 0.01$) in enterocyte NAG synthase activity [measured as previously described (29)], which was 229 ± 36, 72 ± 8.5, 26 ± 3.9, and 28 ± 3.3 pmol/(mg protein·min) (means ± SEM, $n = 5$) for 0-, 7-, 14-, and 21-d-old pigs, respectively. Notably, Yamada and Wakabayashi (29) reported a profound decrease in NAG synthase activity in the small intestinal mucosa of 3-d-old rats, compared with newborn rats. Although the amounts of intestinal CPS-I protein are similar between 2- to 21-d-old pigs on the basis of the enzyme activity measured under in vitro optimal conditions, including an optimal NAG concentration (42), a low level of mitochondrial NAG may limit in vivo intestinal citrulline and arginine synthesis from both glutamine and proline in suckling piglets.

P5C synthase is another key regulatory enzyme in intestinal citrulline synthesis. To determine whether P5C synthase may be activated by NAG, we conducted a study to measure P5C synthase activity in enterocytes from 14-d-old suckling pigs in the presence of 0 or 0.1 mmol/L NAG, by using our established method (16). Our results showed that 0.1 mmol/L NAG increased P5C synthase activity by 124 ± 11% (means ± SEM, $n = 5$). Thus, NAG is a novel activator of P5C synthase in enterocytes. Through modulating P5C synthase and CPS-I activities, mitochondrial NAG levels may play a crucial role in regulating in vivo intestinal synthesis of citrulline and arginine. If this hypothesis is correct, increasing mitochondrial NAG availability may stimulate intestinal synthesis of citrulline and arginine, thereby enhancing endogenous arginine provision.

### *Effects of N-carbamoylglutamate on citrulline and arginine synthesis in incubated enterocytes*

The cytosol of mammalian cells, including enterocytes, contains a high deacylase activity to catabolize NAG (43), thus limiting the use of extracellular NAG to increase mitochondrial NAG concentrations. NCG [an analogue of NAG

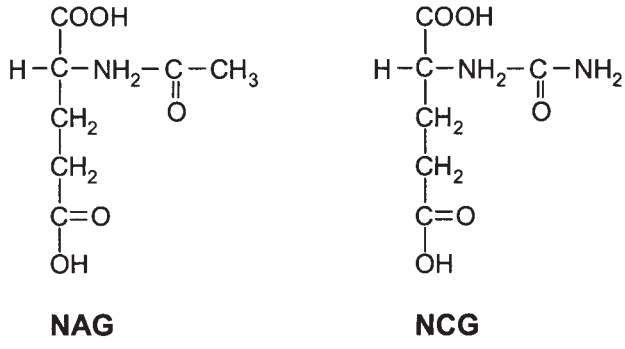


**FIGURE 3** Structures of NCG and NAG. NCG is not degraded by cellular deacylase or amino acid-metabolic enzymes. Therefore, NCG is a metabolically stable analogue of NAG.

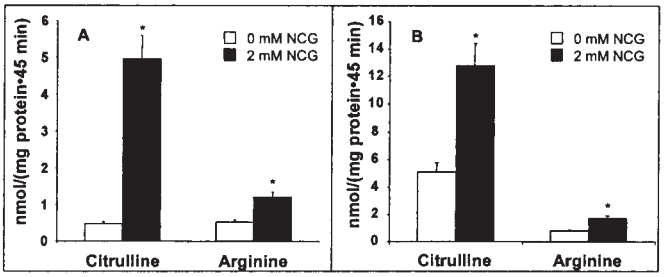


**FIGURE 4** Effect of N-carbamoylglutamate (NCG) on citrulline and arginine synthesis from glutamine (*panel A*) and proline (*panel B*) in enterocytes of 14-d-old sow-reared pigs. Data are means ± SEM, $n = 5$. Jejunal enterocytes were incubated at 37°C for 45 min in Krebs bicarbonate buffer (pH 7.4) containing 2 mmol/L [U-$^{14}$C]glutamine or 2 mmol/L glutamine plus 2 mmol/L [U-$^{14}$C]proline (27) and 0 or 2 mmol/L NCG. Rates of citrulline and arginine synthesis were measured as described by Wu (27). *$P < 0.01$ vs. control (no NCG), as analyzed by paired *t* test (SAS Institute).

(**Fig. 3**)] is not a substrate for deacylase and therefore is a metabolically stable activator of CPS-I (43). Importantly, NCG readily enters cells and mitochondria to exert its effect (43,44). Indeed, the activation of hepatic citrulline synthesis was initially discovered with NCG (45). Additionally, NCG is not toxic to animals on the basis of studies with infants (46) and rats (47) and was used to prevent hyperammonemia, through increasing hepatic urea cycle activity, in human infants with an inherited deficiency of NAG synthase (46). However, an important role for NCG in activating intestinal or endogenous synthesis of citrulline and arginine was not previously recognized. NCG has no other cellular action than serving as an activator of CPS-I (43,44) and intestinal P5C synthase (the present study). Thus, the effect of NCG on intestinal citrulline and arginine synthesis may result from an increase in the availability of both carbamoylphosphate and ornithine (Fig. 1). Further, NCG is not an antibiotic, because it did not kill microbes isolated from the small and large intestines of 7- to 21-d-old pigs (G. Wu, Department of Animal Science and Faculty of Nutrition, Texas A&M University, unpublished results).

On the basis of the reduced concentration of mitochondrial NAG in enterocytes of 7 to 21-d-old pigs (Fig. 2), we hypothesized that metabolic activation of endogenous arginine synthesis may provide a new, effective strategy to augment arginine provision in sow-reared piglets and that this strategy may help overcome the practical limitation of arginine delivery to the neonates. As an initial step to test this hypothesis, we determined the effect of NCG on intestinal citrulline and arginine synthesis in enterocytes from 14-d-old pigs. Consistent with the activation of CPS-I and P5C synthase, the addition of 2 mmol/L NCG stimulated citrulline production from glutamine and proline by 8.7- and 1.6-fold, respectively (**Fig. 4**). NCG also markedly enhanced arginine production from both glutamine and proline by pig enterocytes (Fig. 4). These results are the first to demonstrate the feasibility of enhancing intestinal citrulline and arginine synthesis through the metabolic activation of P5C synthase and CPS-I.

### *Effects of oral NCG administration on plasma arginine concentrations and growth of sow-reared piglets*

Results of our in vitro study are exciting and therefore prompted us to conduct an in vivo study to determine whether NCG can increase plasma concentration of arginine and thus the growth of sow-reared piglets. Four-d-old sow-reared piglets received oral administration of 0 or 50 mg NCG per kg body wt every 12 h (2 times/d) until 14 d of age. This dose of NCG was chosen on the basis of *1*) the previous studies with human infants (46), and *2*) our preliminary finding that oral administration of NCG at 50 to 100 mg/kg body wt twice daily maximally enhanced plasma arginine concentration in 14-d-old sow-reared pigs, compared with 10 and 25 mg NCG/kg body wt.

Concentrations of ammonia or glutamine in the jejunal lumen did not differ ($P > 0.05$) between control and NCG-treated piglets (e.g., 0.21 ± 0.03 vs. 0.23 ± 0.02 mmol/L for ammonia; 2.21 ± 0.28 vs. 2.09 ± 0.34 mmol/L for glutamine; means ± SEM, $n = 5$). This result suggests that NCG did not affect the availability of intestinal ammonia or other nitrogenous precursors needed by gut microbes. However, oral administration of NCG enhanced plasma concentrations of arginine by 68% and prevented the marked postnatal decline in plasma levels of arginine in 14-d-old pigs (**Table 4**), indicating that NCG activates in vivo synthesis of citrulline and arginine by the small intestine. Importantly, NCG treatment had no effect on plasma levels of tryptophan, lysine, or histidine in piglets (data not shown), suggesting that NCG did not affect intestinal absorption of these amino acids. In support of this view, we found that rates of transport of 0.5 mmol/L tryptophan, histidine, or lysine by porcine enterocytes did not differ ($P > 0.05$) between the presence of 0 and 1 mmol/L NCG ($n = 5$).

Using the weigh–suckle–weigh technique (48), we found that milk consumption was not different ($P > 0.05$) between 14-d-old control and NCG-treated piglets [51.6 ± 6.2 vs. 52.3 ± 6.4 g dry matter/(kg body wt·d); means ± SEM, $n = 5$].

## TABLE 4

*Effects of oral NCG administration on plasma arginine concentrations and growth of 4- to 14-d-old sow-reared pigs*[1]

| | Plasma arginine | | Body weight | | |
|---|---|---|---|---|---|
| Piglets | d 4 | d 14 | d 4 | d 14 | Weight gain from d 4 to 14 |
| | *μmol/L* | | *kg* | | |
| Control | 206 ± 8.0 | 142 ± 9.2 | 2.01 ± 0.09 | 4.13 ± 0.19 | 2.12 ± 0.10 |
| NCG | 202 ± 9.1 | 239 ± 11* | 2.05 ± 0.27 | 5.46 ± 0.26* | 3.41 ± 0.15* |

[1] Data are means ± SEM, $n = 10$. Four-d-old sow-reared piglets received oral administration of 0 or 50 mg NCG (dissolved in water) per kg body wt every 12 h (2 times/d at 0730 and 1930). At d 4 and 14 of age, 2 h after suckling and oral NCG administration, respectively, jugular venous blood samples (3 mL) were obtained from pigs for determining plasma amino acids (16); body weights were measured. Data were analyzed by unpaired *t* test (SAS Institute). * $P < 0.01$ vs. the control group.

Remarkably, NCG treatment increased ($P < 0.01$) piglet weight gain by 61% from d 4 to 14 after birth (Table 4). To determine the components of weight gain, we quantified body composition in 14-d-old control and NCG-treated piglets (10 d after initiating NCG treatment), using standard methods (4). Our results showed that the content (%) of water (69.5 ± 0.85 vs. 69.7 ± 0.89), dry matter (30.5 ± 0.85 vs. 30.3 ± 0.89), protein (14.9 ± 0.53 vs. 15.3 ± 0.56), fat (12.1 ± 0.97 vs. 11.6 ± 1.04), and minerals (3.0 ± 0.12 vs. 2.9 ± 0.13) (means ± SEM, $n = 5$) did not differ ($P > 0.05$) between control and NCG-treated pigs. Thus, the weight gain in NCG-treated piglets resulted in part from a greater absolute amount of tissue protein accretion, rather than from a disproportionate change in fat deposition. Our novel findings further support the conclusion that arginine is a major factor limiting the maximal growth of milk-fed young pigs.

NCG offers unique and important advantages over oral administration of arginine or citrulline to sow-reared piglets. First, oral administration of NCG does not affect intestinal absorption of dietary tryptophan, histidine, or lysine in pigs. Second, because of constant activation of intestinal synthesis of citrulline and arginine, oral administration of NCG ensures a balanced supply of arginine to piglets relative to the supply of other basic amino acids from sow's milk during the suckling period. This is particularly important for sow-reared piglets, which suckle about every 1 to 1.5 h. Third, as a metabolic activator of P5C synthase and CPS-I, a low dose of NCG is highly effective in increasing endogenous provision of arginine in piglets. Fourth, NCG is not catabolized by cellular deacylase or amino acid-metabolic enzymes, and thus its in vivo half-life in the intestinal mucosa is expected to be relatively long (perhaps ≥8–10 h). This helps explain our finding that oral NCG administration to piglets twice daily effectively increased in vivo arginine provision (Table 4). Fifth, because NCG can be readily synthesized chemically from glutamate and potassium cyanate in the presence of potassium hydroxide and room temperature (45), its costs can be substantially reduced if large amounts are produced commercially for pork producers. Collectively, our studies demonstrate that NCG is a novel, effective, and low-cost, growth-promoting agent for sow-reared piglets. Thus, oral administration of NCG provides a new, useful tool to inhibit the marked decline in intestinal citrulline synthesis in sow-reared piglets, thereby improving arginine nutrition and enhancing the efficiency of utilization of dietary amino acids for tissue protein accretion. Future studies are necessary to develop more practical and simple approaches (e.g., delivery of an exogenous mammalian NAG synthase gene into enterocytes) than oral administration of NCG to metabolically activate arginine synthesis in sow-reared piglets.

In conclusion, our results indicate that arginine deficiency, owing to its reduced intestinal synthesis, is a major metabolic basis for the submaximal growth of sow-reared piglets and that increasing arginine provision has a great potential to promote the growth of the neonates. Because of a reduced availability of mitochondrial NAG in enterocytes of 7 to 21-d-old sow-reared pigs, the metabolic activation of intestinal citrulline and arginine synthesis with NCG provides a new, effective strategy to augment arginine provision in sow-reared piglets. Our findings not only generate new fundamental knowledge about amino acid utilization by neonatal pigs but also have important practical implications for improving the efficiency of pork production.

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