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Creatine Synthesis Is a Major Metabolic Process in Neonatal Piglets and Has Important Implications for Amino Acid Metabolism and Methyl Balance[1,2]

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Abstract

Our objectives in this study were as follows: *1*) to determine the rate of creatine accretion by the neonatal piglet; *2*) identify the sources of this creatine; *3*) measure the activities of the enzymes of creatine synthesis; and *4*) to estimate the burden that endogenous creatine synthesis places on the metabolism of the 3 amino acids required for this synthesis: glycine, arginine, and methionine. We found that piglets acquire 12.5 mmol of total creatine (creatine plus creatine phosphate) between 4 and 11 d of age. As much as one-quarter of creatine accretion in neonatal piglets may be provided by sow milk and three-quarters by de novo synthesis by piglets. This rate of creatine synthesis makes very large demands on arginine and methionine metabolism, although the magnitude of the demand depends on the rate of remethylation of homocysteine and of reamidination of ornithine. Of the 2 enzymes of creatine synthesis, we found high activity of L-arginine:glycine amidinotransferase in piglet kidneys and pancreas and of guanidinoacetate methyltransferase in piglet livers. Piglet livers also had appreciable activities of methionine adenosyltransferase, which synthesizes *S*-adenosylmethionine, and of betaine:homocysteine methyltransferase, methionine synthase, and methylene tetrahydrofolate reductase, which are required for the remethylation of homocysteine to methionine. Creatine synthesis is a quantitatively major metabolic process in piglets. J. Nutr. 139: 1292–1297, 2009.

Introduction

Creatine, together with creatine phosphate, serves as an important energy buffer in vertebrates. In addition, because of the intracellular distribution of the different creatine kinase isoforms, creatine and creatine phosphate serve as an energy shuttle whereby high-energy phosphates can be efficiently brought from their mitochondrial sites of production to sites of rapid ATP utilization, such as myofibrils, plasma membrane, etc. The creatine/creatine phosphate system does not occur in all cells; rather, it is confined to cells that have high, but variable, energy demands. More recently, it has become apparent that the mitochondrial isoform of creatine kinase plays a special role in maintaining low ADP concentrations and, therefore, in reducing the production of reactive oxygen species by mitochondria. These concepts have recently been reviewed (1,2).

Many vertebrate cells contain large amounts of creatine and creatine phosphate. About 1.7% of the creatine pool is irreversibly converted to creatinine each day and excreted in the urine (2). Therefore, there is a need for continual replacement of this creatine lost as creatinine. There are 2 routes by which creatine may accrete in animals: diet and de novo synthesis. We have calculated the relative importance of these routes of creatine provision in adult humans. Individuals consuming a typical Western, omnivorous diet will receive approximately one-half of their creatine from their diet and the other one-half must be synthesized (3).

Creatine synthesis is a very simple process involving just 2 enzymes (4). It does, however, require 3 amino acids: arginine, glycine, and methionine. Arginine:glycine amidinotransferase (AGAT)[5] (EC 2.1.4.1) transfers an amidino group from arginine to the amino group of glycine to produce guanidinoacetate (GAA) and ornithine:

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[5] Abbreviations used: AGAT, L-arginine: glycine amidinotransferase; BHMT, betaine:homocysteine methyltransferase; GAA, guanidinoacetate; GAMT, guanidinoacetate methyltransferase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase.

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glycine + arginine →ornithine + GAA.

GAA methyltransferase (GAMT; EC 2.1.1.2) employs *S*-adenosylmethionine (SAM) to methylate GAA, producing creatine and *S*-adenosylhomocysteine (SAH):

GAA + SAM →creatine + SAH.

In addition to these 2 enzymes, there is a Na^+-linked creatine transporter that transports creatine into a variety of tissues against very high creatine concentration gradients (as much as 100 to 1 in skeletal muscle) (*5*).

It is likely that the requirement for creatine is proportionally greater in growing animals than in adults, because, in addition to replacing creatine losses to creatinine, there may be a need to provide creatine to the growing tissues. The importance of neonatal creatine metabolism has been emphasized by the discovery of inborn errors of each of the enzymes of creatine synthesis and of the creatine transporter. In addition to hypotonia, infants with these genetic diseases exhibit a common palette of neurological symptoms, including epilepsy, speech delay, and mental retardation. Studies with ^{1}H-NMR revealed a profound depletion of brain creatine (*6*). In the case of infants suffering from defects in AGAT and GAMT, provision of supplementary creatine invariably replenishes brain creatine (6). Usually, creatine supplementation does not entirely reverse the pathology. However, very early provision of exogenous creatine can prevent the neurological effects (*7*). Despite this crucial role in brain development, very little attention has been given to the importance of adequate provision of creatine to the neonate. We used piglets, which are excellent models for human infants with respect to metabolism, nutritional requirements, and development of the gastrointestinal tract (8). Our objectives were to: *1*) determine the rate of creatine accretion between d 4 and 11 in nursing piglets; *2*) identify the sources of this creatine; *3*) measure the activity and tissue distribution of enzymes of creatine synthesis; and *4*) estimate the burden that creatine synthesis places on the metabolism of the 3 amino acids required for creatine synthesis: arginine, methionine, and glycine.

Materials and Methods

Piglets. For the measurement of creatine and protein accretion, Yorkshire-Duroc piglets were obtained from a commercial farm at ages 3–5 d (identified as 4-d-old piglets) and 10–12 d (identified as 11-d-old piglets). For the measurement of enzyme activities, we used 7- to 8-d-old piglets. Immediately upon arrival in the laboratory (within 2 h of removal from the sow), piglets were placed under general anesthesia using 2% halothane delivered in oxygen (1.5 L/min) by mask. Blood samples were taken by cardiac puncture into heparinized tubes and plasma separated by centrifugation at 3000 × *g*; 15 min at room temperature. Plasma and frozen tissues were stored at −20°C until required for analysis. Tissue samples were then taken from skeletal muscle, liver, pancreas, brain, small intestine, and stomach (after these latter 2 organs had been rinsed free of noncellular debris) and were rapidly frozen in liquid nitrogen. The weights of these organs, except for skeletal muscle, were recorded. The entire carcass (excluding removed tissues) was frozen at −20°C and ground (Butcher Boy AA1100) for carcass analyses of protein and total creatine. Arterial blood samples and milk samples were taken from 6 multiparous lactating sows (Landrace × Yorkshire) at mid-lactation (d 10–18 of lactation) as described by Trottier et al. (9). Blood samples were also collected. Plasma was separated and stored at −20°C. Either the Memorial University's Institutional Animal Care Committee (piglets) or the Michigan State University All University Committee on Animal Use and Care (sows) approved all animal protocols.

Analytical methods. Frozen tissues, including carcass samples, were ground with liquid nitrogen to a fine powder in a mortar. One-gram portions of frozen tissue powder were homogenized in 4 mL of 50 mmol/L potassium phosphate buffer, pH 7.0, and the protein content of this homogenate was measured by the Biuret method using 5% deoxycholate to solubilize lipids and bovine serum albumin as a standard. One-gram aliquots of frozen tissue powder were also homogenized with 4 mL of 6% (wt:v) perchloric acid and placed on ice for 15 min and then the precipitated protein was removed by centrifugation at 10,000 × *g*; 10 min at 4°C. The supernatants were neutralized with 50% K_2CO_3/20% KOH, placed in ice for 30 min, and centrifuged at 10,000 × *g*; 5 min at 4°C to remove precipitated salts. Blood plasma was added to an equal volume of 4% (wt:v) perchloric acid and the proteins precipitated, removed by centrifugation, and the supernatant neutralized as described above. Milk was centrifuged at 100,000 × *g*; 1 h at 4°C to float the milk fat, and samples of the aqueous infranatant were deproteinized and neutralized as described for plasma. These neutralized extracts were employed for creatine analysis. Creatine and creatine phosphate were analyzed enzymatically (10), as adapted for use in 96-well plates. Because most of the tissues could not be frozen sufficiently rapidly to prevent anoxic changes in the ratios of creatine:creatine phosphate, we report these data as the sum of creatine and creatine phosphate, referred to as "total creatine."

Enzyme assays. Enzymes were assayed in tissues from 7- to 8-d-old piglets, i.e. at the midpoint of the creatine accretion study. The GAMT activity was measured on the day of the experiment using unfrozen tissue samples using a modification (11) of a method described by Ogawa et al. (12) in which we used HPLC (13) to quantify the creatine produced. For the assay of AGAT, fresh tissue was homogenized 1:5 (wt:v) in 50 mmol/L potassium phosphate buffer (pH 7.4). These homogenates were stored at −70°C and AGAT activity measured within a week as described (14). For the remaining enzyme assays, frozen tissue was homogenized, 1:5 (wt:v), in 50 mmol/L potassium phosphate buffer (pH 7.0) using a Polytron for 20 s at 50% output. The homogenate was centrifuged at 18,000 × *g*; 30 min at 4°C and the supernatant collected for the analysis of enzyme activity. We assayed methionine adenosyltransferase (MAT) using a method based on Mudd et al. (15), using 5 mmol/L dithiothreitol in place of glutathione and 5 mmol/L L-[methyl-^{14}C]methionine (0.1 μCi). The activities of methionine synthase (MS) (16), betaine:homocysteine methyltransferase (BHMT) (17), and methylenetetrahydrofolate reductase (MTHFR) were measured according to published methods (18). All enzyme assays were demonstrated to be linear with time and protein under the conditions used.

Presentation of data. Data are presented as means ± SD. Differences between the 2 age groups were assessed using a Student's unpaired *t* test. Differences of $P < 0.05$ were considered significant.

Results

Body mass was 50% higher in 11-d-old piglets and their whole-body protein content was twice that of 4-d-old piglets (**Table 1**). The total body creatine content was 70% greater in 11-d-old piglets than in 4-d-old piglets and the circulating creatine concentration also was higher in the 11-d-old piglets (**Table 2**). Noteworthy observations were the high creatine content of muscles (skeletal muscle and heart) and the intermediate content of brain and the markedly greater carcass creatine content in the older piglets, largely representing accumulation by skeletal muscle. We found no expansion of the brain creatine pool between d 4 and 11 of life, consistent with the lack of difference in brain mass between 4- and 11-d-old piglets (**Table 1**). We also measured the creatine concentration of sow milk to allow the calculation of the contribution of milk to total creatine accretion; it was 529 ± 85 μmol/L (n = 6).

We measured the activities and tissue distribution of the key enzymes of creatine synthesis in 7- to 8-d-old piglets, i.e. at the

TABLE 1 Body and organ weights and protein contents in 4- and 11-d-old piglets[1]

	4 d old, $n = 4$			11 d old, $n = 5$		
	Weight	Protein	Total protein	Weight	Protein	Total protein
	g	*mg/g*	*g*	*g*	*mg/g*	*g*
Body weight	1800 ± 200	–	–	2700 ± 700*	–	–
Liver	52 ± 12	171 ± 44	9.2 ± 4.5	73 ± 17	164 ± 10	12.0 ± 3.0
Muscle	–	109 ± 22	–	–	188 ± 24*	–
Kidney	12 ± 2	109 ± 26	1.3 ± 0.2	18 ± 4*	141 ± 10*	2.6 ± 0.7*
Pancreas	2.7 ± 1.0	96 ± 24	0.26 ± 0.12	3.8 ± 1.2	130 ± 33	0.48 ± 0.16*
Stomach	8.9 ± 4.4	105 ± 43	0.96 ± 0.61	13.1 ± 4.3	86 ± 19	1.1 ± 0.50
Intestine	71 ± 20	130 ± 9	9.2 ± 2.9	110 ± 27*	129 ± 14	14 ± 50
Heart	14 ± 2	113 ± 11	1.5 ± 0.1	18 ± 4	150 ± 16*	2.7 ± 0.8*
Brain	35 ± 6	90 ± 9	3.1 ± 0.6	36 ± 3	114 ± 23	4.1 ± 1.0
Carcass	1600 ± 200	130 ± 26	205 ± 59	2500 ± 600*	187 ± 45	436 ± 234*
Total	–	–	231 ± 61	–	–	473 ± 240*

[1] Values are means ± SD. *Different from 4 d old, $P < 0.05$.

midpoint of the 4- to11-d-old period we used to characterize creatine accretion (**Table 3**). Because of the requirement of a methyl group from SAM, creatine synthesis is intimately tied to the methionine cycle. We therefore also assayed the activities of enzymes of this cycle (Table 3). The kidney and the pancreas were the only tissues with high activities of AGAT; the liver had a high GAMT activity and the pancreas an intermediate activity. MAT was widely distributed, but the liver had much higher activity than the other tissues examined. Both MTHFR and MS were widely distributed; in each case, the liver exhibited the highest activity, but both the kidneys and the pancreas had appreciable activity. The liver contained a high activity of BHMT that was also found in the kidney. We did not detect BHMT activity in the other tissues examined.

Discussion

The major findings of this study are that piglets accumulate substantial quantities of creatine and that they have high activities of the enzymes of creatine synthesis and of the methionine cycle. From the distribution of the enzymes of creatine synthesis, we suggest that it is likely that piglets synthesize much of their creatine via the renal-hepatic axis, although the role of the pancreas remains enigmatic.

TABLE 2 Organ total creatine contents and concentrations in 4- and 11-d-old piglets[1]

	4 d old		11 d old	
	μmol/g	*μmol*	*μmol/g*	*μmol*
Muscle	17.6 ± 4.2		19.2 ± 6.1	
Liver	1.3 ± 0.6	60.4 ± 24.1	0.8 ± 0.6	62.9 ± 43.3
Kidney	1.2 ± 0.4	13.8 ± 6.2	1.0 ± 0.3	19.4 ± 6.8
Pancreas	1.1 ± 0.7	3.8 ± 3.5	0.7 ± 0.1	2.7 ± 0.5
Stomach	2.3 ± 0.7	19.9 ± 9.0	2.5 ± 1.2	28.0 ± 11.0
Intestine	2.4 ± 1.0	163 ± 46	2.3 ± 0.9	233 ± 143
Heart	10.4 ± 3.6	140 ± 34	12.2 ± 4.3	220 ± 121
Brain	5.8 ± 0.9	207 ± 62	5.9 ± 1.4	206 ± 73
Carcass	9.0 ± 1.6	14,000 ± 3000	10.7 ± 2.0	24,000 ± 6000*
Total		14,600 ± 3000		24,800 ± 6000*
Plasma, *μmol/L*	140 ± 60		380 ± 70*	

[1] Values are means ± SD. *Different from 4 d old, $P < 0.05$.

Sources of creatine in growing piglets. Because piglets may obtain creatine from their diet as well as by de novo synthesis, it is important to estimate the relative contributions of both of these sources to total creatine accumulation. To do so, we assumed the bioavailability of milk creatine to be 100%. It is likely that it is very high. MacNeill et al. (19) determined a bioavailability of 78% in adult humans. It is also known that the uptake of creatine by small intestine is very much higher in suckling rats than in adults (20). However, a bioavailability for milk creatine $< 100\%$ would mean that the piglet's contribution to creatine synthesis would be somewhat higher than we calculated. The calculations of creatine intake and synthesis are outlined in **Table 4**. Milk consumption of 7-d-old piglets has been reported to be ~0.77 L/d (8). Therefore, the estimated creatine intake per piglet between d 4 and 11 of age is 2.8 mmol. During this period, piglets accumulated a net of 10.2 mmol of total creatine (Table 1). Accounting for an irreversible loss of creatine to creatinine of 2.3 mmol over the 7 d (calculated as 1.7%/d of the mean piglet content of creatine between d 4 and 11), we estimated a creatine acquisition of 12.5 mmol/wk. These calculations revealed 2 important features of neonatal creatine metabolism. First, although milk makes a large contribution to creatine accretion by piglets, the origin of the major portion (77%) of creatine must be de novo synthesis by piglets. Second, the net accumulation of creatine in these growing piglets was >4 times the rate of the spontaneous loss of creatine. This emphasized the increased demand for creatine synthesis due to neonatal growth. When expressed in terms of body mass, the mean rate of creatine synthesis between d 4 and 11 was 25.4 $\mu mol \cdot kg^{-1} \cdot h^{-1}$. Our data do not address the origin of milk creatine, whether synthesized in the mammary gland or derived by it from circulating creatine. However, we found the arterial plasma creatine concentration in lactating sows was 292 ± 57 μmol/L ($n = 6$), so the creatine in milk is approximately twice as concentrated as that in plasma.

Enzymes of creatine synthesis and of the methionine cycle. It is of interest to compare the rate of creatine synthesis (25.4 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) (Table 4) with the measured activities of the enzymes of creatine synthesis, also expressed in terms of piglet mass. We found AGAT activities of 84.4 and 91.6 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, respectively, for the kidney and pancreas, hepatic and pancreatic GAMT activities of 44.7 and 0.5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, respectively, and hepatic and pancreatic MAT

TABLE 3 Activities of enzymes of creatine synthesis and homocysteine remethylation in tissues of 7- to 8-d-old piglets[1]

	Liver	Kidney	Intestine	Brain	Muscle	Pancreas
			nmol· min^{-1}·g^{-1}			
AGAT	ND[2]	211 ± 59	ND	ND	ND	1057 ± 216
GAMT	26.8 ± 6.5	1.9 ± 0.4	0.7 ± 0.3	0.35 ± 0.1	0.84 ± 0.04	5.8 ± 1.1
MAT	43.1 ± 4.7	6.1 ± 1.1	4.2 ± 0.9	1.0 ± 0.3	1.1 ± 0.2	4.1 ± 1.2
MTHFR	10.6 ± 0.7	8.2 ± 1.9	4.8 ± 2.1	4.5 ± 1.9	1.7 ± 0.4	8.9 ± 3.2
MS	32.7 ± 2.3	7.3 ± 2.2	0.43 ± 0.45	3.0 ± 1.3	0.73 ± 0.3	2.3 ± 0.7
BHMT	39.2 ± 8.3	2.5 ± 2.2	ND	ND	ND	ND

[1] Data are means ± SD, $n = 4$.
[2] ND, No activity could be detected (<10 nmol·min^{-1}·g^{-1} for AGAT and <1.0 10 nmol·min^{-1}·g^{-1} for BHMT).

activities of 71.8 and 0.36 μmol·kg^{-1}·h^{-1}, respectively. These data have a number of broad implications. First, the activities of both AGAT and GAMT are adequate to account for the estimated rates of creatine synthesis by piglets of this age and that of MAT is adequate to provide the necessary SAM. We do not, of course, expect that the enzyme activities should exactly match the in vivo rates of synthesis, as the activities are measured in vitro under optimal conditions. In addition, MAT must produce SAM for all of the methyltransferase reactions (3) as well as for polyamine synthesis. The relatively high activities of renal AGAT and hepatic GAMT are similar to the distribution in adult rats (4,21). We have recently reported GAA release in vivo by rat and human kidneys and GAA conversion to creatine by rat hepatocytes (22,11). We consider it likely, therefore, that a similar renal/hepatic axis exists for creatine synthesis in piglets. Nevertheless, direct measurement of these interorgan fluxes in piglets is required for confirmation of this hypothesis.

The second major implication of these data relates to the pancreas. The presence of an appreciable activity of AGAT in the pancreas as well as GAMT activity has already been reported for rats (22). Sorenson et al. (23) employed immunofluorescence to locate AGAT to the pancreatic acinar cells; it was absent from islets. When expressed in terms of piglet mass, the pancreatic AGAT activity was comparable to that of the kidneys (91.6 vs. 84.4 μmol·kg^{-1}·h^{-1}). However, the piglet pancreatic GAMT activity (0.5 μmol·kg^{-1}·h^{-1}) was only 1% of that found in the liver. Similarly, the piglet pancreatic MAT activity is sufficient to provide only a little less than 1% of all the SAM required for creatine synthesis. Clearly, the role played by these enzymes in pancreatic physiology remains to be determined.

TABLE 4 Sources of creatine in growing piglets

Milk creatine,[1] *μmol/L*	529 ± 85
Milk consumption,[2,3] *mL/d*	770
Milk creatine intake,[4] *mmol/wk*	2.8
Body creatine accumulation,[4,5] *mmol/wk*	10.2
Spontaneous loss of creatine,[4,6] *mmol/wk*	2.3
Total creatine acquired,[4] *mmol/wk*	12.5
Total creatine synthesis,[4,7] *mmol/wk*	9.7
Creatine supplied in milk, *%*	22.6
Creatine synthesized by piglet, *%*	77.4
Rate of creatine synthesis,[8] *μmol·kg^{-1}·h^{-1}*	25.4

[1] Mean ± SD, $n = 6$.
[2] From (8).
[3] Seven-day-old piglet.
[4] Per piglet between d 4 and 11.
[5] From Table 2.
[6] Calculated as a loss of 1.7%/d of the mean of 4- and 11-d creatine content.
[7] Calculated by subtracting milk creatine intake from the total creatine acquired.
[8] Calculated by dividing the creatine synthesized between d 4 and 11 by the mean weight of piglets in the same interval.

Creatine synthesis and amino acid metabolism. How much of a burden does creatine synthesis place on amino acid metabolism? The issue regarding glycine is straightforward, because the entire glycine molecule is incorporated into creatine. Therefore, between d 4 and 11, piglets incorporated ~9.7 mmol of glycine into creatine at a mean rate of 25.4 μmol·kg^{-1}·h^{-1}. The total intake of glycine over the same period (80 mmol) can be estimated from the glycine composition of mature sow milk (24) and a milk consumption of 0.77 L/(piglet·d) (8). Creatine synthesis, therefore, accounts for ~12% of dietary glycine. In addition, it is clear that considerable glycine synthesis occurs in neonatal piglets. This is evident from the work of Wu et al. (25), who have provided data on the amino acid composition of piglets at term. Glycine accounts for 11.3% of whole piglet protein. Given that our piglets accumulated 242 g of protein over 7 d, we calculated that they deposited ~360 mmol of glycine in their protein, which is >4 times their glycine intake in milk. The glycine used for creatine synthesis is, therefore, only ~2.7% of the net glycine incorporated into protein.

The metabolic burden on methionine and arginine metabolism may also be calculated in a similar way. Between d 4 and 11, we calculated a methionine intake of 27.8 mmol and an arginine intake of 47.7 mmol. Therefore, creatine synthesis amounts to ~35 and 20%, respectively, of the dietary intake of these amino acids. Wu et al. (26) have estimated that ~17% of milk arginine may be used for creatine synthesis in sow-fed piglets. Arginine and methionine, respectively, constitute 6.7 and 1.95% of total piglet body protein (26). We therefore estimated that between d 4 and 11, there was a net deposition of 93 mmol of arginine and 31.7 mmol of methionine in piglet protein. That piglets' arginine deposition into protein was twice their arginine intake in milk reflects the substantial endogenous arginine synthesis in these animals (27). The arginine used for creatine synthesis amounts to ~10.3% of that incorporated into protein. The net deposition of methionine in protein is very similar to our estimates of milk methionine ingestion, which is consistent with the fact that methionine cannot be synthesized in animals. Methionine used for creatine synthesis is ~30.4% of methionine deposited in protein. However, evaluating the burden of creatine synthesis on the metabolism of arginine and methionine is more complex than that of glycine, because the entire methionine and arginine molecules are not incorporated into creatine. In the case of methionine, only the methyl group is incorporated from SAM. In

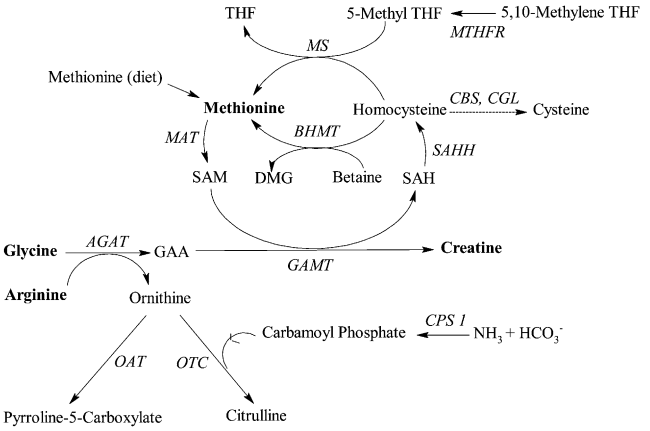

FIGURE 1 Amino acids and creatine synthesis. The production of 1 molecule of creatine requires an entire glycine molecule together with an amidino group (provided by arginine) and a methyl group (provided by SAM). Whether this represents a net utilization of arginine and methionine depends on the occurrence of mechanisms to regenerate arginine from ornithine and methionine from homocysteine. CBS, Cystathionine β-synthase; CGL, cystathionine γ-lyase; CPS 1, carbamoylphosphate synthetase 1; DMG, dimethylglycine; OAT, ornithine aminotransferase; OTC, ornithine carbamoyltransferase; SAHH, SAH hydrolase; THF, tetrahydrofolate.

the case of arginine, only the amidino group is incorporated. In both cases, methionine and arginine can be regenerated by enzymes in their respective cycles.

There are 2 well-described remethylation mechanisms for regenerating methionine from homocysteine (**Fig. 1**). One of these uses 5-methyltetrahydrofolate as the methylating agent and requires the enzymes MTHFR and MS. The other, BHMT, transfers one of the methyl groups from betaine to homocysteine to produce methionine and dimethylglycine. We found appreciable activities of each of these enzymes in piglet livers (17.7, 54.5, and 65.3 μmol·kg^{-1}·h^{-1}, respectively, for MTHFR, MS, and BHMT). Clearly, there are adequate activities of MS and BHMT to support appreciable rates of remethylation. Total hepatic MTHFR activity is lower than that of MS and BHMT, but the standard assay for this enzyme is run in the reverse direction to its in vivo flux. There are also lesser, although substantial, activities of these enzymes in nonhepatic tissues, in particular, kidney, intestine, and pancreas. It appears that piglets have the capacity (measured in vitro) to support substantial rates of remethylation. More direct information on actual rates of remethylation was provided by isotopic experiments in vivo. Riedijk et al. (28) found remethylation rates of 8–14 μmol·kg^{-1}·h^{-1} for 27-d-old piglets. We are aware of no comparable data for younger piglets. Riedijk et al. (28) found transmethylation fluxes in their 27-d-old piglets of 33–40 μmol·kg^{-1}·h^{-1}. Comparison of this rate to the rate of creatine synthesis (Table 3) indicates that creatine synthesis may consume as much as 63–77% of all of the labile methyl groups used by piglets. This is appreciably more than the estimate of 40% in adult humans (3).

The burden imposed by creatine synthesis on arginine metabolism is, also, not clear-cut. AGAT produces ornithine and the key issue lies in the fate of this amino acid (Fig. 1). Ornithine may be converted to citrulline by ornithine carbamoyltransferase and, ultimately, to arginine. However, ornithine conversion to citrulline requires a source of carbamoylphosphate. Carbamoylphosphate synthase 1 is absent from the kidney (29). Alternatively, ornithine may be catabolized via ornithine aminotransferase, which is active in the kidney (30); however, this results in a loss of its potential to be reconverted to arginine. Clearly, this issue requires more work, but the following may be pertinent: *1*) arginine is a conditionally essential amino acid in neonatal piglets, i.e. endogenous arginine synthesis cannot supply all of the arginine required (31); and *2*) arginine may become limiting for growth in neonatal piglets. Plasma arginine decreases 20–40% between the ages of 3 and 14 d (32). The growth of milk-reared piglets between d 7 and 21 can be markedly improved by either provision of supplemental arginine or pharmacological activation of intestinal arginine synthesis (26). These data suggest that the maximal growth of piglets is limited by arginine availability. It may also be noteworthy that preterm infants exhibit hypoargininemia (33). The demand for arginine imposed by high rates of creatine synthesis may well be responsible for the neonatal decrease in circulating arginine levels.

Our measurements reveal that neonatal piglets synthesize most of the creatine that is acquired. This, in turn, has substantial metabolic implications. The rate of creatine synthesis is quite small compared with glycine fluxes and can hardly be considered a major drain on glycine pools. On the other hand, creatine synthesis may well make appreciable demands on arginine metabolism. Clarification of this issue requires further work. Finally, creatine synthesis in neonatal piglets is a major user of labile methyl groups and, clearly, places a very large burden on the dietary provision of appropriate methyl donors (methionine, choline, and betaine) and on the synthesis of new methyl groups via folate-dependent methylneogenesis. We found almost no betaine in either human or sow milk (data not shown). However, Holmes-McNary et al. (34) reported total choline (free and esterified choline) concentrations of ~1.2 mmol/L in human milk. A similar quantity of choline in sow milk would provide a total of ~6.5 mmol to each piglet per week. Milk choline could therefore provide a large portion of the necessary labile methyl groups. However, the degree to which it does so will depend on the extent to which choline is catabolized by piglets compared with its utilization for phospholipid and acetylcholine synthesis.

The contribution of different tissues to creatine synthesis also requires further exploration. Does a renal/hepatic axis for creatine synthesis occur in rapidly growing piglets? What is the role of the pancreas in creatine, or GAA, synthesis in vivo? These issues are currently being addressed.

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