Orotic Acid Excretion and Arginine Metabolism\textsuperscript{1–3}

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Abstract

The urinary excretion of orotic acid, an intermediate in the pyrimidine biosynthetic pathway, is markedly increased in many inborn errors of the urea cycle and in a number of other disorders involving arginine metabolism. Carbamoyl phosphate, which accumulates within hepatic mitochondria in patients with orotic acid transcarbamoylase deficiency, can diffuse to the cytosol and enter the pyrimidine pathway, resulting in greatly increased orotic acid production and excretion. This orotic aciduria also occurs in inborn errors of the mitochondrial orotate/citrulline transporter, arginase, argininosuccinate synthetase, and argininosuccinate lyase. Increased orotic acid excretion is also found in a number of hyperargininemic states, such as lysinuric protein intolerance. However, orotic aciduria should not be used uncritically as an index of arginine deficiency because it is found in patients with arginase deficiency who exhibit hyperargininemia. Increased orotic acid excretion can also arise as a result of impairments of pyrimidine synthesis, whether brought about by a genetic defect (e.g., in UMP synthase) or by drugs that inhibit the terminal part of the pathway (e.g., allopurinol or 6-azauridine). When used appropriately, measurement of urinary orotic acid is a valuable tool for the study of many derangements of arginine metabolism, including arginine depletion, and to assess the efficacy of therapies used to replete this amino acid. J. Nutr. 137: 1656S–1661S, 2007.

Orotic acid is a minor dietary constituent (1). Indeed, until it was realized that it could be synthesized by humans, orotic acid was known as vitamin B-13 (2). The richest dietary sources are cow's milk and other dairy products (3) as well as root vegetables such as carrots and beets (1). Dietary intake probably contributes to a basal rate of orotic acid excretion in urine because fasting decreases excretion by \(50\%\) (4). However, it is now apparent that most urinary orotic acid is synthesized in the body, where it arises as an intermediate in the pathway for the synthesis of pyrimidine nucleotides. This article is concerned with the relation between orotic aciduria and arginine metabolism. In particular, it is concerned with the practical utility of orotic aciduria as a biomarker for arginine depletion. It is necessary, therefore, to discuss the functions of arginine, situations of arginine depletion, and the metabolic origin of orotic acid to describe the relation of orotic aciduria to arginine metabolism.

Functions of arginine

Arginine is 1 of the most physiologically versatile of the amino acids. It is, of course, 1 of the 20 amino acids that are required for protein synthesis, but its importance extends far beyond this (5). Arginine is a critical intermediate in the urea cycle, a source of amidino groups for creatine synthesis, a substrate for nitric oxide synthesis, and a source of ornithine for polyamine synthesis. By virtue of its role as a precursor of NO, arginine plays a key role in vascular function, immune function and brain function. Each of these roles of arginine will become compromised when arginine levels are decreased. However, we do not know the levels of arginine at which each of these processes becomes compromised or, indeed, the order in which they become compromised. In this connection, it is important to bear in mind that orotic aciduria relates to only 1 of these functions, that of urea synthesis.

Arginine depletion

The early work of Rose (6) classified arginine as a dietary non-essential amino acid. However, it is now evident that a better description is that of a conditionally essential amino acid. Arginine is synthesized in vivo, primarily by an interorgan pathway involving the small intestine and the kidney (7). Arginine synthesis uses many of the enzymes of the urea cycle (Fig. 1) and can account, to some degree, for their extrahepatic expression.

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Carbamoyl phosphate synthetase I (CPS I) and ornithine transcarbamoylase (OTC) occur in the small intestine, where they are involved in the production of citrulline, which is released to the hepatic portal vein. Circulating citrulline is taken up by the kidney and converted to arginine, in cells of the proximal tubule, by the sequential action of argininosuccinate synthetase and argininosuccinate lyase. Release of arginine in the renal vein makes it available to the rest of the body (7,8). In this dual-organ pathway, it appears that the intestine is the master, as the kidney can respond to elevated concentrations of citrulline in the plasma by converting them to arginine (Fig. 2). The actual rate of arginine synthesis in humans, however, is thought to make only a minor contribution to body arginine pools (9), and one that does not readily respond to arginine intake in rats (10) or in humans (11).

Clearly, this pathway of endogenous arginine synthesis can produce sufficient arginine for the maintenance of nitrogen balance in healthy adults. We now recognize a number of well-described situations, however, where endogenous arginine synthesis is insufficient and a dietary source of arginine must be provided. Yu et al. (12) showed that endogenous arginine synthesis is unable to keep pace with the accelerated rate of arginine catabolism found in severely burned patients. Impaired intestinal citrulline synthesis, either as a result of intestinal resection in humans (13) or inhibition of the intestinal ornithine transcarbamoylase in rats (14), produces an arginine deficiency that can be corrected by dietary arginine. Intravenous feeding of piglets (which by-passes first-pass gut metabolism) with an arginine-free formula results in hypoargininemia and hyperammonemia (15). Plasma arginine is decreased by ~60% in preterm infants. Wu et al. (16) have characterized this as arginine deficiency. It is known that such infants are susceptible to asymptomatic hyperammonemia that is associated with a moderate increase in orotic acid excretion (17) and responds to arginine therapy (18). Decreased plasma arginine concentrations have also been described in patients with sepsis (19) and cancer (20). Lysinuric protein intolerance, which is caused by a genetic defect in the intestinal absorption and renal reabsorption of cationic amino acids, is characterized by low circulating arginine levels, postprandial hyperammonemia, vascular endothelial dysfunction, and increased orotic acid excretion (21–23).

There are also a number of situations in which hypoargininemia occurs as a result of an elevation of plasma arginase subsequent to the pathological lysis of arginase-containing cells. For example, the plasma concentration of arginine in patients with asymptomatic hyperammonemia that is associated with a moderate increase in orotic acid excretion (17) and responds to arginine therapy (18). Decreased plasma arginine concentrations have also been described in patients with sepsis (19) and cancer (20). Lysinuric protein intolerance, which is caused by a genetic defect in the intestinal absorption and renal reabsorption of cationic amino acids, is characterized by low circulating arginine levels, postprandial hyperammonemia, vascular endothelial dysfunction, and increased orotic acid excretion (21–23).

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asthma is only ~50% of the control level; this is accompanied by elevated plasma arginase (24). Sickle cell disease is also associated with decreased plasma arginine and elevated plasma arginase (as a result of hemolysis). The pulmonary arterial hypertension of this disease has been attributed to decreased NO availability as a result of both decreased arginine levels and inactivation of NO by cell-free hemoglobin (25).

Pyrimidine nucleotide biosynthesis

The synthesis of pyrimidines (UTP, CTP, TTP) begins with the cytoplasmic formation of carbamoyl phosphate from glutamine, bicarbonate, and ATP, followed by the addition of aspartate to produce carbamoyl aspartate, which then undergoes an enzyme-catalyzed cyclization to give rise to dihydroorotate (Fig. 1). These first 3 reactions are carried out by the multifunctional protein that initiates and regulates de novo pyrimidine biosynthesis (CAD). CAD contains active sites for carbamoyl phosphate synthetase II (CPS II), aspartate transcarbamoylase, and dihydroorotase (26). The fourth enzyme, dihydroorotase dehydrogenase (DHODH), which catalyzes the oxidation of dihydroorotate to orotic acid, is an integral protein in the mitochondrial inner membrane, with its substrate binding site facing the intermembrane space (27). Orotic acid is converted to UMP by UMP synthase, another multifunctional protein with both orotase phosphoribosyltransferase and orotidylate decarboxylase activity (26). CAD and UMP synthase are thought to be associated with each other on the outer mitochondrial membrane at sites that are in proximity to DHODH on the inner membrane (27).

Control of pyrimidine synthesis is exerted by negative feedback by UTP and positive feedforward by 5-phosphoribosyl-1-pyrophosphate; both of these controls are exerted on the CPS II site of CAD (26). The most frequently observed inborn error of pyrimidine nucleotide synthesis is a mutation of the multifunctional protein UMP synthase (28). This disorder prevents the conversion of orotic acid to UMP and thus to other pyrimidines. As a result, plasma orotic acid accumulates to high concentrations, and increased quantities appear in the urine. Indeed, urinary orotic acid is so markedly increased in individuals harboring a mutation in UMP synthase that orotic acid crystals can form in the urine (28). The urinary concentration of orotic acid in homozygotes can be of the order of millimoles per millimole creatinine. By comparison, the urinary level in unaffected individuals is ~1 μmol/mmol creatinine (1).

Relation between orotic aciduria and urea synthesis

Orotic acid plays no role in the operation of the urea cycle. Nevertheless, orotic acid excretion is increased in several urea cycle disorders (1). An appreciation of the relation between these 2 processes is, therefore, essential. The first step of urea synthesis in the liver, the synthesis of carbamoyl phosphate, is catalyzed by carbamoyl phosphate synthetase I (CPS I), a mitochondrial matrix enzyme, which condenses bicarbonate, ammonia, and ATP to form carbamoyl phosphate (Fig. 1). CPS I requires N-acetylglutamate (NAG) as an essential allosteric activator. Synthesis of NAG from acetyl CoA and glutamate is catalyzed by NAG synthetase, a mitochondrial matrix enzyme, for which arginine serves as an activator (29). Thus, increased mitochondrial matrix arginine would enhance the production of mitochondrial carbamoyl phosphate and should normally indicate sufficient ornithine for the next step in the cycle. OTC catalyzes the condensation of matrix ornithine with carbamoyl phosphate to give citrulline, which, together with a proton, is transported out of mitochondria, in exchange for ornithine, by the inner membrane transporter ORNT 1 (30,31). Argininosuccinate synthetase produces argininosuccinate from citrulline and aspartate; argininosuccinate is cleaved by argininosuccinate lyase to give arginine (and fumarate), which is hydrolyzed by arginase I to urea and ornithine; this completes the cycle.

The hepatic pool of arginine involved in the urea cycle seems to be somewhat metabolically sequestered. Specifically, Young and co-workers (9), employing stable isotope methodology in humans, have provided evidence that the urea cycle arginine pool is neither in equilibrium with, nor rapidly exchangeable with, plasma arginine or even other hepatic arginine pools. Evidence has been provided that the efficiency of the urea cycle is enhanced by means of metabolic channeling (32,33). The importance of channeling in the urea cycle has recently been emphasized by Watford (34). Such channeling may provide the mechanism whereby the urea-cycle pool of arginine does not rapidly equilibrate with other arginine pools.

Carbamoyl phosphate accumulates within the mitochondrial matrix when there is a mismatch between the fluxes through CPS I and OTC. For example, increased carbamoyl phosphate production (as a result of increased provision of ammonia) in the face of low OTC activity (as a result of either too little OTC activity or too little of the substrate, ornithine) will cause carbamoyl phosphate to accumulate in the matrix. This accumulated carbamoyl phosphate is known to spill over into the cytoplasm (mechanism unknown), where it can enter CAD beyond the control step for pyrimidine nucleotide synthesis (35). This is found in many, but not all, inborn errors of the urea cycle. Specifically, it occurs in deficiencies of ORNT 1, OTC, argininosuccinate synthetase, argininosuccinate lyase, and arginase. It is important to appreciate that flux through the urea cycle is normally much larger than flux through the pathway of pyrimidine nucleotide synthesis so that the diversion of just a fraction of mitochondrial generated carbamoyl phosphate will substantially increase flux through the pyrimidine nucleotide pathway (36).

Orotic aciduria

Once orotic acid reaches the blood, it is efficiently cleared by the kidney. There is evidence for secretion by the tubules, as well as loss by filtration (37). Assessment of urinary orotic acid is facilitated by its remarkable stability. It may even be successfully analyzed in pieces of urine-impregnated filter paper (38,39) that have been mailed to a central laboratory. A variety of analytical techniques provide satisfactory results (1). Analysis of urinary orotic acid has the advantage of providing an integrated picture of orotic acid production over time, rather than the snapshot that is provided by analysis of a single plasma sample (22).

A number of different situations are known where orotic acid accumulates in liver, enters the plasma, and is excreted in the urine, giving rise to orotic aciduria:

**Deficiency of UMP synthase.** This inborn error is known as hereditary orotic aciduria and is unrelated to any abnormality in arginine metabolism (28).

**Deficiency of ornithine transcarbamoylase.** This inborn error leads to excessive carbamoyl phosphate accumulation in liver and intestinal cells, unless the subjects are ingesting a very low-protein diet. Orotic acid accumulates, although not to the same extent as in UMP synthase deficiency. In this situation, the plasma arginine concentration may be low (40).

**Other inborn errors of urea or arginine synthesis.** A deficiency of ornithine synthesis in intestinal cells of the newborn
functional OTC activity because of lack of substrate. In this hy-
urya cycle by regenerating the carrier, ornithine, results in a low
ria is found because carbamoyl phosphate cannot be synthesized.
pace with physiological requirements. However, no orotic acidu-
synthetase, arginine synthesis is seriously compromised.

case. For example, when CPS I activity is deficient because of
arginine deficiency and only in hypoargininemia. This is not the
rulline may be converted to arginine in vivo.
crease orotic acid excretion in these children (44).

Drug-induced orotic aciduria. Both allopurinol and 6-
azaruridine cause increased orotic acid excretion (1). The
isms of these effects are not clear but appear to involve
ions by metabolites of the drugs on pyrimidine synthesis,
ial to the formation of orotic acid.

Specificity and utility of orotic aciduria as an indicator
arginine depletion

To be a generally useful marker of arginine depletion, increased
cretion of orotia c in urine should occur in all instances of
arginine deficiency and only in hypoargininemia. This is not the
case. For example, when CPS I activity is deficient because of
either a mutation in the CPS I gene or deficient activity of NAG syn-
thetase, arginine synthesis is seriously compromised.

Arginine deficiency may result if dietary intake does not keep
pace with physiological requirements. However, no orotic acidu-
ria is found because carbamoyl phosphate cannot be synthesized.
On the other hand, a deficiency of arginase I, which completes the
rea cycle by regenerating the carrier, ornithine, results in a low
functional OTC activity because of lack of substrate. In this hy-
perammonemic situation, the accumulation of carbamoyl phos-
phate leads to elevated orotic acid production in the face of
yperargininemia (47). There is, therefore, no compulsory cor-
relation between orotic aciduria and decreased arginine levels.

In subjects who are deficient in neither CPS I nor NAG syn-
thetase, increased orotic acid excretion is primarily a reflection
of intramitochondrial ornithine availability. Direct evidence for
the importance of the mitochondrial ornithine pool is provided by
patients with hyperornithinemia-hyperammonemia-homo-
citrullinuria syndrome, which arises as a result of a genetic defect
in ORNT 1 (31). This transporter delivers cytoplasmic ornithine
to the mitochondrion in exchange for citrulline. Subjects with
this syndrome develop both hyperammonemia and orotic acid-
ria on challenge with an alanine load, despite their systemic
hyperornithinemia (48). Orotic aciduria, therefore, serves as a
noninvasive measure of the intramitochondrial ornithine pool
and may be employed as an index of the repletion of this pool.
For example, Nagasaka et al. (40) treated 7 boys who had late-
onset OTC deficiency. Their results are shown in Figure 4. Treat-
ment of these patients with arginine caused their elevated
urinary orotic acid levels to decrease, although not to normal
because OTC is still deficient. The increased arginine supply
simply permitted more complete use of the residual enzyme ac-
tivity. Similarly, orotic acid excretion is a useful index of the
efficacy of therapy with urea cycle amino acids in subjects with
lysinuric protein intolerance. In such patients, oral citrulline is
effective in reducing orotic aciduria, whereas oral arginine and
ornithine were less so (22). These differences doubtless reflect
the defective absorption of cationic amino acids (arginine and
ornithine) in this condition as well as the ease with which cit-
rulline may be converted to arginine in vivo.

The above discussion indicates that orotic acid excretion
may be a useful diagnostic tool in some derangements of arginine
metabolism but not in all. As outlined above, there are a number
of situations where hypoargininemia occurs as a result of ex-
trahepatic events (e.g., asthma, sickle-cell disease). This involves
elevated plasma arginase as a result of pathological lysis of cells
that contain substantial activities of arginase I. Unfortunately,
urinary orotic acid does not appear to have been measured in
these situations. Although the hepatic urea-cycle pool of argi-
nine is somewhat segregated from other arginine pools, it is not
absolutely segregated (9), and we may anticipate that it would

![Figure 3](image1.png)

**FIGURE 3** Monitoring of plasma concentrations of orotic acid and arginine over a 3- y period in a patient with argininosuccinic acid synthetase deficiency. Reproduced, with permission, from Sass and Skladal (37).

![Figure 4](image2.png)

**FIGURE 4** Plasma arginine and urinary orotic acid in 7 boys with late-onset OTC deficiency treated with arginine. Control value for arginine was 99 nmol/L, and that for orotic acid was 3 mmol/mmol creatinine. Statistical details are given in the original paper. Redrawn from Nagasaka et al. (40). *Significantly different from “before treatment” (P < 0.01).
decrease as the systemic pools decrease. Evidence for this is provided by studies in patients with short-bowel syndrome. Ingestion of an arginine-free diet by these patients resulted in hypoargininemia and orotic aciduria (13). This shows that the hepatic urea-cycle pool of arginine is not absolutely segregated from other body pools. We suggest, therefore, that future studies on these hypoargininemas of extrahepatic origin should include the measurement of orotic acid excretion so as to determine whether they are accompanied by a secondarily impaired urea cycle. If low arginine is matched by orotic aciduria in these patients, it should be possible to follow the efficacy of their treatment (e.g., with dietary arginine) in this way. Given the stability of orotic acid, this could be done from home; the patient or caregiver would only need to mail a dried, urine-impregnated filter paper to the central laboratory.

Orotic aciduria has a number of causes. It may be brought about as a result of inborn errors in the pathway of pyrimidine synthesis or as a result of drugs that inhibit the distal part of the pathway for pyrimidine synthesis. With regard to arginine metabolism, it is brought about by a mismatch between the in vivo activities of CPS I and OTC. This may occur as a result of some inborn errors of the urea cycle, but not all. It may also occur as a result of arginine depletion brought about by the impairment of endogenous arginine synthesis in the face of insufficient dietary arginine or of increased arginine catabolism. Monitoring of orotic aciduria cannot be employed, therefore, either as a general indicator of arginine depletion or of urea cycle function. However, it can be a very useful index of urea cycle function in situations where flux through OTC is compromised whether as a result of a defect in OTC itself or in ORNT 1 or in 1 of the other urea-cycle enzymes that are necessary to complete the cycle and deliver ornithine to hepatic mitochondria. It may also be useful in conditions of hypoargininemia that originate apart from the urea cycle.

Literature Cited


