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Hydroxyproline metabolism in a mouse model of Primary Hyperoxaluria Type 3

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Abstract

Primary Hyperoxaluria Type 3 is a recently discovered form of this autosomal recessive disease that results from mutations in the gene coding for 4-hydroxy-2-oxoglutarate aldolase (HOGA1). This enzyme is one of the 2 unique enzymes in the hydroxyproline catabolism pathway. Affected individuals have increased urinary excretions of oxalate, 4-hydroxy-L-glutamate (4-OH-Glu), 4-hydroxy-2-oxoglutarate (HOG), and 2,4-dihydroxyglutarate (DHG). While HG and HOG are precursor substrates of HOGA1 and increases in their concentrations are expected, how DHG is formed and how HOG is metabolized to DHG and to oxalate are unclear. To resolve these important questions and to provide insight into possible therapeutic avenues for treating this disease, an animal model of the disease would be invaluable. We have developed a mouse model of this disease which has null mutations in the *Hoga1* gene and have characterized its phenotype. It shares many characteristics of the human disease, particularly when challenged by the inclusion of hydroxyproline in the diet. An increased oxalate excretion is not observed in the KO mice which may be consistent with the recent recognition that only a small fraction of the individuals with the genotype for HOGA deficiency develop PH.

Keywords

Primary Hyperoxaluria; Oxalate; Hydroxyproline; Mouse Model

1. Introduction

The Primary Hyperoxalurias (PH) are rare genetic disorders marked by an increased endogenous oxalate synthesis and a resultant elevation in urinary oxalate excretion, 3 – 10 times above normal. These and other characteristics of the disease have been recently reviewed [1]. This increased oxalate synthesis contributes to kidney stone formation. In

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extreme cases, tissue deposition of calcium oxalate and kidney failure may result. PH Type 1 (PH1) and PH Type 2 (PH2) have been well characterized and are known to result from genetic mutations in enzymes that metabolize glyoxylate. In PH1, alanine:glyoxylate aminotransferase (AGT) is either not functional or is mistargeted to mitochondria [1]. As a result, a peroxisomal pathway converting glyoxylate to glycine is absent. In PH2, the enzyme glyoxylate reductase (GR) is deficient, and the conversion of glyoxylate to glycolate in mitochondria and the cytoplasm is disrupted [2]. PH Type 3 (PH3) is caused by mutations in the mitochondrial enzyme, 4-hydroxy-2-oxoglutarate aldolase (HOGA) [3]. This enzyme is involved in trans-4-hydroxy-L-proline (Hyp) metabolism (Fig. 1), and it catalyzes the cleavage of 4-hydroxy-2-oxoglutarate (HOG) into pyruvate and glyoxylate. We have proposed that a buildup of HOG due to HOGA deficiency may result in an inhibition of GR activity based on results observed in vitro with the purified enzyme [4, 5].

Because Hyp is a major source of the glyoxylate produced in the body each day, we have argued that it makes a significant contribution to the oxalate produced in individuals with PH1 and PH2 [6]. Hyp is produced primarily from endogenous collagen turnover, which is estimated to be 2–3 g/day [7]. As collagen is ~15% Hyp, this turnover results in the release of 300 – 450 mg of Hyp, which must be degraded or excreted as it cannot be used for protein synthesis. Less than 30 mg of Hyp is excreted in urine each day, predominantly in peptides, with <5 mg of free Hyp excreted, indicating that most of the Hyp is metabolized [8]. Diet can also be a source of collagen. A single serving of Jello-brand gelatin contains a gram of collagen, which would contain 150 mg of Hyp. A quarter pound hamburger could contain as much as 6 grams of collagen yielding 780 mg of Hyp, if it were rich in ground ‘gristle’ [9]. Thus, significant levels of Hyp are degraded on a daily basis.

The Hyp catabolic pathway (Fig. 1) involves four enzymatic reactions [7, 10–12]. This metabolism is confined principally to the liver and kidney [7, 13]. The first step of the pathway is the flavin-dependent oxidation of Hyp to 1-pyrroline-3-OH-5-carboxylate (3-OH-P5C) by Hyp dehydrogenase (HYPDH). This intermediate is converted to 4-hydroxyglutamate (4-OH-Glu) by 1P5C dehydrogenase (P5CDH), an NAD^+-dependent enzyme shared with the proline degradation pathway [11]. Aspartate aminotransferase (AspAT) then utilizes oxaloacetate to convert 4-OH-Glu to HOG. Importantly, HOG is then cleaved by HOGA into two fragments, glyoxylate and pyruvate. The glyoxylate can then be converted to glycolate and glycine via GR and AGT, respectively. In PH patients, the glyoxylate produced from Hyp is not efficiently metabolized and oxalate production is increased via the activity of lactate dehydrogenase (LDH).

The development of an animal model would greatly facilitate further investigations of Hyp and glyoxylate metabolism in individuals with PH3. It is not known whether the HOG that builds up is the major source of the increased oxalate synthesis or whether other pathways are involved. Individuals with PH3 have also been shown to synthesize significant amounts of 2,4-dihydroxyglutarate (DHG), presumably derived from the reduction of HOG (Fig. 1) [14]. The pathway that converts HOG to DHG requires investigation, as it could be exploited therapeutically to enhance the disposal of HOG to limit its conversion to oxalate. Through the knockout mouse project (KOMP), we report here the development of a mouse model that has a suitable phenotype for such studies when challenged with Hyp.

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2. Materials and methods

2.1 Chemicals

Reagent grade chemicals were obtained from either Sigma-Aldrich Chemicals (St Louis, MO) or Fisher Scientific (Pittsburgh, PA). Trans-4-L-hydroxyproline for feeding studies was purchased from Sigma-Aldrich. The synthesis of $^{13}C_5$-Hyp has been previously described [13]. DL-4-Hydroxy-2-ketoglutaric acid lithium salt (HOG) was purchased from Sigma-Aldrich. Fluorescein-isothiocyanate-labelled sinistrin (FITC-S) was purchased from Fresenius Kabi Austria GmbH, Graz, Austria.

2.2 Animals

The mouse strain used for this research project was created from embryonic stem cell clone (EPD0380_4_H08) obtained from the NCRR-NIH supported KOMP Repository (www.komp.org) and generated by the CSD consortium. Methods used on the CSD targeted alleles have been published [15]. *Hoga1* heterozygous mice were intercrossed to generate *Hoga1* knock out (KO) and wild type (Wt) mice, and these animals were used to obtain phenotypic data. Mice were genotyped by tail snip DNA, using a polymerase chain reaction (PCR) with primers HOGA1 Forward (binds to 5′ homology arm), 5′-CCCGGGCAGCTGCTCTGCTCCT, HOGA1 Reverse (binds to the 3′ homology arm), 5′-GCCCCGAAACCGCCCAGCAA, and 5′ Universal (LAR3)-reverse, 5′-CACAACGGGTTCTTCTGTTAGTCC-3′. Agarose electrophoresis of PCR products yielded a 597bp product for the wild type allele (primers HOGA1F&R) and a 383bp product for the knock out allele (primers HOGA1&LAR3). Mice were maintained in a barrier facility with a 12 h light/dark cycle and an ambient temperature of 23 ± 1°C and had free access to food and water. All animal studies were approved by the Institutional Animal Use and Care Committee of the University of Alabama at Birmingham.

2.3. Metabolic cage collections

Animals (n = 5–6 per sex/per strain), 12–14 weeks of age, were equilibrated in Nalgene metabolic cages (Nalgene, Rochester, US) for 1 week and had free access to water and a custom calcium deficient, high sucrose basal diet (TD.130032) designed by Harlan Laboratories (Madison, WI), to which calcium chloride was added at 5 mg per gram dry diet. The custom, purified diet contained a very low background oxalate content (12.9 ± 1.1 μg oxalate per gram diet) and is devoid of Hyp. By weight, the diet contains 19.6% protein (whey protein isolate), 57.7% carbohydrate (maltodextrin and sucrose), 6.6% fat (lard and corn oil), and 10.3% cellulose. For Hyp feeding, the custom diet was supplemented with 1% Hyp. After equilibration for 1 week on the diet, 24 hr urines were collected on 1-ml mineral oil to prevent evaporation and 50 μl of 2% sodium azide to prevent bacterial growth. The stability of HOG in urine samples collected this way and stored at −80°C was confirmed. Three to four 24 hr urines were performed for each mouse. Urine collections with creatinine values 25 % lower/higher than mean values for each animal were considered incomplete/over collections and were discarded.

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2.4. $^{13}C_5$-^{15}N-Hyp intravenous infusion

Male Wt and KO mice (n = 4) under isoflurane anesthesia were catheterized via the jugular vein with a funnel catheter (Instech Lab Inc, PA), which was attached to a swivel (Instech Lab Inc, PA) and mounted to the metabolic cage. $^{13}C_5$-^{15}N-Hyp was prepared in 0.9% saline containing FITC-S (0.5 mg/ml) and infused at a rate of 10 μmoles/hr/kg. FITC-S was included to ensure consistency of urine collections and infusion rates between animals. After a three day recovery period, three consistent 24 hr urines were collected from each animal. The 24 hr urines containing less than 75% of FITC-S infused were not included in the data analysis.

2.5. Glomerular filtration rate

A miniaturized Non-Invasive-Clearance (NIC) technique to measure kidney function in conscious mice was applied to determine GFR, as previously described [16, 17]. The NIC-Kidney device (Mannhein Pharma and Diagnostics, Mannheim, Germany) allows for transcutaneous measurement of the elimination kinetics of the fluorescent renal marker FITC-S without surgery or blood and urine collection. Briefly, the NIC device was attached to the back of mice using a double-sided adhesive patch under anesthesia with 2% isoflurane. After a 5-min baseline recording, FITC-S (10–30 mg/100g body weight) was dissolved in 200 μl sterilized saline and administrated intravenously via the tail vein. Mice were allowed to recover from anesthesia and data were acquired for 90–120 minutes. The half-time of FITC-S elimination was determined using the established one compartment model and GFR was calculated.

2.6. Sample preparation

For oxalate determination, part of the urine collection was acidified to pH <1 with hydrochloric acid prior to storage at −80 °C to prevent any possible oxalate crystallization that can occur with cold storage and/or oxalogenesis associated with alkanization. The remaining non-acidified urine was frozen at −80 °C for the measurement of other urinary parameters. Plasma preparations were filtered through Nano-sep centrifugal filters (VWR International, Batavia, IL) with a 10,000 nominal molecular weight limit (NMWL) prior to ion chromatography analysis. Centrifugal filters were washed with 0.1 M HCl prior to sample filtration to remove any contaminating trace organic acids trapped in the filter device. Liver tissue was freeze clamped in liquid nitrogen immediately after sacrifice and stored at −80 °C. Liver tissue (10%) was homogenized in hypotonic lysis buffer containing 25 mM HEPES, pH 7.1, and 0.1% Triton X-100 and extracted with 10% trichloroacetic acid (TCA), with subsequent removal of the TCA as previously described [18].

2.7. HOGA expression

Western blot analysis was performed as previously described [5]. HOGA enzymatic activity was assessed by measuring the production of glyoxylate from the cleavage of HOG in a crude mitochondrial preparation isolated as previously described [19]. 100 μL reactions contained 1% mitochondrial homogenate, 100 mM TRIS pH 8.5, and 0.5 mM HOG. The reactions were run at 37 °C in triplicate for 15 min, and the reaction stopped with the addition of 11 μL 5 M perchloric acid (PCA). Glyoxylate was measured in PCA extracts by

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reversed phase HPLC after derivatization with phenylhydrazine, as previously described [18].

2.8. Analytical Methods

Urinary creatinine was measured in non-acidified urines on a chemical analyzer and oxalate in acidified urine by ion chromatography (IC), as previously described [20]. IC coupled with negative electrospray mass spectrometry (IC/MS) (Thermo Fisher Scientific Inc, Waltham, MA) was used to measure glycolate, HOG and DHG. For glycolate measurement, the IC equipment consisted of a Dionex™ ICS-5000 system with an AS15, 2 × 150 mm, anion exchange column at a controlled temperature of 30 °C and a Dionex™ ERS™ 500 anion electrolytically-regenerated suppressor. A gradient of KOH from 3 to 80 mM over 30 min at a flow rate of 0.3 ml min^{-1} was used to separate sample anions. The mass spectrometer (MSQ-PLUS) was operated as previously described [21]. Samples were diluted in the same amount of $^{13}C_2$-glycolate and selected-ion monitoring (SIM) was performed at the following mass/charge ratios, $^{12}C_2$-glycolate (SIM75) and $^{13}C_2$-glycolate (SIM77). For HOG and DHG quantification, an AS11-HC-4 μm, 2 × 150 mm, anion exchange column was used. A gradient of KOH from 0.5 to 80 mM over 60 min at a flow rate of 0.38 ml min^{-1} was used to separate anions in samples. The MSQ-PLUS mass detector was operated in ESI negative mode, needle voltage 1.5 V, cone voltage 30 V, and a temperature of 500 °C. The column eluent was mixed with 50% acetonitrile at 0.38 ml min^{-1} using a zero dead volume mixing tee prior to entry into the mass spectrometer. Selected-ion monitoring (SIM) was used to measure the isoptomers of HOG and DHG at the following mass/charge ratios; HOG (SIM161), $^{13}C_5$-HOG (SIM166), DHG (SIM163) and $^{13}C_5$-DHG (SIM168). Commercial standards for DHG do not exist, so its levels were estimated using pure standards of 2-hydroxyglutaric acid. Plasma Hyp was measured as previously described [6]. The mole percent enrichment of $^{13}C_5$-^{15}N-Hyp in plasma was measured by gas chromatography-mass spectroscopy by Metabolic Solutions (Nashua, NH). The contribution of Hyp metabolism to urinary oxalate excretion was calculated as previously reported for glycine [22]. A Coomassie Plus protein assay kit (Pierce, Rockford, IL), with bovine serum albumin (BSA) as the standard, was used to determine protein concentrations in tissue preparations.

2.9. Statistical analysis

The mean of at least two 24 hr urine analyte determinations was used to characterize excretions in each mouse. Student's *t*-test was used to compare sex differences and impact of Hyp feeding or $^{13}C_5$-^{15}N-Hyp intravenous infusion. Data are expressed as mean ± SD. The criterion for statistical significance was $P < 0.05$.

3. Results

3.1. Growth, Development, and HOGA expression

Hoga1 KO mice developed similarly to Wt mice with regards to weight gain, urine volume and creatinine output (Table 1). Western blot analysis showed HOGA present in liver mitochondria from Wt animals, but not KO mice (Fig. 2A). Mitochondria from Wt animals possessed HOGA enzymatic activity, but not mitochondria from *Hoga1* KO mice (Fig. 2B).

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3.2. Biochemical Analysis of Hoga1 KO mice

Biochemical analyses of plasma and urine from KO and Wt mice fed a Hyp-free diet are shown in Table 1. DHG levels in plasma and urine were significantly higher in *Hoga1* KO mice compared to Wt animals. There were no other significant differences between *Hoga* KO and Wt animals. Liver HOG and DHG were below detection limits. Interestingly, female animals had higher levels of glycolate in plasma and 24 hr urine collections compared to male animals. The fractional clearance of glycolate (FE-Glc) relative to FITC-S was lower in male *Hoga* KO mice. In both sexes significant reabsorption of glycolate by the kidney occurred. It is also important to note that the *Hoga1* KO mice did not exhibit hyperoxaluria under a Hyp-free diet, a marked contrast to PH3 patients.

3.3. Hydroxyproline feeding

As HOGA is in the pathway for Hyp catabolism (Fig. 1), we investigated how male and female mice metabolized Hyp by supplementing their diets with 1% Hyp. HOG and DHG levels in urine (Fig. 3), plasma HOG and DHG (Table 2), and DHG levels in liver tissue (Table 2) were significantly higher in KO animals compared to Wt mice of both sexes. HOG levels in liver tissue were only higher in female KO mice. Despite the increase in the production of HOG and DHG, as seen in human PH3 patients, the KO and Wt mice excreted similar amounts of oxalate (Fig. 4A). The urinary glycolate levels of Hyp-fed Wt mice of both sexes, however, showed a marked increase (~10-fold for males, ~14-fold for females; Fig. 4B, Table 1). In contrast, the urinary glycolate level of Hyp-fed female *Hoga1* KO mice increased to a lesser extent (~6-fold), and the amount of glycolate in plasma decreased ~ 2-fold (Table 2)

3.4. $^{13}C_5$-^{15}N-Hyp intravenous infusion

To more precisely determine whether the HOGA deficiency altered the metabolism of Hyp to oxalate, glycolate, HOG and DHG, Wt and KO male mice were continuously infused with low levels of $^{13}C_5$-^{15}N-Hyp (Table 3). The infusion with $^{13}C_5$-^{15}N-Hyp enriched plasma Hyp to similar extents in both strains. Urinary $^{13}C_2$-oxalate and $^{13}C_2$-glycolate excretions were also similar in Wt and KO animals. Calculations indicated that the contribution of Hyp metabolism to urinary oxalate excretion was similar in Wt and KO animals. Urinary $^{13}C_5$-HOG was not detected in Wt and KO animals. However, $^{13}C_5$-DHG was detected in urine of KO animals, but not Wt animals. This indicates that the DHG detected in Wt animals on a Hyp-free diet (Table 1; ~12 μg/mg Cr) is not derived from Hyp metabolism.

4. Discussion

Mutations in the gene encoding HOGA were recently identified as causes of PH Type 3 [3]. This disease has an average earlier age of onset and a slower progression than PH1 or PH2 [23]. HOGA is one of two enzymes unique to the Hyp catabolism pathway (Fig. 1), and splits HOG into pyruvate and glyoxylate. There are no known sex differences in urinary excretions in individuals with the disease. Affected individuals have increased excretions of oxalate, 4-OH-Glu, HOG and DHG in their urine [3, 5, 24]. The increases in 4-OH-Glu and HOG are expected, as they represent the substrate and product of aspartate aminotransferase, the enzyme preceding HOGA in the pathway. How HOG is cleaved in the absence of

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HOGA and whether DHG is also broken down and ultimately converted to glyoxylate are open questions.

Chiral analysis of the HOG in the urine of PH3 patients is consistent with the conversion of Hyp to the R-isomer, maintaining the stereochemistry at the 4-position from Hyp (Fig. 1) [14]. The finding of an elevation of two stereoisomers of DHG in that study suggests that more than one enzyme may be able to reduce HOG to DHG, but the identity and localization of these enzymes remain to be determined. The availability of an animal model that provides access to tissue will facilitate the identification of the enzymes involved. It is possible that *Hoga1* KO mice could be useful in the development of specific therapeutic approaches that increase the formation of DHG from HOG and limit its conversion to oxalate.

We obtained mice, developed as part of the Knockout Mouse Project (KOMP), that contained a deletion in their *Hoga1* gene. These mice lacked *Hoga1* gene expression and protein production (Fig. 2). When placed on a Hyp-free diet, the phenotype of these mice was significant only in the excretion of larger amounts of DHG (males 8-fold higher than Wt animals and females 7-fold higher) (Table 1). These observations support endogenous Hyp breakdown making a substantial contribution to urinary oxalate and glycolate levels. DHG levels increased further with Hyp feeding (Fig. 3B, Table 2), and our experiments suggest that DHG may be the main end product of HOG metabolism in the *Hoga1* KO mouse. Surprisingly, oxalate excretion was not elevated even with Hyp feeding (Fig. 4). This phenomenon may in part be due to the presence of AGT in mouse liver mitochondria as weIl as in peroxisomes, as AGT is only present in the peroxisomes in humans. Mitochondrial AGT activity provides the mouse with an additional means of removing glyoxylate other than through GR activity. The combination of AGT activity and a higher conversion of HOG to DHG in mice compared to humans may limit the availability of glyoxylate for conversion to oxalate. It is also possible that the mild phenotype in *Hoga1* KO mice matches the bulk of the individuals with the PH3 genotype. The genotype for PH3 is the most common amongst the various PH types based on an analysis of carrier frequencies in the population [23]. As only a small fraction of these individuals present with disease, most commonly with calcium oxalate kidney stones, the phenotype of the majority of individuals with the genotype is not known. It will be informative to determine their ability to metabolize Hyp and their urinary excretions of oxalate, HOG and DHG.

We have previously suggested that the hyperoxaluria observed in individuals with PH3 is caused by an inhibition of GR activity due to the increased concentration of HOG, based on in vitro observations [5]. An inhibition of GR activity might be expected to decrease the synthesis of glycolate from glyoxylate. The formation of $^{13}C_2$-glycolate from $^{13}C_5$-^{15}N-Hyp, however, was comparable in male Wt and *Hoga* KO mice (Table 3). This contrasted with the lack of $^{13}C_2$-glycolate formation in *Grhpr* KO mice, where the inhibition of glycolate formation and the role of GR are very clear [13]. The inhibition of GR may have resulted in the larger contribution of Hyp metabolism to urinary oxalate excretion in KO mice compared to Wt mice. As discussed above, the presence of AGT in the mitochondria and peroxisomes of the murine liver is a confounding factor. Notably, excess Hyp feeding did result in female *Hoga1* KO animals excreting significantly less urinary glycolate than female Wt animals, supporting the idea that only when HOG levels reach high

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concentrations within both the mitochondrion and cytosol is GR activity significantly inhibited. The lack of a difference in urinary glycolate excretion between Wt and *Hoga1* KO male animals with Hyp feeding may be due to this sex metabolizing glycolate more efficiently than females and thus blunting any urinary glycolate differences between the strains.

The clearance of glycolate relative to FITC-S indicated that ~90% was reabsorbed (Table 1). This is greater than the clearance relative to creatinine we reported in studies with human subjects which indicated that ~40% was reabsorbed [6]. How much of this reabsorbed glycolate is metabolized will require further experimentation, perhaps comparing the glycolate concentration in a renal vein with that in the arterial supply. A possible product of glycolate oxidation in the kidney is glyoxylate, which could result in further oxalate production. While an enzyme that could catalyze this conversion has not been identified, it is possible that long chain hydroxy-acid oxidase (HAOX2) or an aldehyde oxidase that has some affinity for glycolate could be involved. Two brothers who appear to lack functional glycolate oxidase activity with an elevated urinary glycolate but normal oxalate excretion have recently been identified [25]. This finding warrants investigation due to glycolate oxidase being considered as a target enzyme for lowering endogenous oxalate production [26].

The infusion experiments with $^{13}C_5$-^{15}N-Hyp (Table 3) confirm that HOG is broken down to $^{13}C_2$-glycolate and $^{13}C_2$-oxalate despite the absence of HOGA activity in the KO mice. These results suggest that another enzyme with lyase-like activity is able to split HOG into pyruvate and glyoxylate when the HOG concentration increases due to the lack of HOGA activity. Our tissue measurements of HOG and DHG suggest that the pathway converting HOG to DHG is present in the liver. N-acetylneuraminate lyase (NAL) was suggested as a candidate enzyme but we found that the NAL from *Escherichia coli* had minimal activity with HOG as a substrate [5]. Enzymes that could potentially recognize HOG as a substrate for conversion to DHG include 2-oxoglutarate dehydrogenase (OGDH), a 2-oxoglutarate dehydrogenase-like enzyme (OGDHL), or a dehydrogenase E1 transketolase domain containing protein (DHTK1) [27]. Work in our labs and others are in progress to determine the alternative metabolic pathways for HOG and DHG and the flux of labeled Hyp in normal and PH1-3 patients.

In summary, normal individuals and mice excrete very low levels of HOG in urine (Table 1) [5, 14]. HOG excretion is elevated in subjects with PH3 [5, 14], but not in *Hoga1* KO mice until they are challenged with Hyp feeding. Female *Hoga1* KO mice show the greatest response, and could be the model of choice for monitoring HOG production. Elevated DHG excretions are observed in both PH3 subjects and *Hoga1* KO mice and are substantially increased in *Hoga1* KO mice with Hyp feeding. This pattern of excretions indicates that both sexes of the *Hoga1* KO mouse will be useful models to study both DHG formation and the hydrolysis of HOG to pyruvate and glyoxylate. Decreasing the breakdown of HOG and increasing the formation of DHG from HOG are potential therapeutic strategies for decreasing oxalate synthesis in individuals with PH3. In addition, these studies support the need to develop animal models of PH3 where AGT is not present in the mitochondrion.

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These alternative models have the potential to remove the confounding interpretation of metabolic data and to more closely recapitulate the PH3 phenotype.

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HIGHLIGHTS

- The phenotype of mice lacking HOGA1 is described
- Urinary excretion of oxalate is normal but dihydroxyglutarate is elevated
- KO mice will help elucidate the enzymes splitting HOG and converting HOG to DHG

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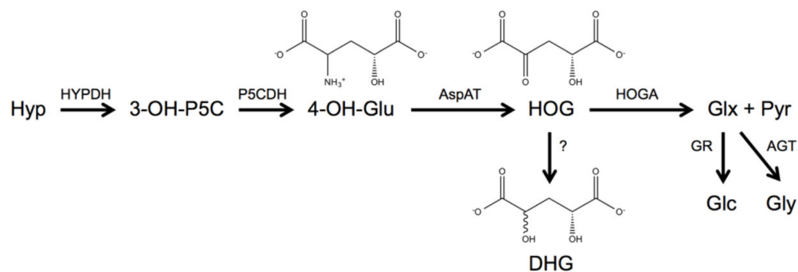

Figure 1.

Hydroxyproline catabolic pathway. The terminal step of the pathway is catalyzed by HOGA and produces glyoxylate (Glx) and pyruvate (Pyr). Glx can be converted to glycolate (Glc) and glycine (Gly) by mitochondrial glyoxylate reductase (GR) and peroxisomal alanine-glyoxylate aminotransferase (AGT), respectively. Mutations in HOGA result in PH3 and the buildup of 4-OH-Glu, HOG and DHG. HOG has been shown to inhibit GR in vitro. The conversion of HOG to DHG by an unknown enzyme is observed only with excessive Hyp intake or with HOGA deficiency. See main text for additional compound and enzyme abbreviation definitions. The wavy bond on DHG indicates that both the R- and S-isomers have been identified in urine samples from PH3 patients.

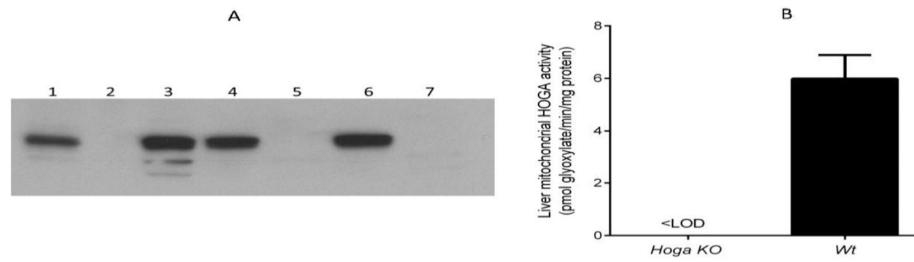

Figure 2.

HOGA protein and activity levels from mitochondria isolated from Wt and *Hoga1* KO mice. (A) Western blot of analysis with polyclonal rabbit antibodies against recombinant human HOGA. Western blot key: 1, human liver; 2, blank; 3, recombinant human *HOGA* protein (32878 Da); 4 and 6, Wt mice; 5 and 7, *Hoga* KO mice. (B) Enzymatic activity expressed as mean ± SD. LOD, 1.9 pmol glyoxylate/min/mg protein.

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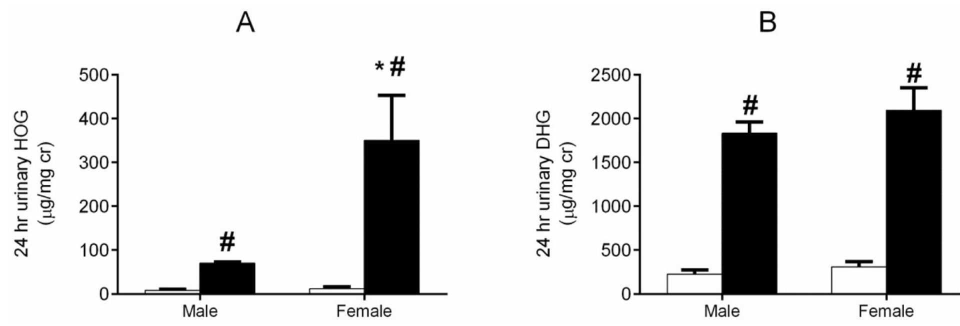

Figure 3.

24 hr urinary HOG (A) and DHG (B) excretion with 1% Hyp feeding in Wt (□) and *Hoga1* KO (■) mice. Data are expressed as mean (SD). *, significant differences between sexes within each strain. #, significant differences between Wt and *Hoga1* KO mice.

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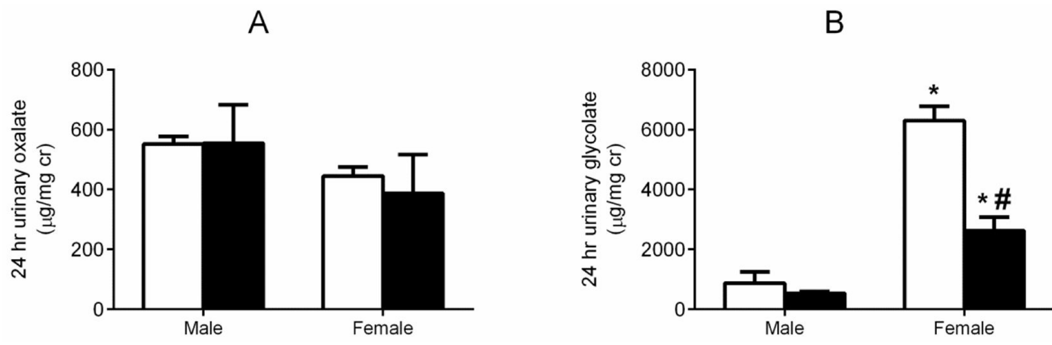

Figure 4.

24 hr urinary oxalate (A) and glycolate (B) excretion with 1% Hyp feeding in Wt (□) and *Hoga1* KO (■) mice. Data are expressed as mean (SD). *, significant differences between sexes within each strain. #, significant differences between Wt and *Hoga1* KO mice.

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Table 1

24 hour urine, plasma, and glomerular filtration rate measurements from Wt and *Hoga1* KO mice fed a purified diet devoid of Hyp.

	Wt		*Hoga1* KO	
	Male	**Female**	**Male**	**Female**
Body weight (g)	28.4±0.1	23.4±1.6*	29.6±2.3	24.8±1.3*
Urine volume (ml)	2.1±0.5	2.5±0.7	2.8±0.3#	2.5±0.7
Urinary creatinine (mg)	0.46±0.02	0.44±0.05	0.49±0.05	0.43±0.06
Urinary oxalate (μg/mg Cr)	74.3±5.6	66.1±4.6*	79.1±10.6	73.1±6.9
Urinary glycolate (μg/mg Cr)	103±4	436±71*	111±11	439±74*
Urinary HOG (μg/mg Cr)	8.8±2.0	8.8±1.8	9.4±1.2	7.6±1.3
Urinary DHG (μg/mg Cr)	13.8±1.2	11.0±1.2*	112±23#	76.0±15.5#
Plasma DHG (μM)	0.11±0.02	0.18±0.19	0.33±0.09#	0.39±0.18
Plasma glycolate (μM)	32.4±6.6	37.5±3.7	29.7±1.4	38.0±4.4*
GFR (μl/min)	203±8	ND	229±26	ND
FE-Glc (%)	9.3±1.3	ND	6.8±1.5	ND

Data are expressed as means ± SD. Student t-test was used to determine significant differences between sexes and phenotype, and P<0.05 was considered significant.

*, significant differences between sexes within each strain.

#, significant differences between Wt and *Hoga1* KO mice. ND. Not determined. HOG was not detected in the plasma of any animals (<0.2 μM).

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Table 2

Liver and plasma measurements in Wt and *Hoga1* KO mice after 1% hydroxyproline feeding.

	Wt		***Hoga1* KO**	
	Male	**Female**	**Male**	**Female**
Plasma HOG (μM)	<0.2	0.69±0.44	5.9±5.1	37±9*#
Plasma DHG (μM)	0.36±0.11	0.52±0.37	7.2±1.6#	8.2±1.9#
Plasma glycolate (μM)	84±2	318±49*	92±6	130±29#
Liver HOG (nmoles/mg protein)	<0.01	<0.01	<0.01	1.0±0.3*#
Liver DHG (nmoles/mg protein)	<0.01	0.1±0.1	0.2±0.0#	1.3±0.4*#

Data are expressed as means ± SD. Student t-test was used to determine significant differences between sexes and phenotype, and $P<0.05$ was considered significant.

*, significant differences between sexes within each strain.

#, significant differences between Wt and *Hoga1* KO mice.

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Table 3

24 hour urinary and plasma carbon 13 measurements in male Wt and *Hoga1* KO mice infused with $^{13}C_5$-^{15}N-Hyp infusion.

	Wt	*Hoga* KO
Plasma Hyp (μM)	25.5± 6.5	22.7±2.6
Plasma $^{13}C_5$-^{15}N-Hyp mole percent enrichment	34.2±6.0	31.8±5.4
Urinary $^{13}C_2$-oxalate (μg/mg Cr)	5.5±1.6	6.8±1.3
Urinary $^{13}C_2$-glycolate (μg/mg Cr)	2.9±0.9	3.5±1.7
Urinary $^{13}C_5$-DHG (μg/mg Cr)	<0.02	8.9±3.5[#]
Contribution of Hyp metabolism to urinary oxalate excretion (%)	22.1±2.2	27.3±5.6