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The Effects of the Inactivation of Hydroxyproline Dehydrogenase on Urinary Oxalate and Glycolate Excretion in Mouse Models of Primary Hyperoxaluria

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Abstract

The major clinical manifestation of the Primary Hyperoxalurias (PH) is increased production of oxalate, as a consequence of genetic mutations that lead to aberrant glyoxylate and hydroxyproline metabolism. Hyperoxaluria can lead to the formation of calcium-oxalate kidney stones, nephrocalcinosis and renal failure. Current therapeutic approaches rely on organ transplants and more recently modifying the pathway of oxalate synthesis using siRNA therapy. We have recently reported that the metabolism of trans-4-hydroxy-L-proline (Hyp), an amino acid derived predominantly from collagen metabolism, is a significant source of oxalate production in individuals with PH2 and PH3. Thus, the first enzyme in the Hyp degradation pathway, hydroxyproline dehydrogenase (HYPDH), represents a promising therapeutic target for reducing endogenous oxalate production in these individuals. This is supported by the observation that

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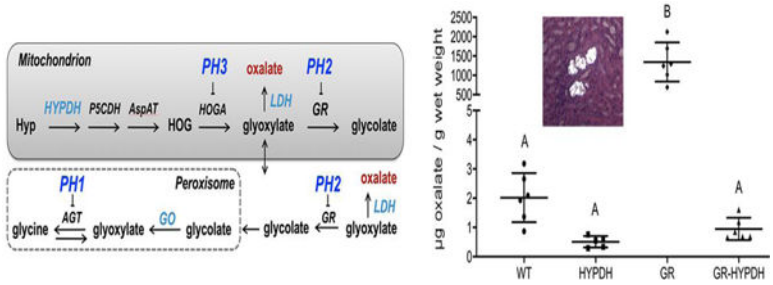
Disclosures

Drs. Holmes and Lowther have a patent pending (Serial No. 62/107,701) on the use of HYPDH inhibitors to treat primary hyperoxaluria.

individuals with inherited mutations in HYPDH (*PRODH2* gene) have no pathological consequences. The creation of mouse models that do not express HYPDH will facilitate research evaluating HYPDH as a target. We describe the phenotype of the *Prodh2* knock out mouse model and show that the lack of HYPDH in PH mouse models results in lower levels of urinary oxalate excretion, consistent with our previous metabolic tracer and siRNA-based knockdown studies. The double knockout mouse, *Grhpr* KO (PH2 model) and *Prodh2* KO, prevented calcium-oxalate crystal deposition in the kidney, when placed on a 1% Hyp diet. These observations support the use of the *Grhpr KO* mice to screen HYPDH inhibitors *in vivo*. Altogether these data support HYPDH as an attractive therapeutic target for PH2 and PH3 patients.

Graphical abstract



1. Introduction

Hydroxyproline (Hyp) is a critical component of collagen, the most abundant protein in the body. Together with glycine and proline it contributes to its fibrillar structure. Hyp is not incorporated into proteins by protein synthesis but is formed in situ in collagen by the hydroxylation of proline. When collagen is broken down, free Hyp and Hyp-containing peptides are released and degraded in both the hepatocytes and the proximal tubules of the kidney [1]. This pathway (Fig. 1) relies upon the unique enzymes, hydroxyproline dehydrogenase (HYPDH; previously known as HPOX and PRODH2) and 4-hydroxy-2-oxoglutarate (HOG) aldolase (HOGA1), together with one enzyme shared with proline metabolism, Δ1-pyrroline-5-carboxylate dehydrogenase (P5CDH) [1, 2]. We have recently analyzed the contribution of Hyp to endogenous oxalate and glycolate synthesis in normal individuals and those with Primary Hyperoxaluria (PH) [3]. This rare disease is caused by a variety of genetic mutations that cause a dramatic increase in oxalate synthesis. In healthy individuals the Hyp contribution to oxalate was 15%. Individuals with PH Type 1 (PH1), who have a deficiency in alanine:glyoxylate aminotransferase activity (AGT), exhibited a similar contribution of 18%. In individuals with PH2, who have a deficiency in glyoxylate reductase (GR) activity, the Hyp contribution to oxalate was 57%. Individuals with PH3 and a deficiency in HOGA1 showed that 33% of the oxalate produced came from Hyp. Thus, blocking the Hyp degradation pathway could be beneficial in all PH patients by reducing the oxalate load on the kidney.

The flux of metabolites and the generation of oxalate in PH3 patients is perplexing, especially since HOGA catalyzes the last step in Hyp metabolism to form glyoxylate. The inactivity of HOGA should prevent oxalate formation from glyoxylate. However, in the

absence of HOGA, HOG and its precursor 4-hydroxyglutamate accumulate [4, 5]. We and others have proposed that the breakdown of HOG to glyoxylate, by pathways not dependent on HOGA activity, is sufficient to cause hyperoxaluria [4, 5]. Moreover, Belostotsky et al. have shown that the cytosol of cultured liver cells contains an aldolase activity capable of metabolizing HOG [4]. We have shown that HOG inhibits GR activity, and this may contribute to the hyperoxaluria in these patients [5]. While research is still needed to understand the altered metabolism in PH3, it is clear that blocking the activity of HYPDH would be especially beneficial for PH3 patients as the formation of HOG and all other metabolites of the Hyp degradation pathway would be prevented.

HYPDH is localized in the mitochondrial matrix and is anchored in the inner mitochondrial membrane [2]. Characterization of the recombinant enzyme demonstrated its specificity for Hyp as a substrate [6]. HYPDH was originally proposed to be similar to proline dehydrogenase (PRODH1), an enzyme involved in the activation of P53 and apoptosis [7]. This response, however, was not confirmed in a more recent study [8]. Overexpression of PRODH1 and HYPDH in cultured cells has been shown to trigger oxidative stress through the generation of reactive oxygen species [7, 9]. It is important to point out that individuals that are deficient in HYPDH have no untoward effects and safely excrete any excess Hyp in their urine [10].

To facilitate research on PH and the development of potential therapies, knockout (KO) mouse models have been developed and include the inactivation of AGT (PH1) [11], GR (PH2) [12], and HOGA (PH3) [13]. In this report we describe the development of a mouse model lacking an intact *Prodh2* gene (the HYPDH protein) and have determined its phenotype with and without a dietary Hyp challenge. The prevention of extensive renal oxalate deposition in mutant mice fed 1% Hyp (both HYPDH KO and GR-HYPDH KO) supports the applicability of these models for screening HYPDH inhibitors to treat PH2 and PH3 patients.

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.

2.2. Animals

All studies were conducted using protocols consistent with local, state and federal regulations as applicable, and with adherence to the NIH Guide for the Care and Use of Laboratory Animals. All studies were approved by the Institutional Animal Care and Use Committee at University of Alabama at Birmingham (UAB), AL. 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.

2.3. Generation Prodh2 knockout mice using CRISPR/CAS methodology

CRISPR targets in exon 2 of the *Prodh2* gene were chosen using the MIT CRISPR design tool, GGCCACAAACTGCCCGTAGA (TGG) and CCTCGGTGGGTACTGCCAAC (AGG) [14]. Single guide RNA (sgRNA) molecules were generated as described earlier [15]. Pronuclear injections into C57BL/6J zygotes were performed with a solution of sgRNAs (25 ng/μl of each guide) and Cas9 capped mRNA (50 ng/μl). Efficiency of nuclease activity of each of the guides was first tested in blastomeres cultured from injected zygotes. The injected zygotes were implanted into pseudo-pregnant CD1 recipients. Genomic DNA obtained from tail biopsies of putative founder (G_0) animals and F1 lines were genotyped in-house (at UAB) using heteroduplex mobility assay and DNA sequencing; subsequent litters were genotyped by Transnetyx (Nashville, TN; Forward Primer Sequence – AGAGGTTTGAGGTAGGGAGAA; Reverse Primer Sequence – GAAGGGTGTTCGAGAAGGAAG). Four mutant alleles were identified in G_0 animals, three of which were deletions (72 bp, 85 bp and 99 bp) spanning the two target sites suggesting that both guides were effective in creating double strand breaks (Fig. 2A). The fourth allele (an indel +1 bp; −15 bp) resulted from activity of the second guide. Two of the deletion alleles (72 bp and 85 bp) were transmitted through the germline. Both alleles showed similar phenotypes but the 85 bp deletion allele, owing to its frameshift effect, was used in all further studies. The truncated *Prodh2* allele produced a band of 380 bp, while the WT allele produced a band of 467bp. These differences enabled the facile screening of the offspring to produce the following homozygous knockout animals: *Prodh2*, and *Grhpr*/*Prodh2*, and *Agxt*/*Prodh2.* The generation and characterization of the *Grhpr* KO mouse and *Agxt* KO mouse have been previously described [11, 12].

2.4. Metabolic cage collections

Animals, 12–16 weeks of age, were singly housed in Techniplast mouse metabolic cages for collection of three consecutive 24-hour urines. 24-hour urines were collected on 1 ml mineral oil to prevent evaporation and 50 μl 2% sodium azide to prevent bacterial growth. Animals had free access to water and a calcium-deficient, high-sucrose basal diet (TD.130032) designed by Envigo (Madison, WI), to which calcium chloride was added at 5 mg per gram dry diet. This custom diet contains 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.

2.5. Necropsy and tissue harvest

After completion of experiments, mice were anesthetized with isoflurane, and necropsy was performed. A terminal bleed was performed by left ventricle aspiration and blood collected in heparinized tubes. Plasma was stored at −80 °C. Liver and kidney were harvested immediately, flash frozen and stored in liquid nitrogen. One kidney from animals fed 1% Hyp was fixed in 4% buffered paraformaldehyde and embedded in paraffin and sectioned with a microtome. Liver and kidney sections were stained with hematoxylin and eosin for histopathology examination by the UAB Comparative Pathology Lab and Gnotobiotic Core.

2.6. Sample preparation and analytical measurements

For oxalate determination, part of the urine collection was acidified to pH <2.0 by adding 50 μL 2 M HCl to 950 μL urine prior to storage at −80 °C. This treatment is necessary to prevent oxalate crystallization that could occur with cold storage and/or oxalogenesis associated with storage under alkaline conditions. The remaining non-acidified urine was frozen at −80 °C for the measurement of other analytes. For measurement of plasma glycolate, samples were first filtered through Nano-sep centrifugal filters (VWR International, Batavia, IL) with a 10,000 nominal molecular weight limit to remove macromolecules prior to ion chromatography coupled with mass spectrometry (ICMS; Thermo Fisher Scientific Inc, Waltham, MA). Centrifugal filters were washed with 10 mM HCl prior to sample filtration to remove any contaminating trace organic acids trapped in the filter device. Plasma and urinary hydroxyproline was measured in 10% (w/v) trichloroacetic acid extracts by the Waters AccQ.Tag method (Milford, MA) according to the manufacturer's instructions. Urinary creatinine was measured on a chemical analyzer, and urinary oxalate by ICMS, as previously described [16]. Glycolate was determined by $^{13}C_2$-glycolate isotope dilution ICMS, with an AS15, 2 × 150 mm, anion exchange column (Thermo Fisher Scientific Inc), using IC and MS settings previously described [16]. Kidney oxalate content was determined by ICMS after grinding tissue to powder in liquid nitrogen and extracting oxalate from powdered tissue with continuous high speed vortexing in acid (one weight tissue + 2 volumes 1 M HCl) for 5 min at room temperature.

2.7. HYPDH protein expression levels via Western blot

Liver tissue was homogenized in iced cold RIPA buffer (Thermo Fisher Scientific Inc.) using probe sonication. The resulting lysate was boiled in Laemmli sample buffer for 5 min. The Western blot procedure and antibodies used have been previously described [17]. Pre-stained Precision Plus Protein Dual Color Standards (Bio-Rad) were used as molecular weight markers.

2.8. Expression of HYPDH in CHO cells

All cell culture reagents were purchased from Invitrogen. Full length HYPDH was cloned into a cDNA™3.1D/V5-His/*lac*Z vector with a BamHI restriction site using a pcDNA™3.1 Directional TOPO® Expression Kit (Cat # K4800–01SC). A CHO cell line expressing HYPDH was generated by stable transformation using Lipofectamine 3000 reagent (Cat # L3000015) following manufacturer's protocol. Transfected CHO cells were selected by limiting dilution and cultured in Ham's F12 medium (Cat # 10565–042) with 10% fetal bovine serum and Penicillin/Streptomycin. Selection pressure was maintained by adding 800 ug/ml G418 Sulfate (Cat # 10131027) to the media [16]. Expression levels of HYPDH were confirmed by Western Blot and found to be stable. CHO cells were received as a generous gift from the Danpure lab [18].

2.9. Statistical analysis

All graphing and statistical analyses were conducted using GraphPad Prism v.6 (GraphPad Software Inc., San Diego, CA). The mean of at least two 24-hr urine analyte determinations was used to characterize excretions in each mouse. Data are expressed as mean ± SD.

Student's *t*-test was used to compare sex and strain differences and impact of Hyp feeding. For some analyses, the Tukey's honestly significant difference (HSD) multiple-comparison tests (after one-way ANOVA) was used. The criterion for statistical significance was $P < 0.05$.

3. Results

3.1. HYPDH KO mice are normal.

Mice deficient in the *Prodh2* gene product HYPDH were generated to evaluate how preventing Hyp metabolism alters metabolite urine profiles. Multiple alleles in exon 2 of the *Prodh2* gene were generated and evaluated (Fig. 2A; see Methods for additional details). Allele 1 was chosen to be propagated, as it contained an 85 bp deletion resulting in a frameshift of the HYPDH protein coding sequence. For simplicity, these animals will be referred to as the HYPDH KO mice. The HYPDH KO animals developed normally and exhibited similar behavior to wild-type (WT) animals. Liver and kidney H&E analysis performed by the UAB Comparative Pathology Lab and Gnotobiotic Core showed normal histology. HYPDH KO mice, both male and female, were similar to WT mice in their weight gain and their urinary volume and creatinine excretions (Table 1). The liver tissue lysates lacked HYPDH expression, when compared to CHO cells that express the full-length HYPDH protein as a positive control (Fig. 2B). As expected, the plasma Hyp and urinary Hyp excretion levels were significantly elevated in HYPDH KO mice compared to WT animals of both sexes (Table 1, $P < 0.001$).

3.2. Male HYPDH KO mice have lower urinary oxalate and glycolate levels.

The excretions of oxalate and glycolate in male WT and HYPDH KO mice are compared in Fig. 3, using a low-oxalate, no-Hyp diet. Male HYPDH KO mice had an 18% lower urinary excretion of oxalate compared with WT mice ($P = 0.002$) and a 20% lower urinary glycolate excretion ($P = 0.014$). Intermediate reductions for these metabolites were not seen in the heterozygous mice (Fig. 3). Urinary oxalate and glycolate excretions in female HYPDH KO mice were not significantly different from WT animals (WT vs. KO, 90 ± 15 vs. 79 ± 7 μg oxalate/mg creatinine, $P = 0.13$; WT vs. KO, 438 ± 83 vs. 360 ± 64 μg glycolate/mg creatinine; $P = 0.058$). A pronounced gender effect was observed with urinary glycolate excretions. Mean excretion was 4-fold higher in WT female mice compared to WT male mice (438 ± 83 vs. 110 ± 22 μg glycolate/mg creatinine, respectively; $P < 0.001$). This 4-fold difference in urinary glycolate excretion was also seen between the female and male HYPDH KO mice.

3.3. HYPDH KO lowers urinary oxalate levels in PH1 and PH2 mouse models.

In order to evaluate the contribution of Hyp metabolism to oxalate and glycolate levels in the PH1 and PH2 mouse models, double KO animals were generated (AGT-HYPDH and GR-HYPDH). The double HOGA-HYPDH KO model was not generated, as the HOGA KO mouse (PH3 model) is not hyperoxaluric and does not recapitulate the human phenotype [13].

As previously reported [11, 12], AGT KO mice and GR KO mice had an increased urinary oxalate excretion compared to WT mice, with male animals excreting a greater amount of urinary oxalate compared with females (Figs. 4 & 5). This gender difference was particularly evident between male and female AGT KO animals (males excreted 2-fold more than females). Independent of the mouse sex or PH-causing gene deletion, the removal of HYPDH reduced urinary oxalate excretions 18 – 30%. Despite these significant reductions, urinary oxalate excretions were still above normal levels. Knockout of the HYPDH enzyme also lowered urinary glycolate levels in the male and female AGT KO mice (Fig. 4), consistent with the contribution of Hyp metabolism to glyoxylate production. It also makes sense that with the loss in GR activity, the GR-HYPDH KO mice do not show changes in urinary glycolate levels (Fig. 5).

3.4. HYPDH KO prevents hyperoxaluria and renal oxalosis upon 1% Hyp feeding.

Hyperoxaluria and the deposition of calcium oxalate crystals in the kidney can be induced in mice and rats with 1% Hyp feeding [17, 19]. Hyp feeding resulted in significant differences in urinary oxalate and glycolate excretion between WT, HTZ and KO animals ($P<0.0001$), with WT and HTZ mice excreting 4 to 6-fold higher urinary oxalate and 9 to 15-fold higher glycolate levels compared to HYPDH KO mice (Fig. 6 A & B).

To determine if the loss of HYPDH can prevent oxalate crystal deposition, male WT, HYPDH KO, GR KO and GR-HYPDH KO mice were fed 1% Hyp for 3 weeks. Whereas profound renal oxalosis was observed with Hyp feeding in GR KO mice, consistent with our previous results [17], kidney oxalate levels in the HYPDH KO and GR-HYPDH KO mice were ~4-fold and ~1,300-fold lower, respectively (Fig. 6C).

4. Discussion

Two enzymes, HYPDH and HOGA, (Fig. 1) have been shown to be unique to the Hyp metabolic pathway shown [1]. Counter-intuitively, a deficiency in HOGA activity can increase oxalate synthesis rather than decreasing it and was identified as the cause of PH3 [20]. PH3 was initially thought to be relatively benign, apart from an increased incidence of calcium oxalate stone disease [21, 22]. It is now evident, however, that PH3 can be associated with advanced kidney disease in some individuals, particularly those with other co-morbidities [23, 24]. Moreover, carrier frequency and prevalence analyses gleaned from available DNA databases indicate that the PH3 genotype is more prevalent than PH1 and PH2 [25]. Whether all individuals with the PH3 genotype have hyperoxaluria, however, has not been ascertained. There is concern that some affected individuals may be currently unrecognized in idiopathic stone disease and chronic kidney disease populations.

Loss of HOGA enzymatic activity results in the buildup of HOG and its precursor 4-hydroxy-glutamate in urine and plasma [5, 26, 27]. HOG has also been shown to be an inhibitor of GR [5], which may increase oxalate synthesis by limiting the conversion of glyoxylate to glycolate. While it is still unclear how this metabolism leads to the hyperoxaluric PH3 phenotype, it is clear that blocking Hyp metabolism by inhibiting HYPDH activity would prevent the formation of glyoxylate, oxalate and all precursors

including HOG. In the current study, we evaluated the metabolic consequences of deleting the HYPDH protein in mouse models of PH.

Consistent with the deletion of HYPDH in humans, the knockout of the *Prodh2* gene in mice had no effect on viability, litter sizes, and behavior. The only consequence of the absence of HYPDH enzymatic activity was a high level of Hyp in urine and plasma (Table 1). When challenged with 1% Hyp diet, the knockout of HYPDH prevents the elevation of urinary oxalate and glycolate (Fig. 6). This finding indicates that complete absence of HYPDH expression in the kidney and liver is required to prevent hyperoxaluria in this extreme challenge model. These findings also support the notion that HYPDH is the only enzyme that is capable of performing the first step of the Hyp degradation pathway. The absence of HYPDH in male mice lowered urinary oxalate and glycolate excretions by 18% and 20%, respectively (Fig. 3), when on a diet low in oxalate and lacking Hyp. These results are consistent with the endogenous contribution of Hyp catabolism to oxalate in male mice (22%) [28]. These findings indicate other sources of oxalate and glycolate remain to be identified. These sources could include glyoxal [29], phosphoglycolate [30], and ascorbic acid [31]. In normal individuals and PH patients of both sexes, the contribution of Hyp to urinary oxalate ranges from 14–47% [3]. Therefore, we were surprised to observe that the deletion of HYPDH in the female mice did not lower urinary oxalate. This latter finding may be related to the lower plasma hydroxyproline (Table 1), pronounced elevation of glycolate production (Table 1) and other metabolic differences in kidney function between male and female mice. Extensive studies are needed to further understand these sex differences in mice. Sex differences have not been consistently observed or extensively evaluated in individuals with PH and have not been reported in European or US PH registries. It was reported that there was no sex difference in the progression to ESRD in the US registry [Zhao F, Bergstralh EJ et al CJASN]. Reasons for this may pertain to the variability in excretions observed in individuals of the same genotype as discussed by Hopp et al. [25].

Importantly, 18–30% reductions in urinary oxalate and glycolate (Figs. 4 & 5) were observed in the male and female, double knockout mice (AGT-HYPDH and GR-HYPDH) on the low-oxalate diet. These reductions in urinary oxalate did not reach the normal range, but nonetheless would represent a significant improvement for PH1 and PH2 patients. The protective effect of the HYPDH KO is further demonstrated by blocking renal oxalosis on the 1% Hyp challenge diet (Fig. 6). Therefore, use of a 1% Hyp diet in HYPDH KO and GR-HYPDH mice should be useful in identifying and validating HYPDH inhibitors that prevent the early stages of calcium oxalate tissue deposition. It should also be noted that for PH3 patients, monitoring the reduction in urinary and plasma levels of HOG, 4-hydroxyglutamate, and dihydroxyglutarate, may also prove to be useful for validation and efficacy studies.

Based on the studies presented herein and the available metabolic tracer studies in humans, inhibition of HYPDH should be of therapeutic benefit to PH2 and PH3 patients [3]. Further support for HYPDH as a molecular target extends from the absence of any pathology in humans who do not express HYPDH [10]. Even the modest reduction in urinary oxalate level afforded to a PH1 patient by HYPDH targeting should be considered, especially given the interrelationship between glyoxylate and glycolate metabolism. The rationale for PH2

and PH3 patients is stronger because of the fact that Hyp metabolism and glyoxylate metabolism occurs in the kidney and liver. This contrasts with PH1 patients, where the metabolic consequences of the AGT defect are liver centric. Given the significantly elevated levels of glycolate in PH1 patients, it is intriguing to speculate that the current GO-targeted siRNA approach by Alnylam Pharmaceuticals [32] may benefit from blocking the production of glycolate via HYPDH inhibition. In a similar manner, blocking the flow of Hyp metabolites to glyoxylate and oxalate may also prove to be useful in ongoing trials by Dicerna Pharmaceuticals that target LDHA [33]. A broader question of interest is why the Hyp catabolic pathway has been preserved, given that the loss of HYPDH activity in mice and human is well tolerated. Cooper et al. suggested it may act as a sensor of excessive extracellular matrix degradation and trigger apoptosis [7]. Recent studies have also shown that Hyp can be degraded in the gut by common anaerobic microbes, suggesting that a symbiotic relationship may exist [34]. Taken together, this discussion points to the need for future studies in evaluating the Hyp degradation pathway, especially as it relates to PH.

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Highlights

- Hydroxproline metabolism contributes to oxalate production
- Hydroxyproline dehydrogenase (HYPDH) is a target for treating primary hyperoxaluria
- Deletion of HYPDH in mice increases hydroxyproline in urine and plasma
- Urine oxalate and glycolate levels can be lowered by deletion of HYPDH
- Loss of HYPDH can protect GRHPR KO mice from calcium oxalate crystal formation

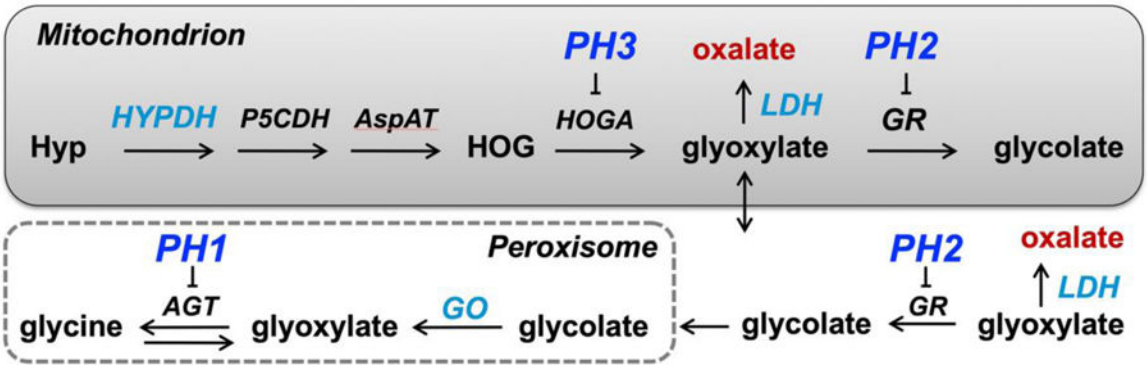

Figure 1.

Hydroxyproline metabolism in hepatocytes and deficiencies associated with Primary Hyperoxaluria. Abbreviations: hydroxyproline dehydrogenase (HYPDH), Δ1-pyrroline-5-carboxylate dehydrogenase (1P5CDH), aspartate aminotransferase (AspAT), 4-hydroxy-2-oxoglutarate (HOG), 4-hydroxy-2-oxoglutarate aldolase (HOGA1), lactate dehydrogenase (LDH), glyoxylate reductase (GR), glycolate oxidase (GO).

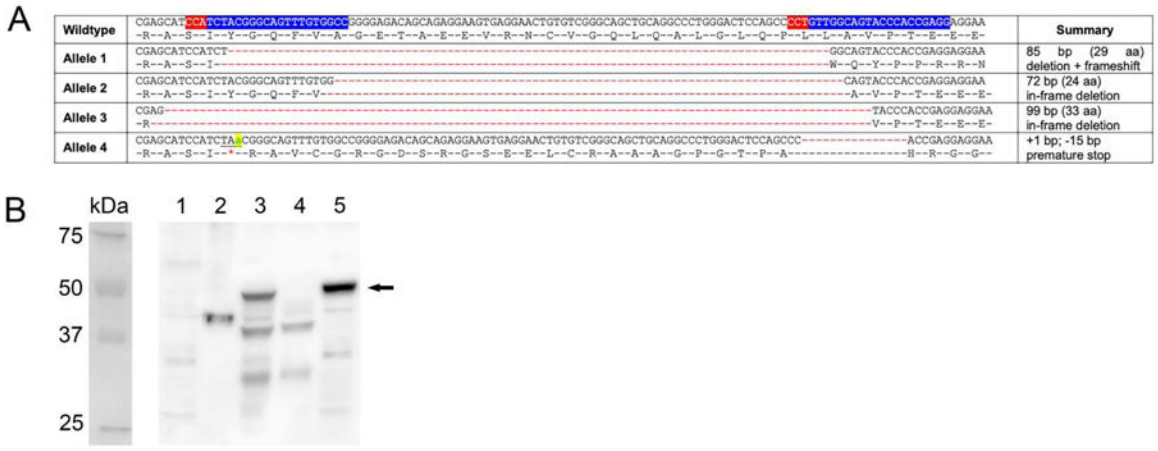

Figure 2.

Confirmation of *Prodh2* mouse KO. Panel A: Genetic analysis of CRISPR-Cas9-derived alleles. Allele 1 was propagated to generate homozygous mice; see text for additional details. The sequences highlighted in blue are the CRISPR targets and those in red are the PAM sites. Panel B: Western blot analysis to confirm loss of HYPDH protein. Left panel, pre-stained markers. Right panel, Western blot; Lane 1, Chinese Hamster Ovary cultured cells (CHO). Lane 2, Recombinant human HYPDH (truncated protein; core domain residues 157–515; 40 kDa). Lane 3, wildtype mouse liver. Lane 4, *Prodh2* KO mouse liver. Lane 5, CHO cells overexpressing full-length HYPDH (50 kDa). Arrow indicates full length HYPDH protein.

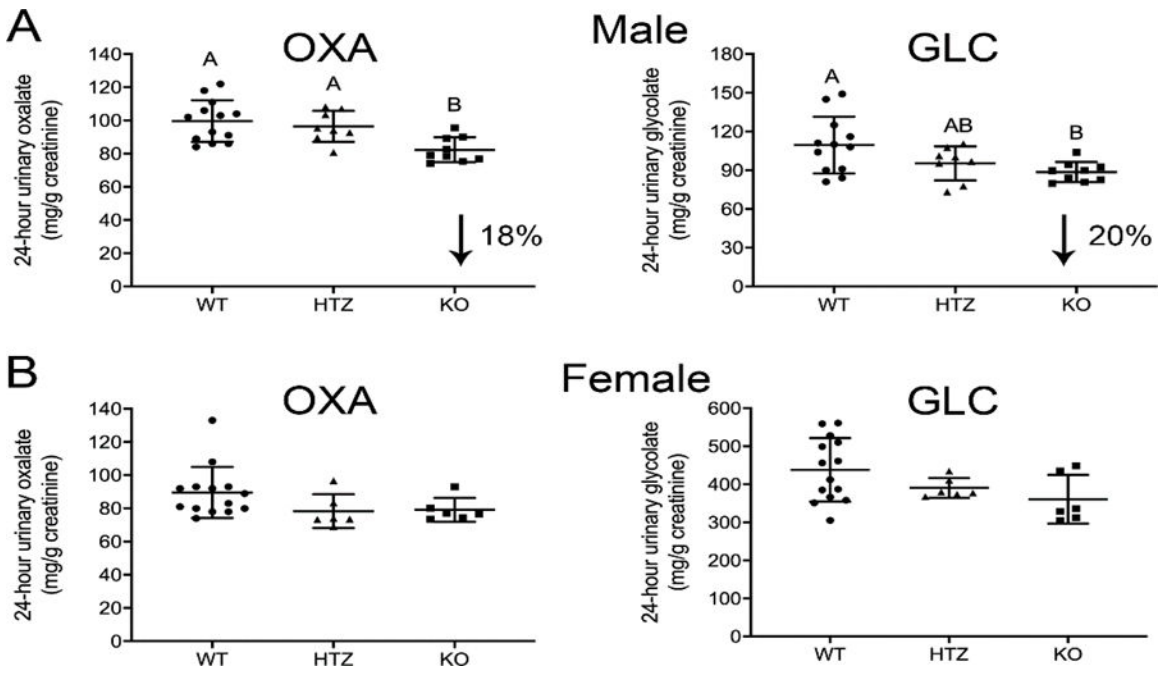

Figure 3.

24-hour urine analyses for HYPDH mouse variants. Panel A: Male mice with wildtype (WT), heterozygous (HTZ) or homozygous (KO) for the deletion of the HYPDH protein. Panel B: Female mice with the same genotypes. Abbreviations used: oxalate (OXA), glycolate (GLC). Abbreviations used: oxalate (OXA), glycolate (GLC). Different capitalized letters indicate the significance ($P < 0.05$) of Tukey's honestly significant difference (HSD) multiple-comparison tests (after one-way ANOVA); see main text for details and P values. Bars represent means ± SD; n= 6 – 14. The percentage reduction relative to the WT animals are indicated where significant.

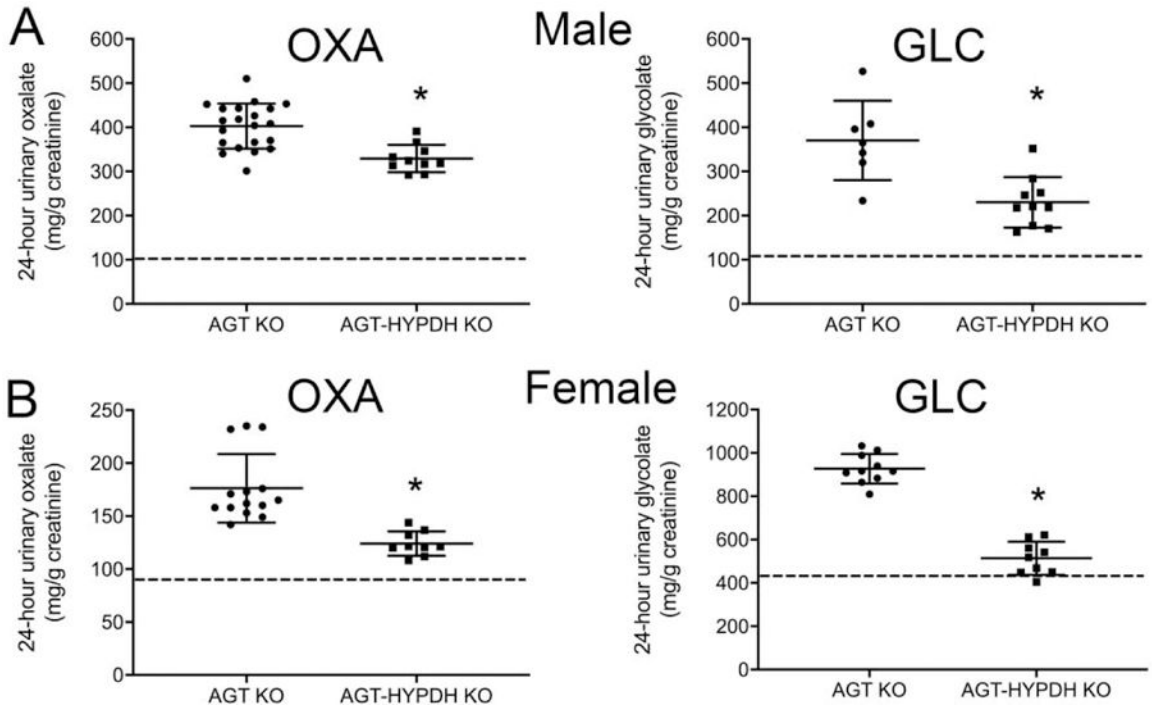

Figure 4.

Urinary excretions of AGT KO mice with and without HYPDH expression. Panel A: Oxalate and glycolate urine levels for male mice. Panel B: the same metabolites for female mice. *P < 0.05 between AGT KO and AGT-HYPDH KO animals analyzed by Student t-test. Bars represent means ± SD; n = 7 – 21. Dotted line represents mean excretions of WT animals.

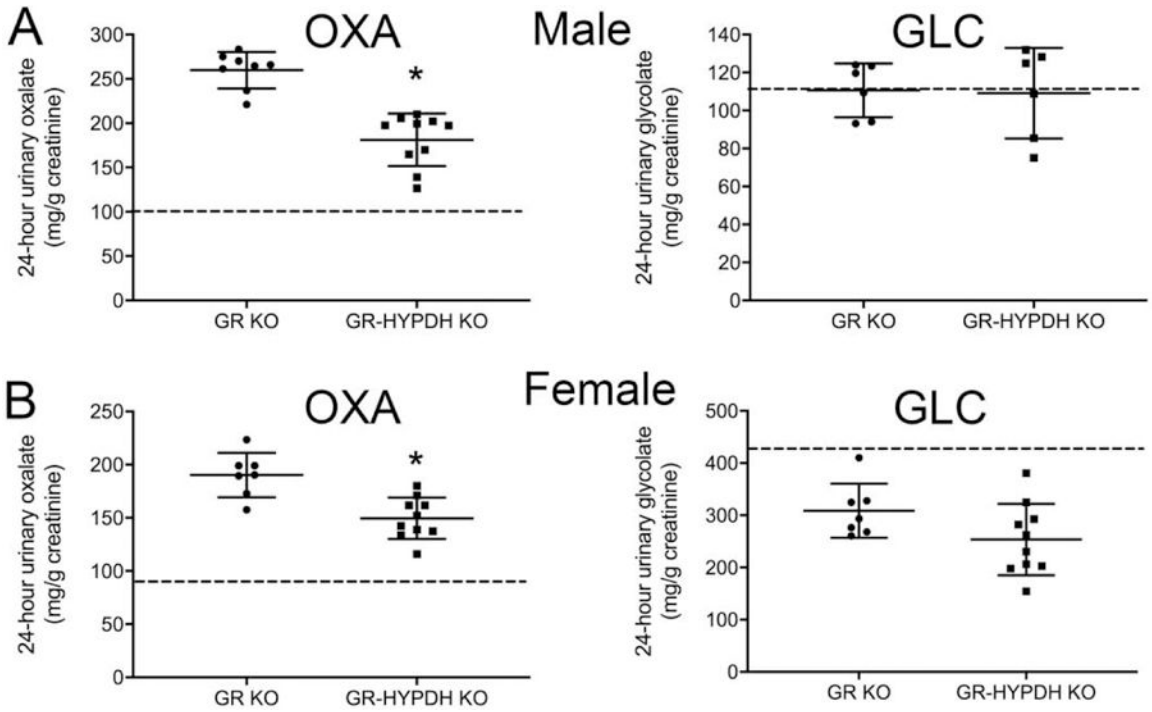

Figure 5.

Urinary excretions of GR KO mice with and without HYPDH expression. Panel A: Oxalate and glycolate urine levels for male mice. Panel B: the same metabolites for female mice. *P < 0.05 between GR KO and GR-HYPDH KO animals analyzed by Student t-test. Bars represent means ± SD; (n = 7 – 10). Dotted line represents mean excretions of WT animals.

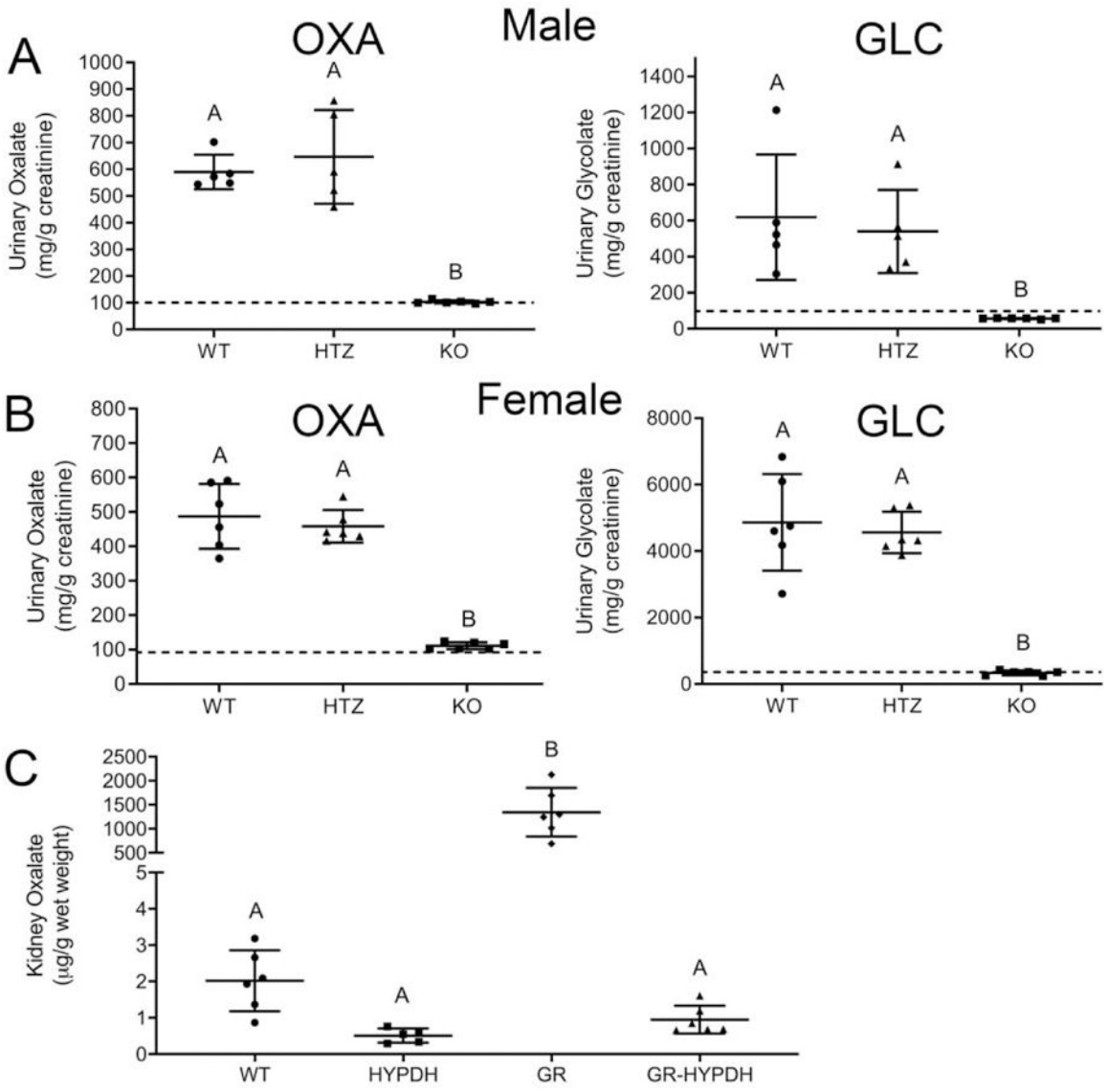

Figure 6.

Assessment of hyperoxaluria and oxalate crystal deposition in a variety of mouse strains fed 1% Hyp. Panel A: 24-hour urine analyses for male mice with wildtype (WT), heterozygous (HTZ) or homozygous (KO) for the deletion of the HYPDH protein. Panel B: Female mice with the same genotypes. Panel C: Kidney oxalate levels in male WT, HYPDH KO, GR KO, and GR-HYPDH KO mice following 1% Hyp feeding for 3 weeks. Different capitalized letters indicate the significance ($P < 0.05$) of Tukey's honestly significant difference (HSD) multiple-comparison tests (after one-way ANOVA); see main text for details and P values. Bars represent means ± SD; n = 5 – 6. The dotted lines represent urinary excretions of WT mice not fed 1% Hyp.

Table 1.

Plasma and 24-h urine parameters on custom-purified, low-oxalate diet in WT and HYPDH KO mice.

Group	MALE			FEMALE		
	WT	HYPDH KO	P value	WT	HYPDH KO	P value
N	13	9		14	6	
Age (weeks)	15 ± 2	14 ± 2	0.75	16 ± 2	16 ± 2	0.79
Body weight (g)	28 ± 3	26 ± 1	0.13	21 ± 1	20 ± 0.8	0.50
24 hr Urine volume (ml)	2.2 ± 0.4	2.5 ± 0.4	0.11	1.8 ± 0.4	2.0 ± 0.6	0.36
24 hr Urinary Creatinine (mg)	0.45 ± 0.05	0.45 ± 0.03	0.71	0.32 ± 0.08	0.30 ± 0.03	0.64
24-hr Urinary Hyp (μg)	<19	992 ± 303	<0.001	<19	466 ± 94	<0.0001
Plasma Hyp (μM)	23±3	479 ± 90	<0.001	22 ± 3	360 ± 45	<0.001

Results presented are means ± SD.