Oxalate Production in Primary Hyperoxaluria Types 1, 2 and 3 and the Role of LDH in the Liver

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Overview

- Hydroxyproline metabolism in liver: enzymes and intermediates and alternative pathways
  - PH and the roles of GO (Glycolate Oxidase) and LDH (Lactate Dehydrogenase) enzymes in the liver
- Genetic mutations associated with PH1, PH2, and PH3 and oxalate production
  - Potential metabolic pathway changes after reducing LDH activity in the liver, including NMD-PH
- LDH (Lactate Dehydrogenase) enzyme isoforms and tissue expression
  - Focus on the LDH5 tetrameric isoform of LDH expressed from the LDHA gene in liver
- Tolerability of LDH5 as a drug target based on humans completely deficient in LDHA gene activity
- Mouse models of PH versus human Primary Hyperoxalurias
- Hypothesis – Reduction of LDH activity in liver might be an effective treatment for all forms of PH

PH and Glycolate Oxidase and Lactate Dehydrogenase in the Liver

GO = Glycolate Oxidase and LDH = Lactate Dehydrogenase

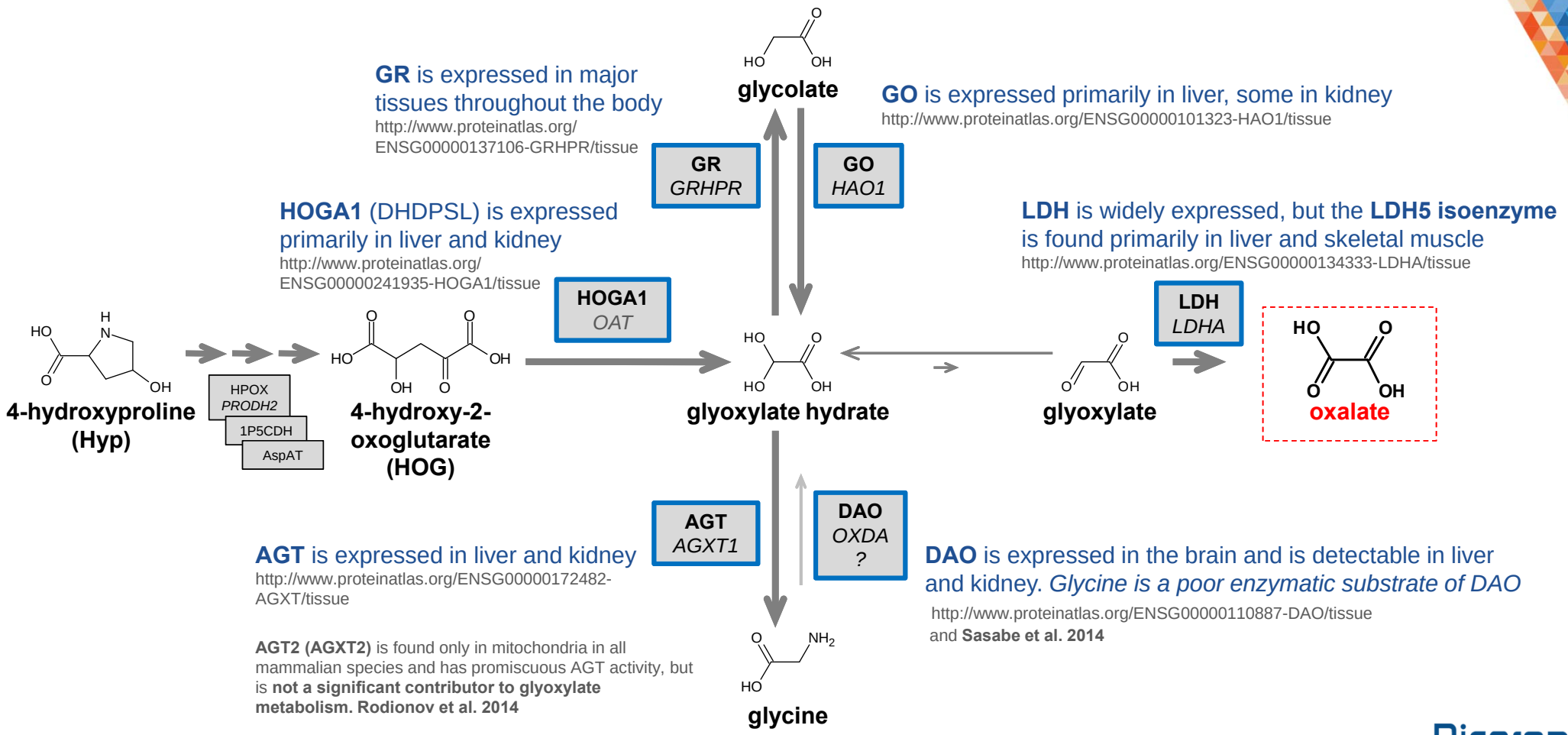
- Focus on *human data and human-derived systems* to understand PH1, PH2 and PH3 (and NMD-PH)
  - Most references in the following figures are based on healthy volunteers or PH patients
  - Some references are based on human cells or purified human proteins under physiological conditions
  - Conflicting results due to species differences or non-physiological conditions have been reduced

- GO activity on glycolate increases the pool of *glyoxylate*, the immediate precursor of oxalate
  - Increased *glyoxylate* is converted to oxalate by LDH (Lactate Dehydrogenase) activity in the liver
  - GO activity on *glyoxylate* is not a significant source of oxalate production
  - GO inhibition should reduce oxalate in PH1, but is unlikely to reduce oxalate in PH2, PH3 or NMD-PH

- Targeting LDH in the liver instead of GO might result in deeper suppression of oxalate production
  - Glycolate and glyoxylate sources other than GO and HOGA1 activities might contribute to oxalate production
  - LDH activity in the liver (LDH5) on *glyoxylate* is the ultimate source of oxalate production in the liver
  - Reduction of LDH activity in the liver is hypothesized to treat all forms of Primary Hyperoxaluria

Hydroxyproline Metabolism and Urinary Oxalate in Humans

Urinary oxalate has multiple sources, but hydroxyproline metabolism is the most significant



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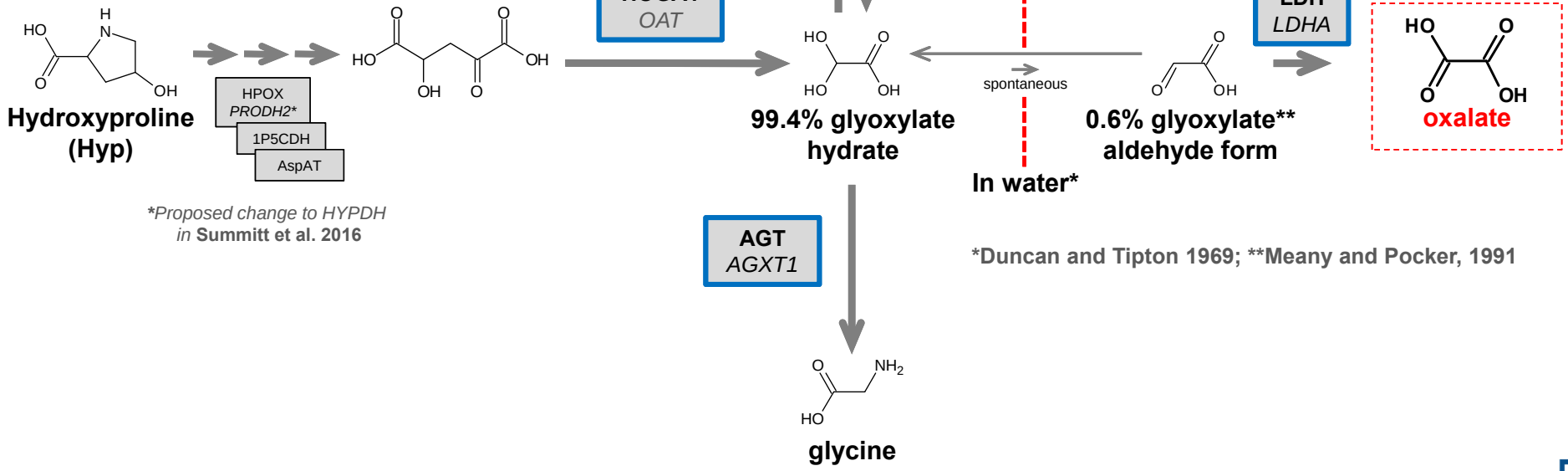
Hydroxyproline Metabolism and Normal Urinary Oxalate in Humans

Urinary oxalate has multiple sources, but hydroxyproline metabolism is considered the most significant

GR preferentially converts glyoxylate to glycolate over LDHA conversion of glyoxylate to oxalate under typical cellular conditions. *Pure human proteins*, **Mdluli (Rumsby) et al. 2005**

"…oxidation of glycolate to glyoxylate is the primary reaction catalyzed by GO, …oxidation of glyoxylate to oxalate (by GO) is most likely not relevant under normal conditions." *Using pure human GO*. **Murray, Holmes and Lowther 2008**

Endogenous + dietary collagen yields an average of **~500 mg of hydroxyproline per day, up to ~280 mg glyoxylate per day**. *In humans*, **Jiang et al. 2011**



Mdluli et al. 2005, Knight et al. 2009, Holmes and Assimos 1998, others

*Proposed change to HYPDH *in* **Summitt et al. 2016**

*Duncan and Tipton 1969; **Meany and Pocker, 1991

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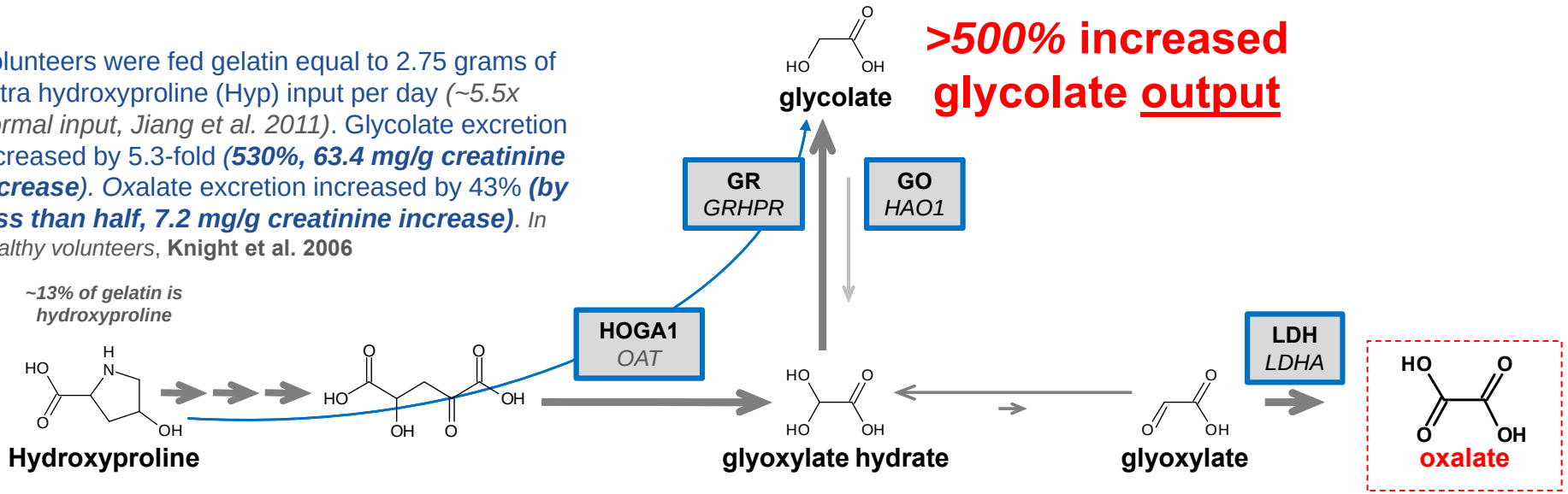
Hydroxyproline Challenge Results in NHV: Glycolate versus Urinary Oxalate

Observations of healthy volunteers fed high levels of hydroxyproline (Hyp) in the form of gelatin

Volunteers were fed gelatin equal to 2.75 grams of extra hydroxyproline (Hyp) input per day *(~5.5x normal input, Jiang et al. 2011)*. Glycolate excretion increased by 5.3-fold ***(530%, 63.4 mg/g creatinine increase)***. Oxalate excretion increased by 43% ***(by less than half, 7.2 mg/g creatinine increase)***. *In healthy volunteers*, **Knight et al. 2006**



~550% more Hydroxyproline input than normal

***<50%* increased oxalate output**

Glycolate output was 10 times greater than oxalate output

*Glycine not measured in **Knight et al. 2006**, supported by healthy volunteer measurements in **Knight et al. 2009***

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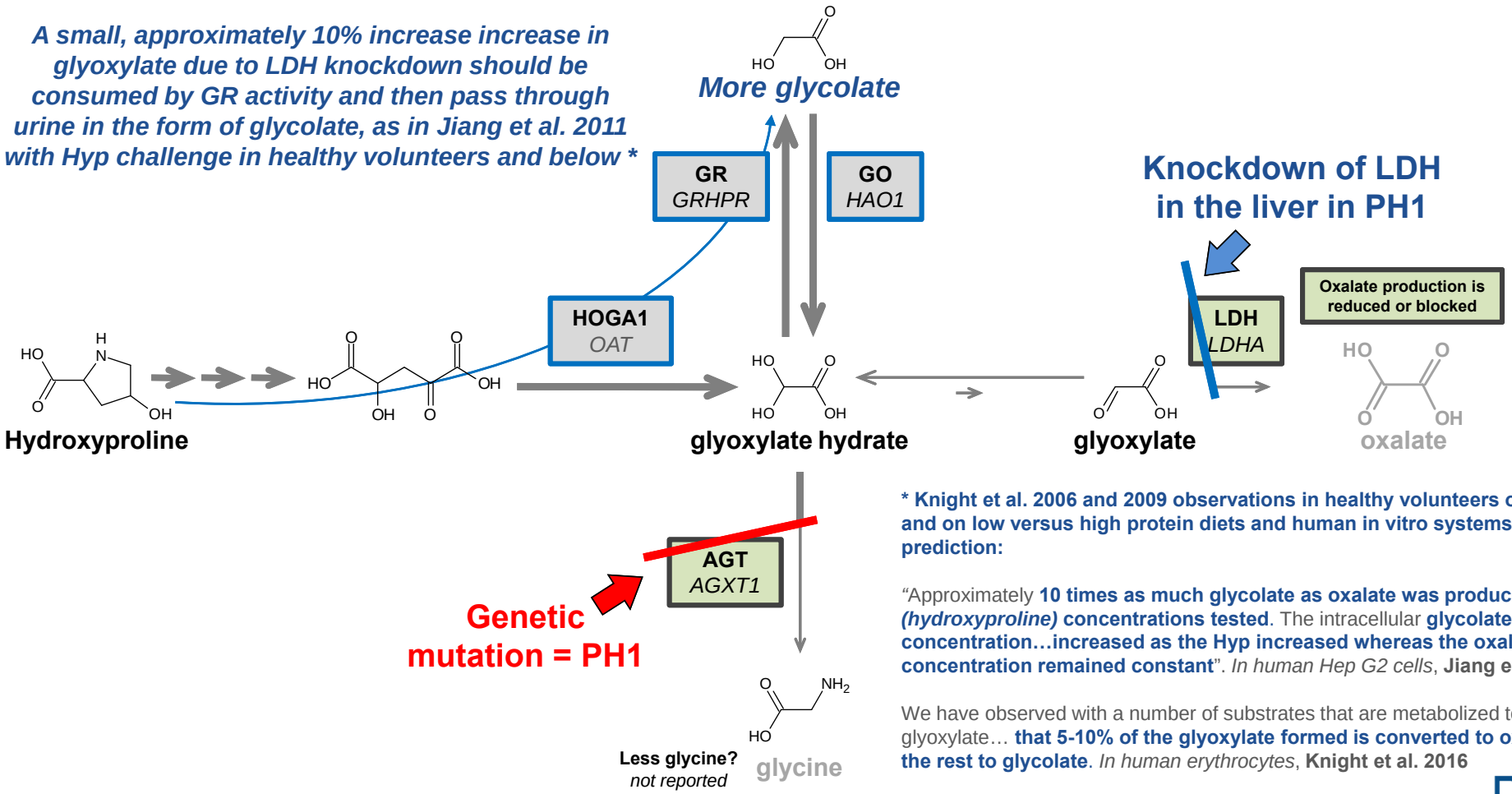
Potential Oxalate Reduction in PH1 Patients with Reduced LDHA in Liver

An insignificant increase in glycolate is predicted when oxalate production is blocked in PH1 patients

A small, approximately 10% increase increase in glyoxylate due to LDH knockdown should be consumed by GR activity and then pass through urine in the form of glycolate, as in Jiang et al. 2011 with Hyp challenge in healthy volunteers and below *



*** Knight et al. 2006 and 2009 observations in healthy volunteers on gelatin and on low versus high protein diets and human in vitro systems support this prediction:**

"Approximately **10 times as much glycolate as oxalate was produced at all Hyp *(hydroxyproline)* concentrations tested**. The intracellular **glycolate concentration…increased as the Hyp increased whereas the oxalate concentration remained constant**". *In human Hep G2 cells*, **Jiang et al. 2011**

We have observed with a number of substrates that are metabolized to glyoxylate… **that 5-10% of the glyoxylate formed is converted to oxalate and the rest to glycolate**. *In human erythrocytes*, **Knight et al. 2016**

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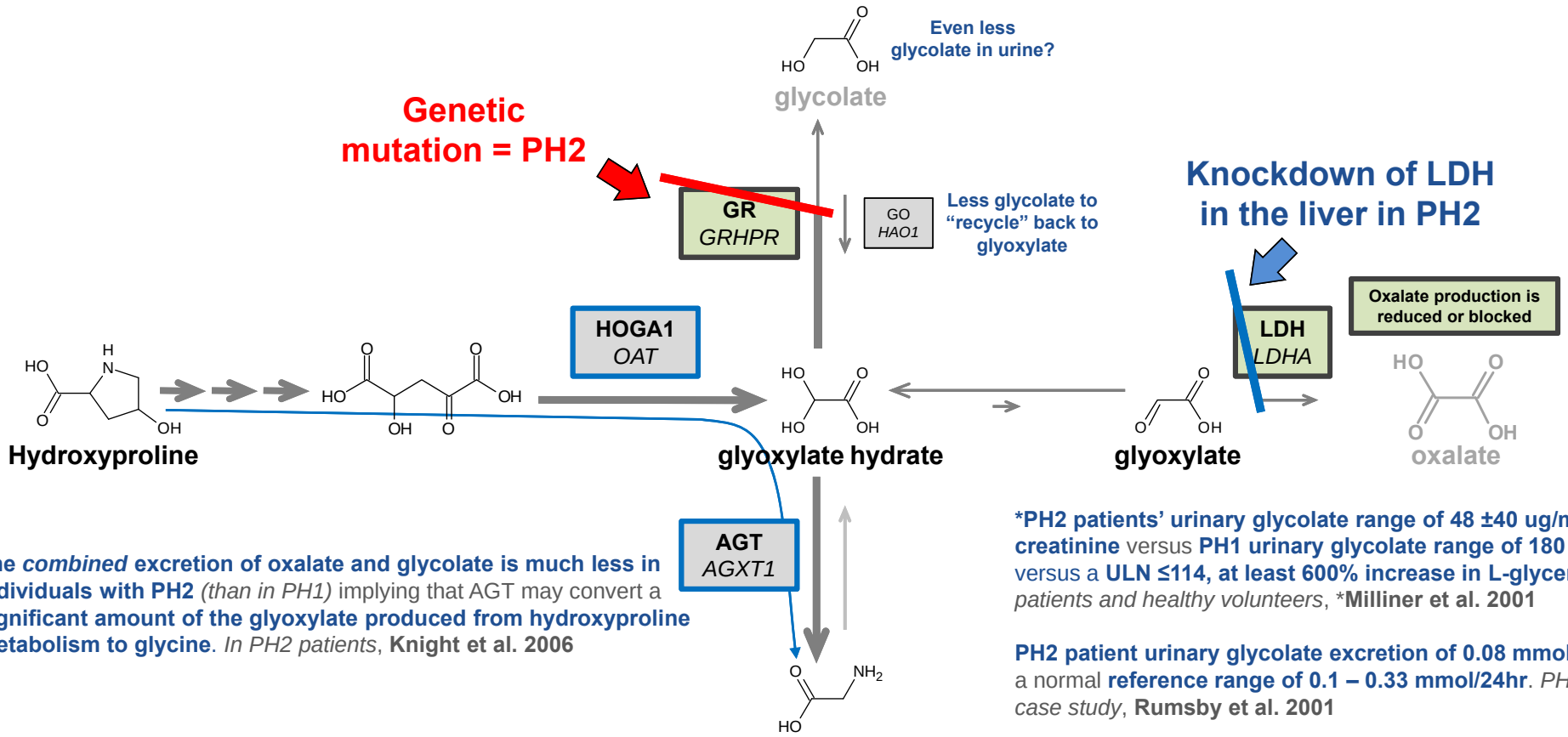
Potential Oxalate Reduction in PH2 Patients with Reduced LDHA in Liver

As the pathway predicts, PH2 patients have lower baseline $U_{glycolate}$ and lower $U_{oxalate}$ than PH1 patients



The *combined* excretion of oxalate and glycolate is much less in individuals with PH2 *(than in PH1)* implying that AGT may convert a **significant amount of the glyoxylate produced from hydroxyproline metabolism to glycine**. *In PH2 patients*, **Knight et al. 2006**

***PH2 patients' urinary glycolate range of 48 ±40 ug/mg creatinine** versus **PH1 urinary glycolate range of 180 ±148 ug/mg** versus a **ULN ≤114, at least 600% increase in L-glycerate**. *In PH patients and healthy volunteers*, ***Milliner et al. 2001**

PH2 patient urinary glycolate excretion of 0.08 mmol/24hr versus a normal **reference range of 0.1 – 0.33 mmol/24hr**. *PH2 patient case study*, **Rumsby et al. 2001**

Possible markers: Glycine, L-glycerate or hydroxypyruvate changes

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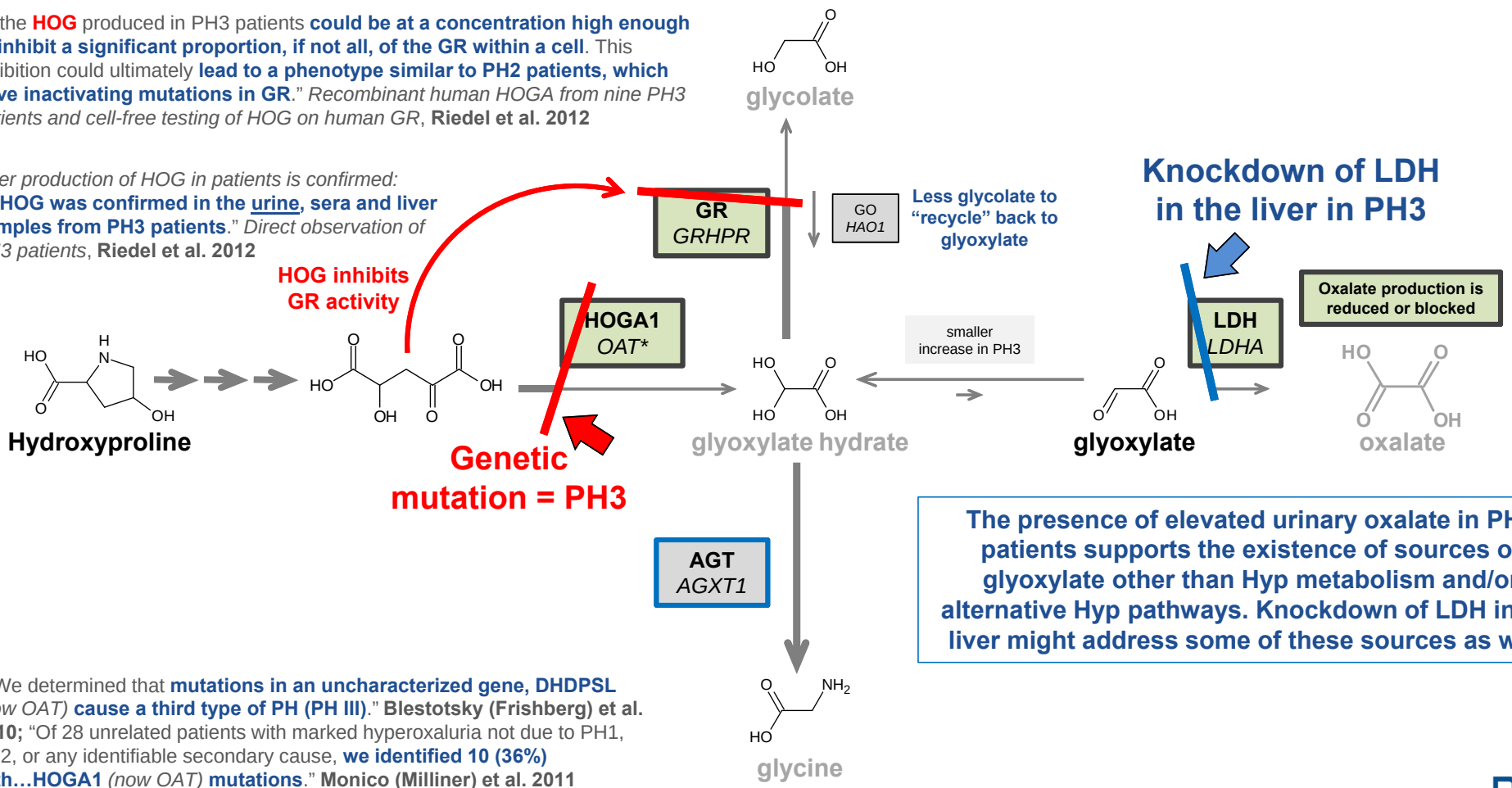
Potential Oxalate Reduction in PH3 Patients with Reduced LDHA in Liver

PH3 patients have a metabolite-induced systemic GR deficiency and a milder phenotype than PH2 patients

"…the **HOG** produced in PH3 patients **could be at a concentration high enough to inhibit a significant proportion, if not all, of the GR within a cell**. This inhibition could ultimately **lead to a phenotype similar to PH2 patients, which have inactivating mutations in GR**." *Recombinant human HOGA from nine PH3 patients and cell-free testing of HOG on human GR*, **Riedel et al. 2012**

Over production of HOG in patients is confirmed:
*"…**HOG was confirmed in the urine, sera and liver samples from PH3 patients**." Direct observation of PH3 patients*, **Riedel et al. 2012**



The presence of elevated urinary oxalate in PH3 patients supports the existence of sources of glyoxylate other than Hyp metabolism and/or alternative Hyp pathways. Knockdown of LDH in the liver might address some of these sources as well.

* "We determined that **mutations in an uncharacterized gene, DHDPSL** *(now OAT)* **cause a third type of PH (PH III)**." **Blestotsky (Frishberg) et al. 2010;** "Of 28 unrelated patients with marked hyperoxaluria not due to PH1, PH2, or any identifiable secondary cause, **we identified 10 (36%) with…HOGA1** *(now OAT)* **mutations**." **Monico (Milliner) et al. 2011**

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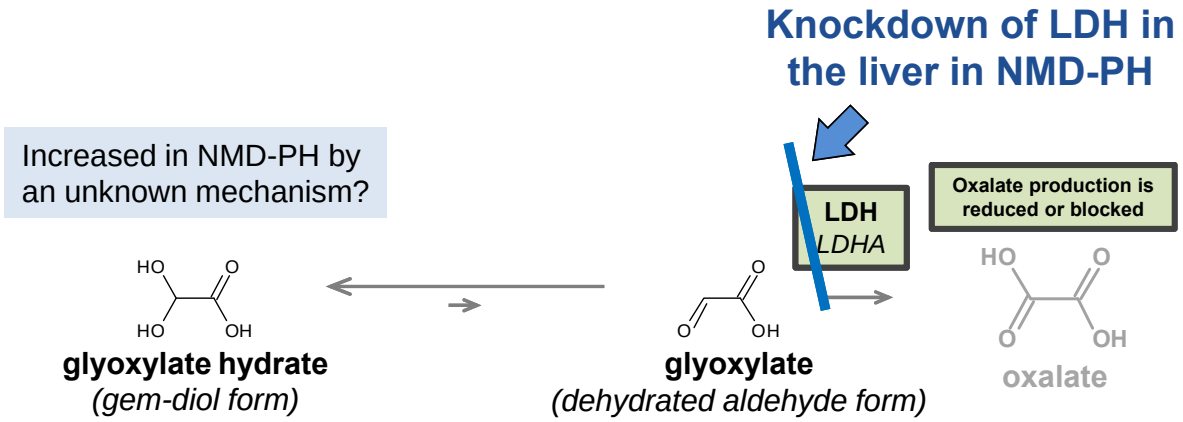
Idiopathic Primary Hyperoxaluria or NMD-PH (No Mutation Detected-PH)

The genetic lesion(s) underlying NMD-PH are unknown, some cases might be occult secondary hyperoxaluria

"Of 28 unrelated patients with marked hyperoxaluria **not due to PH1, PH2, or any identifiable secondary cause**, we identified 10 *(36%)* with HOGA1 mutations." **Monico (Milliner) et al. 2011**

18 out of 28 non-PH1, non-PH2 patients were <u>also</u> non-PH3 patients



***<u>Hypothesis</u>*: In patients with no identifiable source of secondary hyperoxaluria and no known oxalate pathway mutations detected (NMD-PH patients), there are one or more sources of elevated glyoxylate in the liver due to unknown metabolic pathway or intracellular transporter mutations.**

Reduction of LDH activity in the liver of NMD-PH patients might reduce oxalate production.

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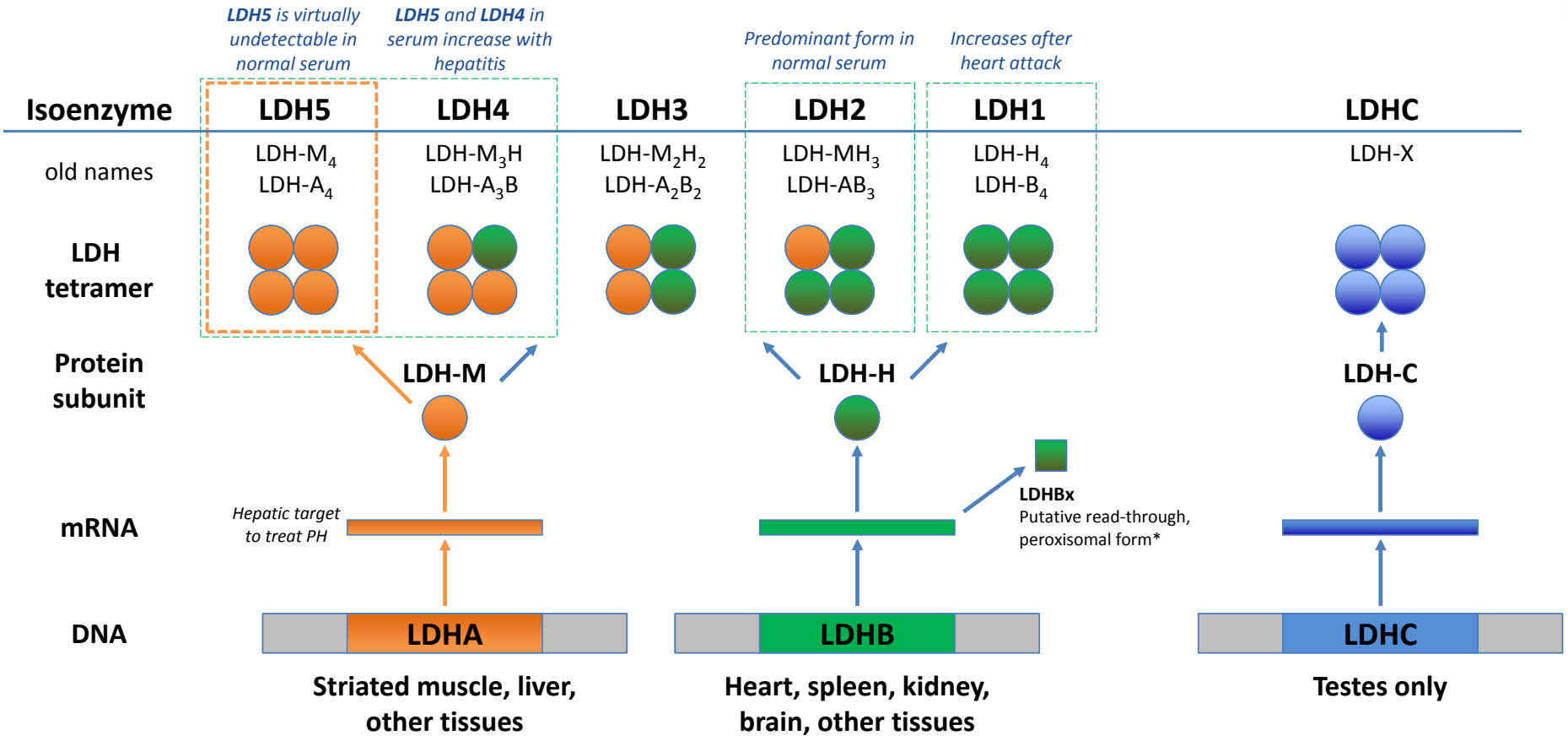
Human Lactate Dehydrogenase (LDH) Proteins and Nomenclature

LDHA and LDHB are expressed at variable ratios throughout the body, LDHC is testes-specific



*Adapted from Valvona et al. Brain Pathology 2016;**26**:3-17 and Granchi et al. Fut Med Chem 2013;**5**:1967-1991 with additional information; * <1% in Schueren et al. eLife 2014;3:e03640*

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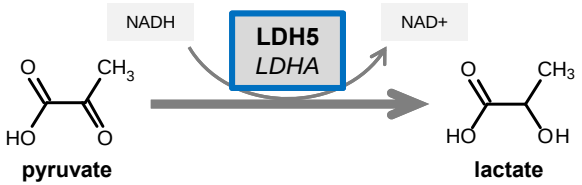
LDH Activity in the Liver is Not Required for Normal Liver Function

LDH5-deficient humans have no detectable hepatic effects or alteration of serum chemistry



- LDHA deficiency has been characterized in humans; these individuals have only LDHB
- Systemic LDHA deficiency does not alter baseline blood chemistry or cause any other effects except rare muscle symptoms due to LDHA deficiency in muscle
- Pharmaceutical companies are researching small molecule inhibitors of LDHA to treat serious diseases other than PH

- Miyajima et al. Characterization of the glycolysis in lactate dehydrogenase A deficiency. Muscle & Nerve 1995;18:874-878
- Takahashi et al. Genetic analysis of a family of lactate dehydrogenase A subunit deficiency. Internal Med 1995;34:326-329
- Pretsch et al. A mutation affecting the lactate Takayasu et al. Hereditary lactate dehydrogenase M-subunit deficiency: lactate dehydrogenase activity in skin lesions and in hair follicles. J Am Acad Dermatol 1991;24:339-342
- Mutation affecting the lactate dehydrogenase locus Ldh-1 in the mouse. Genetics 1993;135:161-170.
- Maekawa et al. Laboratory and clinical features of lactate dehydrogenase subunit deficiencies. Clinica Chimica Acta 1989;185:299-308
- Kanno et al. Lactate dehydrogenase M-subunit deficiency: a new type of hereditary exertional myopathy. Clinica Chimica Acta 1988;173:89-98.
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Future Med Chem. 2013 October ; 5(16): 1967–1991. doi:10.4155/fmc.13.151.

Small-molecule inhibitors of human LDH5

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The latest findings on the role played by human LDH5 (*h*LDH5) in the promotion of glycolysis in invasive tumor cells indicates that this enzyme subtype is a promising therapeutic target for invasive cancer. Compounds able to selectively inhibit *h*LDH5 hold promise for the cure of neoplastic diseases. *h*LDH5 has so far been a rather unexplored target, since its importance in the promotion of cancer progression has been neglected for decades. This enzyme should also be considered as a challenging target due the high polar character (mostly cationic) of its ligand cavity. Recently, significant progresses have been reached with small-molecule inhibitors of *h*LDH5 displaying remarkable potencies and selectivities. This review provides an overview of the newly developed *h*LDH5 inhibitors. The roles of *h*LDH isoforms will be briefly discussed, and then the inhibitors will be grouped into chemical classes. Furthermore, general pharmacophore features will be emphasized throughout the structural subgroups analyzed.

The lack of significant side effects deriving from *h*LDH5 inhibition might be expected considering that people with a hereditary deficiency of the *LDH-A* gene, who consequently completely lack the A subunit, show myoglobinuria (the presence of myoglobin in the urine caused by muscle damage) after intense anaerobic exercise (exertional myoglobinuria), whereas they do not display any symptoms under ordinary circumstances [33–35].

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The Human Oxalate Pathway Compared to Laboratory Research Species

Mouse models do not fully represent human forms of Primary Hyperoxaluria

In Mouse:

- Genetic knockout and RNAi knockdown of *Agxt* (AGT) are both sufficient to induce hyperoxaluria in mice
  - Normal mouse urine chemistry prevents calcium oxalate deposition (crystal or stone formation) unless oxalate production is super-elevated by the addition of ethylene glycol to drinking water
- RNAi knockdown of *Grhpr* (GR) is sufficient to induce mild hyperoxaluria in mice
  - As with humans, knockdown of *Grhpr* results in less elevation of oxalate than *Agxt* knockdown
- We are unable to generate a PH3 model by knocking out *Hoga* in mice
  - Is HOG unable to inhibit GR in mice or is HOG metabolized or excreted differently in mice than in humans?

In Monkey:

- We are unable to create a non-human primate model of PH1 by knocking out *AGXT* in monkeys to date
  - Is the enzymatic capacity of GR and/or transport and consumption of glyoxylate in cynos too great to overcome with AGT knockdown alone? Or is cyno hepatic LDH less efficient at converting glyoxylate to oxalate?
- We are treating monkeys with GalXC-AGXT and GalXC-GRPHR conjugates simultaneously in an attempt to create a "**Super PH**" non-human primate model of Primary Hyperoxaluria
  - We will then test both GalXC-LDHA and GalXC-HAO1 in this model and evaluate oxalate, glyocolate and glyoxylate levels and measure other PH pathway markers

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Conclusions – Reduction of LDH Activity in Liver in All Forms of PH

- Oxalate-generating pathways in humans have been well characterized in healthy volunteers, Primary Hyperoxaluria patients and human-derived experimental systems
- Lactase dehydrogenase acting on glyoxylate might be the primary source of oxalate in all forms of PH
  - LDH5 expressed from the LDHA gene is the primary form of LDH enzyme activity in the liver
- Natural human mutations confirm that systemic (liver and muscle) LDHA deficiency is well tolerated
  - There are no liver-based observations reported in LDHA deficiency, only sporadic muscle observations
  - This suggests that even life long therapy to reduce LDHA activity only in the liver might be well tolerated
- Human data and non-human experimental systems suggest that reduction of LDH in liver might reduce oxalate production in patients with any known form of Primary Hyperoxaluria

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