Hydroxyproline Metabolism and Oxalate Synthesis in Primary Hyperoxaluria

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SUPPLEMENTAL MATERIAL

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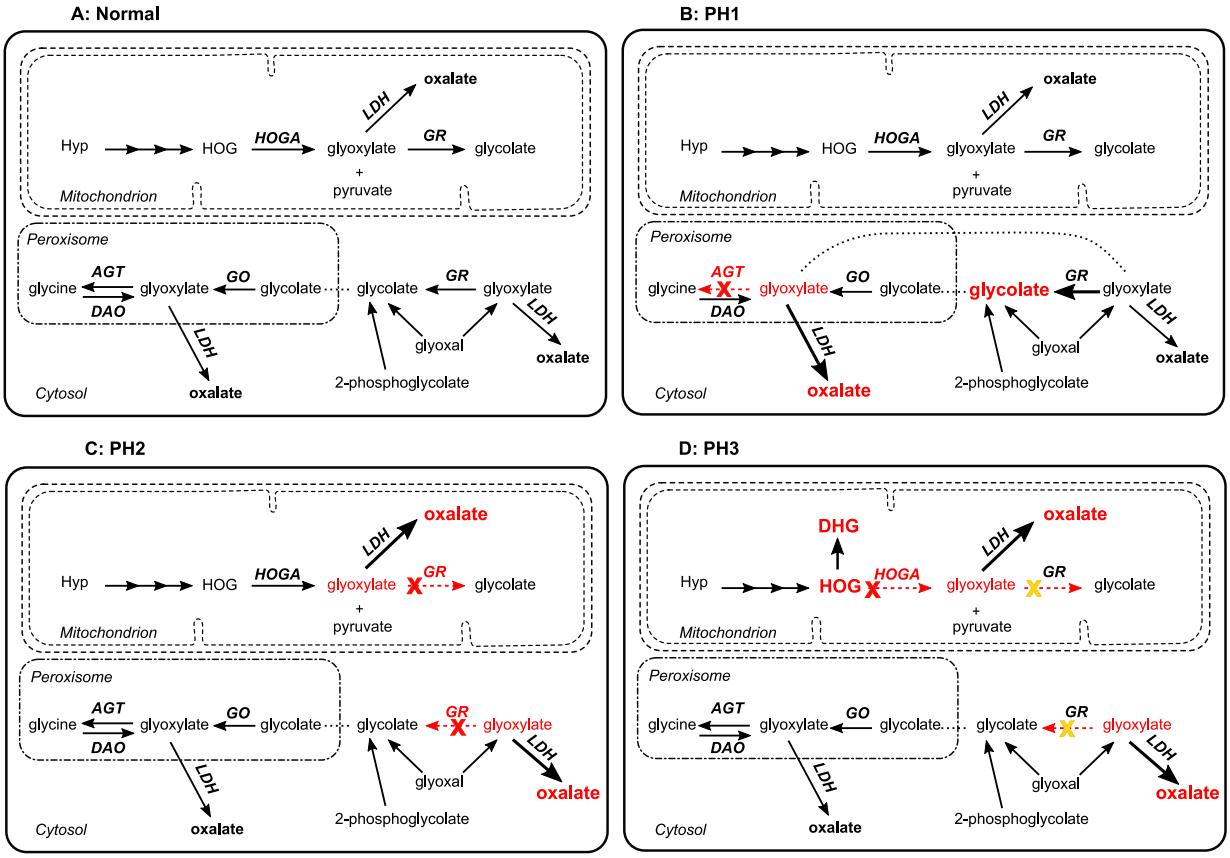


Supplemental Figure 1. Changes in the metabolism of glyoxylate in primary hyperoxaluria. (A): Metabolism in healthy subjects; (B): in PH1; (C): in PH2; (D): in PH3. All enzyme deficiencies (dotted arrows, red cross) and the resulting consequences on metabolites are indicated in red and described below. In PH1 the deficiency in peroxisomal alanine:glyoxylate aminotransferase (AGT) leads to oxidation of glyoxylate to oxalate by LDH and reduction of glyoxylate to glycolate by GR. Glycolate is often (variably) elevated in the urine and plasma of PH1 patients. In PH2, the deficiency in glyoxylate reductase (GR) in mitochondria and cytosol leads to conversion of glyoxylate into oxalate by LDH. GR also catalyzes the conversion of hydroxy-pyruvate to D-glycerate. In PH2 hydroxypyruvate is instead converted to L-glycerate by LDH, and glycerate is typically elevated in the urine of PH2 patients (not shown). In PH3, mutations in 4-hydroxy-2-oxoglutarate aldolase (HOGA) lead to the buildup of 4-hydroxy-2-oxoglutarate (HOG). Excess HOG is reduced to dihydroxy-glutarate (DHG) by unknown enzymes. HOG may inhibit GR (yellow cross) leading to glyoxylate to oxalate conversion by LDH. One or several enzyme reactions could also lead to breakdown of HOG into glyoxylate and oxalate. Hyp: hydroxyproline, LDH; lactate dehydrogenase, DAO: D-amino oxidase, GO: glycolate oxidase. Arrows denote enzyme reactions, dotted lines between metabolites denote movement between intracellular compartments.

SUPPLEMENTAL METHODS: Principles of Metabolic Analysis Using Isotope Tracers

1. GENERAL CONSIDERATIONS

A ***Tracee***: is a naturally occurring compound of interest. The tracee in the current study was hydroxyproline (Hyp).[1]

A ***Tracer*** is a compound chemically and functionally identical to its naturally occurring counterpart (tracee), but has some distinctive characteristic that allows selective detection.[1] The tracer in the current study was Hyp labeled with stable isotopes: $^{15}N,^{13}C_5$-Hyp. The tracer was used in two ways: to determine the turnover of Hyp, by looking at the tracer dilution [equations *(1)* and *(2)*] and to investigate its catabolism into specific products, oxalate and glycolate, by the incorporation of the carbon-13 into both these metabolites [equations *(3)* and *(4)*].

The term ***Enrichment*** refers to the relative amount of tracer to tracee.

The term ***Mole percent enrichment or atom percent enrichment*** (MPE) is defined as the ratio of tracer to the sum of tracer and tracee.[1]

The ***Isotopic steady-state*** is defined as a state where the rate at which the tracer enters a pool is equal to its disappearance from that pool.

2. *HYP TURNOVER*

The measurement of Hyp turnover (**Q**) used the common technique of constant infusion of the tracer with a priming dose to shorten the time to isotopic steady-state.[1] Once isotopic equilibrium is achieved, the various pools containing the Hyp tracer can be simplified to a single pool into which tracer enters and from which sampling occurs (Supplemental Figure 6). If there is no change in the size of the Hyp metabolic pool over the course of the infusion, the rate at which Hyp leaves the pool is equal to the rate at which it arrives, rate of appearance equals rate of disappearance.[1] The general equation describing this is:

$$\textit{Rate of Appearance } (I + B) = \textit{Rate of Disappearance } (S + U_T) = Q \qquad (1)$$

Where **Q** = Flux or turnover

I = Exogenous intake, from the diet

S = Synthesis from Hyp

B = Breakdown into Hyp (collagen being the main source)

$\mathbf{U_T}$ = Catabolism of Hyp into products and excretion of unmodified Hyp

$$U_T = U_H + U_{H>Ox} + U_{H>G} + U_{H>y} + U_{H>z}$$

$\mathbf{U_H}$ = urinary excretion of Hyp (insignificant <5mg per 24 hours)

$\mathbf{U_{H>Ox}}$ = catabolism followed by urinary excretion of Hyp as urinary oxalate

$\mathbf{U_{H>G}}$ = catabolism followed by urinary excretion of Hyp as urinary glycolate

$\mathbf{U_{H>y}}$ = catabolism followed by urinary excretion of Hyp as other forms

$\mathbf{U_{H>z}}$ = catabolism of Hyp as other forms (glycine, CO_2, etc.)

For Hyp, Equation 1 can be simplified because, 1), Hyp is not used for synthesis of proteins (S = 0) and 2) the infusion was done in the fasted state (I=0). Therefore:

$$Q = B = U_T \qquad (1)$$

Q, the Hyp turnover rate (μmol/kg/h) is determined from the dilution of the $^{15}N,^{13}C_5$-Hyp infusion in plasma Hyp once isotopic steady-state is reached.[2-5]

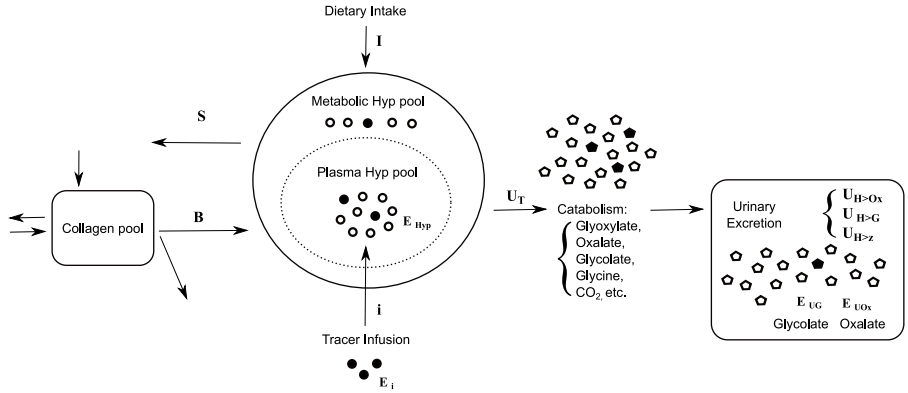
$$Q = i\,[(E_i / E_{Hyp}) - 1] \qquad (2)$$

where **i** = is the $[^{15}N,^{13}C_5]$-Hyp infusion rate (0.75 μmol/kg/h)

$\mathbf{E_i}$ = isotopic enrichment of the $[^{15}N,^{13}C_5]$-Hyp infusate (94%)

$\mathbf{E_{Hyp}}$ = mole percent enrichment of the total Hyp plasma pool with $[^{15}N,^{13}C_5]$-Hyp at isotopic plateau, directly measured by GC/MS on subject samples.

The second term of the equation removes the contribution of the tracer infusion from the Hyp turnover.[2,3]



Supplemental Figure 2. Schematic representation of the isotope tracer model of analysis. *empty circles: Hyp tracee, black circles: Hyp tracer, empty pentagons: unlabeled product, black pentagons : labeled products. Blood and urine sampling allow measurement of the enrichment of the total Hyp pool with $^{15}N,^{13}C_5$-Hyp ($\mathbf{E_{Hyp}}$), and the urinary enrichment of oxalate and glycolate with $^{13}C_2$-oxalate and $^{13}C_2$-glycolate, respectively ($\boldsymbol{E_{UOx}}$ and $\boldsymbol{E_{UG}}$).*

3. CONTRIBUTION OF HYP CATABOLISM TO THE PRODUCTS OXALATE AND GLYCOLATE

The model used for the study of Hyp metabolism to oxalate and glycolate is modeled on the measurement of protein fractional synthesis and breakdown.[1] The absolute synthesis of oxalate from Hyp, or Hyp-derived urinary oxalate, $\boldsymbol{U_{H>Ox}}$, can de defined as

$$\boldsymbol{U_{H>Ox} = Cf * Pool\ size} \qquad \textbf{(3)}$$

Where the pool size is the total amount of urinary oxalate, $\boldsymbol{T\ U_{Ox}}$, and the fractional synthesis is ($\boldsymbol{Cf}$), the contribution of Hyp to oxalate.

The contribution of Hyp to the metabolism of oxalate and glycolate was studied from the perspective of the urinary pool, which is relevant to the disease primary hyperoxaluria and the risk of kidney stones formation.[6] The contribution of Hyp metabolism to urinary oxalate can be defined as the fractional synthesis of product, oxalate, to precursor, Hyp.[4,5] Assuming that during the intravenous infusion of [$^{15}N,^{13}C_5$]-Hyp tracer that, 1), the urinary oxalate pool size is constant, 2), the rate of Hyp catabolism to oxalate is constant, and 3), all oxalate produced endogenously is excreted in urine, at steady-state:

$$\boldsymbol{Cf = E_{UOx} / E_{Hyp}} \qquad \textbf{(4)}$$

Or expressed as percentage contribution:

$$\boldsymbol{C_{\%} = (E_{UOx} / E_{Hyp})*100} \qquad \textbf{(4)}$$

Where $\boldsymbol{E_{Uox}}$ is the steady state enrichment of the total urinary oxalate pool with $^{13}C_2$-oxalate at steady state, and $\boldsymbol{E_{Hyp}}$ is the plasma enrichment with [$^{15}N,^{13}C_5$]-Hyp at steady-state. Thus, if all the oxalate excreted in urine was derived from Hyp breakdown, i.e., contribution of 1, (or expressed as a percentage, 100%), then if one were to increase or enrich the body pool of the tracee Hyp by 10% with intravenous infusion of the [$^{15}N,^{13}C_5$]-Hyp tracer, then at steady state the total urinary pool of oxalate would also become enriched with $^{13}C_2$-oxalate by 10%.

The amount of Hyp-derived urine oxalate in Equation ***(3)*** then becomes

$$\boldsymbol{U_{H>Ox} = (E_{UOx} * T\ U_{Ox}) / E_{Hyp}} \qquad \textbf{(3)}$$

Or using percentage contribution:

Hyp-derived oxalate (mg/g creatinine) = mean hourly urine excretion of total oxalate during infusion in mg/g creatinine x (% Hyp contribution at T360/100) **(3)**

The same principles and equations were applied to determine the net contribution of Hyp catabolism to urinary glycolate excretion and total Hyp-derived urinary glycolate.

4. ASSUMPTIONS OF THE MODEL

1. The single-pool steady-state model assumes that the substrates are distributed in a single pool, which is homogenous and instantly mixed so that the site of infusion and blood sampling do not matter. This simplification allows calculation of turnover without knowledge of the Hyp pool size once steady-sate has been achieved, at which point the tracer is lost at the same rate that it appears, with no more changes in ratio of tracer to tracee with a constant infusion.
2. The size of the body Hyp pool and product pools (urinary oxalate and glycolate) are constant throughout the tracer infusion.
3. There is no isotopic discrimination between tracer $^{15}N,^{13}C_5$-Hyp and tracee Hyp. However, there is equilibration between the plasma pool of the Hyp tracer and the various pools of Hyp. This is achieved by steady-state infusion.
4. There is equilibration between the plasma pool of the Hyp tracer and the various pools of Hyp. This is achieved by steady-state infusion.
5. $^{15}N,^{13}C_5$-Hyp is a valid tracer for urinary oxalate and glycolate. Both the labeled carbons in oxalate and glycolate are derived from Hyp (Supplemental Figure 2).[6]
6. There is no recycling of the carbon13 labeled isotope. This assumption is true as far as Hyp and oxalate are concerned, as Hyp is not synthesized de novo in from its downstream metabolites during the course of the infusion and oxalate is a metabolic end-product, excreted almost entirely (>95%) in urine. For glycolate, the net contribution is determined under these conditions.
7. The fraction of Hyp synthesized to oxalate and glycolate is constant.
8. The maximum contribution of Hyp metabolism to urinary oxalate and glycolate is determined once product plateau has been reached. This was not achieved during the course of the study infusion, leading to underestimation of the contribution.

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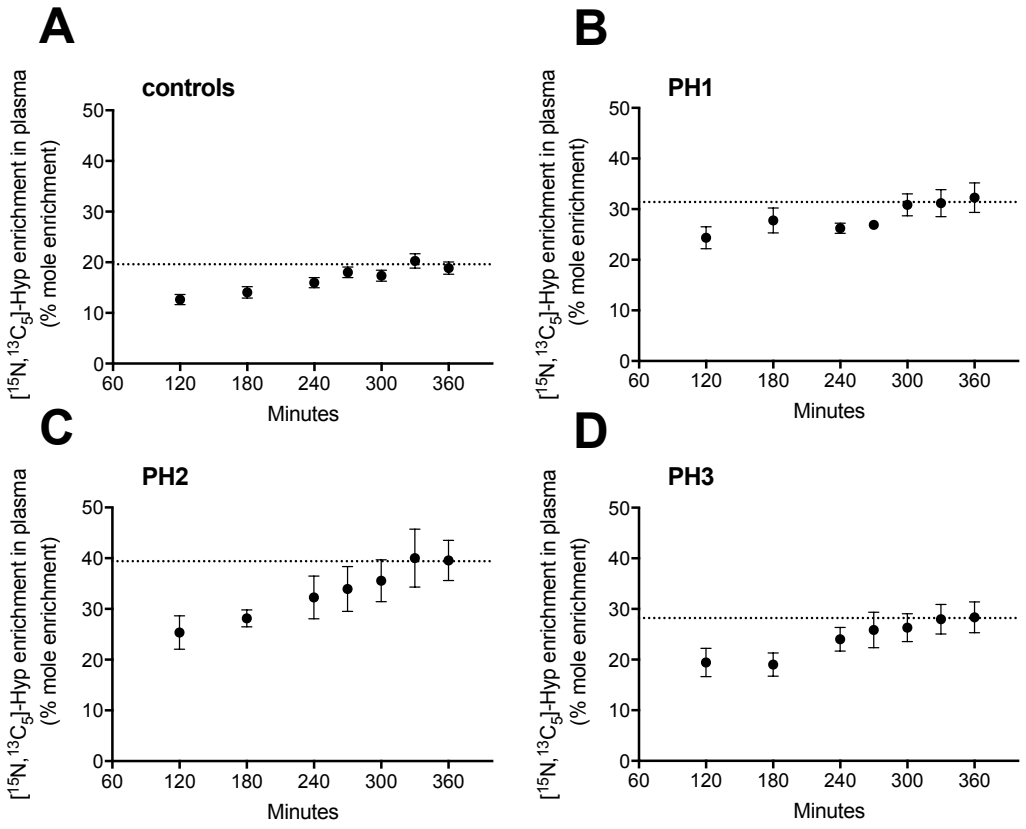
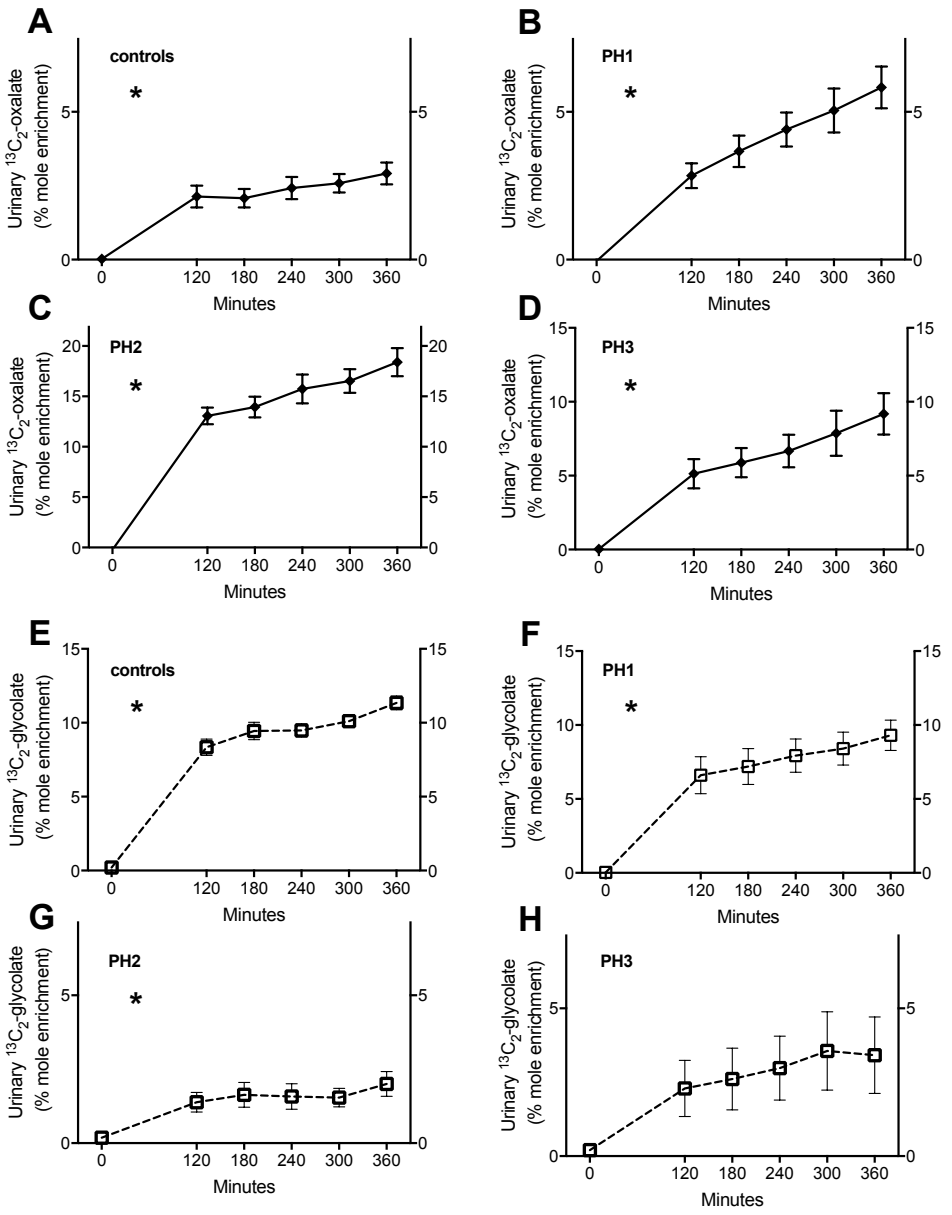
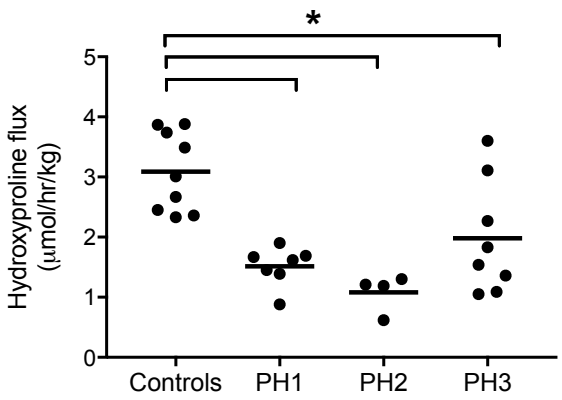


Supplemental Figure 3. Enrichment of plasma [^{15}N,$^{13}C_5$]-Hyp during infusion. (**A)**: healthy controls, (**B)**: PH1 patients, (**C)**: PH2 patients, (**D)**: PH3 patients. Percent mole enrichment of plasma values expressed as mean ± SEM (plasma enrichment with [^{15}N,$^{13}C_5$]-Hyp approached, but did not achieve, steady state after 300 minutes in all groups). The dotted line is the average of the last two values, 330 and 360 minutes, and indicates equilibration of isotope tracer



Supplemental Figure 4. Urinary $^{13}C_2$-oxalate and $^{13}C_2$-glycolate mole percent enrichment in hourly urinary collections with [^{15}N,$^{13}C_5$]-Hyp infusion. (**A-D)**: urinary $^{13}C_2$-oxalate mole percent enrichment; (**E-H)**: urinary $^{13}C_2$-glycolate mole percent enrichment. (**A, E)**: healthy controls, (**B, F)**: PH1 patients, (**C, G)**: PH2 patients, (**D, H)**: PH3 patients. Data expressed as mean ± SEM. ◆, oxalate, solid line. □, glycolate, dotted line. Urinary $^{13}C_2$-oxalate and $^{13}C_2$-glycolate did not reach steady state in the groups. Enrichment significantly increased after infusion with one-way ANOVA (Friedman test): * P<0.05.

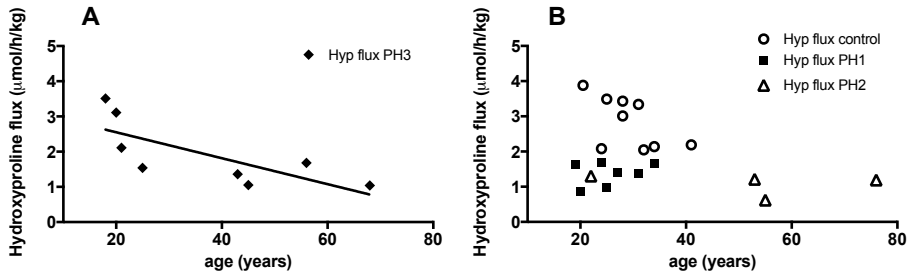


Supplemental Figure 5. Hydroxyproline turnover rate. The hydroxyproline turnover rate, or flux, was determined from the enrichment of the plasma Hyp pool with [^{15}N, $^{13}C_5$]-Hyp infusion at T360. Comparison within groups with Tukey's post-hoc test are only significant with controls, *,*P*<0.05. Line: mean. Individual points: separate subjects.

The whole body turnover rate or flux of Hyp (Q_{hyp}) was calculated as described in Equations 1.

$$Q = i\,[(E_i / E_{Hyp}) - 1] \qquad (1)$$

where "i" is the [^{15}N,$^{13}C_5$]-Hyp infusion rate in μmol/kg/h and E_i /E_{Hyp} is the ratio of isotopic enrichment of the [^{15}N,$^{13}C_5$]-Hyp infusate (E_i, 94%) and the mole percent enrichment of the total Hyp plasma pool with [^{15}N,$^{13}C_5$]-Hyp (E_{Hyp}) (referred to as "enrichment" in the text).



Supplemental Figure 6. Hydroxyproline flux varies with age in patients with PH3**.** (**A**): Linear regression drawn for PH3 (◆, R^2 0.56, *P*=0.034). (**B):** There was no significant correlation in the other subject groups, though age distribution was narrower in control subjects and PH1 (healthy controls (○, PH1 (■) or PH2 (△) patients).

Supplemental Table 1. Individual data for healthy subjects and PH patients included - Baseline.

	BASELINE sex M/F	age	weight (kg)	height (m)	BMI	Genotype		Pyridoxine dose (mg/kg/day)	GFR (ml/min/1.73m²)	24 h Urine Oxalate (mg/g creat)	24 h Urine Glycolate (mg/g creat)	24 h Urine HOG (mg/g creat)	24 h Urine DHG (mg/g creat)	24 h Urine Glycerate (mg/g creat)	FE Glycolate (%)
PH1 SUBJECTS															
mean ± SEM		***25.7 ± 2.1***	***75.4 ± 5.6***	***1.79 ± 0.2***	***24.6 ± 1.1***				***112.1 ± 4.0***	***63.2 ± 23.1***	***92.4 ± 30.8***		***0.9 ± 0.1***	***7.0 ± 3.1***	***30.6 ± 5.2***
median		***25.0***	***74.8***	***1.78***	***23.8***				***115.0***	***52.2***	***44.9***		***0.9***	***5.0***	***36.0***
#10	M	24	75	1.77	23.8	p.Phe152Ile	p.Gly109fs9X	8.0	91	64	45	<3	0.7	7	41
#11	M	31	84	1.93	22.6	p.Pro11fs33X	p.Gly41Arg	2.4	115	23	101	<3	1.1	4	36
#12	M	27	78	1.81	23.9	p.Gly170Arg	p.Asp201Glu	6.4	107	24	32	<3	0.6	<0.7	42
#13	M	25	101	1.81	30.8	p.Gly170Arg	p.Asp201Glu	5.0	115	23	38	<3	0.9	1	43
#14	F	19	60	1.64	22.0	p.Val358fs	p.Gln282fs	No	122	52	232	<3	1.3	22	29
#15	F	20	73	1.69	25.7	p.Phe152Ile	p.Lys227fs	4.1	114	61	25	<3	0.6	6	12
#16	F	34	57	1.56	23.3	p.Pro11fs33X	p.Arg233Leu	7.1	121	195	174	<3	0.9	3	11
PH2 SUBJECTS															
mean ± SEM		***51.5 ± 11.1***	***72.4 ± 10.3***	***1.66 ± 0.3***	***26.2 ± 3.3***				***129 ± 17.7***	***88.9 ± 19.1***	***33.0 ± 5.1***		***1.7 ± 0.2***	***517.8 ± 189.7***	***27.5 ± 3.4***
median		***54.0***	***69.9***	***1.67***	***25.5***				***136.0***	***78.2***	***31.4***		***1.7***	***486.0***	***26.0***
#17	F	53	62	1.59	24.6	p. Asp35fs10X	p. Asp35fs10X	No	133	83	47	<3	2.2	1158	22
#18	F	22	51	1.64	19.1	p. Asp35fsX10	p. Asp35fsX10	No	139	73	30	<3	1.8	607	30
#19	F	55	99	1.68	34.8	p.Asp35fsX10	p.Asp35fsX10	No	164	56	33	<3	1.5	365	36
#20	F	76	78	1.72	26.3	p.Asp35fsX10	p.Asp35fsX10	No	80	144	22	<3	1.3	341	22
PH3 SUBJECTS															
mean ± SEM		***37 ± 6.6***	***91.6 ± 8.5***	***1.75 ± 0.2***	***28.7 ± 2.6***				***97.5 ± 9.7***	***52.4 ± 6.6***	***16.3 ± 5.5***	***28.2 ± 9.1***	***16.4 ± 3.4***	***9.3 ± 5.8***	***65.6 ± 15.1***
median		***34.0***	***87.0***	***1.77***	***27.8***				***86.0***	***54.4***	***10.8***	***18.1***	***14.3***	***3.6***	***65.5***
#21	M	43	137	1.81	41.6	p.Pro190Leu	p.Leu233_Gly234ins17	0.7	84	60	10	68.1	18.6	<0.7	67
#22	M	20	70	1.91	19.1	p.Leu233_Gly234ins17	p.Leu233_Gly234ins17	No	102	33	23	3.2	8.0	<0.7	64
#23	M	56	92	1.78	28.9	p.Arg303Cys	p.Glu315del	No	72	66	9	43.8	18.0	2	127
#24	M	45	103	1.84	30.5	p.Leu233_Gly234ins17	p.Thr280Ile	No	80	50	12	13.4	11.8	4	82
#25	M	25	83	1.76	26.7	p.Arg97Cys	p.Glu315del	No	127	44	52	17.0	-	<0.7	119
#26	M	18	75	1.77	24.0	p.Glu315del	p.Glu315del	No	150	59	12	60.1	42.6	9	33
#27	M	21	65	1.70	22.3	p.Glu315del	p.Glu315del	No	88	24	6	19.1	9.7	1	18
#28	M	68	109	1.74	36.1	p.Glu315del	p.Glu315del	5.5	77	82	7	<3	14.3	32	15
CONTROL SUBJECTS															
mean ± SEM		***29.3 ± 2.0***	***74.3 ± 4.8***	***1.71 ± 0.3***	***25.2 ± 1.2***				***105 ± 6.5***	***18.9 ± 2.2***	***36.1 ± 9.7***		***0.8 ± 0.1***	***3.3 ± 0.8***	***78.3 ± 11.1***
median		***28.0***	***77.7***	***1.71***	***24.8***				***110.0***	***18.7***	***30.3***		***0.9***	***3.7***	***61.0***
#1	F	41	57	1.62	21.6	-	-	-	89	24	-	-	-	-	45
#2	M	34	82	1.71	28.3	-	-	-	84	23	-	-	-	-	52
#3	M	28	75	1.77	23.9	-	-	-	84	14	-	-	-	-	79
#4	M	28	78	1.62	29.5	-	-	-	121	-	-	-	-	-	61
#5	F	32	65	1.66	23.7	-	-	-	114	28	24	<3	1.1	5	61
#6	F	21	49	1.58	19.7	-	-	-	110	15	37	<3	0.6	2	109
#7	M	24	93	1.78	29.6	-	-	-	119	10	73	<3	0.9	4	134
#8	M	31	84	1.79	26.1	-	-	-	87	22	30	<3	1.0	5	116
#9	M	25	85	1.86	24.8	-	-	-	137	15	17	<3	0.7	1	48

"-": analysis not performed. The renal fractional excretion of glycolate (FE Glycolate) was calculated using estimated GFR and the average levels of plasma and urine glycolate between T0 and T360 for each subject. Urine values at baseline are 24 hours urine collections.

Supplemental Table 2. Individual data for healthy subjects and PH patients with [^{15}N, $^{13}C_5$]-Hyp infusion at 0.75 µmol/h/kg.

		PLASMA										CALCULATIONS						
		$^{13}C_2$- enrichment (%)				Total (uM)							Hydroxyproline contribution (%)		Hyp and Non Hyp-derived Urine metabolites (mg/g creatinine)			
		Hydroxyproline		Leucine	Glycolate	Hydroxyproline		Oxalate		Glycolate		Hyp flux (µmol/h/kg)	to Urine Oxalate	to Urine Glycolate	Hyp-derived Oxalate	Non Hyp-derived Oxalate	Hyp-derived Glycolate	Non Hyp-derived Glycolate
		T final	*(T330-T360)*	*T final*	*T final*	*T0*	*T final*	*T0*	*T final*	*T0*	*T final*	*T final*	*T final*	*T final*				
PH1 SUBJECTS																		
	mean ± SEM	***31.8 ± 2.1***		***2.6 ± 0.2***	***6.1 ± 0.5***	***6.1 ± 0.5***	***8.3 ± 0.6***	***6.6 ± 1.6***	***5.7 ± 1.5***	***26.9 ± 10.2***	***25.5 ± 9.6***	***1.5 ± 0.1***	***18.1 ± 1.1***	***29.5 ± 3.3***	***12.8 ± 4.7***	***60.3 ± 23.2***	***16.3 ± 4.0***	***43.3 ± 13.0***
	median	***29.8***		***2.3***	***5.6***	***6.3***	***8.3***	***4.4***	***4.2***	***14.1***	***13.6***	***1.6***	***17.1***	***26.1***	***7.8***	***32.0***	***16.2***	***31.2***
	#10	28.9	26.7	1.7	4.8	8.0	9.9	*	*	22.9	20.4	1.7	16.3	19.8	7.8	39.9	10.5	42.4
	#11	33.0	30.6	2.1	7.6	6.6	9.3	3.7	2.9	14.1	11.0	1.4	20.7	35.0	7.9	30.2	16.8	31.2
	#12	26.6	27.9	2.2	5.7	5.3	8.2	*	*	7.3	8.8	1.9	18.8	42.4	7.4	32	16.2	22
	#13	32.1	32.1	5.0	5.2	6.0	10.3	4.1	3.5	7.2	5.4	1.5	14.1	37.0	3.6	21.8	10.6	18
	#14	29.8	29.7	-	5.6	6.4	8.3	9.5	6.8	48.9	44.8	1.6	17.1	26.1	19.2	93.4	37.6	106.8
	#15	43.2	43.3	2.5	8.5	4.1	5.9	4.4	4.2	10.0	13.6	0.9	22.5	25.6	5.2	18	3.9	11.5
	#16	29.1	29.1	2.4	5.3	6.3	6.8	11.1	11.0	78.2	74.6	1.7	17.1	20.3	38.6	187.1	18.2	71.5
PH2 SUBJECTS																		
	mean ± SEM	***39.6 ± 4.0***		***3.1 ± 0.1***	***2.8 ± 0.7***	***8.4 ± 0.8***	***9.0 ± 1.1***	***4.3 ± 0.4***	***3.6 ± 0.2***	***7.4 ± 1.1***	***4.4 ± 0.5***	***1.1 ± 0.2***	***47.0 ± 2.9***	***5.4 ± 1.3***	***32.9 ± 8.0***	***38.0 ± 11.3***	***1.4 ± 0.4***	***24.0 ± 1.2***
	median	***36.2***		***3.1***	***2.4***	***7.9***	***8.2***	***4.2***	***3.6***	***6.4***	***4.4***	***1.2***	***45.8***	***5.1***	***29.8***	***29.3***	***1.3***	***23.0***
	#17	36.0	35.5	-	1.6	7.6	8.9	4.3	3.3	8.8	6.0	1.2	54.4	4.5	38.4	32.2	1.1	23
	#18	34.5	34.0	-	2.9	7.0	7.4	3.4	3.4	7.6	3.2	1.3	48.9	5.7	21.1	22.1	1.4	22.9
	#19	51.4	51.5	3.0	4.9	8.2	10.0	4.2	3.8	6.2	4.6	0.6	42.1	2.4	19.1	26.3	0.6	22.6
	#20	36.4	36.4	3.2	1.9	10.7	12.1	5.2	4.1	5.4	4.5	1.2	42.6	8.8	52.9	71.2	2.6	27.5
PH3 SUBJECTS																		
	mean ± SEM	***28.3 ± 3.1***		***2.4 ± 0.3***	***0.5 ± 0.1***	***7.5 ± 0.7***	***9.5 ± 0.4***	***4.7 ± 0.7***	***4.5 ± 0.7***	***3.5 ± 0.3***	***3.0 ± 0.3***	***2.0 ± 0.3***	***33.2 ± 5.0***	***12.3 ± 4.8***	***14.8 ± 1.8***	***32.2 ± 4.8***	***2.5 ± 1.3***	***10.3 ± 1.9***
	median	***29.0***		***2.3***	***0.6***	***7.6***	***9.7***	***4.0***	***4.1***	***3.5***	***2.7***	***1.7***	***33.1***	***6.9***	***15.8***	***29.9***	***0.8***	***9.1***
	#21	33.4	31.7	2.3	0.6	8.2	10.9	*	*	5.1	4.9	1.4	35.8	10.9	16.4	29.5	1.3	10.6
	#22	18.3	18.1	-	0.7	9.6	10.2	5.1	4.9	4.1	2.6	3.1	19.7	32.9	8.6	35.1	6.8	13.8
	#23	27.3	28.2	-	0.1	7.0	9.3	8.6	8.3	3.0	3.4	1.8	13.9	2.8	10	61.7	0.3	11.1
	#24	39.1	38.6	-	0.6	5.3	8.2	4.0	3.0	2.8	2.6	1.1	22.6	16.5	8.8	30.2	1.5	7.4
	#25	30.7	29.6	1.7	1.1	5.4	8.1	3.6	3.3	3.4	3.5	1.5	44.4	32.0	18.9	23.6	9.9	21.1
	#26	16.2	16.3	-	0.3	10.0	10.7	3.6	4.1	2.6	2.8	3.6	44.1	1.5	22.3	28.2	0.1	7.5
	#27	23.4	24.0	2.2	0.8	5.4	8.1	3.3	3.1	3.7	1.5	2.3	54.8	0.5	18.1	15	0	5.3
	#28	38.4	38.8	3.3	0.0	8.8	10.4	5.0	4.5	3.5	2.7	1.1	30.4	0.9	15.1	34.5	0	5.5
CONTROL SUBJECTS																		
	mean ± SEM	***18.9 ± 1.1***		***1.9 ± 0.1***	***4.7 ± 0.7***	***10.9 ± 0.4***	***10.1 ± 0.5***	***2.8 ± 0.3***	***3.4 ± 0.5***	***4.5 ± 0.3***	***3.9 ± 0.3***	***3.1 ± 0.2***	***15.1 ± 0.9***	***57 ± 3.1***	***1.6 ± 0.1***	***9.0 ± 0.5***	***13.3 ± 2.7***	***10.0 ± 1.6***
	median	***18.7***		***1.9***	***4.5***	***10.9***	***10.0***	***2.7***	***3.5***	***4.8***	***3.7***	***3.0***	***15.9***	***57.0***	***1.7***	***9.1***	***11.4***	***10.5***
	#1	22.7	23.3	1.8	-	10.9	9.5	1.6	1.6	4.9	4.3	2.4	15.9	56.3	1.8	9.7	8.1	6.3
	#2	22.0	23.2	1.9	-	10.2	9.4	3.4	4.3	5.2	6.1	2.5	20.0	38.4	2.3	9.1	6.6	10.5
	#3	15.3	16.1	2.2	-	11.0	10.6	3.2	3.2	3.4	3.4	3.9	11.7	57.0	1.2	8.7	7.5	5.7
	#4	18.7	18.7	-	-	8.8	10.0	1.6	1.6	5.3	4.3	3.0	16.2	51.1	1.6	8	11.4	10.9
	#5	22.9	24.0	1.9	5.0	10.7	9.1	*	*	5.4	2.9	2.3	16.4	52.0	2	9.9	16.3	15
	#6	15.2	15.1	-	3.3	12.1	10.8	2.8	2.4	4.1	3.0	3.9	13.8	65.4	1.9	11.6	32.2	17.1
	#7	20.6	22.7	-	2.2	10.4	8.5	2.6	3.8	3.6	3.2	2.7	14.9	55.2	1.7	9.6	17.2	14
	#8	15.7	16.5	-	4.0	11.4	10.0	4.2	4.9	3.5	3.7	3.7	16.1	67.3	1.5	7.9	11.9	5.8
	#9	16.6	16.4	-	3.5	12.8	13.4	2.5	5.1	4.8	4.4	3.5	10.9	67.5	0.8	6.3	8.9	4.3

		URINE																			
		$^{13}C_2$- enrichment (%)				Total (mg/g creatinine)														Creatinine (mg/h)	
		Oxalate	Glycolate	HOG	DHG	Oxalate			Glycolate			HOG	DHG	Glycerate	$^{13}C_2$-Oxalate	$^{13}C_2$-Glycolate	$^{13}C_2$-HOG	$^{13}C_2$-DHG		
		T final	*T final*	*T final*	*T final*	*T0*	*T final*	*mean*	*T0*	*T final*	*mean*	*T final*	*T final*	*T final*	*T final*	*T final*	*T final*	*T final*	*T final*	*mean*
PH1 SUBJECTS																					
	mean ± SEM	***5.8 ± 0.7***	***9.3 ± 1.0***			***72.3 ± 24.6***	***65.2 ± 20.4***	***73.1 ± 27.9***	***63.4 ± 23.1***	***54.1 ± 13.6***	***59.6 ± 16.7***		***0.4 ± 0.1***	***2.1 ± 0.4***	***3.3 ± 1.1***	***5.2 ± 1.0***			***54.3 ± 15.7***	***54.2 ± 17.6***
	median	***5.0***	***11.1***			***40.7***	***39.0***	***39.4***	***44.3***	***44.0***	***48.0***		***0.4***	***2.2***	***2.7***	***3.8***			***53.2***	***54.4***
	#10	4.7	5.7	<3.3	<0.8	40.7	48.3	47.6	49.9	44.2	53.0	<3	0.4	1	0.1	5.2	<0.8	<0.2	71.3	68.0
	#11	6.8	11.5	<3.3	<0.8	51.0	38.5	38.1	15.2	64.5	48.0	<3	0.5	2	2.7	7.6	<0.8	<0.2	73.1	73.5
	#12	5.0	11.3	<3.3	<0.8	36.9	37.8	39.4	44.3	31.7	38.1	<3	0.4	3	1.9	3.7	<0.8	<0.2	62.1	54.4
	#13	4.5	11.9	<3.3	<0.8	24.5	29.6	25.3	26.7	30.7	28.6	<3	0.6	1	1.4	3.7	<0.8	<0.2	49.7	72.6
	#14	5.1	7.8	<3.3	<0.8	148.7	87.2	112.6	168.0	126.2	144.5	<3	0.0	3	4.5	10.0	<0.8	<0.2	40.5	36.2
	#15	9.7	11.1	<3.3	<0.8	22.1	35.4	23.3	9.9	19.2	15.4	<3	0.6	2	3.5	2.2	<0.8	<0.2	53.2	42.6
	#16	5.0	5.9	<3.3	<0.8	182.2	179.8	225.7	129.5	62.2	89.7	<3	0.4	3	9.2	3.8	<0.8	<0.2	30.5	31.8
PH2 SUBJECTS																					
	mean ± SEM	***18.4 ± 1.4***	***2.0 ± 0.4***			***69.5 ± 12.2***	***57.0 ± 8.8***	***70.8 ± 18.8***	***33.0 ± 6.8***	***16.7 ± 2.8***	***25.4 ± 1.6***		***1.1 ± 0.2***	***248 ± 84.8***	***10.6 ± 1.5***	***0.3 ± 0.1***			***47.5 ± 22.4***	***38.9 ± 12.7***
	median	***18.2***	***1.8***			***66.0***	***57.0***	***58.0***	***27.9***	***18.0***	***24.2***		***1.1***	***297.1***	***10.9***	***0.4***			***42.8***	***41.3***
	#17	19.6	1.6	<3.3	<0.8	68.9	69.1	70.6	26.3	21.3	24.1	<3	1.4	393	13.8	0.4	<0.8	<0.2	38.0	39.4
	#18	16.8	2.0	<3.3	<0.8	43.8	39.5	43.2	27.8	21.4	24.3	<3	1.0	269	6.8	0.4	<0.8	<0.2	47.5	43.2
	#19	21.7	1.2	<3.3	<0.8	63.0	44.3	45.4	24.8	10.0	23.2	<3	<0.2	5	9.8	0.1	<0.8	<0.2	78.4	51.5
	#20	15.5	3.2	<3.3	<0.8	102.3	75.0	124.1	53.2	14.1	30.1	<3	0.8	325	11.9	0.5	<0.8	<0.2	26.0	21.3
PH3 SUBJECTS																					
	mean ± SEM	***9.2 ± 1.4***	***3.4 ± 1.3***	***19.6 ± 2.3***	***4.0 ± 0.6***	***43.3 ± 6.1***	***49.0 ± 4.0***	***47.0 ± 4.0***	***9.4 ± 2.0***	***13.4 ± 2.6***	***12.8 ± 3.1***	***8.5 ± 2.5***	***11.6 ± 2.3***	***1.6 ± 0.2***	***4.4 ± 0.6***	***0.7 ± 0.3***	***2.3 ± 1.0***	***0.4 ± 0.1***	***78.7 ± 20.8***	***86.2 ± 24.9***
	median	***10.3***	***2.2***	***16.9***	***4.2***	***44.0***	***50.0***	***44.8***	***7.5***	***13.0***	***10.1***	***7.0***	***10.5***	***1.5***	***4.3***	***0.3***	***1.8***	***0.4***	***81.1***	***85.2***
	#21	12.0	3.6	26.7	3.9	39.6	47.7	45.9	9.1	12.7	11.8	22.3	14.6	1	6.0	0.5	8.4	0.6	86.0	126.3
	#22	3.6	6.0	16.9	2.9	44.0	44.4	43.7	19.8	21.3	20.6	12.4	6.1	1	1.6	1.3	2.6	0.2	78.1	79.9
	#23	3.8	0.8	15.7	1.6	62.8	65.8	71.7	7.8	13.6	11.4	12.1	19.9	2	2.6	0.1	2.3	0.3	65.3	103.1
	#24	8.8	6.4	26.2	5.4	44.0	51.5	39.0	7.2	14.5	8.9	3.3	4.7	1	4.6	1.0	1.2	0.3	116.1	102.6
	#25	13.6	9.8	25.1	4.4	13.3	28.6	42.5	16.0	26.2	31.1	8.7	10.4	2	4.0	2.6	3.0	0.5	84.0	83.1
	#26	7.2	0.2	12.8	2.1	46.6	53.0	50.5	6.6	7.2	7.6	5.3	21.6	2	3.9	0.0	0.8	0.5	90.4	87.3
	#27	12.8	0.3	<3.3	5.8	28.8	41.6	33.1	4.8	4.7	5.3	3.5	5.3	1	5.4	0.0	<0.8	0.3	62.2	57.5
	#28	11.7	0.3	<3.3	5.5	67.7	59.4	49.6	4.2	6.9	5.6	<3	10.5	3	7.1	0.0	<0.8	0.6	47.6	50.0
CONTROL SUBJECTS																					
	mean ± SEM	***2.8 ± 0.3***	***10.6 ± 0.5***			***13.2 ± 1.5***	***10.4 ± 1.4***	***10.6 ± 0.6***	***23.9 ± 5.7***	***30.4 ± 7.3***	***23.3 ± 4.0***		***0.4 ± 0.2***	***4.6 ± 1.3***	***0.3 ± 0.1***	***2.4 ± 0.5***			***83.7 ± 32.1***	***76.8 ± 20.7***
	median	***2.8***	***10.6***			***13.9***	***11.0***	***11.3***	***17.4***	***33.0***	***17.6***		***0.4***	***4.0***	***0.2***	***2.3***			***83.5***	***84.5***
	#1		13.5	-	-	-	13.7	11.5	8.7	17.0	14.5	-	-	-	0.6	2.3	-	-	53.2	59.4
	#2	4.4	8.5	-	-	-	11.5	11.3	-	16.3	17.1	-	-	-	0.3	2.4	-	-	82.5	77.0
	#3	1.8	8.7	-	-	-	10.2	9.9	14.7	14.4	13.2	-	-	-	0.1	1.5	-	-	93.6	87.1
	#4	3.0	9.6	-	-	-	9.2	9.6	-	23.1	22.2	-	-	-	0.2	2.5	-	-	133.0	85.7
	#5	3.8	11.9	<3.3	<0.8	13.9	11.9	11.9	26.2	32.6	31	<3	0.4	7	0.9	0.1	<0.8	<0.2	42.2	51.0
	#6	2.1	10.0	<3.3	<0.8	15.5	13.4	13.5	51.7	54.1	49.2	<3	0.6	4	0.2	5.5	<0.8	<0.2	44.1	44.8
	#7	3.1	11.4	<3.3	<0.8	16.4	11.4	11.3	34.4	33.7	31.2	<3	0.3	1	0.4	3.9	<0.8	<0.2	83.5	84.5
	#8	2.5	10.6	<3.3	<0.8	12.1	7.6	9.4	17.4	21.2	17.6	<3	0.5	8	0.2	2.3	<0.8	<0.2	109.3	102.6
	#9	1.8	11.2	<3.3	<0.8	8.0	5.2	7.1	13.9	10.5	13.2	<3	0.3	3	0.1	1.2	<0.8	<0.2	112.2	99.4

Hyp: hydroxyproline; T0: pre-infusion values; T final: post-infusion values at 360 min; urine values are from hourly urine collection except for those labeled “mean”, where the average of hourly values during the infusion was used; “-”: analysis not performed; “*”: data excluded (aberrantly high value most likely due to inappropriate sample handling leading to conversion of ascorbic acid to oxalate).