3. Title: Effect of Glycine, Serine and Hydroxyproline Supplementation with and without Phosphate or Thiamine Hydrochloride on Urinary Crystalluria and Urinary Composition (Ubol Village Infants)

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OBJECTIVE

Some insight into the possible etiology of bladder stone disease was obtained by studies on the influence of a variety of dietary supplements on urine composition. Two series of oral supplementation have been studied. In one study a series of methionine (300 mg), vitamin B_6 (3 mg) and placebo was given to Ubol Village infants. In a second study the infants were given inorganic orthophosphate (600 mg P), dry fat–free milk (600 mg P) and placebo. It was found that oral phosphate supplementation could eliminate the oxalate crystalluria and reduce the occurrence of uric acid crystalluria while supplementation with methionine and vitamin B_6 had no effect. Similar results were also obtained from a study in Chiengmai (Hyper–endemic area).

Since calcium oxalate is one of the main compositions of bladder stone in Thailand, it was of special interest to observe that both the orthophosphate and milk administrations also induced a significant decrease in urinary oxalate.

The higher oxalate excretion by village infants before phosphate supplementation is not likely to be due to higher intake since human milk, glutinous rice, and bananas have not been reported to be high in oxalate. Decreased destruction is not likely to occur since oxalate is believed to be metabolically inert in humans.

Increased formation of oxalate endogenously seems a more probable mechanism to explain higher excretion before P supplementation.

Since the only known precursor of oxalate in mammalion metabolism is glyoxylate, and a number of precursors of glyoxylate are known, including glycine, serine and hydroxyproline, this study is planned to investigate the sources of urinary oxalate and their relationship to dietary phosphate and thiamine hydrochloride by supplementation of glycine, serine and hydroxyproline with and without phosphate and thiamine hydrochloride.

DESCRIPTION

Thirty–five male infants ranging in age from 4 to 15 months who lived in four villages (Nong Jarn, Tung Kun Noi, Nong Kae and Tung Kun Yai) in Ubol Province were studied. There have been histories of bladder stone disease among the inhabitants of the village. Twenty nine infants were breast–fed while the other six were on bottle feeding; however they were all supplemented with glutinous rice since the early months of lives.

L–Glycine, 1.0 gm per day, was given alone orally for 5 days after the control period. The L–Glycine 1.0 gm plus orthophosphate (P ~ 600 mg) or L–Glycine 1.0 gm plus thiamine hydrochloride (50 mg) was administered for 5 days. There was a 3 day lapse between each of the three periods.

L–Serine 1.0 gm per day and hydroxy L–proline 1.0 gm per day were given to the other groups of infants following the same pattern as L–Glycine.

Twenty–four hour urine collections were made during the last period of giving each supplement, and freshly voided early morning urine samples were collected each day by utilizing pediatric urine collecting bags. Qualitative tests for PH, protein, sugar, and microscopic examination were performed daily as previously described.

PROGRESS

Table 1. shows number of subjects, mean ages, weight, height, and sequences of the supplementations.

The occurrence of oxalate crystalluria was studied in the breast–fed infants and shown in Table 2. During the placebo period of each series 18, 13 and 18 percent incidence was found; when L–glycine, L–serine and Hydroxy L–proline were given 36, 50 and 55 percent incidence was found respectively. However, the incidence of crystalluria was reduced to 10, 22 and 14 percent respectively when phosphate was added ta L–glycine, L–serine and Hydroxy L–proline. Adding of thiamine HC1 L–glycine caused no difference in the incidence of oxalate crystalluria; however, slight decrease in incidence was observed when thiamine HC1 was added to L–serine and Hydroxy L–proline.

Table 3. demonstrates the urinary excretion of oxalic acid, pyrophosphate and hydroxyproline in the village breast–fed infants following supplementation with a variety of substances described previously.

Urinary oxalic acid was found to increase with Hydroxy L–Proline administration and to decrease with L–Glycine; no difference was found after L–Serine supplementation. The highest degree of oxalate crystalluria and the increase urinary oxalic excretion after Hydroxy L–Proline supplementation suggested the possibility of Hydroxy L–Proline as an important source of urinary oxalate in the infants. Addition of phosphate to the three regimens reduced the urinary oxalic acid excretion as well as the occurrences of oxalate crystalluria.

Urinary pyrophosphate was uniformly low (1.2–4.1 mg/gm Creatinine) in all control and supplementation periods except those following the addition of orthophosphate supplementation, which exhibit nearly 10 fold increases in pyrophosphate excretion (14.0–15.8 gm/gm Creatinine).

Urinary hydroxyproline, however, did not show any significant difference following supplementation of the three regimens with or without the addition of orthophosphate. It is of interest to observe an increase in the excretion after the addition of thiamine hydrochloride to the three regimens.

Table 4. and 5. demonstrate the effect of the various supplements on the excretion of urinary uric acid, calcium, phosphorus, magnesium, sodium, potassium, and chloride.

Administration of L–Glycine, L–Serine and Hydroxy L–Proline caused no obvious effects on urinary composition, however addition of orthophosphate administration along with the three regimens demonstrated the same changes on urinary composition as were described previously. The changes included a marked increase af total urinary phosphate and a decrease of urinary calcium and magnesium. Urinary sodium and potassium were increased but this was expected since the orthophosphate was given in the form of sodium and potassium salts.

On the other hand, additional Thiamine hydrochloride with those three regimens revealed no significant differences in various urinary composition.

Table 1

Description of Subjects and Types of Supplementation

Ubol Province, Thailand

Village	Subjects No.	Ages Months	Weight Kg	Height Cm	Sequences of Supplementation
B	B_{27}	6	6.15	64.0	Placebo — G — G & PO_4
B	B_{29}	9	6.62	63.0	"
A	B_{10}	10	7.00	69.0	"
C	B_{2}	12	8.95	73.0	"
D	B_{17}	12	7.85	69.5	"
B	B_{28}	12	8.30	70.0	"
C	B_{3}	15	6.05	74.0	"
A	B_{31}	6	7.17	64.0	Placebo — S — S & PO_4
B	B_{30}	7	8.30	69.0	"
C	B_{5}	10	7.05	67.5	"
A	B_{12}	12	8.54	71.0	"
D	B_{19}	14	9.61	73.0	"
D	B_{20}	14	8.30	77.0	"
C	B_{6}	15	10.05	71.5	"
B	B_{31}	15	7.78	70.0	"
A	B_{14}	6	7.10	66.5	Placebo — HP — HP & PO_4
B	B_{32}	7	5.85	62.5	"
D	B_{22}	10	6.55	65.0	"
B	B_{33}	11	8.15	69.0	"
B	B_{34}	13	8.75	74.0	"
C	B_{7}	15	8.62	71.5	"
C	B_{8}	15	8.65	71.0	"
C	B_{1}	6	7.15	60.0	Placebo — G — G & B_1
A	B_{9}	6	7.37	66.5	"
A	B_{16}—a	10	6.95	67.0	"
D	B_{18}	14	7.67	66.5	"
C	B_{4}	5	7.10	63.5	Placebo — S — S & B_1
D	B_{21}	4	5.15	57.0	Placebo — HP — HP & B_1
A	B_{13}	5	5.45	61.0	"
D	B_{23}	13	7.43	70.0	Placebo — G — G & PO_4
A	B_{15}	14	9.20	73.0	"
A	B_{16}	12	8.45	68.5	Placebo — S — S & PO_4
D	B_{24}	14	7.78	70.0	"
D	B_{26}	12	11.30	80.0	Placebo — HP — HP & PO_4
D	B_{25}	13	5.92	66.0	"

a. A = Nong Jarn, B = Tung Kun Yai, C = Nong Kae, D = Tung Kun Noi

b. All subjects were on breast–feeding except the last group who were supplemented with sweetened condensed milk.

c. Each supplementation was given for 5 days with 3 days time lapse in each period.
G = L–glycine, S = L–Serine, HP = Hydroxy–L–Proline 100 Gm/day; PO_4 = Phosphate buffer 600 mg/day

B_1 = Thiamine hydrochloride 50 mg/day.

Table 2

Occurrence of Oxalate Crystalluria in Village Infants* Supplementation with Oxalate Precursor with and without Ortho–Phospate or Thiamine Hydrochloride.

Supplement	No. of Infants	Oxalcrystalluria**			No. of Infants with Crystalluria
		No. of Occurrence	No. of Examination	Percentage	
Placebo	11	12	66	18	6
L–Glycine	11	16	44	36	10
L–Glycine + PO_4	7	3	28	11	2
L–Glycine + B_1	4	6	16	37	3
Placebo	9	7	54	13	6
L–Serine	9	18	36	50	8
L–Serine + PO_4	8	7	32	22	5
L–Serine + B_1	1	1	4	25	1
Placebo	9	10	54	18	6
HO–L–Proline	9	20	36	55	8
HO–L–Proline + PO_4	7	4	28	14	4
HO–L–Proline + B_1	2	1	8	12	1

* All subjects here were on breast–feeding with glutinous rice supplementation.

** Microscopic examination from casual urine samples.

Table 3

Urinary Excretion of Oxalic Acid, Pyrophosphate and Hydroxyproline in Village Infants Following Supplementation with a Variety of Substances

Supplementation	No. of Subjects	Ages Months	Oxalic Acid mg/gm Creatinine	Pyrophosphate mg/gm Creatinine	Hydroxyproline mg/24 hr Urine
Placebo	10	10.2	183.8 ± 61.3	1.7 ± 0.5	20.4 ± 2.6
L–Glycine	10	10.2	116.9 ± 6.6	1.2 ± 0.2	20.3 ± 2.7
L–Glycine + PO_4	7	10.8	51.1 ± 17.8	14.0 ± 1.6	16.8 ± 3.1
L–Glycine + B_1	3	8.7	178.3 ± 40.7	1.9 ± 0.8	27.9 ± 5.3
Placebo	9	10.9	139.7 ± 21.5	1.2 ± 0.2	22.8 ± 2.1
L–Serine	9	10.9	140.6 ± 17.0	1.6 ± 0.6	20.6 ± 2.8
L–Serine + PO_4	8	11.6	90.9 ± 23.7	15.8 ± 1.9	31.4 ± 6.6
L–Serine + B_1	1	5.0	102.9	2.8	39.0
Placebo	9	9.6	139.6 ± 37.5	1.9 ± 0.4	17.2 ± 1.5
Hydroxy L–Proline	9	9.6	185.9 ± 15.8	1.4 ± 0.2	33.2 ± 10.6
Hydroxy L–Proline + PO_4	7	11.0	131.7 ± 29.6	15.8 ± 2.6	25.9 ± 4.3
Hydroxy L–Proline + B_1	2	4.5	386.9 ± 14.5	4.1 ± 3.2	76.1 ± 33.5

Table 4

Effect of Oxalate Precursors Supplementations

and Their Relation to Dietary Phosphate on Various Urinary Composition

Groups of Supplementations	No. of Subj.	Urinary Components (mg/gm Creatinnine) Mean ± S.E.						
		Uric Acid	Calcium	Phosporus	Megnesium	Sodium	Potassium	Chloride
Placebo	7	497.6±129.7	70.7±20.9	272.1± 95.8	119.0±18.4	876.2±359.8	1680.6±585.6	3136.2±1176.4
L—Glycine	7	151.4±49.3	74.6±19.3	142.6± 43.6	106.5±11.7	770.1±211.1	675.0± 97.4	2318.2± 438.6
L—Glycine + PO_4	7	764.2±115.9	23.3± 5.2	4307.5±657.8	82.2±20.7	5557.4±959.7	2135.0±532.9	2420.6± 313.1
Placebo	8	314.2±69.9	68.3±20.1	116.9± 37.0	107.8±19.7	1504.5±362.8	1007.6±188.3	3075.6± 732.5
L—Serine	8	418.8±104.9	92.0±26.6	168.2± 50.8	97.2±10.9	1944.0±541.2	1367.3±383.4	3303.2± 829.7
L—Serine + PO_4	8	821·9±160.3	26.2± 6.7	4346.6±455.8	79.8±12.3	6620.0±556.5	2254.2±257.2	2389.5± 626.6
Placebo	7	453.0±227.8	102.2±23.5	270.2± 71.8	100.4±35.4	1126.5±384.7	1417·2±548.3	6541.1±320.35
Hydroxy L—Proline	7	521.7±104.5	115.1±60.7	206.6± 64.7	104.2±21.1	1782.6±481.9	1558.5±202·1	4065.8± 785.3
Hydroxy L—Proline + PO_4	7	887.0±171.9	30.8± 5.1	4707.3±647.9	59.7±14.1	6007.5±1089.4	2744.3±536.8	2410.1± 947.7

Table 5

Effect of Oxalate Precursors Supplementations and Their Relation to Dietary Thiamine Hydrochloride on Various Urinary Compositions

Groups of Supplementations	No. of Subj.	Urinary Components (mg/gm Creatinine) Mean ± S.E.						
		Uric Acid	Calcium	Phosphorus	Magnesium	Sodium	Potassium	Chloride
Placebo	4	489.2±142.1	146.2±42.7	169.8±100.1	120.4±4.8	709.6±181.5	1636.2±601.8	2508.7±444.9
L—Glycine	4	495.4±182.9	144.2±33.5	137.6±68.3	113.0±13.2	954.6±366.1	1173.5±272.5	2614.5±528.5
L—Glycine + B_1	4	816.1±326.8	222.5±62.5	112.9±35.5	184.0±15.9	1412.8±802.1	1634.7±273.9	8871.9±4535.9
Placebo	1	1173.9±00	51.2±00	4.3±00	150.0±00	579.5±00	1070.8±00	1079.1±00
L—Serine	1	973.6±00	578.9±00	47.3±00	76.3±00	399.4±00	1790.5±00	2053.1±00
L—Serine + B_1	1	32.1±00	410.7±00	7.1±00	162.5±00	188.9±00	1089.2±00	760.0±00
Placebo	2	751.3±18.0	172.8±119.8	18.4±8.2	123.9±27.4	771.4±40.8	2967.6±160.9	2768.5±50.0
Hydroxy L—Proline	2	985.7±85.9	75.7±4.3	85.2±72.1	128.1±14.8	1091.1±319.9	3719.3±331.7	3984.9±321.8
Hydroxy L—Proline + B_1	2	203.2±34.4	28.1±3.0	415.9±362.9	111.4±22.9	500.0±286.2	1558.6±1121.9	2876.0±671.8