Methionine feeding prevents kidney stone deposition by restoration of free radical mediated changes in experimental rat urolithiasis

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Feeding calculi producing diet (CPD) to rats for 4 weeks produced calcium oxalate stones deposition. Supplementation of methionine to CPD (m-CPD) prevented the stone deposition. However the urine pH and excretion of oxalate and calcium in m-CPD–fed rats was still as high as in CPD-fed groups compared to that of the control group. The CPD-fed rats exhibited an increase in liver oxalate synthesizing enzymes and glycolic acid oxidase (GAO) and lactate dehydrogenase (LDH), and these activities were not restored in m-CPD–fed rats. Similarly, the elevated LDH activity and oxalate concentration observed in the kidney of CPD-fed rats were not restored by methionine supplementation. Kidney sub-cellular fractions of CPD-fed rats showed increased susceptibility for lipid peroxidation in presence of iron, ascorbate, and t-butyl hydroperoxide. Antioxidant enzyme activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase and antioxidant concentrations of reduced glutathione, total thiols, ascorbic acid, and vitamin E were significantly decreased, while the xanthine oxidase activity and concentrations of hydroxyl radical, diene conjugates, and hydroperoxides were significantly increased in CPD-fed rats. The susceptibility to lipid peroxidation, activities of antioxidant enzymes, and the concentration of antioxidants were normalized in m-CPD–fed rats, thus suggesting that methionine feeding prevents the stone formation by neutralizing the free radical induced changes.

Keywords: methionine; kidney stone deposition; urolithiasis, rat; calculi producing diet

Introduction

The precise mechanism by which kidney stones originate is still not well understood. For quite some time attention was focused toward the relationship between the concentrations of the precipitating substances in urine and the solubility of the salts to be formed,[1] the role of nucleating substances,[2] the role of crystal aggregation[3] and the part played by inhibitors of crystal formation and aggregation.[4] The possible relationship between cell dysfunction and renal stone formation is yet to be explored. It is suggested that stone formation may be a cellular defect in the handling of oxalate, calcium, phosphate, or magnesium or in response by the kidney to hormones such as parathyroid hormone or calcitonin or 25-hydroxy calciferol. Very little study has been done on the possible relationship between cell dysfunction and renal stone formation.

Many biochemical changes, notably accumulation of oxalic acid in kidney membrane fractions by feeding calculi producing diet,[5] or vitamin B_6-deficient diet,[6] or administration of sodium oxalate,[7] elevated levels of phosphatidic acid[8] and sialic acid[9] in calculus rat kidney and enhanced lipid peroxidation in kidney of sodium glycolate-fed rats,[10] vitamin B_6-deficient-diet–fed rats[11] and red blood cells of kidney stone formers[12] have been reported in our earlier studies. Further citrate[13] or pyrophosphate[14] feeding is shown to prevent stone deposition in stone forming rats. The mechanism of inhibition of stone formation by citrate is found to be mediated through restoration of urinary

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risk factors and not by free radical induced changes.[15] This study presents increased lipid peroxidation reaction in calculus kidney due to depletion of antioxidants and these reactions are normalized by feeding methionine.

Materials and methods

Animals

Adult male Wistar rats weighing approximately 100–120 g were purchased from Forensic Sciences Laboratory (Madras, India) and were housed in the Institute animal room. The animal room was well ventilated and no special arrangements were made for heating and cooling. Animals were given normal chow and water ad libitum for 1 week.

Diet

The rat chow was supplied by M/s. Hindustan Lever Ltd. (Bombay, India). The pelleted feed contained protein (21%), lipids (5%), crude fibre (4%), ash (8%), calcium (1%), phosphorus (0.6%), nitrogen free extract (55%), and provided metabolizable energy of 3600 kcal per kg. The diet was enriched with vitamins and trace elements.

Induction of calcium oxalate stones in rats

Calcium oxalate stones were induced in rats according to the method of Chow et al.[16] The rat chow was powdered and then supplemented with 3% sodium glycolate (wt/wt), mixed well with water, pelleted, and dried. This diet was the calculi producing diet (CPD).

Experimental design

The rats were divided into four groups on the basis of their weight with each group containing twelve animals: group I animals were fed with normal rat chow; group II animals were fed CPD; group III animals were fed with CPD, supplemented with L-methionine (330 mg/100 g feed); group IV animals were fed with normal rat chow supplemented with L-methionine (330 mg/100 g feed).

All groups were fed with food and water ad libitum for 4 weeks.

At the end of the experimental period (24 hr) urine samples of all experimental groups were collected by means of metabolic cages using concentrated HCl as preservative. For the determination of urinary pH, separate samples were collected without HCl. The animals were killed by cervical decapitation. Kidneys and livers were quickly dissected into ice-cold saline. The kidneys were trimmed free of connective tissue and finely minced. A 20% homogenate was prepared in Tris-HCl buffer (0.02 M, pH 7.4) containing 0.25 M sucrose using a Potter-Elvehjem homogenizer fitted with power driven Teflon pestle. The homogenate was subjected to differential centrifugation in a Sorvall ultracentrifuge (model OTD 50) at 4° C. The subcellular organelles were isolated according to the method of Sottacasa[17] and the purity of the fractions were checked using specific marker enzymes.

Lipid peroxidation (LPO) of tissue fractions was measured by the release of thiobarbituric acid reactive substances (TBARS) by the method of Brogan et al.[18] NADPH/ascorbate/ferrous ion/peroxide-induced LPO were measured by the method of Devasagayam and Tarachand.[19] Antioxidant enzymes, superoxide dismutase,[20] catalase,[21] and glutathione peroxidase[22] were determined. Antioxidants, ascorbic acid,[23] total reduced glutathione,[24] vitamin E,[25] and total sulphydryl groups,[26] were determined. Xanthine oxidase[27] and glucose-6-phosphate dehydrogenase[28] were estimated. Hydroxyl radicals were determined according to the method of Puntarulo and Cederbaum[29] and the formaldehyde formed in this process was estimated by the method of Burton.[30] Hydroperoxides were estimated by the method of Beuge and Aust[31] and diene conjugates were analysed by the method of Klein.[32] Tissue oxalate[7] and urinary constituents[13] were determined as described earlier. Lactate dehydrogenase was assayed according to the method of Liao and Richardson[33] using glyoxylate as substrate. Glycolate oxalate was assayed by the method of Lui and Roeless.[34] Protein was estimated by the method of Lowry et al.[35]

Histopathological studies were carried out as described earlier.[7]

Statistical analysis was done by Student *t* test.

Results

CPD-fed rats appeared normal except that they showed decreased body weight gain (*Table 1*). The weight of liver and kidney and their protein concentration did not change significantly in the rats fed with CPD when compared with normal diet-fed rats. Feed-

Table 1 Body weight, tissue weight, and tissue protein concentration of control and experimental rats

Particulars	Control	CPD	CPD + methionine	Methionine
Body weight (g)	148.0 ± 16.0	125.0 ± 12.0	145.0 ± 13.0	149.0 ± 15.0
Weight of liver (g)	5.80 ± 0.35	4.80 ± 0.60	5.30 ± 0.51	5.56 ± 0.45
Protein (mg/g wet tissue)	163.0 ± 16.6	152.2 ± 18.2	160.0 ± 13.5	166.5 ± 15.0
Weight of kidney (g)	1.35 ± 0.15	1.15 ± 0.13	1.31 ± 0.09	1.30 ± 0.18
Protein (mg/g wet tissue)	155.5 ± 15.1	144.0 ± 15.0	159.0 ± 13.8	152.6 ± 15.7

Values are mean ± SD for twelve animals in each group.

Table 2 Urinary pH and excretory levels of calcium, phosphorus, magnesium, oxalate, and citrate in control and experimental rats

Particulars	Control	CPD	CPD + methionine	Methionine
Calcium	2.05 ± 0.20	15.5 ± 0.25***[a]	14.0 ± 0.30***[a]	1.95 ± 0.20
Phosphorus	9.80 ± 0.25	3.10 ± 0.25***[a]	3.00 ± 0.30***[a]	9.50 ± 0.30
Magnesium	1.90 ± 0.03	1.05 ± 0.05***[a]	1.10 ± 0.08***[a]	2.10 ± 0.35
Oxalate	0.27 ± 0.05	2.82 ± 0.08***[a]	2.30 ± 0.05***[a]	0.25 ± 0.03
Citrate	3.80 ± 0.28	2.50 ± 0.20***[a]	2.20 ± 0.25***[a]	3.35 ± 0.30
pH	6.35 ± 0.30	8.71 ± 0.35***[a]	8.64 ± 0.40***[a]	6.20 ± 0.25

[a] Significantly different compared with control.
*** $P < 0.001$.
Values are expressed as mg/24 hr/rat: Values are mean ± SD for twelve animals in each group.

Table 3 Oxalate synthesizing enzymes of liver and kidney and kidney oxalate content of control and experimental rats

Particulars	Control	CPD	CPD + methionine	Methionine
Liver				
Glycolic acid oxidase	1.32 ± 0.12	2.40 ± 0.18***[a]	2.45 ± 0.19***[a]***[b]	1.40 ± 0.09
Lactate dehydrogenase	1.80 ± 0.12	2.99 ± 0.18***[a]	2.70 ± 0.15***[a]**[b]	1.59 ± 0.12
Kidney				
Lactate dehydrogenase	1.95 ± 0.16	3.51 ± 0.20***[a]	3.06 ± 0.15***[a]**[b]	2.24 ± 0.10
Total oxalate content	0.55 ± 0.15	0.96 ± 0.20***[a]	0.90 ± 0.15***[a]	0.50 ± 0.18

[a] Significantly different compared with control.
[b] Significantly different compared with CPD.
*** $P < 0.001$.
** $P < 0.01$.
Lactate dehydrogensase activity is determined using glyoxylate as substrate and is expressed in terms of units/mg protein (1 unit = the amount of enzyme that brings about a change in O.D. of 0.01/min). Glycolic acid oxidase is expressed as nmol of glyoxylate formed/mg protein. Oxalate content is expressed in mg/g wet tissue. Values are mean ± SD for twelve animals in each group.

ing methionine, a supplement for the cellular –SH compounds, with CPD (m-CPD), to rats caused them to regain the body weight compared with the CPD-fed rats. Feeding methionine alone did not affect the body weight gain or tissue protein concentration.

Feeding CPD for 4 weeks induced calcium oxalate deposits in the renal tissues of all 12 rats.[9] It is significant to note that no calcium oxalate deposition was observed in the kidneys of rats fed m-CPD. Histologic examination of stone-forming rat kidney revealed cystic dilations of tubules and few tubular dilations with complete occlusions. Feeding m-CPD revealed no pathological changes.

Excretion of oxalate and calcium

The urinary excretion of calcium, oxalate, phosphorus, magnesium, and citrate of control and experimental rats are given in *Table 2*. The normal excretion of calcium was 2.05 mg/24 hr/rat and this value was significantly enhanced to 15.5 mg/24 hr/rat ($P < 0.001$) in CPD-fed rats. The normal excretion values of phosphorus, citrate, and magnesium were 9.8 mg, 3.8 mg, and 1.9 mg/24 hr/rat, respectively and all of these values decreased significantly ($P < 0.001$) to 2.82 mg, 2.5 mg, and 1.05 mg/24 hr/rat, respectively in CPD-fed rats. Oxalate excretion was 0.27 mg/24 hr/rat for the control group and this value was increased nearly 10-fold in CPD-fed rats. Supplementation of methionine in CPD did not restore their excretion.

The urine pH of control rats was acidic in nature (6.35 ± 0.30) while that of CPD-fed rats was alkaline (8.71 ± 0.35). This alkaline pH was maintained even after feeding methionine (8.64 ± 0.40).

Oxalate synthesizing enzymes

Liver is the main tissue to synthesize oxalate endogenously. The activities of oxalate synthesizing enzymes, glycolic acid oxidase (GAO) in liver and lactate dehydrogenase (LDH) in liver and kidney of control and experimental rats are given in *Table 3*. The CPD-fed rats exhibited an 80% increase in GAO activity over that of the control group and GAO activity remained elevated significantly in the m-CPD–fed rats. Liver and kidney LDH activities, using glyoxylate as the substrate, were increased by approximately 90% and 60%, respectively in the CPD-fed rats compared with control. This enhanced LDH activity was not restored to normal in either liver or kidney in m-CPD–fed rats. The enzyme activity observed in this group was still higher than that of control rats suggesting that LDH activity was only partially restored.

Total oxalate concentration in control kidney was

Table 4 Effect of enzymatic and non-enzymatic peroxidation in rat kidney nuclear, mitochondrial, and microsomal fractions

Stimulant	TBARS release (nmol/mg/protein/20 min)			
	Control	CPD	CPD + methionine	Methionine
Nuclear				
None	1.06 ± 0.08	1.88 ± 0.14***[a]	1.15 ± 0.07***[b]	1.07 ± 0.08
NADPH	3.21 ± 0.15	4.59 ± 0.36***[a]	3.24 ± 0.11***[b]	3.20 ± 0.25
Ascorbate	3.57 ± 0.20	6.77 ± 0.31***[a]	3.63 ± 0.15***[b]	3.58 ± 0.21
$FeSO_4$	5.68 ± 0.49	8.05 ± 0.70***[a]	5.67 ± 0.20***[b]	5.53 ± 0.20
t-BH	4.05 ± 0.38	6.09 ± 0.54***[a]	4.12 ± 0.11***[b]	4.03 ± 0.12
Mitochondria				
None	2.05 ± 0.20	3.20 ± 0.29***[a]	2.14 ± 0.13***[b]	2.00 ± 0.16
Ascorbate	6.57 ± 0.57	9.91 ± 0.79***[a]	6.60 ± 0.54***[b]	6.57 ± 0.40
$FeSO_4$	11.02 ± 0.61	19.04 ± 1.65***[a]	11.69 ± 0.62***[b]	11.03 ± 0.65
t-BH	5.10 ± 0.59	9.17 ± 0.52***[a]	5.16 ± 0.44***[b]	5.02 ± 0.40
Microsomal				
None	3.80 ± 0.30	5.08 ± 0.42***[a]	3.82 ± 0.25***[b]	3.70 ± 0.21
NADPH	5.71 ± 0.38	11.96 ± 0.90***[a]	5.83 ± 0.36***[b]	5.63 ± 0.34
Ascorbate	20.85 ± 1.26	38.74 ± 2.80***[a]	23.17 ± 1.90***[b]	20.45 ± 1.70
$FeSO_4$	31.00 ± 2.78	47.92 ± 5.60***[a]	32.02 ± 3.60***[b]	29.55 ± 3.00
t-BH	7.13 ± 0.63	12.48 ± 0.80***[a]	7.17 ± 0.50***[b]	7.10 ± 0.67

[a] Significantly different compared with control.
[b] Significantly different compared with CPD.
*** $P < 0.001$.
Values are mean ± SD for six animals in each group. 0.2 mmol/L of NADPH, ascorbate and $FeSO_4$, and 0.1 mmol/L t-BH were used.

0.55 mg/g wet tissue and this value was significantly increased to 0.96 mg/g wet tissue ($P < 0.001$). This elevated level was not restored to normal by methionine supplementation.

Lipid peroxidation

Ascorbic acid, a precursor of oxalate biosynthesis and oxalate have been shown to increase lipid peroxidation in tissues non-enzymatically.[36] The susceptibility of subcellular organelles to lipid peroxidation was studied in presence of NADPH (enzymatic) and ascorbate, iron, organic peroxides like tBH (non-enzymatic) as stimulators. The data are given in *Table 4*. The basal TBARS levels were 1.06, 2.05, and 3.8 nmol/mg protein for control rat nuclear, mitochondrial, and microsome fractions, respectively. These values were significantly increased in the presence of stimulators in all three fractions of all the groups. Among them, Fe^{2+} stimulated the lipid peroxidation maximally in all three fractions of all groups. The degree of stimulation was in the order of Fe^{2+} > ascorbate > tBH. However, all three fractions of CPD-fed rats were found to be more susceptible for lipid peroxidation in presence of ascorbate or iron than control rat fractions. (Ascorbate stimulated 5- and 7.5-fold and iron 8- and 9.5-fold in control and CPD-fed rat microsomes, respectively). But in contrast, m-CPD–fed rat fractions behaved similarly to control rat fractions suggesting that feeding methionine protected the membranes from oxidative damage.

Antioxidant enzymes

Table 5 presents the activities of kidney SOD, catalase, and glutathione peroxidase of control and experimental rats. Mn-SOD, Cu-Zn SOD, catalase, and glutathione peroxidase activities were significantly decreased ($P < 0.001$; $P < 0.01$; $P < 0.001$; $P < 0.001$, respectively) in the CPD-fed rats compared with control rats. Feeding m-CPD restored both Cu-Zn SOD and Mn-SOD, catalase, and glutathione peroxidase to normal.

Hydroxyl radicals, diene conjugates, hydroperoxides, and iron

Table 6 presents the concentration of hydroxyl radicals, diene conjugates, hydroperoxides, and iron in the kidney of control and experimental rats. The concentrations of hydroxyl radicals and hydroperoxides were enhanced 1.6- and 3-fold, respectively, in CPD-fed rats. It is very interesting to note that feeding m-CPD restored these values to normal. The ratio of A_{233}/A_{215} for the measure of diene conjugates was significantly high in the CPD-fed rats when compared with that of control. This elevated level was restored completely in m-CPD–fed rats. Iron concentration was not significantly altered in experimental conditions.

Superoxide and NADPH generating enzymes

The activity of superoxide generating enzyme, xanthine oxidase, and NADPH-producing enzyme, glucose-6-phosphate dehydrogenase are given in *Table 7*. Xanthine oxidase activity was significantly high in CPD-fed rats ($P < 0.001$) compared with control rats. This elevated activity was not restored to normal in m-CPD–fed rats. In contrast, a highly significant decrease in G6PD activity was noted in CPD fed rats ($P < 0.001$) compared to control rats. This activity was normalized in m-CPD–fed rats.

Table 5 Activities of antioxidant enzymes of control and experimental rat kidney

Particulars	Control	CPD	CPD + methionine	Methionine
Cu Zu-SOD	5.59 ± 0.60	4.32 ± 0.34**[a]	5.50 ± 0.45**[b]	5.29 ± 0.50
Mn-SOD	2.45 ± 0.21	1.98 ± 0.12***[a]	2.35 ± 0.15***[b]	2.40 ± 0.19
Catalase	38.10 ± 3.50	23.68 ± 3.60***[a]	35.50 ± 3.50***[b]	36.40 ± 3.50
Glutathione peroxidase	4.25 ± 0.33	2.70 ± 0.15***[a]	4.14 ± 0.25***[b]	4.30 ± 0.28

SOD activity is expressed in units/mg protein (1 unit = amount of enzyme that inhibits the autoxidation reaction by 50%). Catalase activity is expressed as μmol of H_2O_2 consumed/mg protein/minute. Glutathione peroxidase activity is expressed in terms of μg of reduced glutathione utilized/min/mg protein.
[a] Significantly different compared with control.
[b] Significantly different compared with CPD.
*** $P < 0.001$.
** $P < 0.01$.
Values are mean ± SD for twelve animals in each group.

Table 6 Concentration of hydroxyl radicals and hydroperoxides of control and experimental rat kidney

Stimulant	Control	CPD	CPD + methionine	Methionine
Hydroxyl radical	2.55 ± 0.25	3.75 ± 0.35***[a]	2.14 ± 0.25***[b]	2.20 ± 0.15
Hydroperoxides	2.05 ± 0.09	5.55 ± 0.25***[a]	2.30 ± 0.10***[a]	1.98 ± 0.086
Diene conjugates	0.42 ± 0.05	0.62 ± 0.04***[a]	0.45 ± 0.05	0.38 ± 0.05
Iron	12.00 ± 0.80	11.90 ± 0.75	10.58 ± 0.80	10.50 ± 1.05

OH radicals are expressed as nmol of formaldehyde formed/min/mg protein. Hydroperoxides are expressed in μg of the t-butyl hydroperoxide/mg protein. Diene conjugate is the ratio of A_{233}/A_{215}. Iron is expressed in nmol/mg protein.
[a] Significantly different compared with control.
[b] Significantly different compared with CPD.
*** $P < 0.001$.
Values are mean ± SD for twelve animals in each group.

Table 7 Activities of xanthine oxidase and glucose-6-phosphate dehydrogenase of control and experimental rat kidney

Particulars	Control	CPD	CPD + methionine	Methionine
Xanthine oxidase	0.70 ± 0.05	1.22 ± 0.06***[a]	1.05 ± 0.05***[a]	0.74 ± 0.04
Glucose-6-phosphate dehydrogenase	2.55 ± 0.25	1.90 ± 0.15***[a]	2.40 ± 0.10***[b]	2.40 ± 0.09

Activities are expressed in terms of units/mg protein (1 unit = the amount of enzyme that brings about a change in O.D. of 0.01/min).
[a] Significantly different compared with control.
[b] Significantly different compared with CPD.
*** $P < 0.001$.
Values are mean ± SD for nine animals in each group.

Antioxidants

To determine whether the increased susceptibility to lipid peroxidation was associated with depletion of antioxidants, the concentrations of reduced glutathione, total thiols, ascorbic acid, and vitamin E were determined in experimental rats and the data are given in *Table 8*. All the above antioxidants were significantly low in concentrations ($P < 0.001$) in CPD-fed rats compared with that of control rats. However feeding m-CPD restored the levels of all the antioxidants to near normal.

Discussion

Rats fed CPD show decreased body weight gain without change in organ weights or protein content of the tissues. Similar observations have been reported by Chow et al.[16] All rats fed CPD showed calcium oxalate deposition in renal tubules by histologic examinations. Supplementation of CPD with methionine abolishes the stone desposition completely and the loss of body weight.

Feeding CPD increases the excretion of oxalate and calcium and decreases phosphorus, magnesium, and

Table 8 Concentration of antioxidants in control and experimental rat kidney

Particulars	Control	CPD	CPD + methionine	Methionine
Reduced glutathione	2.50 ± 0.21	1.48 ± 0.10***[a]	2.35 ± 0.20***[b]	2.56 ± 0.23
Total thiol	11.05 ± 1.10	7.93 ± 0.55***[a]	10.92 ± 0.73***[b]	10.14 ± 0.77
Ascorbic acid	1.29 ± 0.08	0.80 ± 0.06***[a]	1.35 ± 0.09***[b]	1.32 ± 0.075
Vitamin E	0.85 ± 0.056	0.45 ± 0.04***[a]	0.75 ± 0.05***[b]	0.84 ± 0.07

[a] Significantly different compared with control.
[b] Significantly different compared with CPD.
*** $P < 0.001$.
Concentrations are expressed as μg/mg protein. Values are mean ± SD for twelve animals in each group.

citrate. Increased excretion of precipitating ions and elevated urine pH decreased excretion of inhibitors are known favorable conditions for stone formation. However, supplementation of methionine does not normalize the urine pH or the excretion of the ions. Elevated level of oxalate in urine has been found in stone patients,[37] pyridoxine deficient rats,[38] and sodium glycolate fed rats.[13] Increased excretion of oxalate by feeding methionine is correlated with the increased activities of oxalate synthesizing enzymes. This suggests that the inhibition of stone formation by methionine is not associated with either, through the inhibition of oxalate synthesizing enzymes (*Table 3*) or restoration of risk factors concentrations in urine (*Table 2*).

To determine whether the mechanism of inhibition of stone formation by methionine is due to inhibition of free radical mediated reactions, further studies have been undertaken.

Significant formation of TBARS by enzymatic (NADPH-induced) and non-enzymatic (ascorbate-iron, t-BH–induced) lipid peroxidation is noted in all subcellular fractions of CPD-fed rats, suggesting increased membrane susceptibility.

The enhanced lipid peroxidation and susceptibility to lipid peroxidation by enzymatic and non-enzymatic systems in CPD-fed rat kidney may be due to either (a) increased promoters of lipid peroxidation or (b) decreased antioxidant protection. Several promoters such as iron and copper have been shown to promote lipid peroxidation.[39] In addition, oxalate has been shown to stimulate lipid peroxidation in rats as a result of either feeding sodium glycolate or vitamin B_6-deficient diet or administration of sodium oxalate.[10,40,41] The present study, (*Table 3*) as well as our earlier studies have shown accumulation of oxalate in kidney subcellular fractions[5] in CPD-fed rats. The elevated lipid peroxidation reaction observed in CPD-fed rat kidney may be associated with the increased levels of promoters.

α-Tocopherol, ascorbic acid, and reduced glutathione levels are significantly low in CPD-fed rat kidney and this is an interesting observation. These three antioxidants are interrelated for recycling processes. Recycling of tocoperoxyl radicals to tocopherol is achieved by reaction with ascorbic acid.[42] The dehydroascorbic acid formed in the above reaction is reduced to ascorbic acid by a non-enzymatic reaction with reduced glutathione.[43] McCay et al[44] have shown the presence of a labile glutathione-dependent factor, which cycles the tocopheroxyl radicals to tocopherol. If recycling of tocopheroxyl radicals to tocopherol is a major mechanism for maintenance of tissue tocopherol levels, deficiency of ascorbic acid is expected to result in depletion of tissue tocopherol. Since we have observed a significant decrease in ascorbic acid level, recycling of tocopheroxyl radicals to tocopherol must have been hindered, resulting in elevated lipid peroxidation reactions. A similar observation has been reported in vitamin B_6-deficient rat kidney.[45] Reduced glutathione maintains cell membrane sulphydryl groups and other structural proteins in the stable form NADPH, which is required for GSH generation (via glutathione reductase) and is supplied by glucose-6-phosphate dehydrogenase.[46] The decreased activity of G6PD observed in CPD-fed rats may decrease the generation of NADPH and thereby decrease the reduction of oxidized glutathione to reduced glutathione.

Accumulation of H_2O_2 is highly toxic to cells. Catalase and glutathione peroxidase are involved in the elimination of H_2O_2. The decreased activity of glutathione peroxidase in CPD-fed rats may be correlated to decreased availability of its substrate, reduced glutathione. Catalase requires NADPH for its regeneration from inactive form[47] and hence, the decreased catalase activity in CPD-fed rats may be associated with low NADPH availability. Further catalase has been shown to be inhibited by oxalate[41] and the observed decreased activity in CPD-fed rats may also be associated with the enhanced level of oxalate. The accumulation of H_2O_2 formed by the reactions lead to increased formation of hydroxyl radicals either with iron by Fenton type reaction or with iron in presence of superoxide by Haber-Weiss reaction. This is supported by the fact that xanthine oxidase that produces superoxide anions,[48] is increased in CPD-fed rats. The enhanced levels of hydroxyl radicals increase diene conjugates, hydroperoxides, and TBARS in CPD-fed rats. Due to the decreased antioxidant levels, the above reactions are not neutralized and show enhanced susceptibility to lipid peroxidation in presence of promotors of lipid peroxidation. As the concentrations of antioxidants are restored to normal level in m-CPD–fed rats, the increased susceptibility to lipid

peroxidation is now prevented. Similar protective role by feeding methionine has been reported in ethanol-induced[49] or lanthanum chloride and neodymium chloride[50] -induced lipid peroxidation.

Lipid peroxidation is also implicated in a number of pathogenic processes.[51-53] Peroxidized microsomal membranes show increased permeability for calcium.[54] Ischemic insult or toxic killing increases the cellular and mitochondrial calcium in kidney by free radical damages.[55,56] These reactions are most likely to be present in conditions like CPD feeding, favoring stone formation, and absent in m-CPD feeding and thereby favoring protection from stone formation.

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