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CITRATE AND MINERAL METABOLISM: KIDNEY STONES AND BONE DISEASE

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1. ABSTRACT

Citrate is a weak acid that is formed in the tricarboxylic acid cycle or that may be introduced with diet. In the present paper all the mechanisms involved in intestinal absorption, renal handling and modulation of citrate will be reviewed. The evaluation of plasma citric acid is scarcely used in the diagnosis of human diseases. On the contrary urinary citrate excretion is a common tool in the differential diagnosis of kidney stones, renal tubular acidosis and it plays also a role in bone disases. Therefore the importance of hypocitraturia will be reviewed with regard to bone mass, urine crystallization and urolithiasis. Finally particular attention will be paid to the incidence of hypocitraturia and to the therapy with citrate salts, both in kidney stone disease and in osteopenia.

2. GENERAL PHYSIOLOGICAL CONCEPTS

Citrate is a weak acid that is formed in the tricarboxylic acid cycle or that may be introduced by exogenous sources. The molecular weight of citric acid is 189 KDa and its pKa values are respectively 2.9, 4.8 and 5.6. At pH 7 citrate exists principally (> 90%) as a trivalent anion; the divalent form of citrate becomes significantly greater at a more acidic pH (1). The extracellular (plasma) concentration of citrate is about 0.1 mM/l. Most of the citrate in the blood circulates unbound and the remaining quota is complexed to calcium, potassium and sodium (2,3). Although some of the tricarboxylic acid cycle enzymes are found in the cytosol, the main localization of these enzymes is in the mitochondria. The initial step of the cycle is catalyzed by the enzyme citrate synthase. The citrate synthase reaction involves the condensation of the acetyl moiety and the alpha-keto function of the dicarboxylic acid oxaloacetate. It has been suggested that the activity of this enzyme may be modulated by ATP, NADH, succinyl-CoA and long-chain acyl-CoA derivatives. It is most probable that the primary regulator of the citrate synthase reaction is the availability of its two substrates, acetyl CoA and oxaloacetate. The citrate molecule is involved not only in energy production by means of tricarboxylic acid cycle but also in several metabolic pathways:

1. Source of cytosolic reducing equivalents for reductive biosynthesis
2. Carbon source for cytosolic biosynthetic processes (e.g. fatty acids, sterols)
3. Regulator of other metabolic steps: e.g. phosphofructokinase (-), acetyl CoA carboxylase (+)

Acetyl CoA is an impermeable substance but it can transfer the 2-carbon fragment (acetyl group) from the mitochondrial compartment to the cytosol, where acetyl moieties are required for fatty acid or sterol biosynthesis. Intramitochondrial acetyl CoA is converted to citrate in the citrate synthase reaction of the Krebs cycle (Figure 1); subsequently the citrate is exported to the cytosol on the tricarboxylate transporter in exchange for a dicarboxylic acid such as malate. Intracellular citrate may be metabolized not only in the mitochondria by the tricarboxylic acid cycle but also in the cytosol via the ATP citrate lyase. This enzyme converts citrate to acetyl CoA and oxaloacetic acid, at the expense of an ATP molecule, in the ATP: citrate lyase reaction (Figure 2) (4,5).

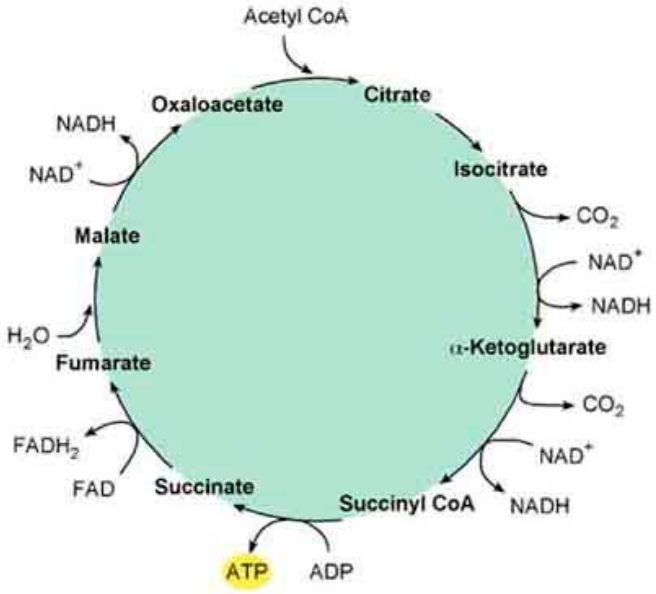

Figure 1. Tricarboxylic acid cycle (Krebs cycle).

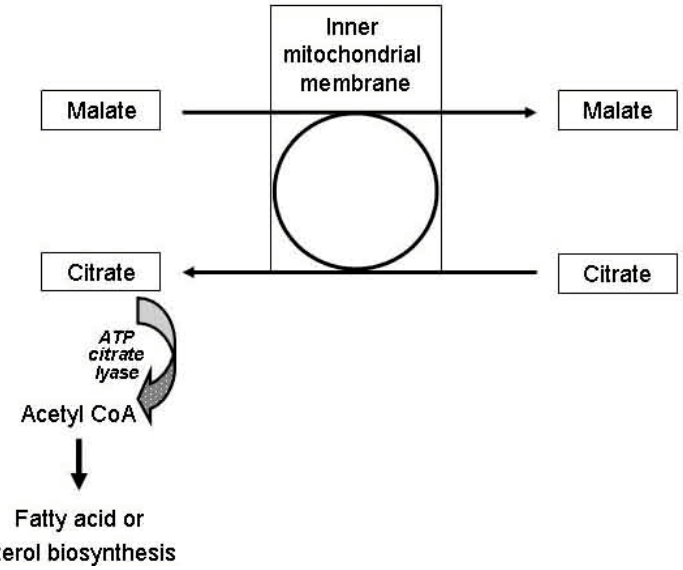

Figure 2. Conversion of intracellular citrate to acetyl CoA by citrate lyase reaction in the cytosol.

Melnick *et al.* showed that the kidneys have a large amount of adenosine triphosphate citrate lyase (ATP citrate lyase) that may contribute to cholesterol and lipid synthesis from cytosolic citrate (6). The activity of ATP citrate lyase in the renal cortex is increased in animals by chronic metabolic acidosis as well as potassium deficiency (6). Also a diet rich in carbohydrates and low in fats induces a large increase of ATP citrate lyase mRNA (7). In the animals submitted to this kind of diet, glucose metabolites and insulin were the main factors inducing the increased activity of ATP citrate lyase (8,9).

Citrate has mostly been evaluated in urine owing to its inhibitory role in calcium stone formation. Urinary excretion of citrate represents the loss, as an anion, of a potential base that could be metabolized to bicarbonate (10). The role of citrate on acid-base regulation is particularly evident in experimental conditions (11-15). Oral citrate undergoes mostly a metabolic process resulting in the production of bicarbonate that may provide dietary alkali or increase the urinary pH and consequently urinary citrate excretion (mainly linked to alkaline load) (10).

3. RENAL HANDLING OF CITRATE

Plasma citrate is filtered at the glomerulus and then reabsorbed mainly in the proximal tubule (16). Roughly 10-35 percent of the total filtered load is excreted in urine (17-19). Citrate is the most numerous of the organic acids and anions present in the urine (15). Citrate, as well as other dicarboxylate intermediates of the citric acid cycle, is reabsorbed at the apical membranes of

proximal tubule by means of two different sodium-dependent dicarboxylate transporters. These transporters are known as NaDC1 and NaDC3 (sodium-dicarboxylate cotransporter 1 and 3) and show a different affinity for their substrates (low for NaDC1 and high for NaDC3). NaDC1 is expressed in the intestine and kidney whereas NaDC3 is present also in the brain, liver and placenta (20). The substrates of the Na-dicarboxylated cotransporters are carried as free, uncomplexed dicarboxylic acids; therefore also citrate is transported as divalent citrate^{2-} (21-23). Trivalent citrate^{3-} may play a role in inhibiting transport at high concentrations (24). Citrate forms complexes with divalent cations such as calcium and magnesium. Calcium and magnesium, by reducing the amount of free citrate, seem to decrease citrate transport (24,25). The final concentration of citrate in urine derives mainly from its reabsorption and metabolism in the proximal tubule that seems to be the only segment involved in processing the luminal or extracellular citrate (16,26-28). In physiologic conditions tubular secretion of citrate has not been proved: therefore urinary citrate represents the amount of filtered load that has not been reabsorbed (1,17). The citrate reabsorbed from the lumen of proximal tubules and the small amount deriving from the peritubular vessels, undergoes a metabolic process resulting in the formation of CO_2 and H_2O, making a small but definite contribution to the energy supply of the kidney (18). *In vivo* most of the rebasorbed citrate is metabolized to CO_2, whereas *in vitro* a significant percentage of citrate is metabolized to glucose and other metabolites (17,19). Citrate undergoes a complete oxidation within the mitochondria and the utilization of renal cell citrate is modulated by its transport inside the mitochondria by means of a tricarboxylic acid transporter (4,18). Moreover, citrate may be metabolized to oxaloacetate and Acetyl-COA by means of citrate lyase (30) and this metabolic process seems to be particularly relevant for fatty acid synthesis. Inside the tubular cell, the citrate amount may increase due to decreased metabolic consumption (e.g. metabolic alkalosis) or by increased citrate synthesis or both (mechanisms) (17,29). Nevertheless, most of the modifications in the amount of intracellular citrate have been ascribed to modifications of citrate consumption(18).

4. GASTROINTESTINAL ABSORPTION OF CITRATE

The usual dietary intake of citrate is about 4 grams (31) and the oral intake of citrate in humans, induces an increase in serum citrate level within 30 minutes (25,32).

Citrate is absorbed from the diet in the small intestine by means of the Na^+/dicarboxylate cotransporter similar to that described in the kidney. Furthermore, the same cotransporter permits the recovery of citrate secreted in pancreatic and gastric juices (33,34). In contrast to renal tubular cells, the enterocytes of the small intestine transport citrate out of the cells in the intestinal lumen, as proved in experimental studies (35,36).

The relative contribution to the plasma citrate concentration of dietary intake and citrate deriving from body tissue such as bone, is not well established. The intestinal absorption of citrate is rapid and almost complete both in healthy subjects and in patients with idiopathic hypocitraturia (37). Morevover the absorbed citrate quickly undergoes a metabolic process that causes an alkaline load that is considered to be mainly responsible for urinary citrate excretion after oral citrate administration (10,32,38). While a low gastrointestinal citrate absorption is not known as a risk factor for renal stone formation, more importance has been attributed to a decrease in the gastrointestinal absorption of alkali that may induce a condition of true hypocitraturia (39).

Finally, it is important to underline that the contemporary administration of citrate and aluminum salts, may increase the intestinal absorption of aluminum, and this possibility must be carefully evaluated in patients with impairment of renal function (40).

5. CITRATE TRANSPORT IN RENAL TUBULAR CELLS

Citrate is filtered at the glomerular level and then undergoes tubular reabsorption primarily in the convoluted and straight segments of the proximal tubule, whereas a tubular transport process does not seem to be present in the thick ascending limb and cortical collecting duct (16). Citrate transport across the apical membrane in proximal tubular cells is coupled with the movement of sodium and citrate secretion does not seem to be present (16). Although the trivalent citrate is the most common ionic species, citrate is mainly transported in a divalent form (1,21,41). Sodium is a critical factor for dicarboxylate transport. The binding of three sodium ions induces a conformational change that increases the substrate affinity (42,43).The transport of three sodium ions and one divalent citrate is an electrogenic process resulting in the net movement of one positive charge. Lithium presents an inhibitory effect by means of a competition with sodium in the Na^+/dicarboxylate cotransporter (44). At the basolateral membrane citrate is transported by means of an electroneutral sodium-dependent transport mechanism, unaffected by pH (45). These transport traits suggest that trivalent citrate may be transported at a peritubular level and experimental studies in cultured cells have suggested the existence of a selective transport system for organic tricarboxylate (46). Transport of citrate across the basolateral membranes seems to contribute in a relevant manner to tubular cell metabolism (18,47).

6. FACTORS MODULATING URINARY CITRATE EXCRETION

Urinary citrate excretion *in vivo* is modulated by several physiological factors including GFR and acid-base status (Table 1) (48). Many experimental and clinical studies have also attributed a significant role to the nutritional state, caloric intake, carbohydrate, sodium chloride intake per day, negative potassium balance and various hormones (49-58). Hormones modulating bone turnover such as estrogens, parathyroid hormone (PTH) and vitamin D seem to increase urinary citrate excretion (57,59-

Table 1. Factors modulating urinary citrate excretion

GFR
• Acid-base status
• Nutritional state
• Caloric intake
• Carbohydrate intake
• Sodium chloride intake
• Potassium intake and balance
• Protein intake
• Hormones (e.g. estrogen, PTH, calcitonin, vitamin D_3, growth hormone)
• Diarrhea and/or malabsorption
• Drugs (calcium, lithium, acetazolamide, ethacrynic acid, converting enzyme inhibitors)
• Transport competitors: succinate, malate, fumarate
• Metabolic inhibitors: fluorocitrate, malonate, maleate
• Oral intake of citrate

63). Potassium deficiency may influence citrate metabolism inducing a decrease in citrate excretion probably by means of intracellular acidosis in the epithelial cells of renal proximal tubules (52). Acidosis, acid loads, chronic diarrhea and/or malabsorption, starvation, and some drugs, such as acetazolamide, ethacrinic acid and the angiotensin converting enzyme (ACE) inhibitors seem to work in the same way, that is decreasing urinary citrate excretion. In contrast, alkalosis, dietary alkali loads, transport competitors or metabolic inhibitors increase urinary citrate excretion (10).

In this paper only some among the various factors that can influence urinary citrate excretion will be examined. Generally, the acid-base state is considered one of the most critical factors modulating citrate excretion, which decreases in conditions associated with acidosis and increases in conditions of alkalosis. The pathogenetic mechanisms responsible for these changes have been described by several authors. Simpson suggested that an increase in cellular pH may decrease the entry of citrate, by means of a mitochondrial membrane tricarboxylate carrier, into the mitochondria (4,18). Consequently, the increased cytoplasmic concentration of citrate reduces the cellular transport of citrate, possibly via a modification of the citrate gradient. The opposite sequence of events has been suggested for metabolic acidosis (18). According to these mechanisms, several authors have found an increased cellular citrate concentration in the presence of metabolic alkalosis, but this increase could be the consequence of both a decreased citrate oxidation and an increased citrate synthesis from other metabolic precursors (4,17,18,29). Similarly, in the presence of metabolic acidosis, the decrease in cellular citrate concentration may derive from an increased activity of cystoplasmic ATP citrate lyase without modifications of the mitochondrial transport or the enzyme regulating the synthesis of citrate (30). Finally, some data indicate that changes in citrate cellular concentration do not correspond to modifications of citrate reabsorption (4,29,64). Furthermore, acid-base changes may modulate urinary citrate excretion by means of a direct effect on the brush border membrane. In fact, it has been shown that luminal pH is an important determinant in proximal tubular citrate reabsorption and that a low pH stimulates citrate transport by modifying $citrate^{2-}$ to $citrate^{3-}$ ratio (21,24). Thus the concentration of transported anion (divalent) will increase about tenfold as the pH is reduced from 7.6 to 6.6. Experimental studies *in vitro* have shown that a peritubular or intraluminal pH decrease, from 7.4 to 7.2, was followed by a lowering in citrate tubular reabsorption (23)**.** In animals fed chronically with an acid load an increase of citrate transport in membrane vescicles was observed whereas an alkali load did not modify the citrate transport (65). Moreover, an increase in ATP citrate lyase was observed with chronic acid loads (30). An increase in apical membrane transport of citrate was observed also in starvation (66). Sato *et al.* proved that when alkalosis was experimentally induced by a low potassium diet, a decrease in urinary citrate excretion was obtained (67). These data suggest that urinary citrate excretion is affected by potassium depletion that may act by means of different mechanisms such as a reduced intracellular pH or an increased luminal H^+ secretion linked to hypokalemia (52,68). Furthermore, Levi *et al.* observed that *in vitro* chronic potassium depletion increases citrate transport at the brush border membrane level, probably increasing the number of citrate transporters (69). Acetazolamide, an inhibitor of carbonic anhydrase, decreases urinary citrate excretion and sometimes may promote calcium stone formation (70). Acetazolamide induces urinary alkalinization together with systemic metabolic acidosis. The hypothetical mechanisms by which acetazolamide causes a decrease in urinary citrate seem to be linked both to cellular and luminal pH changes (10). Thiazides lower urinary citrate excretion by inducing a negative potassium balance and eventually an intracellular acidosis; this potassium wasting is reversed better by administration of potassium citrate than potassiun chloride (71). Recently, some authors have pointed out that angiotensin converting enzyme inhibition may cause hypocitraturia both experimentally and in humans (6,72). The hypocitraturia induced by angiotensin converting enzyme inhibitors is probably linked to a decrease in the intracellular pH of the proximal tubule by means of an inhibition of Angiotensin II-induced stimulation of the apical membrane Na/H exchanger. In this type of hypocitraturia, likely in metabolic acidosis and hypokalemia conditions, hypocitraturia may be due to an enhanced tubular reabsorption of citrate. Furthermore, an increase in ATP citrate lyase activity may contribute to the hypocitraturic effects of angiotensin converting enzyme inhibitors (6).

Also starvation may induce a decrease of urinary citrate excretion through an increase of the number and/or activity of citrate transporters in the apical membrane (66). A positive relationship between the urinary excretion of divalent cations and citrate has been described by several authors (18,25,53,57); thus an increased calcium and magnesium excretion has been found associated with a contemporary increase of urinary citrate excretion. In contrast, an increase in citrate excretion seems to induce an increased urinary excretion of calcium (73,74). This

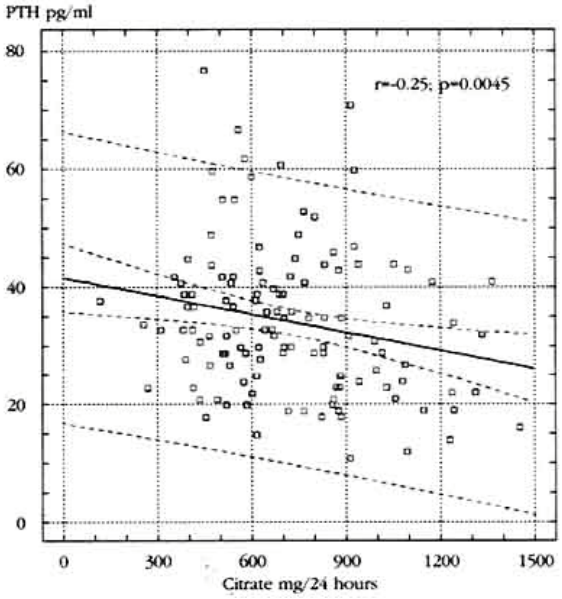

Figure 3. Regression of PTH on urinary citrate excretion in 124 healthy women (From 80, with the permission of the Publisher).

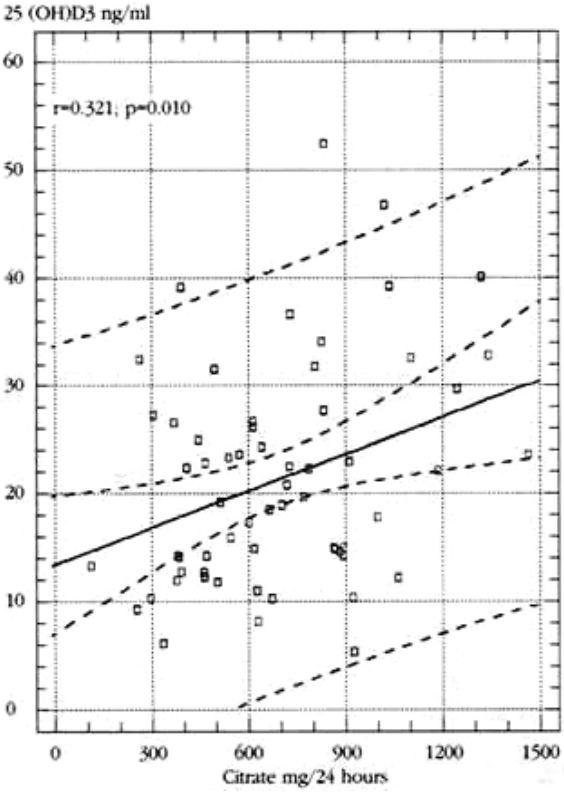

Figure 4. Regression of 25(OH)D3 on urinary citrate excretion in 60 healthy females (From 80, with the permission of the Publisher).

relationship is linked to the formation of calcium-citrate complexes that are not easely reabsorbed (2,3) and this mechanism was observed experimentally in isolated vescicles of the brush border membrane (24). Experimental studies have shown that lithium administration induces an increased urinary excretion of both citrate and other metabolites of the Krebs' cycle by means of an inhibition of the dicarboxilate transporter both in the brush border membrane and at the basolateral membrane (44,75,77). In contrast, clinical data have not shown an increase in urinary citrate excretion in patients treated with lithium salts; these results have been confirmed experimentally in animals chronically treated with lithium (78,79).

A direct role of parathyroid hormone and vitamin D on citrate metabolism and excretion has been described (18,57,60-63). Furthermore we found a negative correlation between urinary citrate excretion and PTH (Figure 3) in a group of healthy females (80). Our clinical findings agree with the experimental work of Luben and Chon that showed that PTH induces demineralization, the synthesis of hyaluronate and the inhibition of citrate excretion (81). In the same paper, we found a positive correlation between citrate and 25 (OH)D3 serum levels ($p = 0.019$) (Figure 4). The latter result seems to be correlated to the observations of Price *et al.* in which rat osteosarcoma cells, with osteoblastic phenotype, responded to 1,25(OH)2D3 and vitamin D3 with an increase in citric acid secretion (82).

The role of sex hormones in modulating the incidence and the onset of urolithiasis may be indirectly inferred by the higher incidence of renal stone disease in males than in females with a reported ratio of about 1:2 or 1:3. Furthermore renal stone formation increases after menopause (83). A higher incidence of renal stone disease in males (with a peak incidence in middle age), has been indicated also by several other authors (84,85). Furthermore, epidemiological studies suggest that stone disease in females has a biphasic behaviour with one peak at about 30 years of age and a second peak at about 55 years of age (86). The role of sex hormones on citrate modulation in women was suggested by the increase in renal stone formation after menopause and the inhibition of calcium oxalate crystal growth in the urine of premenopausal women on oral contraceptives (87). Moreover, Dey J *et al.* showed an increased citrate excretion in postmenopausal women on estrogen therapy as well as an increased inhibition of crystal agglomeration (88).

The influence of estrogens on urinary citrate excretion has been suggested by several authors who report a decrease in urinary citrate excretion in normal postmenopausal females (without any other known cause of hypocitraturia) (59,89). In a previous work we found that urinary citrate excretion in postmenopausal females was significantly lower than that of premenopausal females ($p < 0.011$) and tendentially lower than that of males ($p < 0.11$) (Figure 5). Moreover, postmenopausal females showed a citrate concentration significantly lower than both males ($p < 0.003$) and premenopausal females ($p < 0.0001$) (Figure 6). Finally, premenopausal females had a urinary citrate concentration tendentially higher than males, even though this result did not reach a statistical significance (Figure 6). Schwille *et al.* observed that young females show a higher urinary citrate excretion although they did not observe any

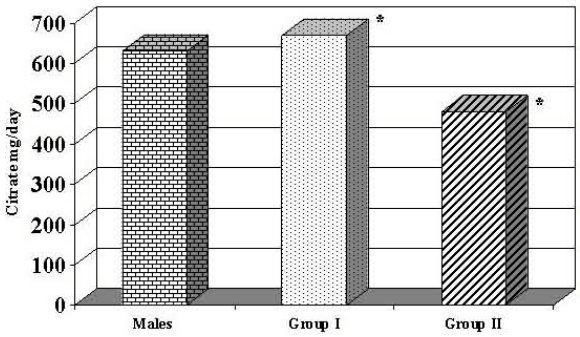

Figure 5. Citrate excretion in healthy males, premenopausal healthy females (Group I) and postmenopausal healthy females (Group II) (From 80, with the permission of the Publisher). Postmenopausal females showed a citrate excretion significantly lower than premenopausal females (*p = 0.01), whereas this value was not significantly different in comparison with males (p =0.11).

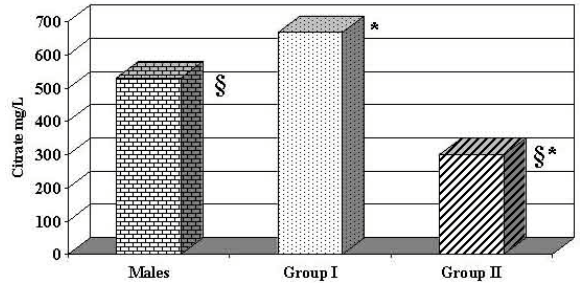

Figure 6. Citrate concentration in healthy males, premenopausal healthy females (Group I) and postmenopausal healthy females (Group II) (From 80,Vol with the permission of the Publisher). Postmenopausal females showed a citrate concentration significantly lower than both males (§p = 0.003) and premenopausal females *p = 0.0001.

age-dependency in female citrate excretion (90). On the contrary an increased citrate excretion, linked to age, was observed in males (91). These data suggest that the postmenopausal loss of female sex steroids may be involved in the control of urinary citrate excretion. The influence of estrogens on urinary citrate excretion may explain the different results obtained by various authors when comparing the citrate excretion in males and females. In fact, premenopausal stone-forming females show greater values of citrate concentration/excretion than males; on the contrary stone forming males have similar values to postmenopausal females (Figure 5 and 6).

Furthermore, several metabolic precursors, metabolic inhibitors and drugs are able to modify citrate excretion (10,48,70,92). Finally, citrate excretion is significantly influenced by the amount of citrate intake as suggested by Pak; according to this author some of the absorbed citrate escapes *in vivo* oxidation and appears in urine (93).

A reduced urinary citrate excretion has been reported in several pathological conditions (Table 2). However, in many patients without any other metabolic alteration, the pathogenesis of hypocitraturia was not clearly identified. Recently, some pathogenetic mechanisms of idiopathic hypocitraturia have been identified in high sodium intake, in animal protein excess and in reduced net gastrointestinal absorption of alkali (93). The presence of a large amount of citric acid in bone and the possible relationship between citric acid and calcification mechanisms were described by Dickens in 1941 (94). Furthermore, the interaction of citrate with calcium and hydroxyapatite suggests a role for citrate in calcium mobilization from the bone induced by vitamin D and PTH (95). Finally, estrogen loss induces both negative changes in external calcium balance (50% due to decreased intestinal calcium absorption and 50% due to increased urinary calcium loss) and a decrease of hepatic synthesis of calcium transport globulin and of total but not free $1,25(OH)_2D_3$ (96).

7. CITRATE AND BONE MASS

As far back as 1941 Dickens recognized that citrate was a major component of normal bone and that its metabolism occured mainly in the bone tissue (94). Citrate is also a well known inhibitor of crystal nucleation and growth in renal stone disease and therefore is widely used as a prophylactic agent against recurrent calcium oxalate nephrolithiasis (97-99). Renal stone disease is often a multisystem disease with bone loss as a frequent feature (100). One of the major causes of osteopenia in these patients appears to be the presence of idiopathic hypercalciuria which can be a consequence either of enhanced intestinal calcium absorption, renal tubular calcium leak or high bone resorption. The long lasting hypercalciuria and the low calcium diet, that is still frequently recommended for stone formers, may induce a negative calcium balance, which, in turn, may lead to bone loss (101). Many other conditions, such as high sodium and animal protein intake (see below), can contribute to bone loss both in idiopathic calcium stone formers and in normal subjects (101-103). Renal calcium excretion is proportional to sodium excretion; approximately 1 mmol of calcium is excreted for every 100 mmol of sodium excreted (104). Sellmeyer *et al.* observed that a net deficit of 1 mmol/day of calcium would lead to the loss of one third of the calcium contained in an adult skeleton in approximately two decades (103). Also a high consumption of animal proteins may lead to bone loss; in fact the metabolism of sulphur-containing amino-acids methionine and cystine generates sulphuric acid and it is believed that this acid load must be buffered, at least in part, by skeletal labile bases (alkaline salts of calcium), thus leading to bone loss (102,105).

In mammals urinary citrate excretion is modulated by different physiological and pathological conditions (Table 1 and 2).

Some evidence exists as to a positive correlation between bone mineral density and citrate excretion. Citraturia is commonly reduced in renal tubular acidosis and calcium oxalate nephrolithiasis and in these diseases a

Table 2. Main causes of hypocitraturia

1. Idiopathic renal hypocitraturia	
2. Secondary hypocitraturia:	
Renal diseases	
	Distal renal tubular acidosis (complete or incomplete form)
	Chronic renal failure
Gastrointestinal diseases	
	Inflammatory bowel diseases
	By-pass or ileal resection
	Chronic diarrhoeal states
	Reduced citrate or alkali intestinal absorption
Drugs	
	Thiazides
	ACE-inhibitors
	Acetazolamide
	Ethacrynic acid
Dietary	
	Excessive protein intake
	Excessive NaCl intake
	Low alkali and potassium intake
Hypokalemia /intracellular potassium depletion	
Starvation	
Strenous exercise (lactic acidosis)	

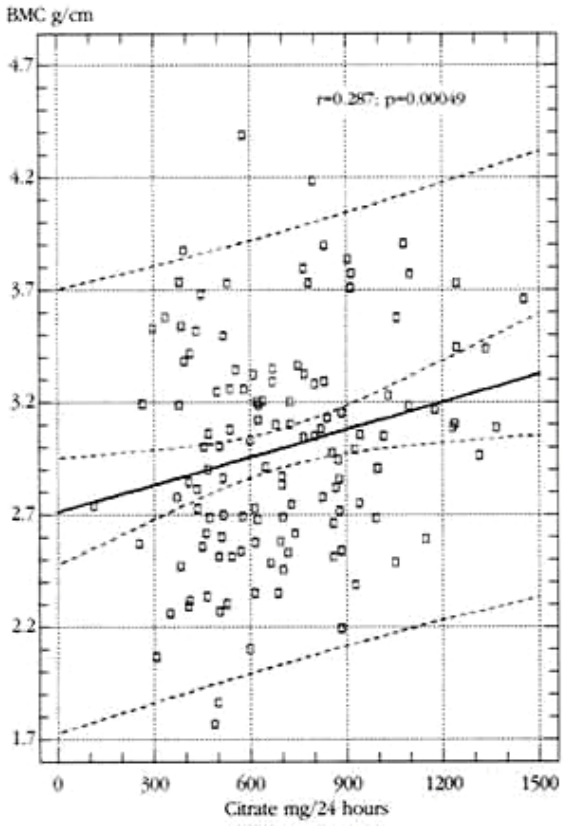

Figure 7. Regression of bone mineral content at distal radius level (BMC) on urinary citrate excretion in 127 healthy females (From 80, with the permission of the Publisher).

reduction of bone mass has also been described (93,106,107).

In a previous work we demonstrated a positive correlation between urinary citrate excretion and radius densitometric values, in a group of 127 pre- and postmenopausal females (Figure 7 and 8) (80). This observation was reinforced by data showing a strong relationship between urinary citrate excretion and the prevalence of vertebral fracture in postmenopausal women (108). In this study we enrolled 49 postmenopausal osteopenic women, as consecutive outpatients; 32 of them presented at least 1 vertebral fracture, while 17 had none. Among the fractured women, 22 showed urinary citrate levels < 400 mg/24h and the remainder had higher values. In the group of non fractured females, 2 were hypocitraturic while 15 were not. Citrate excretion, osteocalcin and glomerular filtration rate (which was within the normal range in all the patients) were significantly lower in fractured females than non-fractured females. PTH was higher in the group of fractured women than non-fracutred women, while radius bone mineral content, bone mineral density and t-score were significantly lower in the group of fractured women than non-fractured women. We also performed a stepwise logistic regression, whose aim was to find the biochemical parameter which related best with vertebral fractures: citrate showed the highest statistical significance (p=0,001). We finally performed Fisher's exact Test which showed that the highest number of fractured women was in the hypocitraturic group (p=0,0001) (108).

The next step was to determine whether potassium alkali administration can reduce bone loss, both in stone formers and in normal people.

In 1994, Sebastian and co-workers demonstrated that the administration of potassium bicarbonate to postmenopausal women induces a positive calcium balance, reduces bone resorption and increases bone formation via a neutralization of endogenous acid load (109). Similar conclusions were obtained also by Sellmeyer *et al.* who found that potassium citrate supplementation reduced urinary calcium loss and bone resorption in a group of 60 postmenopausal women fed with a high sodium diet (103). Finally Pak and co-workers showed that potassium citrate, administered to reduce stone formation, induced a slight increase in lumbar bone mineral density of 3.8%, for a mean treatment period of 44 months (100).

In conclusion, as Dickens observed many years ago, citrate plays an important role in bone metabolism. In fact, notwithstanding the lack of larger observations, urinary citrate excretion correlates with bone mass and, perhaps, with vertebral fractures and therefore may be considered a biochemical risk factor of these conditions. Moreover citrate alkali administration seems to increase bone mass, both in normal subjects and in stone formers, thus adding an additional therapeutic tool for the

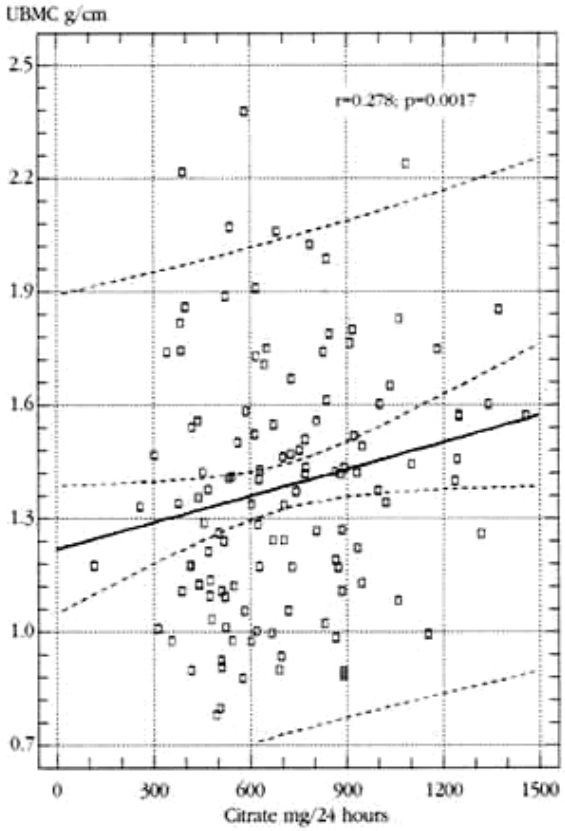

Figure 8. Regression of bone mineral content at ultradistal radius level (UBMC) on urinary citrate excretion in 120 healthy females (From 80, with the permission of the Publisher).

treatment of osteoporosis, particularly in hypocitraturic subjects.

8. CITRATE EFFECTS ON URINE CRYSTALLIZATION

The principal action of citrate in the prevention of kidney stone disease is the formation of soluble complexes with calcium in urine, which cause a reduction in the ionic calcium concentration and in the urinary saturation of calcium oxalate (CaOx) and calcium phosphate (CaP) (110). Furthermore, citrate directly inhibits the spontaneous nucleation and the crystal growth of CaOx and CaP (111-113). Furthermore, Kok *et al.* observed that the diluted urine of stone formers inhibits calcium oxalate monohydrate crystal growth and agglomeration at a lower degree than the urine of normal subjects (114). These authors showed also that citrate inhibits calcium oxalate monohydrate crystal aggregation (115). Agglomeration represents the process of random crystal clumping that takes place in the early phases of stone formation *in vivo*; on the contrary, the term aggregation indicates a spatially well oriented form of crystal adherence (114). Finally, the well known power of citrate to inhibit calcium oxalate crystal growth and aggregation seems to be linked to a direct effect on the crystal surface rather than to a modification of the calcium ion concentration (116).

Bisaz *et al.* reported that citrate is responsible for 50% of the inhibitory activity against CaP precipitation in normal urine (117). Also Lieske and Coe indicated that urinary citrate has a strong inhibitory effect on CaP crystal growth (116). Moreover, citrate presents an inhibitory action on the heterogeneous nucleation of CaOx induced by monosodium urate (118). Finally, citrate seems to play a key role in the opposite behaviour of Tamm-Horsfall protein (THP) in crystallization processes, both on its promoting[120] and inhibiting actions on aggregation processes in stone formation (119,121,122). THP is produced at the distal tubule level by epithelial cells and is the main component of urinary casts (123). Moreover, THP is a component of stone matrix. Although the biological role of THP is not completely understood, some authors have suggested that the daily urinary excretion of THP may be an indicator of functioning nephron mass (124,125). The dual behaviour of THP seems to be a function of pH and of the ionic strength of the urine; citrate seems to play a critical role in THP behaviour, by decreasing the viscosity of this protein via a formation of complexes with calcium ions (126). In fact, Hess *et al.* observed that THP had an inhibitory effect on calcium oxalate aggregation when citrate was present, and the opposite effect (e.g. promoter) when citrate was absent (127). A similar result was obtained in an indirect way, by Ganter *et al.* who showed that the inhibitory activity of THP, on calcium oxalate crystallization processes, was higher in healthy subjects than in stone forming subjects (125).

9. URINARY CITRATE EXCRETION AND KIDNEY STONES

Urinary citrate excretion *in vivo* is modulated by several physiological factors and drugs (see former sections). The hormones that seem to be most involved in urinary citrate excretion are sex and calcitropic hormones. While there is general agreement on the positive effect of estrogen on urinary citrate excretion, contrasting results have been obtained as to the effect of PTH. In fact, some experimental and clinical data seem to suggest that PTH has a stimulatory effect on citrate excretion (57,128). In contrast, Nicar *et al.* found normal urinary citrate excretion in patients with primary hyperparathyroidism and Smith *et al.* described low citrate excretion in subjects with primary hyperparathyroidism and renal stones (128,129). Finally, in 1995 we found a negative correlation between PTH levels and urinary citrate excretion (Figure 3) (80).

Decreased urinary citrate excretion in patients with renal stones was reported for the first time by Boothby and Adams in 1934 and successively confirmed by Kissin and Locks in 1941 (130,131). These observations were ignored for several years and mainly attributed to urinary tract infection and postrenal bacterial consumption of citrate (132,133). In 1962 Hodgkinson suggested that a condition of hypocitraturia, defined as a urinary citrate excretion lower than 400 mg/day in males and 200 mg/day in females, may be present in stone formers with urinary tract infection (56). In the last 4 decades numerous work performed both on free or fixed diet conditions, with few exceptions, have described a reduced urinary citrate

Table 3. Urinary citrate excretion (mmol/day) in healthy subjects and in stone formers considered separately according to sex (patients were maintained on a free diet)

Healthy subjects		Stone formers				
Males	**Females**	**Males**	**Females**	**p**	**Year**	**Reference**
2.94 ± 1.0§	3.79 ± 1.2&	2.22 ± 1.1§	2.42 ± 1.2&	§0.049 &0.02	1962	(56)
1.69 ± 0.9*	2.92 ± 1.4*	1.25 ± 1.0*	1.17 ± 1.2*	* 0.001	1976	(137)
(*Age 20-40*) 2.27 (*Age >40*) 2.92*	(*Age 20-40*) 3.23° (*Age >40*) 1.95*	(*Age 20-40*) 1.62 (*Age >40*) 1.95*	(*Age 20-40*) 1.79 (*Age >40*) 1.95*	0.01 0.001	1979	(90)
3.26 ± 1.28*		2.45 ± 1.18*		0.001	1981	(138)
3.16 ± 1.1*		2.48 ± 1.0*	2.73 ± 1.3	*0.001	1981	(139)
2.86 ± 0.9§	3.27 ± 0.7§			§0.05		
2.54 ± 0.6§		1.59 ± 0.9§		<0.05	1982	(140)
3.35 ± 1.1	3.08 ± 1.2	2.86 ± 1.2	2.94 ± 1.2	N.S.	1985	(91)
2.89 ± 0.9&	3.86 ± 1.3&	2.73 ± 1.2	2.92 ± 1.2	0.02	1986	(142)
3.28 ± 1.7§	3.48 ± 1.35*	2.55 ± 1.34§	2.35 ± 0.9*	§0.05 *0.001	1987	(143)
2.99 ± 0.83§	3.86 ± 1.04*	2.26 ± 1.20§	2.59 ±1.59*	§0.05 *0.001	1989	(19)
2.11 ± 1.2#	2.00 ± 1.4	1.39 ± 1.3#	1.45 ± 1.2	0.01	1989	(147)
3.35 ± 0.17§		2.73± 0.14§		0.05	1991	(144)

Statistically significant differences between healthy and stone forming subjects of the same sex. § p =0.05; & p = 0.02; # p = 0.01; *p = 0.001; N.S. = Not Significant

Table 4. Urinary citrate excretion (mmol/day) in healthy subjects and stone formers maintained on a controlled diet

Healthy subjects	Stone formers	Year	Reference
3.54 ± 0.44	2.63 ± 0.23	1971	230
3.40 ± 1.58	2.69 ± 1.55	1982	135
Males: 3.08 ± 1.01	2.08 ± 0.94	1983	128
Females: 3.65 ± 1.44	(hypercalciuric)		

excretion and/or a hypocitraturia in stone formers when compared with normal subject (19,25,63,128,134-144). On the contrary, there is no general agreement on the differences in urinary citrate excretion linked to sex, age and on the incidence of hypocitraturia. These differences may arise from the evaluation of stone forming and normal subjects without differentiating the subjects according to sex and age and from the different criteria used to define the condition of hypocitraturia.

Some of these results are summarized in tables 3 and 4; the works quoted in these tables were performed on patients mantained on a free diet or a controlled diet, in order to avoid the influence of dietary components on urinary citrate excretion.

Rudman *et al.* found a significantly higher citrate excretion in healthy subjects than in stone formers; furthermore, a supplementation of 4.5 grams of sodium citrate to the diet, did not increase the urinary citrate excretion (140). The same authors, evaluating the renal handling of citrate (citrate clearance and tubular reabsorption), suggested that hypocitraturia was the consequence of an abnormally high net tubular reabsorption in the presence of a normal citrate filtered load. Finally, these authors showed a negative correlation between citrate and urinary phosphate excretion (r= -0.77) and found hypocitraturia (urinary citrate excretion < 215 mg/day) associated with hypercalciuria in one third of the stone forming males. Wikstrom *et al.* evaluated the citrate urinary excretion in 167 consecutive stone formers and in a control group of 200 healthy subjects and found a significant difference between the two groups ($p < 0.05$) (138). However, these authors measured urinary citrate excretion in the two groups, without considering males and females separately. Tiselius, observed that urinary citrate excretion was significantly higher in healthy subjects than in stone-forming males ($p < 0.001$); however, citrate excretion was greater in females than in stone-forming males although this difference did not reach statistical significance (139). Furthermore, the same author considered a group including 30 normal males and 30 normal females with the same age distribution, and found that citrate excretion was greater in females than in healthy males ($p < 0.05$). Parks and Coe observed a significant difference between females and males; healthy women had a greater citrate excretion than stone forming females. Healthy males had values of urinary citrate excretion similar to those of stone-forming females; furthermore, urinary citrate excretion was similar both in healthy and stone forming males (145). Minisola *et al.* described the presence of a significant difference in the excretion of urinary citrate between normal and stone forming subjects with a greater excretion in females than in males (146). On the contrary, Nikkila *et al.* described a significant difference in citrate excretion between normal and stone- forming males ($p < 0.01$) but

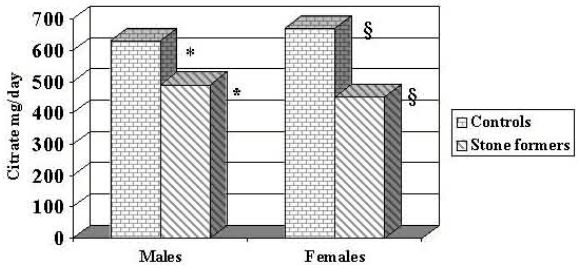

Figure 9. Urinary citrate excretion in stone formers and in healthy subjects (modified from 143). Urinary citrate excretion was significantly lower both in stone-forming males (*p < 0.05) and females (§p < 0.001).

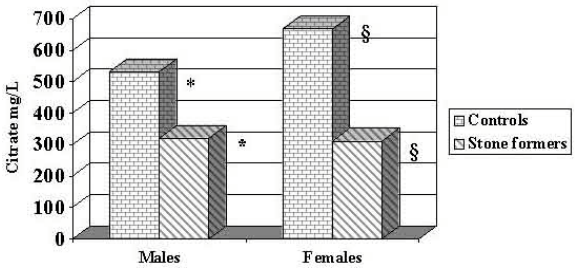

Figure 10. Urinary citrate concentration in stone formers and in healthy subjects (modified from 143). Urinary citrate concentration was significantly lower in both stone-forming males (*p < 0.001 and females (§p < 0.001).

not in females (147). A possible explanation of these results may be found in the higher number of males studied. In 1985 Caudarella *et al.* found a significant difference in urinary citrate excretion between healthy subjects and calcium stone formers ($p < 0.05$) (141). However, authors of the same group found a significant difference in both citrate excretion and concentration between healthy and stone-forming subjects of the same sex (Males: $p < 0.05$. Females: $p < 0.001$); on the contrary, no differences between men and women of the two groups were observed, but it must be underlined that in this paper the pre and postmenopausal females were considered as a whole (Figure 9 and 10) (143). Mean values of citrate excretion were significantly higher in premenopausal than in post-menopausal females ($p < 0.01$) (Figure 5) (143). In a recent paper, evaluating the 24-hourchemistry and the risk of kidney stones between women and men, Curhan did not find any significant differences between males and females nor between patients and controls in the three groups evaluated (148). However, this paper had some problematic features: in fact, the age of the three groups was different and the citrate excretion was evaluated only on a single 24-hour urine collection and both the collection system used in this study as well as the analytic method were different from those commonly used. However, in contrast to the literature data, the hypocitraturia frequency was similar in the three groups without any difference even between stone formers and control groups. Moreover, Asplin *et al.* did not find any significant difference in citrate urinary excretion between seventeen stone forming females and a control group matched for sex and age (149). Nevertheless, these authors showed that urine citrate molarity is an important correlate of the upper limits of metastability (ULM) for calcium-phosphate in patients and healthy subjects, and for ULM for calcium oxalate only in stone formers. The authors suggested that citrate may be a true molecular determinant of ULM and therefore may prevent stone formation (149). Some authors chose to evaluate the urinary citrate/creatinine ratio in both normal and stone-forming subjects separately according to sex(19,142,145,147,150). Nikkila *et al.* found that females showed a higher ratio than males in the control group (Females= 0.21; males:0.15); furthermore, this ratio in stone forming females (0.13) was similar to that of normal men whereas stone forming males had the lowest ratio (0.08) (147). In this study, a statistically significant difference was observed only between control and stone-forming males. Parks *et al.* as well as Minisola *et al.* found similar results showing that females have a higher citrate/creatinine ratio than males both in healthy and stone forming subjects (19,142). In all these studies a higher citrate excretion was observed in females of the control group according to the results of several authors, as well as a lower excretion of urinary citrate in stone forming subjects of both sexes.

Considering these data all together, sex and age appear to influence urinary citrate excretion. In fact some authors observed a higher citrate excretion in females of the control group, especially younger subjects and a tendency to increase with aging (91,151-153). Vahlensieck *et al.* examined 150 healthy men and women to evaluate the influence of age and sex on citrate urinary excretion; however, to avoid the influence of the diet the study was performed both on free and controlled diets (154). On both standard and free diets, females showed a significantly higher urinary citrate excretion than males and subjects under 20 and over 60 years of age showed a significantly reduced citrate excretion. In contrast, in stone forming subjects the authors did not observe any difference between the sexes owing to a significantly decreased citrate excretion in women; moreover, healthy females showed a greater excretion of citrate than stone-forming females (154). Male sex is usually considered a risk factor for kidney stones. In fact, the ratio male to female in stone formers, evaluated in different countries, ranges from 2:1 to 3:1 (155-157). When considering children or postmenopausal females, the frequency of kidney stone disease in the two sexes was not different (158). On the contrary a different ratio (about 3:1) was evident considering premenopausal females (159). Furthermore, a change in stone composition was described in pre and postmenopausal females; in fact postmenopausal females mainly form CaOx stones (158,160). Iguchi *et al.* experimentally observed a decrease of urinary oxalate excretion in oophorectomized rats treated with estrogens (161). These results suggest that the reduced production of female sex hormones will promote stone formation, non only by a decrease in citrate excretion, but also by an increase in urinary oxalate excretion.

The role of sex hormones on urinary citrate modulation in females is indirectly inferred by the increase of renal stone formation after menopause and also by the

Table 5. Frequency of hypocitraturia in patients with nephrolithiasis

Frequency %	Year	Reference
55	1983	128
29.2	1985	91
8.0	1986	163
12.6	1986	141
50	1987	235
47	1987	154
46.56	1991	231
34	1991	144
68.3	1994	232
29.2	1994	233
32.8	1996	192
29	1997	162

protective effect of hormonal therapy in premenopausal females (87). Furthermore the highest citrate values are reached together with the peak of estrogen, during the menstrual cycle (53). Finally, in a recent paper, Hess *et al.* confirmed the previous findings of the literature, that females excrete more citrate than males (162).

10. INCIDENCE OF HYPOCITRATURIA

The prevalence of hypocitraturia among stone formers ranges from 12% up to 63% (Table 5). Some possible explanations for these different rates may be:

1. The different values used to indicate true hypocitraturia; in fact, Nicar *et al.* defined hypocitraturia as a 24 hour citrate excretion lower than 320 mg (or 1.7 mmol) while other authors fixed the limit below the normal range (90,91,128,137,163).
2. The evaluation of hypocitraturia as a metabolic derangement alone, or together with other metabolic alterations. For example in 1994 we found a hypocitraturia frequency, considered as a single metabolic alteration, of 12%, whereas, when considering hypocitraturia together with hypercalciuria and/or hyperuricosuria, this rate increased to 32% (164).

The influence of the diet on urinary citrate excretion as relevant modifications of daily citrate excretion can occur when subjects are on their home diet.

Following these considerations Pak proposed, in 1994, the use of a "functional" definition of hypocitraturia which is a citrate excretion lower than 320 mg/day (165). This low normal limit was formerly estabilished in Pak's laboratory, for men and women without any relationship to age (128). Moreover, some authors proposed a different cutoff to define hypocitraturia. In fact, hypocitraturia was defined as a urinary excretion lower than 115 mg/day in males and lower than 200 mg/day in females by Menon and Mahle (63). Laminski *et al.* did not agree with these values as they did not find any difference in urinary citrate excretion between stone formers and healthy subjects of both sexes (166). Höbart and Hofbauer proposed a cut off for citrate excretion of 1.5 mmol/day; these authors found a higher rate of hypocitraturia in recurrent stone formers (41%) than in single stone formers (29%) (144). Hess *et al.* suggested a different limit for urinary citrate excretion for males (1.7 mmol/day) and females (1.9 mmol/day) (162). The Southwestern study group proposed as a limit for the diagnosis of hypocitraturia, a urinary citrate excretion lower than 220 mg/day, although this value was open to criticism because it did not consider sex and/or age (167).

Some authors pointed out that stone formers with other metabolic alterations showed a urinary citrate excretion lower than patients without detectable abnormalities as well as stone formers with a very active calcium stone disease and/or low urinary citrate/calcium ratio (168-170). The daily urinary citrate to calcium ratio was evaluated by several authors suggesting that this ratio can be used as a risk factor for stone formation. Welshman and McGeown found that calcium/citrate ratio was different in normal subjects and stone formers as well as in males and females; in fact, they found a calcium/citrate ratio in stone forming and normal subjects of 4.52 and 3.02 respectively; in stone forming females this ratio was 3.54 and 1.41 in healthy females (137). Similar results were obtained in successive studies (144,147,169). Finally, Parks *et al.* examined citrate/calcium ratio in 13 studies in which patients were mantained on their home diet, and they found a sharp separation between renal stone formers and healthy subjects (145). Stone-forming females have higher urinary calcium and a lower citrate than control females, whereas stone forming males presented a significantly higher urinary calcium excretion than normal subiects but only a small decrease of citrate excretion. Furthermore, also the studies in which the patients ate fixed diets, showed, generally, a lower urinary citrate excretion in stone formers than in healthy subjects and these differences persisted when the subjects were considered separately according to sex (145).

11. PATHOPHYSIOLOGY OF HYPOCITRATURIA

Although, as shown above, many factors can modulate citrate excretion *in vivo* (Table 1 and 5), perhaps the most important one can be considered the acid-base status. Systemic alkalosis causes a marked increase in citrate excretion and, in contrast, systemic metabolic acidosis leads to a decrease in urinary citrate excretion. Consequently, some pathological conditions characterized by acidosis are associated with nephrolithiasis (renal tubular acidosis, malabsorption syndrome, thiazides-induced hypokalaemia and urinary tract infection). Some experimental data pinpoint the mitochondria as the site where citrate excretion and metabolism is regulated by acid-base changes. The inner mitochondrial mambrane is passively impermeable to most anions and citrate is transported across it by a tricarboxylic carrier. This carrier is stimulated by low intracellular pH and bicarbonate concentration, whereas the opposite conditions induce its inhibition. Accordingly, the inhibition of citrate oxidation at high pH and bicarbonate level is the consequence of the decreased entry of citrate into the mitochondrial matrix space. There is also evidence that bicarbonate enhances the removal of citrate from the mitochondria. During metabolism, the matrix space has a higher pH than the exterior, due to an active pumping out of the hydrogenion

ions; the resulting electrical and pH gradient across the inner mitochondrial membrane is the driving force for ATP synthesis (18). Metabolic acidosis inducing both a lowering of cytoplasmatic pH and of serum bicarbonate concentration, increases the pH gradient that, in turn, favours the citrate influx into the mitochondrial matrix compartment (167). Citrate undergoes metabolism to a large extent in the mitochondria of the proximal tubular cells, with final production of carbon dioxide and glucose (167). Citrate excretion is about 25% of the total citrate filtered load (2.2 micromol/min): either a decreased renal citrate load or increased citrate metabolism may reduce urinary citrate excretion (167,171). The diseases associated with metabolic acidosis induce a decrease of urinary citrate excretion. For example patients with distal renal tubular acidosis have a significant reduction of urinary citrate excretion and thus a tendency to calcium oxalate and calcium phosphate stone formation (172). Moreover, in these patients urinary pH is alkaline in spite of the systemic metabolic acidosis that directly induces low urinary citrate excretion. Thiazide therapy induces hypocitraturia by means of hypokalemia and the resulting intracellular acidosis (see above). Also diarrhea causes acidosis by means of a pathogenetic mechanism which is similar to thiazide. Inflammatory bowel diseases (IBD) can promote calcium stone formation through other mechanisms, such as dehydration, increased intestinal oxalate absorption and decreased urinary magnesium excretion (173). Furthermore, patients with IBD have a negative calcium balance which is followed by severe osteoporosis. In these patients a supplementation with calcium citrate has been proposed as the preferential treatment for kidney stone disease, although clinical trials are still lacking (167). Although several causes for low urinary citrate excretion has been identified (Table 2), in many patients with nephrolithiasis the exact cause of hypocitraturia is still unknown. Some hypothetical mechanisms of idiopathic hypocitraturia are proposed and include the influence of the diet, the intestinal absorption or the renal tubular resorption of citrate.

11.1. Sodium-induced hypocitraturia

Several authors have found a negative relationship between the intake of NaCl and urinary citrate excretionin patients maintained on both free and controlled diet (93,174,175). Benazzi *et al.* showed that the amount of NaCl in the diet may influence citrate metabolism in man as shown previously in rats (51,175). In fact, urinary citrate excretion decreased significantly after the addition of 6 grams of NaCl/day to a free choice diet both in normal and stone-forming subjects.The same authors suggested that the changes in proximal tubular reabsorption of Na and filtered fluid induced by NaCl are associated with increased citrate reabsorption. A similar result was obtained by Pak in 14 normal subjects, maintained on a constant sodium intake (50 mEq/day) when the sodium intake was increased to 300 mEq/day (93). In fact, urinary citrate excretion decreased significantly from 3.14 to 2.50 mmol (593 to 473 mg/day) ($p < 0.05$). The author also found a decreased venous blood bicarbonate concentration together with an increased serum chloride level and urinary pH. These results suggested that sodium-induced volume expansion with a consequent increase of urinary bicarbonate excretion, causes a mild metabolic acidosis, which, in turn, accounts for hypocitraturia (93). Sakhaee *et al.* observed that a diet with high NaCl content induced a 20% decrease in urinary citrate excretion without any modification of plasma pH or potassium values (174). Recently, Melnick *et al.* have suggested that a decrease in angiotensin II levels is responsable for hypocitraturia in subjects eating a high salt diet or treated with enalapril (72).

11.2. Animal protein excess

Hypocitraturia may follow an excessive intake of animal proteins. In fact animal proteins cause a renal acid load by means of sulfate generation due to sulfur oxidation of aminoacids contained in the proteins themselves. In 15 patients eating for a period of 12 days a vegetarian diet followed by an animal protein diet, Breslau *et al.* observed, in the second period, both higher urinary sulphate excretion and net acid excretion together with a lowering of urinary pH, suggesting that an acid load was delivered; also urinary citrate, was significantly reduced ($p< 0.02$) (176). Vahlensieck *et al.* found hypocitraturia in 47% of the stone formers examined on a free diet whereas hypocitraturia was observed only in 17% of the stone formers on a standard diet (balanced diet with an animal protein content of 60 grams/day) (154). This diet leads to an average increase of 25% in citrate excretion in both men and women.

11.3. Net Gastrointestinal absorption of alkali

As renal citrate excretion is modulated by acid-base balance a dietary intake of alkali or acid is likely to modify urinary citrate excretion. A simple and accurate method was proposed by Oh for the calculation of net gastrointestinal absorption of alkali (177). Using this formula, Pak showed that in normal subjects, urinary citrate was directly correlated with the net gastrointestinal absorption of alkali (93). These results suggested that dietary alkali or acid absorption is a critical determinant of citrate excretion and that hypocitraturia may result from reduced net gastrointestinal absorption of alkali. Our observations showed in a large group of calcium stone formers a positive correlation between gastrointestinal alkali absorption and citrate urinary excretion ($p= 0.00024$) (178). Furthermore, in patients with ulcerative colitis we observed that after surgical treatment there was a further decrease in urinary citrate excretion, probably due to a contemporary reduction of gastrointestinal alkali absorption (173).

Moreover, some patients may present a primitive intestinal malabsorption of citrate. This possibility was suggested by Cowley *et al.* who observed in stone formers, a smaller citratemic response in comparison to healthy subjects, following oral citrate assumption (179).

12. CITRATE TREATMENT OF KIDNEY STONE FORMERS

Several reports have proved the need for selective long-term medical treatment for the prevention of stone formation in patients with recurrent calcium lithiasis. In fact, new stone formation continued in 39% of the patients

Table 6. Alkali citrate treatment: main clinical indications

1. Idiopatic calcium oxalate stone disease with hypocitraturia alone or with other metabolic alterations
2. Idiopatic calcium oxalate stone disease without hypocitraturia
3. Uric acid nephrolithiasis
4. Hyperuricosuric calcium nephrolithiasis
5. Cystine kidney stones
6. Secondary hypocitraturia
a. Distal renal tubular acidosis (complete or incomplete)
b. Nephrolithiasis associated with inflammatory bowel diseases
c. Nephrolithiasis associated with by-pass or ileal resection
d. Chronic diarrhoeal states or malabsorption
e. Therapy with drugs inducing hypocitraturia and hypokalemia (e.g. thiazides, ACE-inhibitors, acetazolamide)
f. Clinical conditions associated with hypokalemia
g. Excessive dietary acid load
7. Residual renal stone fragments after extracorporeal shock wave lithotripsy

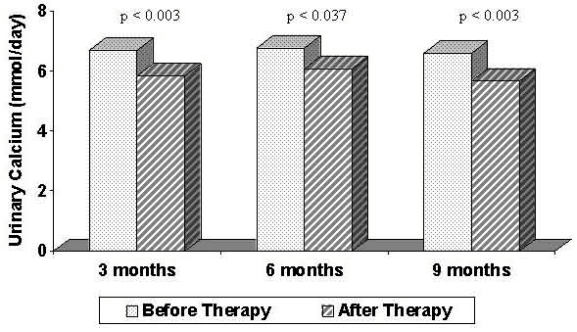

Figure 11. Urinary calcium excretion before and after potassium-citrate therapy (From 164, with the permission of the Publisher).

during conservative or placebo trials and 69% of untreated stone formers, however, needed at the end surgical treatment (180). On the other hand only 2% of the patients receiving medical therapy required further surgical treatment (180). In the last two decades alkaline salts have become a common prescription in patients with recurrent calcium stone disease and in several other pathological conditions associated or not with calcium stones (Table 6) (93,181-184). In fact alkaline citrate, by increasing urinary pH, decreases the calcium oxalate crystallization processes. Furthermore, the increased citrate excretion raises calcium citrate complex formation in urine with a lowering of the relative supersaturation ratio with respect to CaOx (185-190). The most commonly used salts are potassium citrate, sodium citrate, calcium citrate and potassium-magnesium citrate, although potassium citrate is usually the preferential treatment. In fact, the use of sodium citrate is limited by the increased sodium load, which induces or worsens hypercalciuria, whereas a decrease in urinary calcium concentration has been described with potassium citrate (191). Also in our work (164), a significant decrease in urinary calcium excretion was observed after 9 months of therapy (Figure 11), while we were not able to find the same result in a successive paper in which the treatment was significantly longer (48 months) (192). In this paper a significant decrease was observed only after 1 year of therapy and successively, it tended to increase again. Fuselier *et al.*, observed a small, but significant, decrease in urinary calcium excretion during treatment periods ($p= 0.0475$) (193). Furthermore, in patients with hypertension, sodium intake must be restricted, as well as in patients with calcium oxalate lithiasis treated with thiazide. In fact, sodium loads induce a hypercalciuria that is uncontrollable with thiazide (167). Also calcium citrate, increases urinary citrate excretion in non-stone-forming subjects, although with this salt urinary calcium excretion increases significantly (194). Calcium citrate is not usually used for calcium lithiasis treatment, whereas it is usually prescribed as a calcium supplement. Calcium citrate seems to be better absorbed at an intestinal level than other calcium salts. Calcium absorption from calcium citrate was significantly higher (27%) than that from calcium carbonate (22%), both on an empty stomach or co-administered with meals (195). Furthermore calcium citrate is a soluble calcium source and thus it is absorbed in a normal way in subjects with increased gastric pH (196). The intestinal absorption of calcium citrate is greater than other supplements, as calcium is absorbed both by means of an active intestinal transport (as calcium ion) and by a paracellular pathway (as calcium-citrate complex) (197). However, the simultaneous increases in urinary citrate, largely due to the alkali load, decrease the risk of stone formation. Two studies confirmed this concept: in the first, premenopausal stone-forming females, treated with calcium citrate supplements did not show any increase in urinary CaOx and CaP saturations (198). In the other study, non stone-forming patients chronically taking calcium citrate did not show any modifications in urinary chemistry indicating an increased lithogenetic risk (199).

Moreover, the formation product of CaOx rose during treatment, indicating that the increased citrate excretion enhanced the inhibitory activity against CaOx crystallization. Thus, calcium citrate may not be attendant with the risk for stone formation that is usually associated with calcium supplementation.

In 1992, Pak *et al.* compared the effect of both potassium-magnesium citrate and potassium citrate on some urinary parameters and the urinary saturation of CaOx in two groups of subjects: the first included calcium stone formers and the second healthy volunteers (200). Urinary pH was significantly higher during potassium-magnesium citrate than during potassium citrate therapy. Urinary magnesium increased significantly during potassium-magnesium citrate treatment but not during potassium citrate therapy. Urinary citrate excretion was higher during potassium-magnesium citrate treatment and the difference between the two supplements was

Table 7. Short-and long-term studies showing the efficacy of alkaline citrates

Citrate salt	Dose/day	Tipe of study	Follow-up	Year	Reference
Na-K-citrate	10-27 mmol	NRT	8 days	1981	236
K-citrate	30-100 mEq	NRT	1-1.42 years	1983	186
Na-K-citrate	88 mmol	NRT	0.7-7.4 years	1984	237
K-citrate	60 mEq	NRT	at least 1 week	1984	188
K- citrate	30-60 mEq	NRT	4-20 months	1985	238
K- citrate	60-80 mEq	NRT	3-8 months	1985	180
Na-K-citrate	7.5 g	NRT	4 weeks	1985	239
K-citrate	10-33 mmol	NRT	3 years	1985	190
K-citrate	60-80 mEq	NRT	34 months	1985	180
K-citrate	10-33 mmol	NRT	2 years	1985	190
K- citrate	60 mEq	NRT	1 week	1986	240
K-citrate	10-27 mmol	NRT	2.13 years	1986	189
K-citrate	20-27 mmol	NRT	2.35 years	1986	218
Na-K-citrate	10 g	NRT	18 months	1990	234
K-citrate	10-33 mmol	NRT	1-4.33 years	1990	181
K- citrate	80 mEq	NRT	1-18 days	1991	214
K- citrate	20-40 mEq	NRT	7-34 months	1993	241
K-citrate	1 mEq/kg	NRT	3-9 months	1994	164
K-Citrate	5 g	NRT	48 months	1996	192
K-Citrate	60 mEq	RCT double-blind	3 years	1993	97
Na-K-Citrate	78 mEq	PRT	3 years	1994	98
K-Mg-citrate	63 mEq	RTC double blind	3 years	1997	99
k-Citrate	6-8 g	PRT	12 months	1994	228
k-Citrate	30-90 mEq	NRT	6-53 months	1998	193

NRT = Non Randomized Trials; RCT = Randomized Controlled Trial; PRT = Prospective Randomized Trial

statistically significant ($p < 0.02$). Finally, urinary saturation of CaOx and uric acid declined significantly during potassium-magnesium citrate therapy and minimally during potassium citrate therapy. The authors concluded that this supplement was more efficient than potassium citrate. These results were confirmed by Ettinger *et al.* who showed a decrease of the recurrence rate of stones in idiopatic calcium stone formers chronically treated with potassium-magnesium citrate (201). Schwille *et al.* proved that magnesium-alkali citrate induced anti-CaOx crystallization effects through the combined direct and indirect action of magnesium, citrate and the stabilization of urinary pH (202). In the presence of less acidic renal tubular fluid, citrate reabsorption decreases. This effect, together with the contemporary progressive citrate deprotonation, causes a greater intraluminal concentration of charged citrate, which presents higher inhibitory properties on crystallization processes (99,203-206). Potassium-magnesium citrate seems to be particularly recommended for the treatment of patients with a decreased urinary excretion of citrate, magnesium and potassium. Some authors assume that potassium-magnesium citrate will become the supplement of choice in the therapy of calcium oxalate and uric acid nephrolithiasis (167).

Several experimental and clinical studies examined the effect of alkaline citrate on urine composition and the risk of crystallization of CaOx and CaP, as well as on the clinical course of calcium stone disease and the effects of different dose regimens. In table 7 the results of some short and long term treatment are summarized. Citrate is a well known inhibitor of the crystallization of calcium salts by means of the mechanisms described above, (e.g. increased urinary citrate excretion and increased urinary citrate concentration, inhibiting the various steps of CaOx and CaP crystallization) (184,207-211). Alkaline citrate salts induce a significant increase in urinary citrate excretion mainly by means of an alkaline load (80%) and of the consequent rise of pH inside the tubular cells; furthermore a small fraction of absorbed citrate (about 20%) is excreted directly in urine.

There is general agreement about some urinary modifications, such as the increase of urinary pH (Figure 12) and potassium and citrate urinary excretion (Figure 13 and 14) (192,212-214). Furthermore, our data showed the persistence of increased urinary citrate excretion considering the mean of "Delta values" between basal and follow-up periods (Figure 15). In contrast, when the results of "Delta values" were examined separately, patients showed a different behaviour; namely, the "Delta value" of the 5^{th}, 50^{th} and 95^{th} percentiles of each follow-up showed an nonhomogenous behaviour with some patients showing a negative "Delta value". In particular the 5^{th} percentile showed only a temporary increase of citrate excretion and then returned to values similar to or lower than basal values (Figure 16) (192). These results suggest that the behaviour of patients with hypocitraturia is heterogeneous and that only 20% of them tend to reach the mean value of the urinary citrate excretion of the stone former group. Recently, Fuselier *et al.* confirmed our observation that in 21% of the patients treated with potassium citrate, urinary citrate excretion does not rise (193,211). The authors confirm the need for a careful follow-up of the patients treated with potassium citrate in order to identify patients requiring a more aggressive medical therapy and to properly modify the dose of alkali salts in patients that need chronic medical treatment (193).

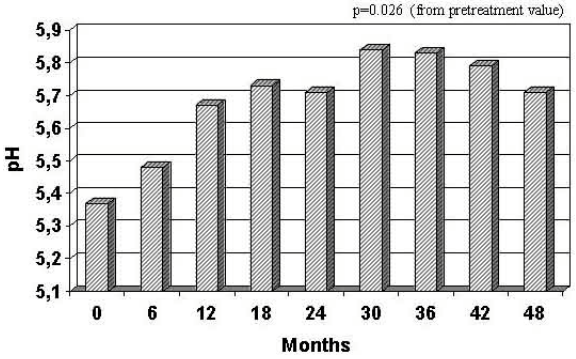

Fig.12. Urinary pH after potassium citrate therapy (modified from 192).

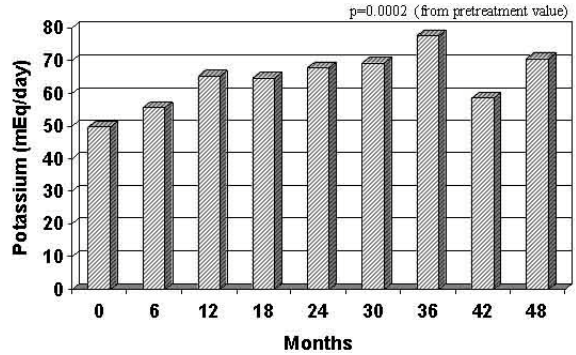

Figure 13. Urinary potassium excretion after potassium citrate therapy (modified from 192).

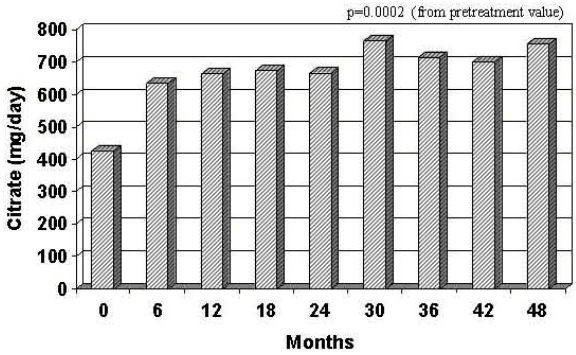

Figure 14. Urinary citrate excretion after potassium citrate therapy (modified from 192).

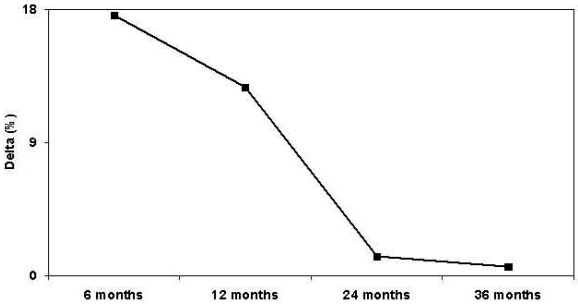

Figure 15. Trend of percentage delta in the follow-up period indicating a progressive decrease when compared vs precedent control (modified from 192).

In patients treated with potassium citrate, no differences were observed for oxalate, uric acid, urine volume and creatinine excretion rate before and during the follow up (164,192,193). Therapy with potassium citrate decreases the supersaturation ratio with respect to CaOx (185,212,215-217). Potassium citrate does not usually induce an increase in the relative supersaturation ratio of brushite as it reduces urinary calcium excretion. However an excessive amount of potassium citrate may increase the relative supersaturation ratio of brushite and this effect is irrespective of whether potassium citrate is taken with meals or not (212,215,217,218). Sodium citrate induces a small decrease in supersaturation with respect to CaOx because it does not decrease urinary calcium excretion, thus causing an increase of the supersaturation with respect to CaP salts (145). Also Lemann observed a more favourable effect of potassium salts in comparison with sodium salts. Since urinary calcium was also reduced with potassium bicarbonate, calcium balance appeared still improved with potassium salt (219). Furthermore, in patients with distal renal tubular acidosis, potassium citrate treatment contributed to the improvement of calcium balance, both by increasing intestinal calcium absorption, by means of a $1,25(OH)_2D_3$ independent mechanism and by decreasing urinary calcium excretion. According to some authors, the decreased calcium excretion can be explained by an increased calcium reabsorption in the distal tubule induced by metabolic alkalosis as well as by the increased luminal pH (220,221). The final effect of chronic treatment with potassium citrate and other alkaline salts may be a positive calcium balance as shown by a small but significant increase of bone mineral density in stone forming females (109,222). A similar result was obtained by Sebastian *et al.* who treated a group of healthy postmenopausal women with potassium bicarbonate improving calcium balance through an interaction of bone remodelling phases (109).

Several studies *in vitro* showed that citrate inhibits struvite formation through the chelation of magnesium, disruption of the hydrogen and ionic binding of this mineral and coating of the surface of struvite crystal (223-225). This latter effect prevents the further growth of struvite crystals *in vitro*. Furthermore, Wang *et al.* showed that citrate added to the urine or taken orally strongly delays urease-induced crystallization in human urine despite the increase in pH (226). Clinical studies showed that potassium citrate therapy could prevent the recurrence of infection stones following extracorporeal shock wave lithotripsy (ESWL) (227,228). In a successive study, Fine *et al.* proved that citrate therapy improved the clearance of residual fragments of CaOx and infection stones (75%) compared to the control group (32%). Moreover, crystal growth was also retarded in patients treated with citrate (5%) in comparison with the control group (47%) (229).

Although several studies are reported in the literature, there are only three randomized trials on the recurrence rate in calcium stone formers treated with both potassium citrate and magnesium-potassium citrate, and only one on the clearance of fragments from the kidney after ESWL treatment (Table 7). In all these studies the treatment with different alkaline citrates (Barcelo *et al.* K-

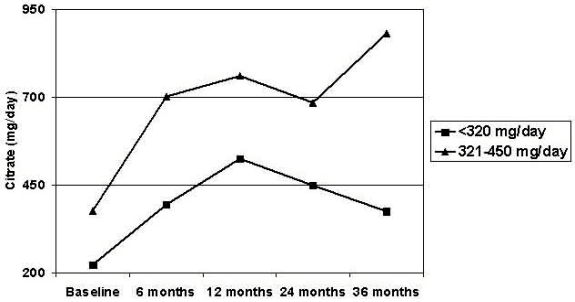

Figure 16. Trend of citrate excretion after potassium citrate therapy in patients with different baseline values of citrate (modified from 192).

itrate 60 mEq/day, Hofbauer Na-K-citrate 78 mEq/day, Ettinger K-Mg-Citrate 63 mEq/day) reduced the stone recurrence rate and the rate of stone-free patients was 72, 31 and 87 per cent respectively. Furthermore, stone formation rate decreased from 1.2 to 0.1, from 2.1 to 0.9 and from 0.57 to 0.008 respectively.

The small number of randomized trials may be explained both by the observation that citrate therapy is well tolerated and by the personal feeling of most of the authors that believe it is not ethically correct to leave patients without treatment, since numerous studies have shown the positive effect of potassium citrate therapy in patients with nephrolithiasis.

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