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URINARY CITRATE, BONE RESORPTION AND INTESTINAL ALKALI ABSORPTION IN STONE FORMERS WITH FASTING HYPERCALCIURIA

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Abstract

Reduced citrate in urine and increased fasting excretion of calcium are abnormalities frequently reported in stone forming (SF) patients. Increased dietary acid (or reduced alkali) introduction or absorption may be a potential cause of both these pathological findings. To test this hypothesis, we studied 64 SF patients {32 with fasting hypercalciuria (FH) and 32 without FH (NFH)}. After a basal evaluation for nephrolithiasis, while on a 500 mg calcium diet, they were evaluated for: (**1**) daily intestinal alkali absorption (IAA), by urinary electrolyte excretion; (**2**) basal concentrations of PTH, calcitonin (CT) and 1,25(OH)2-VitD; (**3**) oral calcium load for evaluation of changes in calcium and hydroxyproline urinary excretions; (**4**) intestinal calcium absorption (18 patients), with double curve analysis (stable Sr as tracer); and (**5**) changes in citrate excretion after an alkali load (50 mEq of a mixture of calcium gluconate, lactate and carbonate) in 10 patients.

The results demonstrated: (**1**) FH stone formers had reduced citrate excretion and lower mean IAA levels than NFH stone formers; (**2**) FH stone formers also had higher bone resorption levels with lower PTH and higher CT levels; (**3**) IAA levels were related to both citrate excretion and bone turnover indices; and (**4**) the increases in citrate excretion after oral alkali load were strictly related to basal IAA values (index of alkali absorption and/or generation after oral load), demonstrating that a different absorptive capacity of alkali rather than a different dietary content may underlie these metabolic abnormalities.

Key Words: Intestinal alkali absorption, citrate, bone resorption, hydroxyproline, fasting hypercalciuria, calcitonin, parathyroid hormone, urolithiasis, intestinal calcium absorption, vitamin D.

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Introduction

Reduced urinary citrate excretion is a common trait among patients who form stones [9, 21, 22]. Acidosis is the main factor in causing hypocitraturia encountered in distal renal tubular acidosis [16], chronic diarrheal states [19], and thiazide-induced hypokalemia [20]. However, in most hypocitraturic stone formers (SF), no exact cause can be identified. Increased dietary intake of protein, mainly composed of sulphur-containing amino acids, resulting in acid load, has been suggested as a putative factor in "idiopathic" hypocitraturia [3]. Another possible explanation is that low alkali intake or absorption might be the causative factor. In 1987, Cowley *et al.* [6] found a reduced citraturic response after an oral citrate load in SF. Since Oh [17] introduced a simple and reliable method for the calculation of net gastrointestinal alkali absorption (IAA), it has been possible to directly relate citrate excretion with IAA. Indeed, Pak [18] demonstrated that IAA, after an oral citrate load, was reduced in some SF, suggesting reduced intestinal absorption. The first aim of the present study was to confirm or otherwise test this hypothesis, by directly measuring citrate excretion after an oral alkali load other than citrate.

It has also been suggested that increased levels of bone turnover (BT), independent of PTH secretion, might play a causative role in fasting hypercalciuria (FH) of SF [2, 13, 14], especially in conditions of relatively low calcium intake [12]. In this pathophysiologic set, a relative hypoparathyroidism has also been reported [8]. Notwithstanding a well known role of calcitonin (CT) in controlling BT rate [7], after the initial finding by Ivey *et al.* [10] of increased CT levels in hypercalciuria, little attention has as yet been paid to this hormone in FH SF. The second aim of our study has been to further investigate the CT role, if any, in FH of SF.

Furthermore, it has also been demonstrated that increased dietary acid load is able to induce increased calcium excretion in urine together with increased bone resorption [1, 11]. The final aim of the present investigation was to assess whether the alkali composition of the

Table 1. Main clinical data of the two groups of patients (mean ± standard deviation, sd; ns = not significant).

	N	CrCl (ml/min)	Age (years)	BMI	Sex (F/M)	Activity (no. Stones/ last 2 years)
NFH	32	106 ± 4.0	39.9 ± 10.7	25.0 ± 0.18	14/18	3.1 ± 4.0
FH	32	104 ± 3.8	44.3 ± 12.7	24.6 ± 0.18	15/17	2.8 ± 1.0
p		ns	ns	ns	ns	ns

Table 2. Daily urine parameters and calculated IAA values in FH and NFH patients (mean ± sd).

	NFH (n 32)	FH (n 32)	p value
u-Ca mmol/day	4.6 ± 1.8	5.95 ± 2.73	< 0.05
uNa mmol/day	140 ± 68	170 ± 89	ns
uMg mmol/day	4.0 ± 1.5	4.5 ± 2.1	ns
u-Pi mmol/day	26.1 ± 11.3	25.7 ± 11.9	ns
u-Ox mmol/day	360 ± 155	398 ± 202	ns
u-Cit mmol/day	3.68 ± 1.85	2.28 ± 1.37	< 0.05
uUA mmol/day	4.0 ± 4.9	3.7 ± 1.4	ns
uSulf mmol/day	21.1 ± 6.9	21.3 ± 6.7	ns
uUrea mmol/day	328 ± 143	379 ± 136	ns
IAA mEq/day	31.2 ± 19.4	15.3 ± 18.3	< 0.05

diet might also play a role in increased bone turnover of FH.

Patients and Methods

Among the patients evaluated for active urolithiasis in our Nephrology Unit in the last 4 years, we enrolled 64 patients (29 females, 35 males; ages 19-63 years), on the basis of the presence of hypercalciuria, defined as a daily urinary calcium excretion > 0.1 mmol/kg bw/day (bw = body weight), on a free diet and the common composition of the expelled stones (calcium oxalate). None of them suffered from primary hyperparathyroidism, overt renal tubular acidosis, or medullary sponge kidney disease; no drug known to affect calcium metabolism was consumed by any patient, at least in the two months preceding the study. At least 2 months had elapsed from the last expelled stone or urological intervention.

For all 64 patients, after 15 days on an ambulatory diet, with a moderately restricted calcium content (about 500 mg), daily urines were collected on two consecutive days for the assessment of: calcium, sodium, potassium, magnesium, chloride, phosphate, sulphate, citrate, oxalate, uric acid, urea, creatinine and the calculation of

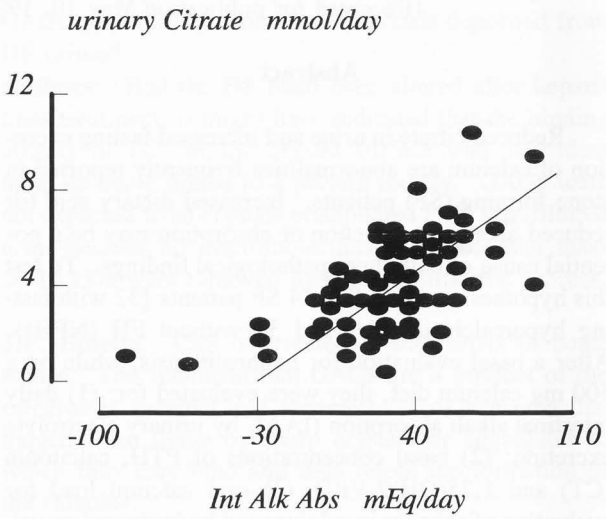

Figure 1. Correlation between IAA values and urinary citrate excretion. A significant linear direct correlation was evident (Cit = 2.65 + 0.018 x IAA; r = 0.382, p < 0.001).

intestinal alkali absorption (IAA; see below). On the third day, after 12 hour of complete fast, freshly voided 2-hour urines were analysed for: calcium, phosphate, hydroxyproline and creatinine. A blood sample was also obtained for evaluating: calcium, phosphate and the other electrolytes, acid-base balance, creatinine, intact PTH, monomeric CT and 1,25(OH)2-VitD. Then an oral calcium load (OCL) {1 g of calcium ion, given as a mixture of calcium gluconate, lactate and carbonate (Calcium Sandoz)} was performed and in the urines collected for the following 4 hours, calcium, phosphate, hydroxyproline and creatinine were evaluated. A blood sample was also obtained 2 hours after OCL for the evaluation of: calcium, bicarbonate, PTH and CT. According to fasting calcium excretion values (Ca/Cr above or below 0.310 mmol/mmol), the patients were defined as respectively fasting hypercalciuric (FH) and not fasting hypercalciuric (NFH) SF; by chance, 32 of them were FH and 32 NFH.

In addition, in 18 (9 FH and 9 NFH) of these patients, intestinal calcium absorption (ICaA) was measured by a double curve analysis, utilizing stable

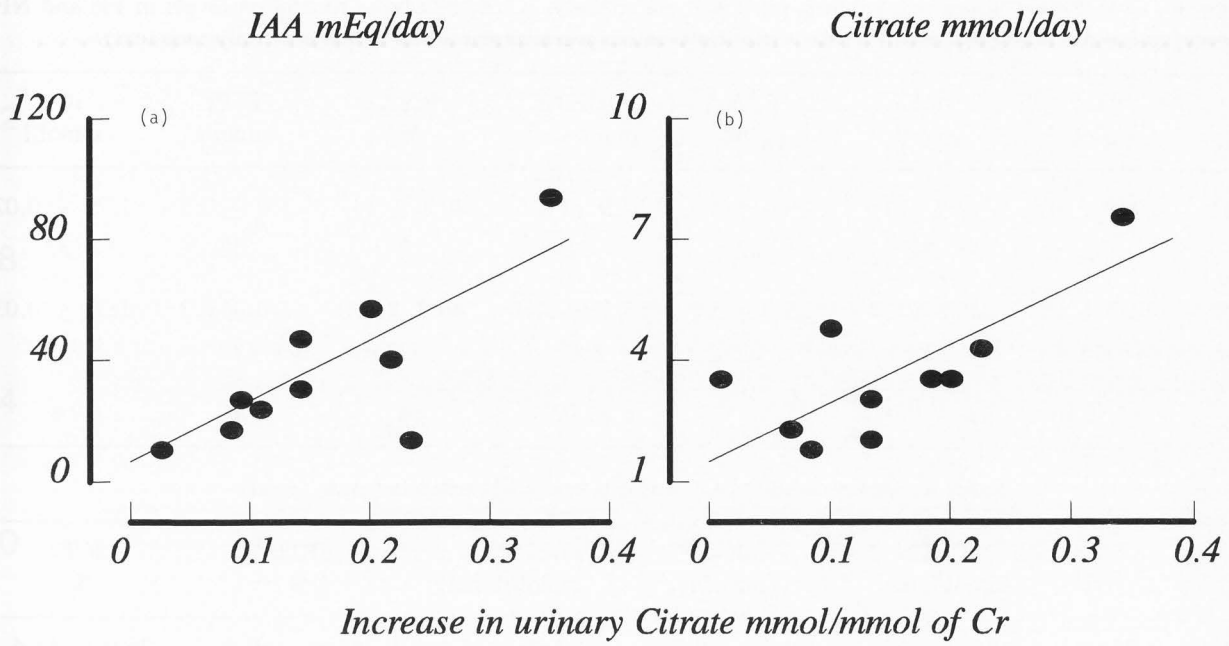

Figure 2. Correlations between the increase of citrate excretion after OCL and both daily urinary citrate (**a**) and IAA (**b**). A significant positive linear relationship was evident in both cases (daily Cit = 0.037 + 0.033 x dCit; r = 0.717, $p < 0.01$; IAA = 0.028 + 0.003 x dCit; r = 0.742, $p < 0.01$).

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strontium as tracer, according to a method already described [15]: briefly, 2.5 mmol and 1.25 mmol of stable strontium were given, on two separate days, respectively by mouth and intravenously in bolus; then multiple blood samples were obtained at 0, 5, 10, 15, 30, 60, 120, 180, 240, 360, 480, 600 minutes for the measurement of Sr [4] and the calculation of true intestinal absorption was obtained by integrating the two curves [15].

Furthermore, in 10 of these patients, urinary citrate and intestinal alkali absorption were evaluated before and after OCL, which also represents an alkali load of 50 mEq.

Intestinal alkali absorption (IAA) in the daily urine of all 64 patients and in urine collected before and after OCL, in 10 patients, were calculated according to the formula suggested by Oh [17]:

$$IAA = (Na + K + Ca + Mg) - (Cl + 1.8\ iP)$$

where Na, K, Ca, Mg, and Cl are expressed as mEq and iP (inorganic phosphate) as mmol, excreted in daily urines.

Tubular threshold of phosphate (TTPi) was calculated by urinary excretion of phosphate and creatinine in fasting urine, according to Walton and Bijvoet [23].

Electrolytes, creatinine, urea and uric acid in serum and urine were measured by standard methodology (autoanalyzer, absorption spectrophotometry, flame photometry).

Strontium in serum was measured by atomic absorption spectrophotometry [4].

Urinary oxalate and citrate were measured by column gaschromatography (Hewlett-Packard 5890 II; Supelco SPB-5), in urine collected under 6N HCl.

Hydroxyproline in urine was determined by liquid chromatography (Beckman System Gold), after acid hydrolysis.

PTH was measured by intact-PTH immunoradiometric assay (IRMA, NICHOLS Institute Diagnostic, San Juan Capistrano, CA, USA), utilizing two different policlonal antibodies, purified by affinity chromatography, specific for 39-84 and 1-34 regions. The intra-assay coefficient of variation was 2.4% and the inter-assay coefficient 5.6%.

Calcitonin was analyzed by radio-immunoassay with double antibodies in liquid phase (NICHOLS). This assay has been proven to be specific for monomeric CT (the active hormone) [5], with an intra-assay coefficient of variation of 3.3 %, and an inter-assay coefficient of 5.5%.

For 1,25(OH)2-vitamin D determination, a 10 ml blood sample was collected with heparin, immediately centrifuged at 4°C and stored at -20°C, until the assay was performed (within 20 days). After acetonitrile extraction, in the presence of tritiated calcitriol (as an indicator of extraction efficiency), plasma sample was submitted to a "phase switching" procedure, using a single C-18OH cartridge (BondElut) for subsequent separation

Table 3. Calciotropic hormones, calcium-phosphate parameters, ICaA, and bone resorption levels in FH and NFH patients, in basal conditions (mean ± sd). The ICaA values were measured in 18 patients only (see text).

	PTH pg/ml	CT pg/ml	1,25 pg/ml	OHP/Cr mmol/mol	ICaA %	TTPi mmol/l	iCa mmol/l
NFH	27.6 ± 10	12.9 ± 12	35.6 ± 11	13.5 ± 4.6	40.1 ± 14	1.0 ± 0.3	1.29 ± 0.02
n =	32	32	32	32	9	32	32
FH	20.7 ± 11	28.8 ± 24	38.8 ± 14	18.1 ± 11.1	46.3 ± 8	1.0 ± 0.2	1.31 ± 0.03
n =	32	32	32	32	9	32	32
p	< 0.01	p < 0.05	ns	p < 0.01	ns	ns	ns

Table 4. Main results of OCL in FH and NFH patients (mean ± sd).

	dCa/Cr mmol/mmol	dCa mmol/l	dOHP/Cr mmol/mmol	dPTH %	dCT %
NFH (n = 32)	0.185 ± 0.17	0.12 ± 0.10	-4.0 ± 7.7	-44.9 ± 60.4	26.0 ± 45.4
FH (n = 32)	0.169 ± 0.27	0.47 ± 0.47	-4.9 ± 8.5	-34.3 ± 48.9	9.9 ± 34.5
p	ns	ns	ns	ns	ns

of 25-(OH)D, 24,25-(OH)2D and 1,25-(OH)2D with isopropanolol-hexane; then the final product was dried by nitrogen stream and measured by radioreceptorial method (RRA, NICHOLS). Each sample was analyzed in duplicate. The recovery for this metabolite was between 60 and 80% and each sample was corrected for its own recovery. The intra-assay coefficient of variation was 10.0% and the inter-assay coefficient of variation was 14.0%.

Statistics were calculated by Anova, paired T test and linear regression analysis utilizing a BMDP computer statistical package on an Olivetti M-300-10 PC.

Results

The main clinical data of the FH and NFH patients are shown in Table 1. No differences were indicated between the two groups as regards age, sex distribution, renal function (evaluated by creatinine clearance), stone activity and body mass index (BMI).

Table 2 summarizes the main 24-hour urine parameters and the calculated values of IAA in the 2 groups. FH patients had significant higher calcium, lower citrate urinary values and significant lower IAA levels, with no significant difference as regards the other parameters. Furthermore, the IAA values were significantly and positively correlated with citrate urinary excretion (Fig. 1). Citrate excretion values did not appear significantly correlated with either urea or sulfate daily urinary excretion.

The main clinical data of the 10 patients (6 males, 4 females; 44.9 ± 8.7 years in age) in whom citrate and IAA were measured before and after OCL were as follows: creatinine clearance 103.7 ± 18.2 ml/min; BMI 24.8 ± 3.5; and activity (number of stones / last two years) = 4.9 ± 5.5. Both urinary citrate and IAA significantly increased after OCL (Cit/Cr: 3.91 ± 0.22 versus 2.37 ± 1.47 mmol/mmol; $p < 0.001$; IAA: 2.6 ± 2.2 vs 0.9 ± 1.3 mEq/hr). The increase of citrate excretion was significantly and positively related to the daily levels of both urinary citrate (Fig. 2a) and IAA (Fig. 2b). On the other hand, no correlation was found between the increase of IAA after OCL and basal IAA values.

The basal levels of calciotropic hormones and related data, ICaA, and BT are shown in Table 3. FH SF patients had higher bone resorption (as measured by OHP/Cr), with lower PTH and higher CT levels than NFH SF. No differences were evident in VitD status, ICaA or calcium-phosphate parameters. The concentrations of CT were significantly and directly related to daily urinary calcium excretion (Fig. 3a) and inversely to citrate excretion (Fig. 3b). Furthermore, the levels of CT and hydroxyproline excretion were inversely related to IAA basal values (Fig. 4).

The main results of OCL in the two groups of patients are shown in Table 4. No significant difference was evident between the two groups of patients, however CT changes after OCL tended to be higher in NFH, and

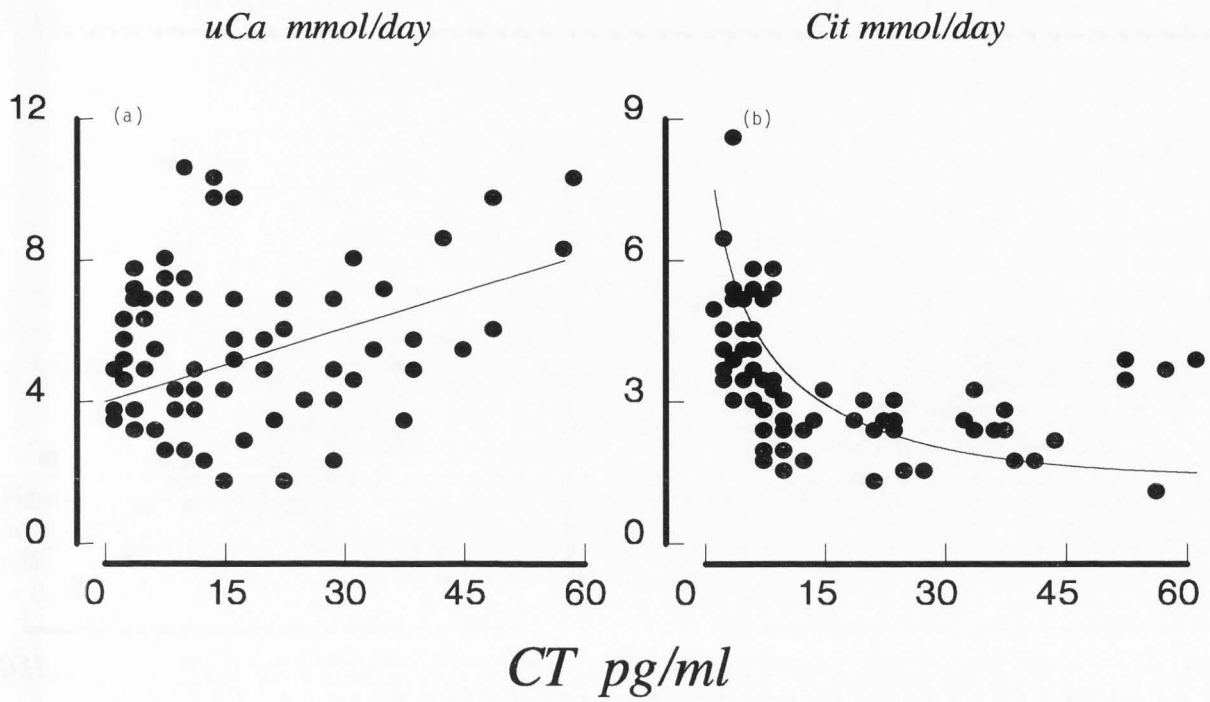

Figure 3. Correlations between basal CT serum levels and daily urinary excretions of calcium (**a**) and citrate (**b**). A direct linear correlation was evident between CT and daily calciuria (uCa = 4.9 + 0.022 x CT; r = 0.238, $p < 0.05$) and an indirect hyperbolic correlation with urinary citrate (lnCit = 1.66 - 0.26 x lnCT; r = 0.443, $p < 0.001$).

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the percentage CT increase was inversely correlated with basal levels of CT (Fig. 5).

Discussion

An overt cause of reduced citrate excretion, which frequently occurs in the urines of SF patients, is often lacking. Considering that both intra and extracellular acidosis is the common factor in all the conditions known to be accompanied by hypocitraturia [16, 19, 20], it is conceivable that increased acid or reduced alkali intake in the diet might be the causal factor of the "idiopathic" form of hypocitraturia. The method suggested by Oh [17] for calculating IAA, by simply measuring electrolyte excretion in daily urines, has led to the possibility of testing the above hypothesis. Indeed, the data from Pak [18] clearly suggest that IAA affect the levels of urinary citrate, and our results confirm these data (Fig. 1). However, these results may be a consequence of either reduced alkali content in the diet or reduced alkali transport efficiency in the intestine. In our patients, no correlation was found between urinary citrate and urea and sulphate daily output (indices of protein and acid content of the diet), suggesting that the alkali food content is not the main cause in this respect. Indeed, Cowley *et al.* [6], utilizing a citrate load, found reduced citrate excretion in stone formers and, more recently, Pak [18] indirectly confirmed these results demonstrating increased IAA after oral citrate load. However, in the first case [6], the increase in citrate excretion was obtained during the specific load of the same alkali (i.e., citrate) and this finding is not necessarily indicative of citraturic response after an aspecific alkali load, and in the second study [18], the response to alkali load was assessed as the increase in IAA, evaluated with Oh method, which is not the recommended method to measure alkali absorption when an acid-base perturbation is going on, as during an alkali load [17]. We tested the increase of citrate excretion and of IAA after an alkali load of 50 mEq, represented by calcium gluconate, lactate and carbonate, and found that the increase of citrate excretion after OCL was direct correlated with basal IAA. These finding, in our opinion, support the hypothesis that IAA is mainly dependent on the intestinal transport capacity of the intestine, after an oral alkali load, and not on the alkali content of the diet. The lack of correlation between basal IAA values and changes in IAA after OCL confirm that the measurement of IAA by Oh's method during an ongoing perturbation of acid-base balance is not reliable, as stressed by Oh himself [17].

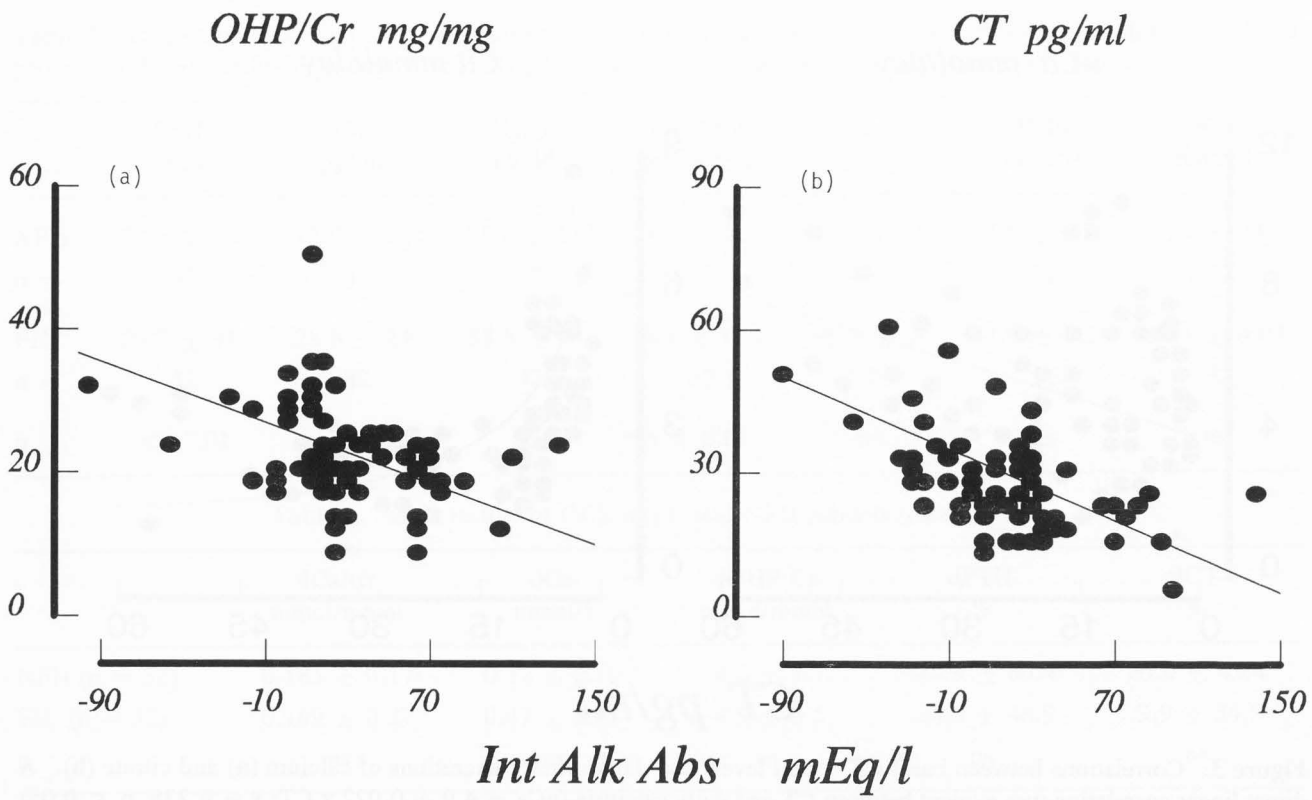

Figure 4. Correlations between IAA levels and fasting hydroxyproline urinary excretion (**a**) and basal CT serum concentrations (**b**). An inverse correlation was evident in both cases (OHP = 21.7 - 0.07 x IAA; r = 0.345, $p < 0.01$; CT = 19.4 - 0.15 x IAA; r = 0.400, $p < 0.01$).

Evidence has been provided that fasting hypercalciuria is often accompanied by increased bone resorption indices and reduced PTH levels [2, 12, 13, 14]. Little attention has as yet been paid to the CT role in this pathophysiological condition, after a previous finding of increased CT levels in hypercalciuria by Ivey *et al.* [10]. Indeed, our data confirm higher values of CT in patients with FH, and its values were significantly related to both calcium (positively) and citrate (negatively) urinary excretions (Fig. 3). A possible explanation for all these findings may be an increased dietary calcium absorption, possibly due to increased VitD bioavailability, which might cause increased calcium excretion, stimulated CT and suppressed PTH and perhaps by a concomitant increased content of protein and acid load in the diet, reduced citrate excretion and increased bone resorption. This hypothesis is well supported by Hess's data [8] which demonstrated that the relative hypoparathyroidism of hypercalciuric stone formers might be explained by increased levels of VitD and secondary increased calcium absorption. However, in our patients, the levels of VitD were completely overlapping in the two groups of SF, with or without fasting hypercalciuria. Furthermore, there were no differences in ICaA in the 18 patients for whom it was measured, nor in the increase of calcium excretion after OCL between FH and NFH stone formers. The apparent discrepancies between our results and Hess's [8] are at least in part due to a different calcium intake in the two studies (reduced in our, free in Hess's) and a different method of classification (our patients were divided on the basis of the presence or otherwise of FH, Hess's patients on the presence or otherwise of daily hypercalciuria). An alternative explanation might be that the different alkali absorption by the intestine might play a role.

Indeed, another interesting finding of our study was that both citrate and IAA were significantly lower in FH patients. Furthermore, IAA values were inversely correlated to both hydroxyproline and CT levels. Taken together all the results might suggest that in FH, reduced IAA might induce not only a reduction in citrate excretion, as suggested by Pak [18], but also an increased bone resorption, secondary to the well known effect of acid-base balance perturbations on bone resorptive rate [11]. The increased bone resorption in turn might sustain increased calcium excretion in fasting urines and a resetting of parathyroid glands and thyroid C-cells with a consequent compensatory reduced PTH and increased

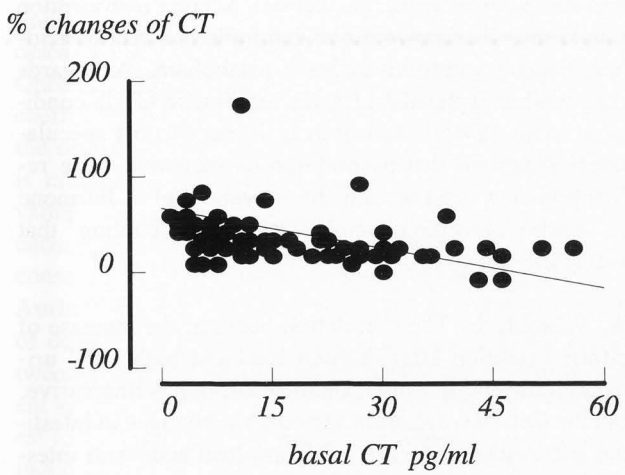

Figure 5. Correlation between the percentage changes inCT after OCL and its basal values. An inverse linear relationship was evident (dCT% = 29.0 - 0.37 x CT; r = 0.277, p < 0.02).

CT secretion. This resetting of secretion is, at least in part, supported by the finding of the reduction of CT secretory response to OCL proportional to the basal CT levels: i.e., the higher the basal secretory rate the lower the stimulated secretory response. However, to further validate this last hypothesis, studies specifically directed to measuring the CT secretion rate in these kinds of patients are needed.

Conclusions

Citrate excretion is mainly controlled by intestinal alkali absorptive capacity. Stone formers with fasting hypercalciuria, together with higher BT and relative hypoparathyroidism, present lower urinary citrate, higher urinary calcium and higher CT levels; reduced IAA levels might be the link for all these findings.

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Discussion with Reviewers

D.M. Wilson: According to the discussion by Oh [17], the alteration in IAA could be related to an abnormality in GI absorption but could also reflect cellular changes or bone buffering. Is it possible that the calculated change in IAA is a consequence of altered bone buffering or cellular metabolism?

Authors: What you say is true and is affirmed in the **Discussion**. In fact, the method of calculation for alkali absorption suggested by Oh [17] is usable only in the basal conditions and when it is presumed that there are no changes in bone and/or cellular buffering capacity. Indeed, our data show that IAA, measured by that method before and after alkali load (a condition that may be accompanied by a change in the cellular buffering) does not correlate with the IAA values measured in basal conditions.

H.-G. Tiselius: The authors state that an increased IAA, in addition to a reduced excretion of citrate, leads to hypercalciuria brought about by an increased bone resorption and an increased calcitonin concentration. The patients in this study, however, did not differ in 1,25-vitamin D levels or in calcium absorption. It would be interesting if the authors, in more detail, could describe how the different findings in these patients can be explained from a reduced IAA.

Authors: As stated in the text, lower IAA values were associated with lower citraturia and higher bone turnover indices. The hypothesis suggested is that a lower availability of alkali from intestine would determine, on one hand, lower excretion and/or renal production of citrate, and on the other hand, an increase in bone reabsorption due to the well-known direct and indirect effect of acid-base balance variations on bone metabolism. As regards the possible explanation for the increase in CT in conditions of increased bone turnover, it may be only speculatively supposed that a condition of increased bone resorption may up-regulate the secretion of a hormone (CT) which has the physiological role of "cooling" that metabolic process.

A. Trinchieri: The correlation between the increase of citrate excretion after calcium load and both basal urinary citrate and intestinal alkali absorption is impressive, but the lack of correlation between the changes in intestinal alkali absorption after calcium load and basal intestinal alkali absorption could be due to the very low number of patients in which urinary citrate was evaluated. Furthermore, it would be interesting to distinguish the effect of an alkali load between hypercalciuric and non-hypercalciuric stone formers.

Authors: We are in total agreement that, given the relatively low number of observations (10) on IAA before and after alkali load, the absence of a correlation may not necessarily be true, but we have to comment on our statistical data as they stand.

W.C. de Bruijn: As regards the stone formers (SF), please give an indication as to which stones were formed (calcium-oxalate, struvite?) and comment on the homogeneity of this population in this respect.

Authors: As stated in the text, all the SF patients studied had expelled stones prevalently composed of calcium oxalate.

W.C. de Bruijn: Various tests are performed with blood and urine samples from these SF, but can you, more explicitly, give the number of patients related to each graph.

Authors: As stated in the text, the total population studied was 64 patients and basal IAA had been measured in all; the 18 patients who had also had their intestinal calcium absorption measured belonged to the first population; in 10 of these patients citraturia and IAA were also measured both before and after alkali load. Thus, the graphs in Figures 1, 3, 4 and 5 show the observations in the total population (1 observation per patient); graph In Figure 2 shows the observations in the 10 patients who had their IAA and citraturia measured before and after alkali load.

W.C. De Bruijn: In most graphs, a linear relation is assumed to be present and calculated and the coefficients given in the legends. But in Figure 3b suddenly a logarithmic relation is presented. What was the basic as-

sumption to ask the computer to draw these linear relations and why not so in Figure 3b, and why is not in all cases a non-linear relation is assumed and/or tested. Once a (linear) relation is acquired, the correspondence coefficient (r) is indicative for the value one can give to the observed relation. In most cases shown, this value is rather low (ranging from 0.238 - 0.742) whereas a value around unity is acquired for a perfect correspondence. Please comment on this aspect and discuss the consequences.

Authors: The significance of the coefficient "r" is that of defining, as is known, the degree of correlation between two variables, of which one is taken as independent and the other dependent, the correlation being greatest for values of "r" close to unity. Of course, when the independent variable is the only one (or in any case preponderant), in order to determine the variations in the dependent variable (e.g., time versus a tracer concentration, the concentration of a chemical substratum versus the final product of a chemical reaction, etc.) for a correlation to be "good", the value of "r" must really be near unity. In multifactor biological systems (like those explored in clinical, physiopathological, epidemiological studies, etc.), "r" values are obviously much lower (there being many other factors, known and unknown, that cannot be considered in every single analysis): statistical significance tests intervene precisely to prove, or not, whether the relation existing between the variables examined is significant, weighing it by the number of observations and the spread of data around the interpolating line.

As regards the type of interpolation, when two variables are put in relation to one other, the function (given that there is one) that best interpolates the observed points should always be sought. It is natural that the linear function is the first to be explored but we always try the interpolation of at least the most common functions (hyperbolic, parabolic, logarithmic, exponential). Of course, in the end, the one that provides the highest "r" value will be chosen, to indicate a better interpolation of the way the relation is going.