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## Comparison of Semi-Empirical with Computer-Derived Methods for Estimating Urinary Saturation of Brushite

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### Abstract

**Purpose**—The Equil 2 computer program has been questioned by the new Joint Expert Speciation System program (Mayhem Unit Trust and Council for Scientific and Industrial Research, Pretoria, South Africa) for estimating the urinary saturation of stone forming salts to gauge the propensity for stone formation. To attempt resolution the supersaturation index according to the Joint Expert Speciation System and the relative saturation ratio according to Equil 2 were compared with the semi-empirically derived concentration-to-product ratio.

**Materials and Methods**—Data were obtained from a recent article in *The Journal of Urology*®, in which pH, calcium and citrate were varied over a wide range in 72 urine samples. We calculated the relative saturation ratio and the supersaturation index of brushite, and compared them with the available concentration-to-product ratio derived from the growth or dissolution of synthetic brushite.

**Results**—The mean concentration-to-product ratio did not differ from the supersaturation index but the concentration-to-product ratio and the supersaturation index were significantly lower than the relative saturation ratio ( $p < 0.004$ ). On the saturation value and urinary variable plot the relative saturation ratio could be readily distinguished from the concentration-to-product ratio because it was consistently and significantly higher. While the supersaturation index pattern was similar to the concentration-to-product ratio, the supersaturation index was slightly lower at high urinary pH and calcium, and slightly higher at lower urinary pH and calcium ( $p < 0.001$ ). When the  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  complex was deleted from the Joint Expert Speciation System, the corrected supersaturation index was not significantly different from the relative saturation ratio determined by Equil 2.

**Conclusions**—The relative saturation ratio overestimates brushite saturation by about 80%. The supersaturation index yields a good approximation of brushite saturation at modest degrees of saturation but it overestimates saturation at low pH or calcium (low saturation) and underestimates it at high pH or calcium (high saturation).

### INTRODUCTION

The use of outcome such as stone events requires long-term follow-up and is often impractical. Thus, the determination of urinary saturation of stone-forming salts is useful in gauging the propensity for stone formation and monitoring response to treatment.<sup>1</sup> In the late 1960s, computer programs were developed to calculate ionic activities of stone constituents;<sup>2,3</sup> urinary saturation was then estimated from activity products. In 1985, the Equil 2 computer program was introduced as an improved method for calculating urinary saturation of stone salts,<sup>4</sup> including brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) believed to be a precursor phase of hydroxyapatite.<sup>5</sup> By then, a detailed analysis of urine for stone-forming constituents and ancillary components was

being undertaken in stone research laboratories. By using such data, Equil 2 program derived concentration of  $\text{Ca}^{2+}$  (by subtracting known soluble complexes of calcium) and  $\text{HPO}_4^{2-}$  (from dissociation of phosphate and deletion of soluble phosphate complexes from total phosphate). The ionic activities of  $\text{Ca}^{2+}$  and  $\text{HPO}_4^{2-}$  were then calculated as an inverse function of ionic strength (derived from urinary components).

Since then, Equil 2 has been widely disseminated, becoming the tool of choice for estimating urinary saturation in both research and commercial clinical laboratories. Thus, this method has been used to assess stone-forming propensity of certain metabolic conditions (distal renal tubular acidosis<sup>6</sup> and bowel diseases<sup>7</sup>), dietary factors<sup>8</sup> (salt, calcium, animal proteins and fruit juices), and drugs (thiazide and alkali therapy).<sup>6,8,9</sup> Much of our current knowledge on the physicochemistry of stone formation and drug action has depended on the saturation data derived from Equil 2.

In 2006, Rodgers et al.<sup>10</sup> introduced a new computer program JESS (Joint Expert Speciation System) to calculate urinary saturation of stone-forming salts. In contrast to EQUIL 2, the JESS software recognizes additional soluble complexes –  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  (dicalcium-dihydrogen phosphate) and  $(\text{CaCitPO}_4)^{4-}$  (calcium phosphocitrate). About 25% of urinary calcium at baseline and greater than 50% of calcium after alkali treatment was reported to be complexed as  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  and  $(\text{CaCitPO}_4)^{4-}$ . The formation of these complexes is dependent on pH and citrate. Thus, over a wide range of pH produced by alkali treatment, urinary saturation of brushite calculated by JESS was reported to be much lower and less dependent on pH, compared to that obtained by EQUIL 2.<sup>10</sup> A current challenge in urolithiasis research is to ascertain the relative merits of the two computer programs.

In 1969, we introduced a semi-empirical approach for measuring brushite saturation, based on the ratio of activity products or concentration products before and after incubation with synthetic brushite.<sup>3</sup> Although this method revealed a more direct estimate of saturation from the extent of actual growth (supersaturation) or dissolution (undersaturation) of added brushite, we abandoned it in the mid-80's due to its complexity (requiring 2 days of incubation).

Recently, we simplified the method for the concentration product ratio (CPR) by reducing the time of incubation with synthetic brushite to 5 hours.<sup>11</sup> To test the new approach, we assessed the effect of varying urinary pH, calcium and citrate on brushite saturation. In this report, we took the data from the aforementioned study, and calculated brushite saturation values by Equil 2 as relative saturation ratio (RSR) and by JESS as supersaturation index (SI). We then compared RSR and SI with the corresponding CPRs.

## METHODS

### Data

From our recent publication in this Journal,<sup>11</sup> we took values for CPR of brushite obtained by a new, simplified method over a wide range of pH (by titration with dilute HCl or KOH to 5.67, 6.0, 6.35 and 6.7), calcium (by addition of calcium chloride to 2.5, 3.75, 5.0, and 6.25 mmol/L) and added citrate (0, 1, 2, and 3 mmol potassium citrate/L). In this manuscript, the corresponding RSR and SI of brushite were calculated by Equil 2 and JESS, respectively.<sup>4, 10</sup> In 6 urine samples tested at four ranges of pH, calcium or citrate ( $n = 72$ ), CPR was compared with SI and RSR. Moreover, the JESS software program was modified by deleting  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  complex alone, or both  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  and  $(\text{CaCitPO}_4)^{4-}$  complexes. SI obtained with and without deletion of above complexes was then compared with RSR. For technical reasons, we could not add the above complexes to the Equil 2 program.

**Statistical analysis**—The significant difference in saturation ratios at each level of urinary variable (pH, calcium or citrate) was assessed by repeated measures analysis of variance using a mixed linear model approach. In the whole group of 72 samples, or the subgroup of 24 samples (for each urinary variable), each 24-hour urine collection had been adjusted to yield 4 ranges of urinary pH, calcium or citrate. Thus, a mixed linear model was used to assess differences in saturation ratios in the whole group or subgroups. The subject was included as a random effect in these models to account for the lack of independence between observations obtained from the same individuals. Adjustments for multiple comparisons were made using the adjust=simulate option of SAS Proc Mixed LSMEANS.<sup>12</sup>

The slopes of the relationship between brushite saturation and varying pH, calcium and citrate were determined; they were compared between the saturation variables by using a random coefficient model. For data derived from changes in urinary pH, calcium and citrate, taken separately or combined, the dependence of RSR and SI on CPR was determined by linear regression analysis. Only the relationship for the combined data are presented here, since the same pattern was observed for separate urinary variables. Statistical analysis was performed using SAS 9.1.3 (SAS Institute, Cary, NC).

## RESULTS

### Comparison of CPR with RSR and SI

Individual values for CPR are compared with corresponding RSR and SI in Fig. 1. For each variable (pH, calcium or citrate), data from 24 samples (6 urine samples at four ranges) are shown. For all three variables, CPR was lower than RSR, but similar to SI, in most of the samples. When the group means were compared for each variable, CPR was nearly identical and non-significantly different from SI ( $p > 0.9$ ). Both CPR and SI were significantly lower than RSR ( $p < 0.003$ ).

### Effect of varying pH, calcium and added citrate on CPR, RSR and SI

In Fig. 2, the relationship between brushite saturation and urinary variable is displayed. For all three methods of assessing saturation, a direct association was depicted between brushite saturation and urinary pH or calcium, and an inverse association was shown between brushite saturation and urinary citrate. This pattern of change in RSR was distinct from that of CPR or SI, with RSR being significantly higher than CPR or SI ( $p = 0.05$ – $0.001$ ) at most levels of all three urinary variables ( $p < 0.0001$  from linear model). However, the pattern of change in SI merged with that of CPR, especially with added citrate (Fig. 2, right). Compared to CPR, SI tended to be slightly lower at high ranges of urinary pH and calcium, and slightly higher at lower ranges (Fig. 2, left and middle). The slope of the lines for SI was less steep than those for CPR ( $p < 0.003$ ).

### Dependence of RSR and SI on CPR

For the combined data ( $n = 72$ ), RSR was highly correlated with CPR, with the intercept near zero and the slope of 1.79 (Fig. 3). Thus, at a broad range of brushite saturation, RSR was about 80 percent higher than CPR.

SI was also highly correlated with CPR. However, the intercept was near 1 and the slope was much lower at 0.53 (Fig. 3). Thus, SI closely approximated RSR at modest degrees of brushite saturation, but was higher than CPR at low saturation ranges and lower than CPR at high saturation ranges.

### Comparison of RSR and SI before and after deletion of complexes

Computations were made from combined data compiled from all urine samples at four ranges of pH, calcium and citrate ( $n = 72$ ). SI was not significantly different from CPR ( $p = 1.0$ ), but RSR was significantly higher than CPR ( $p < 0.0001$ ) (Fig. 4). When  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  complex was deleted from JESS program, the corrected SI increased significantly from the original value ( $p < 0.0001$ ), to attain a value slightly lower numerically but not significantly different than RSR ( $p = 0.9$ ). When both  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  and  $(\text{CaCitPO}_4)^{4-}$  complexes were deleted, the corrected SI was numerically higher but not significantly different than RSR ( $p = 0.8$ ).

## DISCUSSION

From data derived from a recent report in this Journal,<sup>11</sup> urinary saturation of brushite was calculated by Equil 2 and JESS computer programs<sup>4,10</sup> as RSR and SI, respectively, during wide changes in urinary pH, calcium and citrate. RSR and SI were then compared with the corresponding semi-empirically derived CPR. Overall, SI provided a good approximation of CPR, but RSR did not.

The JESS program differs from Equil 2 by inclusion of additional soluble complexes.<sup>4,10</sup> Principal complexes relevant to calculation of brushite saturation are:  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  and  $(\text{CaCitPO}_4)^{4-}$ . By diverting some of total calcium and phosphate to soluble ligands, these complexes reduce the amount of ionized calcium and phosphate and hence the saturation of brushite. Thus, Rodgers et al.<sup>10</sup> reported a much lower value for brushite saturation when calculated by JESS rather than by Equil 2.

In contrast to the above computer-derived methods, CPR is obtained semi-empirically,<sup>11</sup> where the ratio of concentration products are obtained by a simple computation, before and after experimentally incubating urine with synthetic brushite. CPR has a “physicochemical reality,” since supersaturation is obtained from the actual growth of added brushite, and undersaturation by dissolution of brushite. Thus, we believe that CPR of brushite can be used to validate the results of computer-derived approaches.

This study disclosed that RSR yields a poor approximation of CPR, being higher by 70–80 percent and showing wider variation with changes in urinary pH, calcium or citrate. In contrast, SI gave a good approximation of CPR. For all 72 samples and for each set of separate urinary variables ( $n = 24$ ), the mean SI values were nearly identical and not significantly different from corresponding CPR values. On closer inspection, SI was slightly lower at higher levels of pH and calcium, and slightly higher at lower levels, compared with CPR. Though small, the above differences were significant ( $p < 0.001$  for the slopes). These effects canceled each other to yield the group mean values of SI that were nearly identical to CPR.

The above findings (by random coefficient model) were affirmed by a different statistical analysis (regression analysis). While both RSR and SI were highly correlated with CPR, the slope for RSR was higher at 1.79 with the intercept originating near zero. The results indicated that RSR overestimated CPR by about 80 percent at a wide range of brushite saturation. In contrast, the slope for SI was much lower, and the intercept was near 1. SI was therefore higher than CPR at low ranges of brushite saturation, and lower than CPR at high ranges of saturation. Brushite saturation is decreased at low urinary pH and calcium, and increased at high urinary pH and calcium. Thus, at low ranges of brushite saturation (corresponding to low urinary pH and calcium), SI probably overestimates brushite saturation. Conversely, at high ranges of brushite saturation (high urinary pH and calcium), SI probably underestimates it.

To understand the basis for the above results, SI was recalculated after omitting key soluble complexes contained in JESS but not in Equil 2 program. For all urine samples, the deletion

of  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  complex from the JESS program yielded SI of brushite that approached the corresponding RSR value. The deletion of both  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  and  $(\text{CaCitPO}_4)^{4-}$  complexes produced SI value slightly higher numerically than the RSR value. Thus, the overestimation of brushite saturation by RSR is probably due mainly to the omission of  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  complex in Equil 2. Other complexes or varying stability constants employed by the two programs may account for the different slopes of the relationship between brushite saturation and urinary variables. To be sure, actual identification of these complexes in urine would be required. Future studies must also focus on why SI deviates from CPR at extremes of urinary pH, calcium or brushite saturation.

The method for CPR is too labor intensive for routine clinical application, even though it has been simplified.<sup>11</sup> We suggest that in a routine setting, RSR may be substituted by SI to estimate brushite saturation in urine. Alternatively, RSR might be corrected by dividing it by 1.8. However, the use of CPR is recommended for research purposes, until SI method is further refined or validated.

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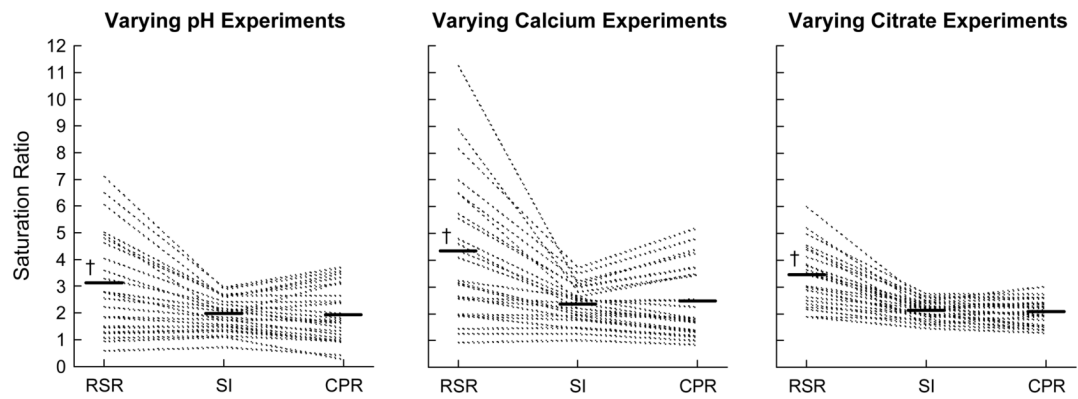
## ABBREVIATIONS USED

CPR	Concentration product ratio, a measure of brushite saturation derived by a semi-empirical method
RSR	Relative saturation ratio, a measure of brushite saturation calculated by Equil 2 computer program
SI	Supersaturation index, a measure of brushite saturation calculated by JESS computer program
JESS	Joint Expert Speciation System, a new computer program for estimating urinary saturation of stone salts
$\text{Ca}^{2+}$	Ionized calcium
$\text{HPO}_4^{2-}$	Ionized divalent phosphate

## References

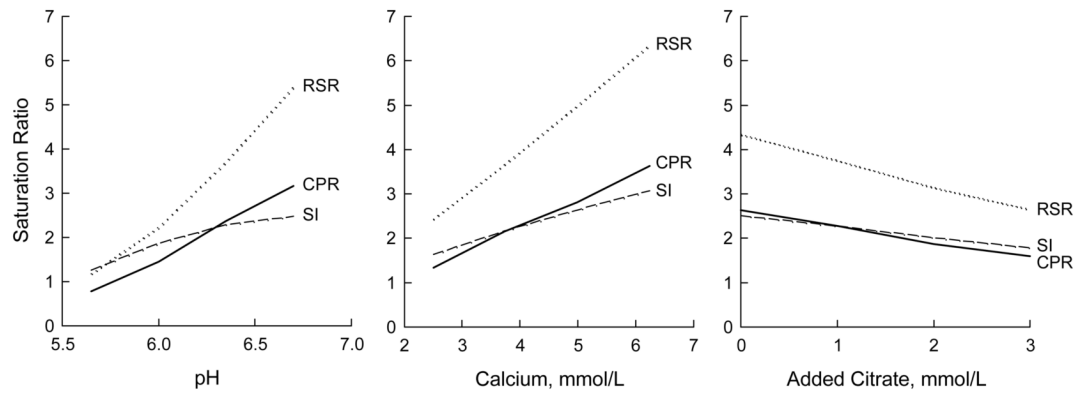
1. Parks JH, Coward M, Coe FL. Correspondence between stone composition and urine supersaturation in nephrolithiasis. *Kidney Int* 1997;51:894. [PubMed: 9067927]
2. Robertson WG, Peacock M, Nordin BEC. Activity products in stone-forming and non-stone-forming urine. *Clin Sci* 1968;34:579.
3. Pak CYC. Physicochemical basis for the formation of renal stones of calcium phosphate origin: calculation of the degree of saturation of urine with respect to brushite. *J Clin Invest* 1969;48:1914. [PubMed: 5822595]
4. Werness PG, Brown CM, Smith LH, Finlayson B. EQUIL 2: a basic computer program for the calculation of urinary saturation. *J Urol* 1985;134:1242. [PubMed: 3840540]
5. Francis MD, Russell RG, Fleisch H. Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcifications in vivo. *Science* 1969;165:899. [PubMed: 5816326]
6. Preminger GM, Sakhaee K, Skurla C, Pak CYC. Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. *J Urol* 1985;134:20. [PubMed: 4009822]

7. Asplin JR, Coe FL. Hyperoxaluria in kidney stone formers treated with modern bariatric surgery. *J Urol* 2007;177:565. [PubMed: 17222634]
8. Wabner CL, Pak CYC. Effect of orange juice consumption on urinary stone risk factors. *J Urol* 1993;149:1405. [PubMed: 8501777]
9. Parks JH, Worcester EM, Coe FL, Evan AP, Lingeman JE. Clinical implications of abundant calcium phosphate in routinely analyzed kidney stones. *Kid Int* 2004;66:777.
10. Rodgers A, Allie-Hamdulay S, Jackson G. Therapeutic action of citrate in urolithiasis explained by chemical speciation: increase in pH is the dominant factor. *Nephrol Dial Transplant* 2006;21:361. [PubMed: 16249202]
11. Pak CYC, Rodgers K, Poindexter JR, Sakhaee K. New methods for assessing crystal growth and saturation of brushite in whole urine: effect of pH, calcium and citrate. *J Urol*. Accepted, October 2008.
12. Littell, RC.; Milliken, GA.; Stroup, WW.; Wolfinger, RD.; Schabenberger, O. *SAS for Mixed Models*. 2. SAS Institute, Inc; Cary, NC: 2006.



**Figure 1.**

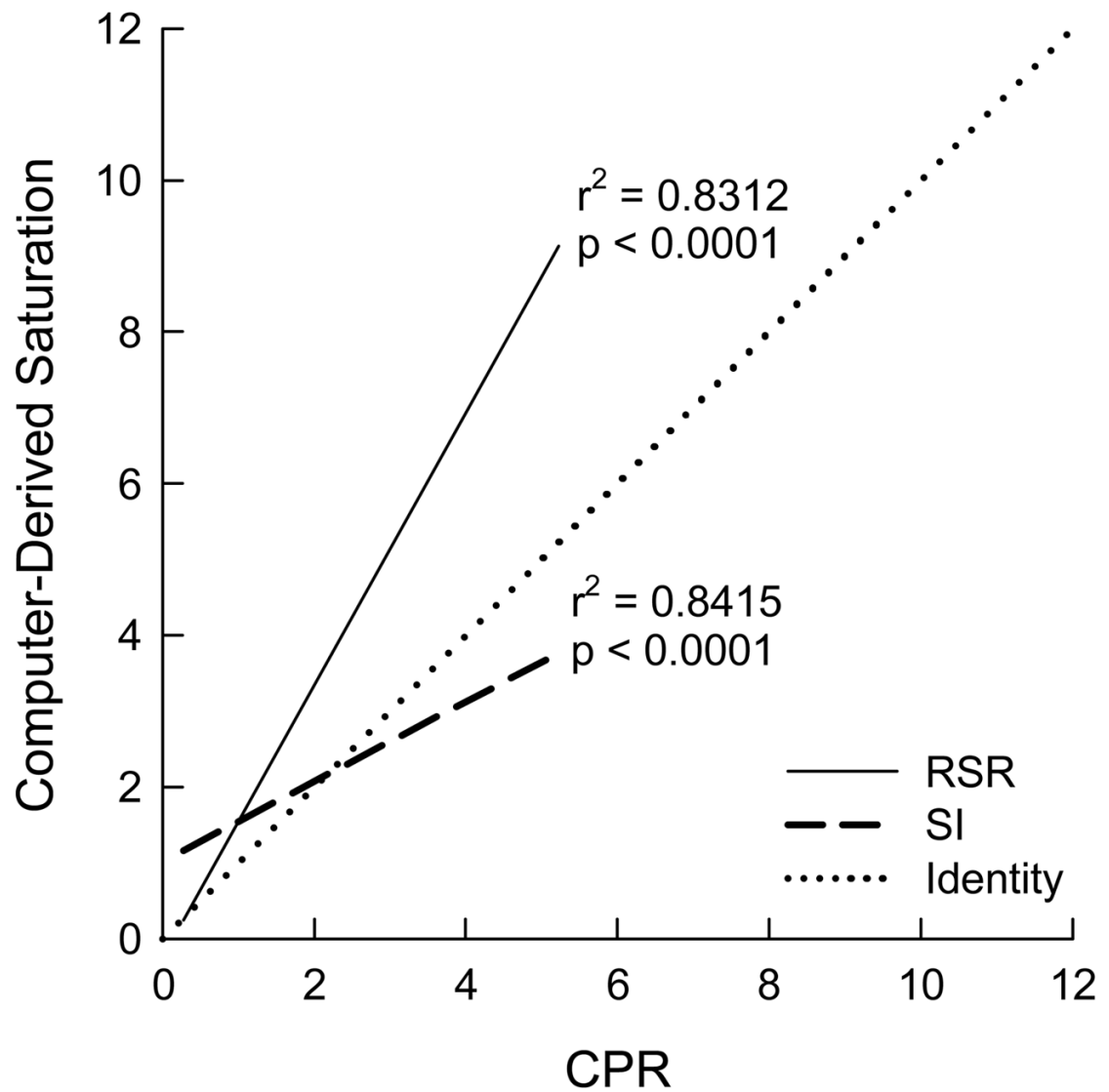
Individual depiction of RSR, SI and CPR during variations in pH, calcium and citrate. Values from same subjects are connected by dotted lines. Statistical significance between RSR and CPR or between RSR and SI is indicated by † for  $p < 0.004$ , with adjustment for multiple testing and accounting for the correlation of repeated measurements within subjects. Horizontal bars indicate group means. Saturation ratio = brushite saturation.



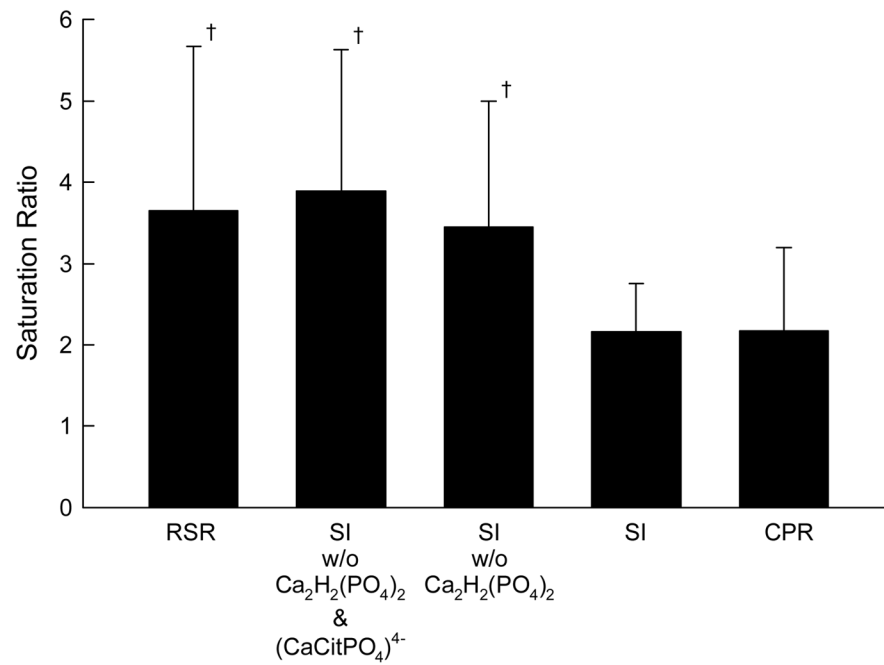
**Figure 2.**

The relationship between brushite saturation (RSR, SI and CPR) and urinary variables (pH, calcium and citrate). RSR, SI and CPR were measured in each urine sample. For each urinary variable, 6 urine samples were adjusted to yield four levels of the said variable. The interaction between the type of saturation measurement and urinary concentration was significant for all three urinary variables ( $p < 0.01$ ). The lines connect the group means.





**Figure 3.** Dependence of RSR on CPR and of SI on CPR. Dotted line indicated identity. Regression lines for RSR vs. CPR (solid) and SI vs. CPR (dashed) are displayed.



**Figure 4.**

Comparison of group mean RSR versus group mean SI, before and after deletion of  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  alone and both  $\text{Ca}_2\text{H}_2(\text{PO}_4)_2$  and  $[\text{CaCitPO}_4]^{4-}$ . Data for all three urinary variables at four levels ( $n = 72$ ) were utilized. Statistical significance between CPR and RSR, between SI and RSR, between CPR and SI without complexes, or between SI alone and SI without complexes, is indicated by † for  $p < 0.0001$ . Vertical bars above shaded blocks indicate mean  $\pm$  SD. w/o = without.