

## Influence of grapefruit-, orange- and apple-juice consumption on urinary variables and risk of crystallization

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Alkalinizing beverages are highly effective in preventing the recurrence of calcium oxalate (Ox), uric acid and cystine lithiasis. The aim of the present study was to evaluate the influence of grapefruit-juice and apple-juice consumption on the excretion of urinary variables and the risk of crystallization in comparison with orange juice. All investigations were carried out on nine healthy female subjects without any history of stone formation and aged 26–35 years. Each juice was tested in a 5 d study. During the study, the subjects received a standardized diet. Fluid intake of 2.75 litres was composed of 2.25 litres neutral mineral water, 0.4 litre coffee and 0.1 litre milk. On the fourth and fifth day 0.5 litre mineral water was partly substituted by 0.5 or 1.0 litre juice under investigation respectively. The influence on urinary variables was evaluated in 24 h urine samples. In addition, the BONN risk index of CaOx, relative supersaturation (RS)<sub>CaOx</sub> crystallization was determined. Due to an increased pH value and an increased citric acid excretion after consumption of each juice, the RS<sub>CaOx</sub> decreased statistically significantly ( $P < 0.05$ ) for grapefruit juice, but not significantly for orange and apple juice. The BONN risk index yielded a distinct decrease in the crystallization risk. We showed that both grapefruit juice and apple juice reduce the risk of CaOx stone formation at a magnitude comparable with the effects obtained from orange juice.

**Grapefruit juice: Apple juice: Orange juice: Relative supersaturation: Alkalinizing beverages: BONN risk index**

A high fluid intake is the first general advice given to patients in the prevention of stone recurrence, irrespective of stone composition. An increase of fluid intake is associated with a reduced risk for kidney stone formation, reported by several authors (Curhan *et al.* 1996, 1998; Hesse *et al.* 1993; Hesse & Siener 1997). Urine dilution causes a lowering of the concentration of constituent ions and thus a decrease of the super-saturation of the stone-forming salts. Depending on the stone composition, several fluids have been found to be suitable, e.g. mineral water, orange juice, apple juice, fruit and herbal teas (Vahlensieck, 1986; Hesse *et al.* 1993; Wabner & Pak 1993). Alkalinizing beverages such as orange juice are highly effective in the metaphylaxis of calcium oxalate (Ox), uric acid and cystine lithiasis.

Curhan *et al.* (1996, 1998) investigated the influence of beverages on the risk of kidney stone formation in two prospective studies. Their investigations resulted in the postulation of grapefruit juice and apple juice being risk-increasing beverages in respect to CaOx formation. Their results were based on an evaluation of questionnaires without any measurements of urinary composition. These results, however, cannot be understood without any further information about the changes in urinary composition.

Therefore, the results lead to the question: why does the ingestion of grapefruit juice causes an effect that is different from that of other juices with a high content of citric acid? There must be a constituent of grapefruit juice be responsible for this effect. To find an answer to this question some authors investigated the influence of grapefruit juice on urinary composition (Goldfarb & Asplin, 2000, 2001; Trinchieri *et al.* 2002). Unfortunately these investigations did not lead to meaningful results. The present study was undertaken to overcome these shortcomings by evaluating the influence of grapefruit juice and apple juice on urinary composition and therefore on risk of crystallization. The effects of both juices were compared with those obtained from the ingestion of orange juice. All juices were tested on the same persons and under the same dietary conditions.

### Materials and methods

All investigations were carried out using nine female volunteers with no history of urolithiasis or other renal disorder. The mean age was 29 (range 26–35) years. All subjects received a standardized diet formulated according to the dietary recommendations of the German Society of

Nutrition (Deutsche Gesellschaft für Ernährung, 1996). The diet consisted of normal food items to ensure consistency of the investigation results. The same type and amount of food was consumed for 5 d (103.0 mg Ox/d). Sports were not permitted during the experimental phase. Volunteers were supplied with breakfast, and lunch, both were ingested in our hospital. The other foods and the collecting bottles were given with full instructions. Substitutions were not allowed.

Fluid intake of 2.75 litres was composed of 2.25 litres neutral mineral water, 0.4 litre coffee and 0.1 litre milk. On the loading days (fourth day 0.5 litre, fifth day 1.0 litre) mineral water was partly substituted for by the juice tested. For details of the composition of grapefruit, orange and apple juice see Table 1. The subjects received juices with 100% fruit content and without any additives (grapefruit juice from 'master product', apple juice from 'master product' (Hamburger Warencenter, Hamburg, Germany) and orange juice from Krings, Herrath).

The 24 h urine samples were divided in two portions after each voiding. One fraction was stored at 4°C without any preservation. This fraction was used for the determination of the urinary pH value and the BONN risk index (BRI; for details, see later). The other fraction was also stored at 4°C and mixed with 10 ml HCl (250 ml/l) to preserve the urine. Urine samples were tested for the presence of blood and infection. Nitrite-positive and haematuric samples were discarded. In addition, volume, specific gravity (urinometer) and pH (potentiometer) were recorded. The analytical methods used had the following relative CV (%): Na, K, and Ca (flame photometry) 1.3; Mg (xylyl-dyl-blue reaction) 0.3;  $\text{NH}_4^+$  (ion selective electrode) 1.5; chloride (coulomb metric titration) 2.0; inorganic phosphate (phosphate molybdate reaction) <5; inorganic sulfate (nephelometry) <5; creatinine (Jaffé reaction) 2.0; uric acid (uricase method) <5; citric acid (citrate lyase method) 1.6; oxalic acid (HPLC enzyme reactor method) 0.5 (Hesse *et al.* 1997; Hönow *et al.* 1997; Hesse & Bach, 1982).

The relative supersaturation (RS) for CaOx was calculated using the EQUIL 2 program (Finlayson, 1977; Werness *et al.* 1985). The Wilcoxon matched-pairs signed-rank test, was used testing two matched samples (control and loading day) as a non-parametric test of significance. The significance level was set at 5%.

The BRI were determined according to Laube *et al.* (2000). The BRI is a newly established method for CaOx crystallization risk determination from unprepared native

urine samples (Laube *et al.* 2001, 2002). In a standardized experimental procedure, the BRI is derived from the initial urinary concentration of free ionized Ca ( $\text{Ca}^{2+}$ ): amount of ammonium Ox which has to be titrated to that urine ( $\text{Ox}^{2-}$ ) in order to induce a precipitation of CaOx salts, i.e.  $\text{BRI} = \text{Ca}^{2+}/\text{Ox}^{2-}$ .

In the present study,  $\text{Ca}^{2+}$  was measured by ion-selective electrodes. The moment of crystallization was detected by a laser-probe device (MTS-Messtechnik Schwartz, Dusseldorf, Germany).

The advantage of the BRI in the evaluation of the crystallization risk is the fact that all urinary components contribute their individual effects, which may promote and/or inhibit both salt precipitation and/or particle aggregation in the experiment.

## Results

The main results are reported in Table 2. The volumes of the 24 h urine samples did not differ from day 3 to day 4 and 5. This represents the standardization of the volunteers. The dosage of 0.5 litre (and 1.0 litre) orange juice leads to an alkalization of the 24 h urine, whereas only the 1.0 litre dosage of apple juice caused a significant increase of the pH value. Only a slight increase of pH value was observed after ingestion of 0.5 or 1.0 litre grapefruit juice. The differences were not statistically significant. The citric acid excretion increased statistically significantly ( $P < 0.05$ ) in all juices tested at both dosages. The increases of pH values and of the citric acid excretions are shown in Fig. 1. The Ox excretions did not change during experimental phase. The excretion of Ca was significantly lower ( $P < 0.05$ ) after the ingestion of 0.5 litre orange juice and of 0.5 litre grapefruit juice. However, the differences could not be confirmed after the ingestion was elevated to 1.0 litre. The urinary Mg excretion increased only with 1.0 litre grapefruit juice. Uric acid excretion was not influenced after ingestion of any of the juices. On the basis of changed urinary composition there is a decrease in  $\text{RS}_{\text{CaOx}}$  after ingestion of all juices. This finding was statistically significant ( $P < 0.05$ ) only for grapefruit juice (0.5 litre).

The BRI decreased after the intake of 1.0 litre orange juice by 0.5 units, after intake of 1.0 litre grapefruit juice by 0.4 units and after intake of 1.0 litre apple juice by 0.5 units. These decreases are significant ( $P < 0.01$  for grapefruit juice and  $P < 0.05$  for orange juice and apple juice). Results are reported in Fig. 2.

## Discussion

Grapefruit juice and orange juice contain about 10 mg citric acid/g. The high content of citric acid causes an increased citric acid excretion and is responsible for the alkalizing effect. The elevated citric acid excretion due to the ingestion of apple juice, however, was unexpected and cannot be explained at the moment. The alkalizing effect and the increased citric acid excretion lead to a decreased  $\text{RS}_{\text{CaOx}}$  and may have mainly caused the decreasing of the BRI values.

These results are in accordance with the results reported for orange juice by Wabner & Pak (1993). However, these

**Table 1.** Composition of the juices tested (mg/l)

	Grapefruit juice	Apple juice	Orange juice
pH value	31.1	34.3	37.2
Na	115	< 110	< 110
K	1127	1084	1638
Mg	79	50.4	81
Ca	80	48	124
Citric acid	14120	44	8972
Ascorbic acid	400	14	420
Oxalic acid	2	10	2

**Table 2.** Urinary variables in 24 h urine samples during, before and after fruit juice ingestion†  
(Mean values with their standard errors for nine subjects)

	Orange juice						Grapefruit juice						Apple juice					
	Control		0.5 litre		1.0 litre		Control		0.5 litre		1.0 litre		Control		0.5 litre		1.0 litre	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
pH value	6.08	0.09	6.42**	0.08	6.66**	0.08	6.29	0.10	6.32	0.12	6.42	0.10	6.20	0.08	6.20	0.09	6.38*	0.09
Volume (litres)	2.805	0.126	2.638	0.111	2.606	0.113	2.767	0.159	2.633	0.110	2.716	0.055	2.706	0.179	2.658	0.127	2.806	0.167
Ca (mmol/d)	4.04	0.48	3.58*	0.39	3.70	0.40	3.73	0.43	3.15*	0.33	3.62	0.35	4.01	0.52	4.21	0.64	4.02	0.53
Mg (mmol/d)	4.52	0.36	4.25	0.36	4.81	0.76	4.28	0.34	4.27	0.39	5.11*	0.48	4.06	0.43	4.37	0.48	4.12	0.36
K (mmol/d)	52.2	5.1	70.3*	4.8	70.2	5.8	49.6	3.2	63.2*	4.9	66.5*	5.1	53.9	5.1	60.7	6.0	67.2*	5.4
Oxalate (mmol/d)	0.180	0.014	0.197	0.020	0.189	0.019	0.172	0.009	0.177	0.008	0.180	0.008	0.203	0.013	0.200	0.019	0.206	0.015
Citrate (mmol/d)	3.215	0.418	3.893*	0.489	4.364*	0.542	2.791	0.443	3.364**	0.466	4.119**	0.574	3.203	0.729	3.881*	0.795	4.552**	0.767
Uric acid (mmol/d)	3.17	0.11	3.37	0.17	3.11	0.20	3.16	0.16	3.26	0.10	3.26	0.12	2.95	0.14	2.98	0.16	1.12	0.07
RS <sub>CaOx</sub>	1.48	0.27	1.31	0.18	1.24	0.19	1.29	0.17	1.14*	0.14	1.18	0.15	1.87	0.48	1.67	0.35	1.47	0.35
RS <sub>Uric acid</sub>	0.80	0.13	0.446*	0.11	0.25**	0.04	0.56	0.11	0.59	0.16	0.43	0.12	0.61	0.11	0.61	0.11	0.44	0.11
RS <sub>Brushite</sub>	0.32	0.06	0.48	0.09	0.57*	0.11	0.40	0.06	0.44	0.10	0.44	0.07	0.40	0.08	0.41	0.11	0.35	0.06
RS <sub>Struvite</sub>	0.016	0.005	0.047*	0.015	0.093*	0.030	0.034	0.009	0.051	0.016	0.048	0.012	0.019	0.006	0.020	0.006	0.023	0.006

RS, relative supersaturation; Ox, oxalate.

Mean values were significantly different from those of the control: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

† For details of subjects, juices and procedures, see Table 1 and pp. 295–296.

results are different from those reported by Curhan *et al.* (1996, 1998). They found an increasing risk of stone formation for a daily intake of 240 ml grapefruit juice or apple juice. Apple juice intake was correlated directly with stone formation only for men, and not for women. It should be notified that they did not analyse any urinary variables or lithogenic constituents.

Goldfarb & Coe (1999) postulated that grapefruit juice, if it stimulates stone formation, must have a significant effect on urinary composition different from that other citrus juices, or that the measurement of urinary composition in short-term studies fail to predict long-term outcomes accurately.

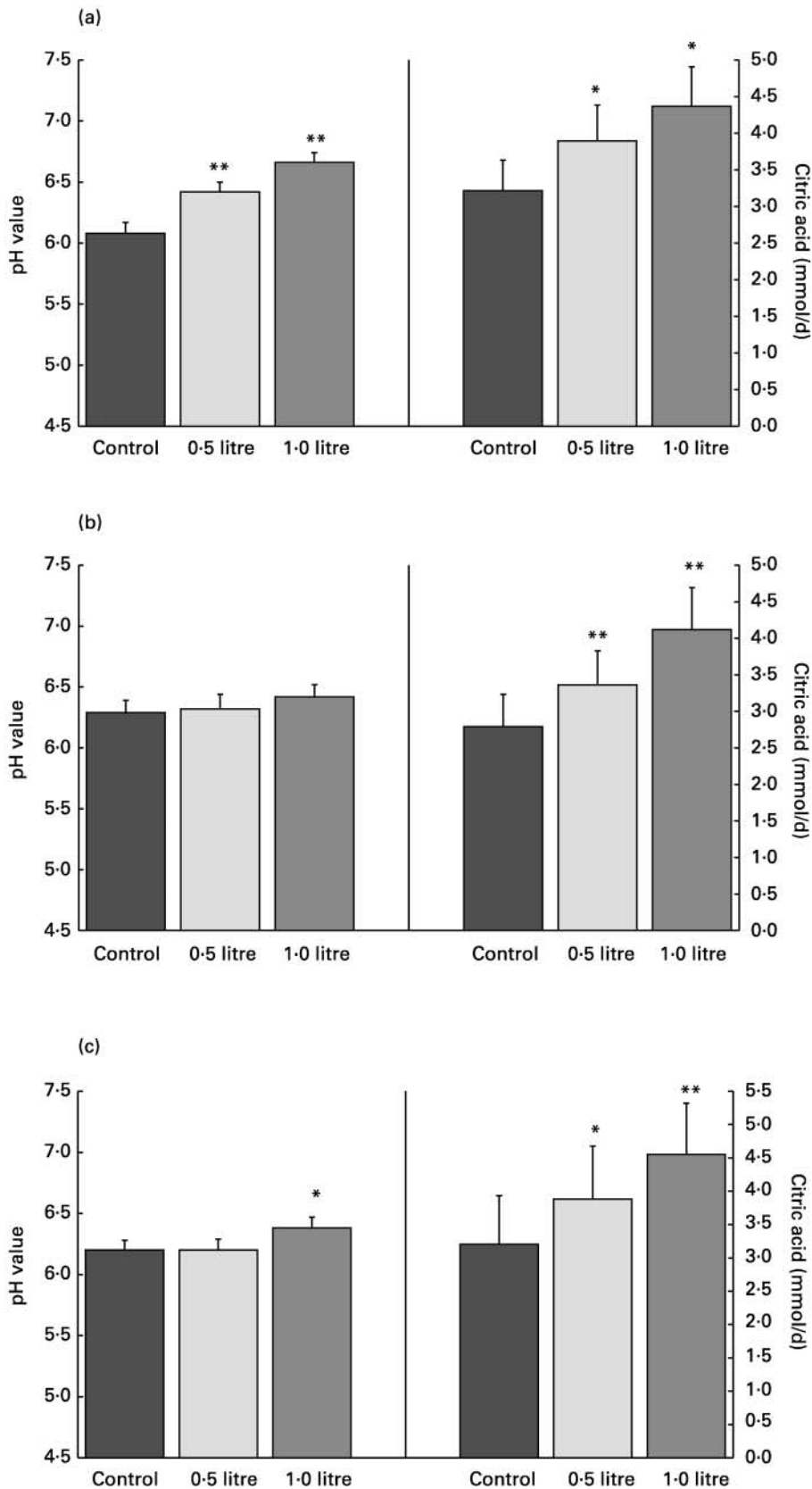
Goldfarb & Asplin (2001) determined in urine the upper limits of metastability, based on a modified crystallization experiment described by Nicar *et al.* (1983). For that purpose, each urine sample was centrifuged, pH adjusted and then preserved with sodium acetate. After this preparation, a crystallization experiment on these strongly altered urine samples was performed in order to obtain metastability.

It is clear that the metastability results cannot reflect the urinary situation in total. The BRI, however, takes into account the effects of all urinary constituents in their native ratio and native chemical environment: only unprepared urine samples are investigated. In urine compositions in which those substances not considered by metastability determination contribute a 'non-average' effect to the total crystal formation risk, the result of metastability cannot sufficiently reflect that situation as well as the BRI does. Only full consideration of all urinary substances, in particular of the macromolecular constituents that may have strong effects on the stone formation processes, allows reflection of all grapefruit-related influences on urinary composition, including those influences that are still unknown.

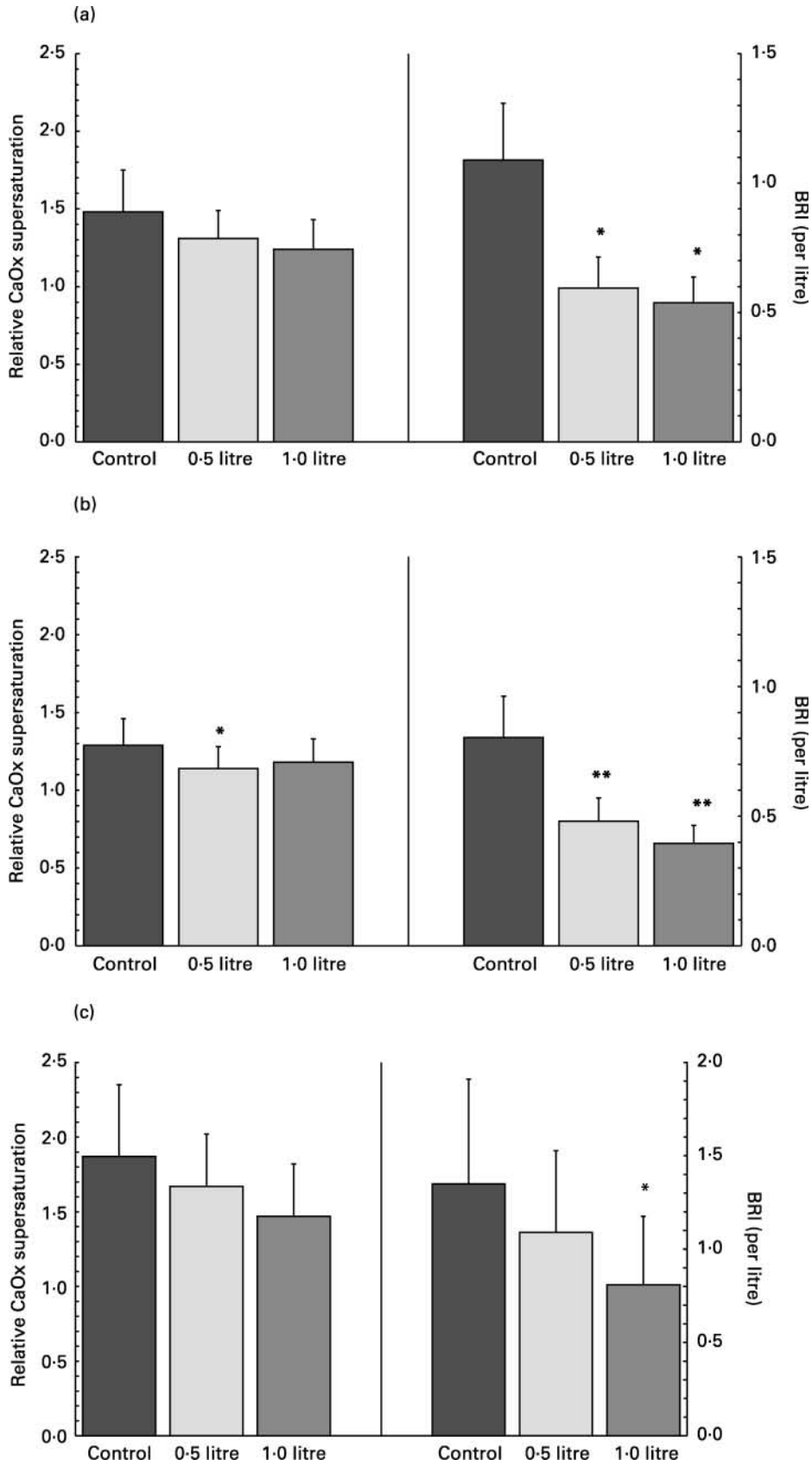
Changes in RS<sub>CaOx</sub> reliably reflect changes in the composition of the major urinary constituents. Goldfarb's investigations (Goldfarb & Coe, 1999; Goldfarb & Asplin, 2000, 2001) and our present results revealed only small effects of grapefruit juice on urinary RS<sub>CaOx</sub>. This is mainly caused by small changes in urinary pH and citric acid concentrations after grapefruit ingestion.

Grapefruit juice is well known to increase the absorption of many drugs (Weber *et al.* 1996; Ameer & Weintraub, 1997). Many studies have shown that the substance naringin (one of the main flavonoids), through its inhibitory effects on the cytochrome P450 enzymes, is responsible for the grapefruit–drug interactions (Fuhr & Kummert, 1995). It has to be investigated in what way substances that appear through a delayed metabolism in the urine have an influence on the crystallization. The Ox excretion will not be influenced through the inhibition of the cytochrome P450 enzyme. This was confirmed also by the results of the present study.

The influence of grapefruit contents themselves on the crystallization has not been examined until now. However, naringin is excreted unchanged only in small quantities. Naringin appeared in urine (5–57%) after a median lag time of 2 h as naringenin glucuronides (Fuhr & Kummert, 1995).



**Fig. 1.** Effect of orange juice (a), grapefruit juice (b) or apple juice (c) on urine pH value and citrate excretion. For details of fruit juices, subjects and procedures, see Table 1 and pp. 295–296. Values are means with their standard errors shown by vertical bars ( $n$  9). Mean values were significantly different from those of the control: \* $P < 0.05$ , \*\* $P < 0.01$ .



**Fig. 2.** Effect of orange juice (a), grapefruit juice (b) or apple juice (c) on urine relative supersaturation with calcium oxalate (Ox) and BONN risk index (Laube *et al.* 2000, 2001). For details of fruit juices, subjects and procedures, see Table 1 and pp. 295–296. Values are means with their standard errors shown by vertical bars ( $n$  9). Mean values were significantly different from those of the control: \* $P < 0.05$ , \*\* $P < 0.01$ .

## Conclusions

Analysis of urinary composition and the BRI yielded a distinct decrease in the risk of CaOx crystallization. Therefore, we suggest that there is no component of grapefruit juice responsible for an increasing risk of stone formation. It can be supposed that women who form stones would react as did those studied here. Thus, we assume that ingestion of grapefruit juice, orange juice and apple juice are effective measures in metaphylaxis of CaOx-stone disease. However, further research on the stone-former is needed to confirm these findings; subsequent studies are necessary to explain which substances of grapefruit juice lead to results described by Curhan *et al.* (1996, 1998).

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