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Does Chronic Cola Consumption Increase Urinary Stone Risk? Evidence from the *Drosophila* Model of Urolithiasis

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Abstract There are rising public health concerns about the links between consumption of sugar-sweetened beverages and weight gain, obesity, and other metabolic problems. Sodium citrate (Na-citrate) is used as an additive in colas and various commercial drinks worldwide. Although potassium citrate (K-citrate) has been prescribed and well accepted by urologists for treating urinary stone disease (urolithiasis), the clinical role of Na-citrate has not been well established. We investigated the effects of Na-citrate and cola on the treatment of urolithiasis with an emerging translational model – *Drosophila melanogaster*. *Drosophila* medium containing 0.5% ethylene glycol (EG) was used as a lithogenic agent for calcium oxalate (CaOx) crystal formation in *Drosophila* Malpighian tubules. Cola (25%) and Na-citrate (2% and 4%) were added to the fly medium for urolithiasis treatment. Medium containing K-citrate (2% and 4%) was used as a positive control. After 3 weeks of treatment, the Malpighian tubules were dissected, removed, and processed for polarized light microscopy examination; fly lifespan was also monitored in different groups. Cola failed to reduce CaOx crystal formation, whereas Na-citrate and the positive control K-citrate significantly reduced EG-induced CaOx crystal formation in *Drosophila*. Administration of either Na-citrate or cola did not inhibit *Drosophila* lifespan. Consumption of cola exerts no detectable change in the lithogenic agent associated with CaOx stone formation in the *Drosophila* model. By contrast, Na-citrate had an inhibitory effect on EG-induced CaOx crystal formation, albeit a lower inhibitory rate upon comparison with K-citrate.

Keywords: *cola, drosophila, sodium citrate, calcium oxalate, urolithiasis*

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1. Introduction

Soft drinks and various beverages make up an increasing percentage of energy intake, and there are rising public health concerns about the links between consumption of sugar-sweetened beverages and weight gain, obesity, and other metabolic problems [1,2,3,4,5]. Cola is an internationally popular carbonated soft drink whose contents include caffeine, phosphoric acid, sugar, and citric acid [6,7]. One major focus of concern is the potential for adverse side effects caused by consuming cola in large quantities over time [8,9,10]. Serial reports indicate that side effects may be attributed to caffeine, phosphoric acid, and excessive sugar intake, the latter of which may cause obesity and contribute to subsequent chronic conditions including urinary stones [11].

It is postulated that excessive long-term intake of cola may cause hypokalemia myopathy and alkalosis due to high levels of caffeine [12]. The current understanding of the effects of cola on urolithiasis is unknown, although an indirect link may exist, as urinary alkalosis may be related to stone formation [13,14]. By contrast, it has been

previously reported that cola contains low levels of oxalate, which may not increase the likelihood of stone formation [15,16]. In one such study, the Rodgers group analyzed the urinary biochemistry of volunteers who drank 2 liters of regular cola over a period of 24 h. Their findings indicated unfavorable changes to risk factors associated with calcium oxalate (CaOx) stone formation [17]. However, there was insufficient clinical evidence to directly correlate long-term intake of cola beverages with stone formation. Clinical studies for urinary stone risk factors of cola intake are currently ongoing, but have yet to yield concrete results [15,17,18,19]. For example, Ferraro *et al*. showed that consuming sugar-sweetened cola had a 23% higher risk of developing kidney stones, whereas consumption of artificially sweetened cola was associated with a trend to reduced risk [19].

Sodium citrate (Na-citrate) is a common additive of commercial cola and juice beverages. Citrate is believed to retain properties for the effective prevention of stone disease [20], and as such, potassium citrate (K-citrate) has become a common stone prevention drug in clinical settings. However, Na-citrate has been deemed less effective by some clinical studies, preventing its use in this context. A study by Sakhaee *et al*. found that both Na-citrate and K-citrate were effective in preventing the buildup of uric acid, however, Na-citrate was less effective [21].

A subsequent clinical study by Preminger *et al*. found that, although Na-citrate was able to increase urinary citrate excretion rate, it was not effective at preventing stone formation in patients with distal renal tubular acidosis [22]. Na-citrate also enhanced brushite and sodium saturation, but was not able to reduce urinary CaOx saturation. However, in a randomized, placebo controlled clinical trial, Allie-Hamaulay and Rodgers demonstrated that Na-citrate may alter the risk factors for CaOx stone [23]. Because Na-citrate is common and inexpensive, there is ample opportunity to investigate its efficacy as a preventive aid for CaOx stone formation.

We have developed a novel, cost-effective fruit fly model for the study of nephrolithiasis [24]. To address questions related to soft drinks, we conducted an animal study by applying our well-established fruit fly model to investigate the possible effects of cola and Na-citrate for treatment of CaOx-related urolithiasis.

2. Materials and Methods

2.1. Fly Stocks and Rearing Conditions

We used wild type adult male flies, *Drosophila melanogaster* CS, in these experiments. We used 150 to 180 flies in each group, bred in plastic vials containing standard fly medium at 25°C and 60% humidity with a 12 h light-dark cycle. The formula of standard fly medium consisted of 6.7 g agar, 21.7 g yeast, 13.1 g sugar, and 66.6 g corn syrup, with the addition of water to a final volume of 1 L.

The solution was heated in the microwave, and after cooling to 85°C, 13.3 mL 99% alcohol and 3.4 g β-hydroxybenzoic acid methyl ester was added. Then, 10 mL of medium was decanted into a 50-mL test tube, and left to return to room temperature before its storage at 4°C. It should be noted that freshly prepared solution was ready for use only within a 2-week interval.

2.2. CaOx Formation in *Drosophila*

The details for the breeding of lithogenic flies are in accordance with our previous study. In brief, a solution of 0.5% ethylene glycol (EG, Sigma, St Louis, USA) was used as a lithogenic agent for CaOx crystal formation in Malpighian tubules. Na-citrate (2% and 4%) and cola (25%) were added to the fly medium (wt/vol%) and used as test agents, while K-citrate medium additives (2% and 4%) were used as positive controls (n = 150 – 180 for each group).

The flies were subjected to CO_2 narcotization, and the Malpighian tubules were dissected, removed, and processed for polarized light microscopy examination 3 weeks after breeding. Intra-tubule crystals were processed with scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) to confirm the composition as CaOx [24,25,26,27].

2.3. Polarized Light Microscopy

The Malpighian tubules were dissected and immediately observed under normal and polarized white light with an Olympus BX51 (Hicksville NY, USA) optical microscope after the CaOx crystal induction period. Pertinent aspects of the tubules were photographed with Kodak ProImage100 film, and scales were obtained with the projection of a micrometric slide under the same conditions used in the illustrations [24,25,26,27].

2.4. Lifespan Assay

Prior to conducting the lithogenic assays, we studied the effects of cola and Na-citrate on the flies' survival. To set up these lifespan assays, new emergents were collected under light CO_2 anesthesia. Foam plugs were used in place of cotton ones, and food vials were kept horizontally to prevent weaker flies from accidentally adhering to food or foam.

Survivors in each vial were counted, and dead flies were removed daily. Survivorship was compared and tested for significance with log-rank tests, and lifespan curves were from pooled counts of a large number of vials ($n \cong 150$) [24,25,26,27].

2.5. Statistical Analyses

Crystal formation rate was analyzed by the Chi-square test. For comparison between two lifespan curves, we determined the *P* value in the log-rank test. All statistics were performed using the Sigma Stat software (SPSS; Systat Software, USA).

3. Results

3.1. CaOx crystal Formation

EG-induced crystal formation in *Drosophila* Malpighian tubules was clearly observed using microscopy (Figure 1) and was previously identified as CaOx.

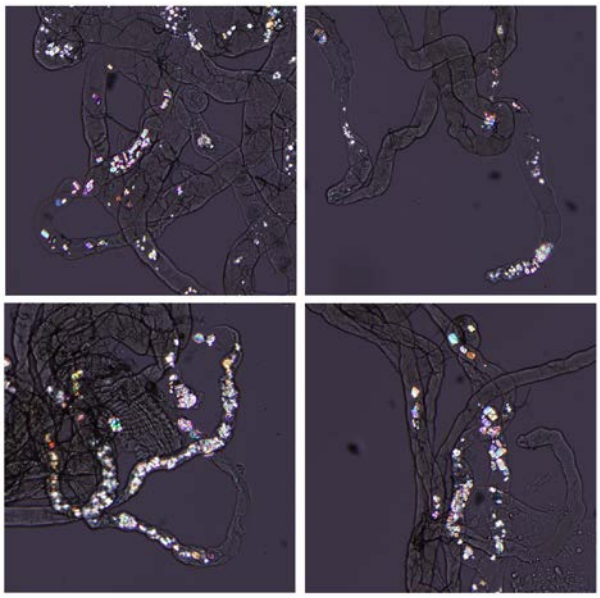

Figure 1. EG-induced CaOx crystal deposition in the Malpighian tubules of *Drosophila*. The images show representative polarized microscopy for the flies with 0.5% EG-induced crystal formation in Malpighian tubules

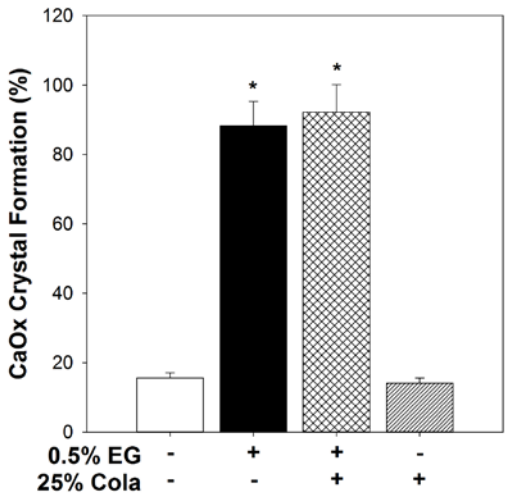

Figure 2. Crystal formation in 0.5% EG and/or 25% cola treated *Drosophila* (n ≅ 150 for each group). *P < 0.05, compared to the control

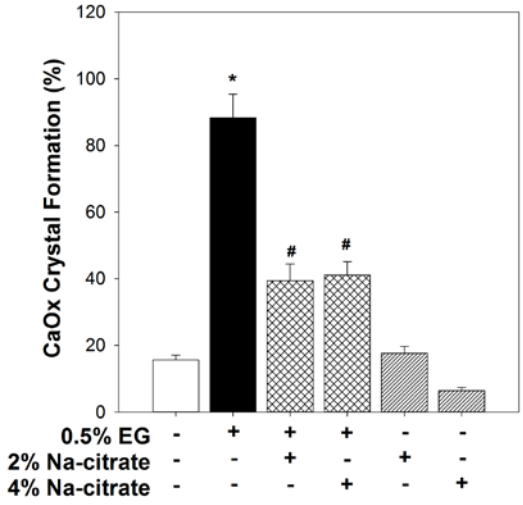

Figure 3. Crystal formation in 0.5% EG and/or Na-citrate (2% and 4%) treated *Drosophila* (n ≅ 150 for each group). *P < 0.05, compared to the control. #P < 0.05, compared to the 0.5% EG-treated group

The rates of CaOx crystal formation in the control group and 0.5% EG group were 15.6% and 88.3%, respectively. The rate of crystal formation in the group of EG+cola was 92.2% and was not different from the lithogenic group (P = 0.245; Figure 2). Single added agents such as cola (Figure 2) and Na-citrate (Figure 3) without EG did not increase the rate of crystal formation. Both K-citrate (Figure 3) and Na-citrate (Figure 4) revealed significant inhibitory effects on crystal formation (P = 0.026).

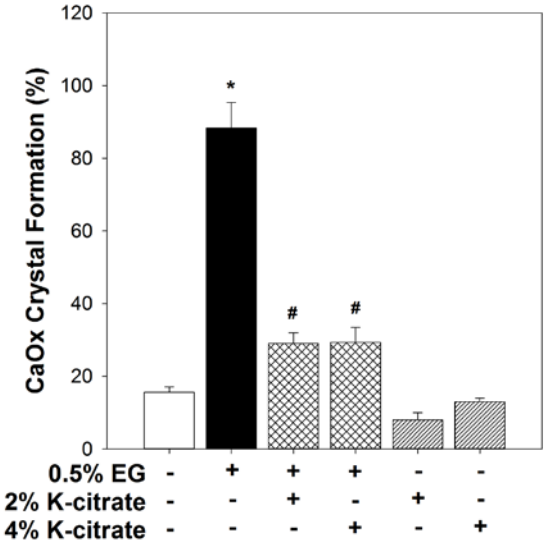

Figure 4. Crystal formation in 0.5% EG and/or K-citrate (2% and 4%) treated *Drosophila* (n ≅ 150 for each group). *P < 0.05, compared to the control. #P < 0.05, compared to the 0.5% EG-treated group

3.2. *Drosophila* LIFESPAN

To test whether the effects of cola or Na-citrate dosage were associated with an increased mortality rate, the lifespans of *Drosophila* were measured after supplementing their food with cola or Na-citrate. The mean life span was not significantly reduced by administration of both agents, and K-citrate comparing with control group (Figure 5).

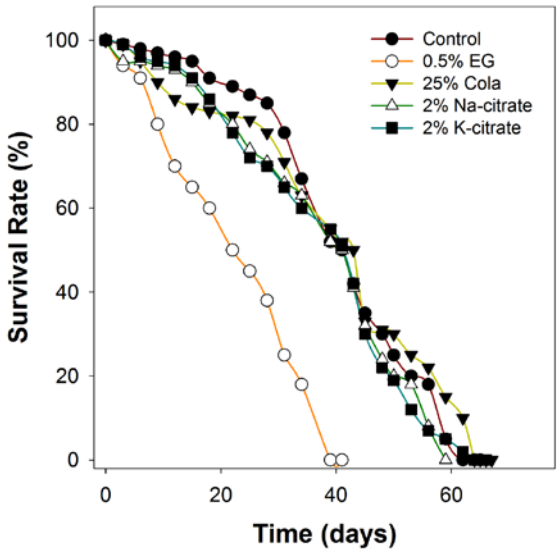

Figure 5. Lifespan of 0.5% EG, 25% cola, 2% Na-citrate, and 2% K-citrate-treated flies

4. Discussion

According to the emerging translational *Drosophila* model of urolithiasis, cola did not promote or inhibit CaOx crystal formation. Administration of 2% Na-citrate exerted an inhibitory effect estimated at 39.4% on EG-induced CaOx crystal formation, similar to that of K-citrate administration (29.0%).

Cola is a common commercial drink that is distributed worldwide [28]. Na-citrate has long been used as a drink additive, yet there is concern about its side effects in a long-term beverage. In this study, there was no difference in the lifespan of *Drosophila* treated with Na-citrate or cola compared with control treatments. Moreover, the addition of cola neither prevented nor enhanced the effects CaOx crystal formation in the Malpighian tubules. Cola contains glucose/fructose, caffeine, water and some confidential components [29]. Long-term consumption of high sugar beverages may contribute to many diseases such as insulin resistance, obesity, hypertensive cardiovascular disease, and kidney disease [30]. However, there was no reported association of the cola with patients' health [31,32]. Caffeine is one of the major contents of cola that has previously been reported responsible for the formation of CaOx stones [12]. Interestingly, Curhan *et al.* conducted a 6-year follow-up study by means of a semi-quantitative food frequency questionnaire that found that those who drink a caffeine-containing beverage daily lowered their risk of stone recurrence by 10% [33]. Furthermore, Herrel *et al*. studied urinary stone risk factors in 13 participants (3 controls) with cola consumption and found no detectable change. They concluded that cola consumption did not contribute to the risk of developing urinary stone disease [15]. Therefore, it is possible that in a clinical context, cola may not exhibit the lithogenic effect that we have shown with our *Drosophila* data.

The clinical, therapeutic effect of K-citrate in preventing CaOx crystal formation has been well documented and confirmed [34]. Gao *et al*. reported that K-citrate significantly increased the successful expulsion rate of melamine-induced urinary calculi [35]. K-citrate has been used clinically for its inhibitory effects on the accumulation of calcium and uric acid stones [36], and because these effects are due to citrate regardless of sodium or potassium. Our results showed Na-citrate having an inhibitory effect similar to that of K-citrate, which argues the potential for its future use in a clinical context. In a study of the alkali action on urinary crystallization of calcium salts by Preminger *et al*., K-citrate, but not Na-citrate, had an effect on the retardation of CaOx saturation in urine. Thereafter, K-citrate has been used clinically as a preventive drug for CaOx stone recurrence, which has been widely reported [22]. Owing to its common use in drinks as an additive, we wonder whether Na-citrate has any contrasting effects; our results showed a therapeutic effect. Therefore, further study of the clinical effects of Na-citrate is warranted to clarify its role in the prevention of CaOx stone recurrence.

Our study has several advantages, yet is not without limitations. We have applied a novel animal model [37], which readily provided a large number of test subjects. Moreover, the experimental period was short and the crystal components in the Malpighian tubules were easily detected through SEM with EDS. In addition, the results of our study were consistent with data from rats and humans [38]. However, the weakness of non-mammalian *Drosophila* model is still a limitation of our study; flies are invertebrate animals, and as such, our model may not be fully indicative of the data we would obtain from mammals. Further studies are warranted to confirm the insect data, especially considering that we provided cola in the fly food daily; this may have exerted additive effects that may not be translatable to human drink habits, unless cola were to be consumed daily.

5. Conclusions

Chronic cola load alone does not affect crystal formation. Na-citrate and K-citrate have a similar inhibitory effect on CaOx crystal formation in our fly model. The ability to readily manipulate and quantify stone formation in these *Drosophila* models presents a unique opportunity to increase our understanding of urolithiasis.

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