# Dietary Potassium Citrate Does Not Harm the pcy Mouse

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**Formation of multiple cysts in the kidneys occurs in several inherited diseases and often leads to terminal kidney failure. Because there is no definitive therapy to halt or slow the progression of renal cystic disease in people, numerous studies have examined possible therapies in animal models. Autosomal-dominant polycystic kidney disease (ADPKD) in the Han:SPRD rat is ameliorated when alkalinizing citrate salts are provided in drinking solutions. By contrast, pcy mice with cystic disease fare worse with the same treatment. We tested the hypothesis that pcy mice ingesting citrate salts in the feed would not be adversely affected by this treatment. Male homozygous pcy mice were given regular feed or 6% potassium citrate-supplemented feed and ad libitum access to water starting at 3 weeks of age. The survival curves of the treated and untreated mice were not significantly different. We conclude that treatment with potassium citrate in the feed does not affect the progression of renal cystic disease in the pcy mouse. This model closely resembles human adolescent nephronophthisis (NPHP3). Based on these findings, citrate treatment cannot be recommended for NPHP3. The fact that it did no harm, however, removes a significant barrier to its consideration as a therapy for ADPKD.**
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**Key words:** autosomal-dominant polycystic kidney disease; citrate; cystic kidney disease; mouse kidney; nephronophthisis

## Introduction

Formation of multiple cysts in the kidneys occurs in several inherited diseases and often leads to terminal kidney failure (1). Because there is no definitive therapy to halt or slow the progression of renal cystic disease in people, numerous studies have examined possible therapies in animal models (2). We found that dietary potassium citrate was strikingly effective in ameliorating autosomal-dominant polycystic disease (ADPKD) in the Han:SPRD rat (3–5). The same treatment, however, appeared to worsen the condition of the pcy mouse (6), which in the past was thought to be an animal model for ADPKD in people (7, 8). The finding that pcy mice fared worse on this treatment called into question the wisdom of testing it in patients with ADPKD, and we therefore undertook this work to determine whether potassium citrate therapy is indeed detrimental to the pcy mouse.

In our previous study of potassium citrate treatment in pcy mice (6), we administered the chemical in drinking water, at the same concentration as we had given the Han:SPRD rat (3). The pcy mice failed to thrive, and we hypothesized that they had an aversion to the potassium citrate/citric acid solution because of its taste. In this work, we therefore took a different approach, and provided potassium citrate in the feed, with free access to plain water. With this protocol, we found no detrimental effect of potassium citrate on the pcy mice.

A recent study has shown that the *pcy* gene in the pcy mouse is orthologous to the gene for human adolescent nephronophthisis (*NPHP3*) (9), suggesting that the pcy mouse is a better model for NPHP3 than for ADPKD. This result and our present findings that potassium citrate does not harm the pcy mouse lead us to recommend that potassium citrate, or an alkalinizing diet, be considered for the treatment of ADPKD.

## Materials and Methods

**Animals and Treatment.** Studies were done on male homozygous CD1-pcy mice. Nineteen mice were fed normal Teklad 7017 sterilized chow (Harlan, Madison, WI) and 17 mice were fed the same feed supplemented with 6% tripotassium citrate, starting at 3 weeks of age. All animals were allowed free access to sterilized water to drink. At 3 months of age, all of the mice, except for five which were male breeders on regular feed, were weighed, and food and water intakes for one week were measured. Survival of the mice was followed. Early in the study, one of the potassium citrate-treated mice developed a prolapsed penis and was euthanized at 182 days of age. We subsequently found that

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a prolapsed penis is probably a sign of general debility, as it developed several weeks before death in many of the mice. Though we had sacrificed this mouse, we did not count it as censored, but included it as having died at 182 days so as to put our data in the least favorable light. With subsequent mice, we treated the discomfort caused by a prolapsed penis with daily application of ointment to the affected area.

After survival data were collected on 22 of the mice, the remaining animals (ages 6–11 months) were sacrificed and measurements were made of body weight, kidney wet and dry weights, and blood urea nitrogen concentration (BUN). For these measurements, the mice were anesthetized with halothane, and a retro-orbital blood sample was collected into heparinized capillary tubes. The anesthetized mice were sacrificed by cervical dislocation. Both kidneys were dissected free, decapsulated, and weighed. Dry weight was determined after heating the kidneys in an oven for 16 hrs at 120°C. BUN was determined by the method of Chaney and Marbach (10).

The care and treatment of the mice were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and local institutional guidelines.

**Statistical Analysis.** Data are presented as means ± SD. If homogeneity of variances could be assumed, the data were analyzed by an ANOVA. If the variances were heterogeneous, then the Welch-Satterthwaite $t'$ test was used. Survival data within each group were analyzed by the Kaplan-Meier method, and the treated and untreated groups were compared by the log-rank test with the Yates correction (11). $P < 0.05$ was considered significant.

## Results

When the mice were 3 months old, we measured body weight and daily food and water intakes (Table 1). The intake of potassium citrate averaged 23 g/m$^2$ of body surface area and was identical to the dose consumed by rats (3). The mice on the potassium citrate-supplemented feed weighed, on average, 14% less than those on the normal feed. Food intake per 100 g body weight was identical in both groups, but the mice on the potassium citrate-supplemented feed drank about 50% more water. By providing the potassium citrate in the feed, we achieved a high level of intake of potassium citrate in the mice without any deleterious effects.

**Table 1.** Measurements on Male pcy Mice at 3 Months of Age[a]

| | Regular feed | 6% potassium citrate feed | *P* value |
|---|---|---|---|
| Body weight (BW), g | 24 ± 1.9 | 21 ± 3.6 | <0.01 |
| Daily food intake, g/100 g BW | 13 ± 1.2 | 13 ± 1.7 | NS |
| Daily water intake, ml/100 g BW | 23 ± 3.8 | 35 ± 8.3 | <0.001 |

[a] Values are means ± S.D. for 14 mice on regular feed and 17 mice on 6% potassium citrate feed.

**Table 2.** Measurements on 6–11-Month-Old Male pcy Mice[a]

| | Regular feed | 6% potassium citrate feed | *P* value |
|---|---|---|---|
| Age, days | 272 ± 55 | 265 ± 47 | NS |
| Body weight, g | 19 ± 4.0 | 17 ± 1.1 | NS |
| Kidney weight, mg | 917 ± 175 | 1,122 ± 234 | NS |
| Kidney weight/ body weight, % | 4.87 ± 0.41 | 6.63 ± 1.50 | NS |
| Kidney tissue, % water | 88.5 ± 0.5 | 89.9 ± 1.5 | NS |
| BUN, m*M* | 29.4 ± 13.7 | 16.7 ± 4.7 | <0.05 |

[a] Values are means ± SD for eight mice on regular feed and six mice on 6% potassium citrate feed.

Table 2 summarizes measurements on mice at 6–11 months of age. Body weights were significantly lower in both untreated ($P < 0.01$) or potassium citrate-treated ($P = 0.05$) mice than at 3 months of age (Table 1). There were no significant differences in any of the variables measured in Table 2, except for the BUN, which was lower in the treated mice. Fig. 1 shows survival data. There was no significant difference between the survival curves of the treated and untreated mice.

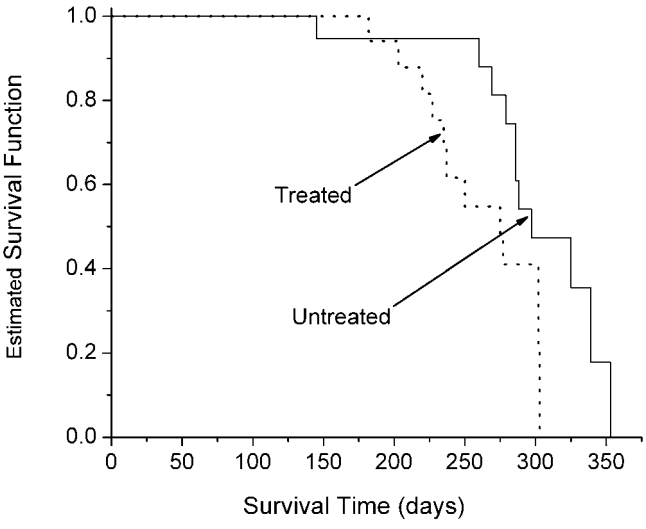


**Figure 1.** Survival of male pcy mice on regular feed (solid line) and on 6% potassium citrate-supplemented feed (dashed line). The survival function was calculated by the Kaplan-Meier product-limit estimate. The untreated group included 19 mice, 8 of which were censored, and median survival was 297 days. The treated group included 17 mice, 6 of which were censored, and median survival was 275 days. Comparison of the two survival curves using the log-rank test, with the Yates correction applied because the data were discrete, yielded $z < 1.71$, so the curves were not significantly different ($P > 0.05$).

## Discussion

This study demonstrates that potassium citrate administration is not harmful to pcy mice with cystic kidney disease. The main difference between this and our earlier study on this mouse model (6) is that the potassium citrate was provided in the feed, not in the drinking solution. In our earlier study, mice on the potassium citrate solution clearly failed to thrive. At 10 weeks of age, their body weight averaged 36% less than untreated animals; one treated mouse died at 7 weeks of age. We assumed at the time that the mice could not tolerate a high intake of potassium citrate because of their cystic disease. Subsequent work with rats, however, convinced us that there could have been another reason for their failure to thrive. We started 3-month-old Han:SPRD rats on a potassium citrate drinking solution, and they drastically reduced their fluid intake. They became ill and, indeed, within 2 weeks, two of four animals had died (unpublished observations). Subsequently, we sweetened the potassium citrate solution with sucrose, and the rats drank this solution readily. We then recognized that the 1-month-old pcy mice in our earlier experiments (6), like the 3-month-old rats, may have had an aversion to the potassium citrate solution and had done poorly because they were not drinking enough. We therefore decided to revisit the question in the pcy mice, and provided potassium citrate in their feed. As our present results show, they tolerated this well.

The BUN in pcy mice, although higher than in normal mice (6), was significantly lower in treated than in untreated animals (Table 2). The reduced BUN could be interpreted as indicating a beneficial effect of potassium citrate treatment on renal function; however, there are other possible explanations. A lower BUN might reflect the higher water intake (Table 1) and hence higher urine output and urea clearance in the treated animals. Also, the alkalinizing citrate salt treatment would counteract the metabolic acidosis and increased rate of protein catabolism associated with renal failure, thereby resulting in a lower BUN (12). The lower BUN, therefore, may not indicate better renal function.

Kidney weight was not significantly affected by citrate treatment (Table 2). In a rat model of ADPKD, citrate treatment strikingly improved renal function (glomerular filtration rate) without preventing an increase in kidney size (3). Therefore, kidney size is not necessarily a reliable indicator of renal function or of citrate's beneficial effect.

In this study, we succeeded in administering potassium citrate to pcy mice without causing morbidity or premature mortality. Even with this method, however, the citrate was not beneficial to these mice, and, in contrast to the Han:SPRD rat (4, 5), survival was not increased. Similarly, in PCK rats, treatment with citrate had no effect on kidney weight or BUN (13). However, in diphenylthiazole-induced PKD in Sprague-Dawley rats, citrate treatment reduced cyst formation and maintained renal function (14). It is not clear why citrate helps in some models of renal cystic disease but not in others. Cyst formation may be the end result of numerous disorders, and the pathways or nephron segments primarily affected in the various diseases may differ.

The recent demonstration that the genes for human NPHP3 and murine pcy have a common ortholog suggests that the pcy mouse may be a more appropriate model for NPHP3 than for ADPKD (9). The pcy mouse shows several features that are similar to those found in patients with NPHP3 (15, 16), including autosomal recessive inheritance, late onset of end-stage renal failure (median age of 19 years in people and adulthood in mice), cysts at the corticomedullary junction, and remarkably similar kidney histology. Overall kidney size, however, is normal or reduced in patients, but is enlarged in the mouse. On the basis of our findings on the mouse, potassium citrate therapy cannot be recommended for treating patients with NPHP3.

Earlier findings that treatment of pcy mice with citrate (6) or bicarbonate (17) solutions had detrimental effects have discouraged clinical trials with alkalinizing salts in ADPKD patients. If this treatment were distinctly harmful in any animal models of renal disease, we would be reluctant to recommend clinical studies. The harmful effect in the earlier mouse experiments might have been due to administration of the salts in drinking solutions. If the mice indeed disliked the taste, they may have then limited their fluid intake and become dehydrated. Recent studies have demonstrated that blockade of vasopressin receptors in the kidneys has a striking beneficial effect in mouse and rat models of renal cystic disease (18, 19). By inference, dehydration, which results in elevated plasma vasopressin levels, might be expected to exacerbate renal cystic disease; this idea would have to be tested in future experiments. In any case, this study shows that potassium citrate *per se* is not harmful to the pcy mouse. This finding thus clears the path to testing alkalinizing therapy in human ADPKD.

There is one group of ADPKD patients who have been treated for many years with potassium citrate, namely those with renal stone disease. Because ADPKD patients are more susceptible to renal stone disease than others, a large population of such patients exists. There are no reports that this treatment is detrimental to them; furthermore, their histories might yield clues as to the efficacy of potassium citrate or an alkalinizing diet in retarding the progress of renal decline. In addition, many people now adhere to a strictly vegan diet, and, among ADPKD patients, they would be expected to have the most alkalinizing diet attainable without added alkali therapy. The medical records of these two groups of patients may reveal whether or not potassium citrate can extend the time to end-stage renal disease in patients with ADPDK.

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1. Grantham JJ, Cowley BD Jr, Torres VE. Progression of autosomal dominant polycystic kidney disease to renal failure. In: Seldin DW, Giebisch G, Eds. The Kidney. Physiology and pathophysiology (3rd ed.). Philadelphia: Lippincott Williams & Wilkins, pp2513–2536, 2000.
2. Qian Q, Harris PC, Torres VE. Treatment prospects for autosomal-dominant polycystic kidney disease. Kidney Int 59:2005–2022, 2001.
3. Tanner GA. Potassium citrate/citric acid intake improves renal function in rats with polycystic kidney disease. J Am Soc Nephrol 9:1242–1248, 1998.
4. Tanner GA, Tanner JA. Citrate therapy for polycystic kidney disease in rats. Kidney Int 58:1859–1869, 2000.
5. Tanner GA, Tanner JA. Dietary citrate treatment of polycystic kidney disease in rats. Nephron Physiol 93:14–20, 2003.
6. Tanner GA, Vijayalakshmi K, Tanner JA. Effects of potassium citrate/citric acid intake in a mouse model of polycystic kidney disease. Nephron 84:270–273, 2000.
7. Takahashi H, Calvet JP, Dittemore-Hoover D, Yoshida K, Grantham JJ, Gattone VH. A hereditary model of slowly progressive polycystic kidney disease in the mouse. J Am Soc Nephrol 1:980–989, 1991.
8. Nagao S, Hibino T, Koyama Y, Marunouchi T, Konishi H, Takahashi H. Strain differences in expression of the adult-type polycystic kidney disease gene, *pcy*, in the mouse. Exp Anim 40:45–53, 1991.
9. Olbrich H, Fliegauf M, Hoefele J, Kispert A, Otto E, Volz A, Wolf MT, Sasmaz G, Trauer U, Reinhardt R, Sudbrak R, Antignac C, Gretz N, Walz G, Schermer B, Benzing T, Hildebrandt F, Omran H. Mutations in a novel gene, *NPHP3*, cause nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. Nat Genet 34:455–459, 2003.
10. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem 8:130–132, 1962.
11. Glantz SA. Primer of Biostatistics (5th ed.). New York: McGraw Hill, pp387–415, 2003.
12. May RC, Kelly RA, Mitch WE. Mechanisms for defects in muscle protein metabolism in rats with chronic uremia. Influence of metabolic acidosis. J Clin Invest 79:1099–1103, 1987.
13. Kamura K, Torres VE, Ishibashi M, Sasaki A, Joh K, Harris PC, Yamada K. Renal functional abnormalities in the PCK rat, a model of human ARPKD. J Am Soc Nephrol 13:115A, 2002.
14. Takashi Y, Fumio I, Yukinari O, Shigeru H, Chika K, Hiroshi T. What are the effects of citrate on experimental polycystic kidney disease? Eur Urol Suppl 1:142, 2002.
15. Omran H, Fernandez C, Jung M, Häffner K, Fargier B, Villaquiran A, Waldherr R, Gretz N, Brandis M, Rüschendorf F, Reis A, Hildebrandt F. Identification of a new gene locus for adolescent nephronophthisis, on chromosome 3q22 in a large Venezuelan pedigree. Am J Hum Genet 66:118–127, 2000.
16. Omran H, Häffner K, Burth S, Fernandez C, Fargier B, Villaquiran A, Nothwang H-G, Schnittger S, Lehrach H, Woo D, Brandis M, Sudbrak R, Hildebrandt F. Human adolescent nephronophthisis: gene locus synteny with polycystic kidney disease in pcy mice. J Am Soc Nephrol 12:107–113, 2001.
17. Torres VE, Cowley BD Jr, Branden MG, Yoshida I, Gattone VH. Long-term ammonium chloride or sodium bicarbonate treatment in two models of polycystic kidney disease. Exp Nephrol 9:171–180, 2001.
18. Gattone VH II, Wang X, Harris PC, Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. Nature Med 9:1323–1326, 2003.
19. Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH II. Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. Nature Med 10:363–364, 2004.