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HIGH URINARY FLOW ACCELERATES RENAL INJURY IN YOUNG RATS WITH PARTIAL UNILATERAL URETERAL OBSTRUCTION

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ABSTRACT

Purpose: We studied the effect of dietary manipulation and high urine flow on neonatal partial ureteral obstruction in a weanling rat preparation.

Materials and Methods: A total of 40, 3-week old Sprague-Dawley rats underwent unilateral ureteral obstruction by burial of the right ureter in the psoas muscle and 13 underwent sham operation. Low, high and normal salt, and high sucrose diets were administered for 2 months. The glomerular filtration rate of each kidney was measured by iothalamate clearance. Intrapelvic pressure and renal blood flow were measured before and after acute volume loading.

Results: Fluid intake and urine output were 8 to 10-fold greater in animals on high salt and high sucrose diets compared to those in rats on normal and low salt diets. Hydronephrosis was observed only in rats with partial obstruction and high urine flow. No difference in renal weight was noted. Relative glomerular filtration rate of the partially obstructed kidney was maintained when urine flow was normal but decreased significantly with high urine flow. Total glomerular filtration rate also decreased with high urine flow. Intrapelvic pressure was elevated significantly at baseline in partially obstructed kidneys with high urine flow. All kidneys with partial obstruction had significantly increased intrapelvic pressure with volume loading. Renal blood flow was not significantly decreased in rats with high urine flow.

Conclusions: Chronic high urine flow causes loss of renal function in partially obstructed weanling rat kidneys. Research should be done to determine whether human infants with hydronephrosis and partial ureteral obstruction would benefit from the prevention of increased fluid and salt intake.

KEY WORDS: ureter; salts; rats, Sprague-Dawley; hydronephrosis; kidney

The proper management of hydronephrosis detected prenatally is controversial because to our knowledge it is not possible currently to predict whether hydronephrosis would stabilize, resolve spontaneously or progress to require surgical intervention. A critical issue is whether hydronephrosis indicates clinically significant obstruction.[1] In the majority of cases hydronephrosis resolves or stabilizes without significant complications.[2] Although Peters took the approach that all patients with hydronephrosis should be offered early repair to prevent acidification or concentrating defects that are not readily measured by renal scanning,[3] Koff and Campbell pointed out that relatively few with unilateral partial obstruction require surgery when they are properly observed.[4] Koff et al proposed 2 types of partial ureteropelvic junction obstruction, namely intrinsic and extrinsic.[5] In the intrinsic type obstruction is fixed and as urine flow increases, renal pelvic pressure increases in a linear fashion. In the extrinsic type the degree of obstruction increases with elevated urine flow. Therefore, flow decreases as renal pelvic pressure increases. In either case the prevention of high urine flow would prevent the onset of elevated intrapelvic pressure and progressive hydronephrosis.

Unfortunately to our knowledge no studies have evaluated the effect of diet on urine flow and hydronephrosis. Since most patients with asymptomatic, prenatally detected hydronephrosis initially undergo observation, we determined whether high urine flow has a role in renal damage due to partial ureteral obstruction. We hypothesized that a chronic increase in urine flow increases hydronephrotic injury, a high salt diet accelerates renal injury in partially obstructed kidneys and a low salt diet protects against renal injury. Results would be useful clinically for advising parents on the proper diet for their child with hydronephrosis who is under observation, and would also explain why the condition of some patients with hydronephrosis improves and that of others deteriorates.

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MATERIALS AND METHODS

We used 3-week-old, newly weaned Sprague-Dawley rats for this experiment. Housing, diet and surgical procedures were approved by the Committee on Animal Research at the University of California, San Francisco. A total of 40 rats underwent exposure of the right ureter, followed by burial of the ureter in the psoas muscle according to the technique of Ulm and Miller.[6] This maneuver created partial unilateral ureteral obstruction. Another 13 rats underwent sham operation with exposure of the right ureter without burial in the psoas muscle. Three salt diets were administered, including low with 0.07, high with 0.9 and normal with 0.4 gm. sodium chloride per 100 cc.[7] The latter group served as a control. Chronic high urine output that was not due to salt intake was modeled by feeding rats a high sucrose solution of 1 gm./100 cc, resulting in a 7-fold increase in urine output.

Rats were placed into metabolic cages every 2 weeks to monitor 24-hour intake, urine output and weight. They were anesthetized with intraperitoneal thiopental 2 months after surgery. The glomerular filtration rate, renal blood flow and intrapelvic pressure were measured before sacrifice.

The glomerular filtration rate of each kidney was measured by iothalamate clearance. The jugular vein was cannu-

lated with polyethylene tubing and iothalamate was infused at a rate of 12 ng. per minute for 1 hour. The corresponding femoral vein and ureter were then cannulated to obtain serum and urine samples. When the kidney was experimentally obstructed, the ureter was cannulated below the level of obstruction. Renal blood flow was measured using an ultrasonic Doppler flow transducer. Intrapelvic pressure at baseline was determined by cannulating the ureter with polyethylene tubing. Saline (5% of body weight) was infused for acute volume expansion. After volume loading intrapelvic pressure was measured. In addition, the kidneys were harvested and wet weight was determined.

The glomerular filtration rate was calculated after high pressure liquid chromatography assay of serum and urine iothalamate.[8] Data are presented as the mean plus or minus standard deviation. Statistical analysis was done using sequential Student's t tests for each pair.

RESULTS

High salt and high sucrose intake individually and together induced an 8 to 10-fold increase in urine flow. There was no difference in urine output in the groups fed high salt, high sucrose or high salt and high sucrose diets. High urine flow resulted in marked dilatation of the renal pelvis of the partially obstructed kidney, compared to only mild dilatation of the renal pelvis when normal urine flow was maintained. No difference in sham operated or obstructed renal weights was noted in the normal and high urine flow groups.

The obstructed kidney contributed significantly less to glomerular filtration rate in all groups with high urine flow (fig. 1). In addition, the total rate was significantly lower in rats with unilateral obstruction and high urine flow than in those on a normal or low salt diet (fig. 2). Baseline intrapelvic pressure was significantly higher in the partially obstructed kidneys of animals with high urine flow than in sham operated and partially obstructed kidneys in rats on a low or normal salt diet (fig. 3). The change in intrapelvic pressure after acute hydration was significantly increased in the obstructed kidneys in all groups but rats on a high salt intake continued to have greater intrapelvic pressure (fig. 4). Although there was a trend toward higher renal blood flow after acute volume loading in partially obstructed kidneys, this difference was not statistically significant compared to that in sham operated kidneys. There were no statistically significant changes in absolute renal blood flow in any group.

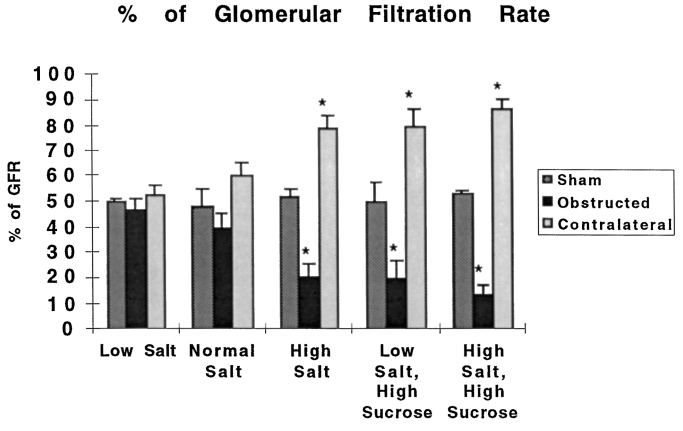

FIG. 1. Relative glomerular filtration rate (*GFR*) in sham operated, partially obstructed and contralateral kidneys. In high urine flow diets of high salt, high sucrose, high salt and high sucrose partially obstructed kidney contributed significantly less and contralateral kidney contributed significantly more than sham operated kidney. Asterisk indicates $p < 0.05$ versus sham operated group.

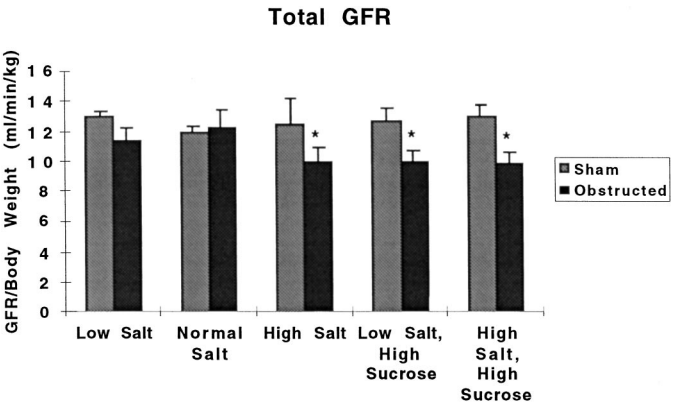

FIG. 2. Total glomerular filtration rate (*GFR*) in sham operated versus partially obstructed groups. In high urine flow diets partially obstructed rats had significantly lower total glomerular filtration rate than sham operated rats. Asterisk indicates $p < 0.05$ versus sham operated group.

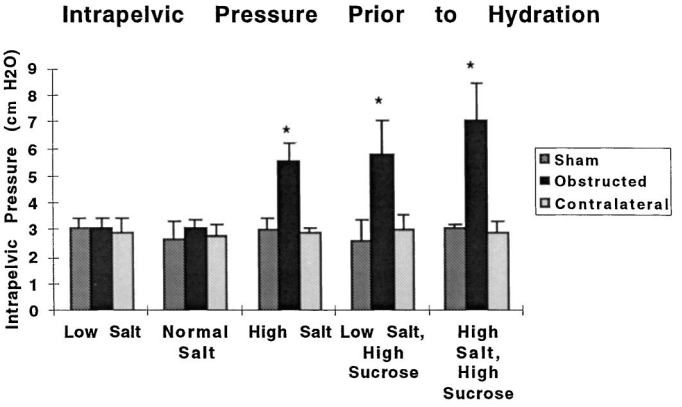

FIG. 3. Baseline renal pelvic pressure. Partial obstruction was created in all but sham operated rats yet baseline renal pelvic pressure significantly increased only when urine flow was increased by dietary manipulation. Asterisk indicates $p < 0.05$ versus sham operated group.

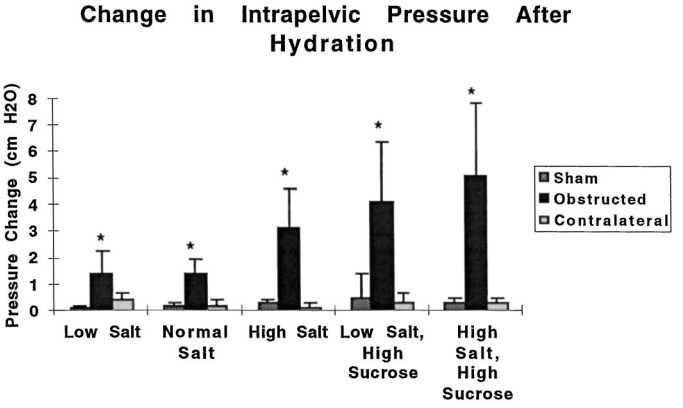

FIG. 4. Renal pelvic pressure significantly increased in all partially obstructed kidneys regardless of diet, confirming partial obstruction. Asterisk indicates $p < 0.05$ versus sham operated group.

DISCUSSION

Although many studies have been performed on the effect of high versus low salt diet for managing hypertension or proteinuria, to our knowledge none has evaluated the effect of a high versus a low salt diet in the setting of obstruction or high urine flow only without salt loading. We report that in young rats with partial unilateral ureteral obstruction

chronic high urine flow secondary to dietary manipulation may cause renal injury. In contrast, in our preparation of 2 months of partial obstruction the relative and total glomerular filtration rates were maintained, provided that urine flow was normal. Similarly although baseline renal pelvic pressure equilibrated by 2 months after unilateral obstruction under normal circumstances, baseline renal pelvic pressure in the animals with high urine flow remained elevated.

High salt and high sucrose diets increased fluid intake and urine output 8 to 10-fold. It appears that the increased salt and sucrose diets caused an obligatory increase in fluid intake, as shown by Walker and Olson.[7] However, rats on a low salt diet did not have decreased fluid intake or urine output to below normal levels and they also maintained similar relative renal weight. These data raise the question of whether the harmful effect of a high salt diet is due to salt or urine volume. Is there truly a salt sensitive effect due to having fewer nephrons, as proposed by Brenner et al?[9] Our high sucrose group allowed us to discriminate the effect of high urine flow only from that of high salt intake. No difference was noted in the high salt and high sucrose groups, implying that it is primarily increased flow that damaged the kidney in our preparation.

The relative glomerular filtration rate in obstructed kidneys did not decrease in the low salt group but decreased 20% in the normal salt group, which was not statistically different from that in the sham operated group. In the high urine flow groups a 60% to 80% loss of absolute glomerular filtration rate was noted in obstructed kidneys with a compensatory increase of 20% to 30% in the absolute rate in contralateral kidneys, resulting in an overall 20% loss of the total glomerular filtration rate. Josephson et al reported a 10% decrease in the relative glomerular filtration rate in neonatal rats[10] and up to a 30% decrease in the relative rate in 6-week-old rats with partial unilateral ureteral obstruction.[11] However, Claesson et al believed that parenchymal atrophy did not progress after the initial 3 weeks.[12] Stenberg et al used a model of partial unilateral ureteral obstruction in weanling rats and observed a 50% decrease in the relative glomerular filtration rate after 1 year of partial obstruction with a contralateral 16% increase.[13] The marked difference in relative glomerular filtration rates was explained by postulating that weanling rats are more susceptible to damage from obstruction compared to neonatal or 6-week-old rats. Our findings show more loss of the glomerular filtration rate in obstructed kidneys than in any other study. This difference is likely due to additional damage caused by high urine flow and high renal pelvic pressure. A low salt diet may be an advantage for preserving the relative glomerular filtration rate, whereas a normal salt diet resulted in a slight decrease in the relative rate that was not statistically significant, as in other studies.

The overall glomerular filtration rate was maintained in rats with normal but decreased by 20% in those with high urine flow. Josephson et al reported no overall rate decrease in newborn and 6-week-old rats that underwent partial unilateral ureteral obstruction using the same technique.[10,11] Stenberg et al also noted no overall decrease in the glomerular filtration rate in weanling rats that underwent burial of the ureter in the psoas muscle.[13] Similarly Chevalier observed no decrease in the glomerular filtration rate after mild partial unilateral ureteral obstruction was created in neonatal guinea pigs.[14] Our results confirm those of previously published studies, illustrating that the overall glomerular filtration rate is maintained in partial unilateral ureteral obstruction when urine flow is normal. Loss of the overall glomerular filtration rate in rats with high urine flow is likely due in some way to elevated intrapelvic pressure.

The fact that rats with normal urine flow maintain normal baseline intrapelvic pressure while those with elevated urine flow have elevated pressure implies that elevated flow, irrespective of whether it is salt or sucrose induced, is responsible for elevated pressure. One would guess that chronic high flow prevents the equilibration in renal pelvic size and compliance that may otherwise occur. All partially obstructed kidneys had a significant increase in intrapelvic pressure after acute hydration regardless of diet, confirming that true obstruction was created.

Renal blood flow was maintained in all dietary groups and was similar in the sham operated, obstructed and contralateral kidneys. Renal blood flow was increased in obstructed high flow kidneys, although this change was not statistically significant. As we showed previously in fetal lambs, partial ureteral obstruction does not necessarily lead to a decrease in renal blood flow, although that was the classic finding in complete ureteral obstruction models.[15] The effect of partial unilateral ureteral obstruction on renal blood flow depends on the animal age, species, severity of partial obstruction and normal renal innervation. Josephson noted a 12% decrease in renal blood flow in neonatal rats.[10] Chevalier and Kaiser observed elevated renal blood flow in neonatal guinea pigs with mild partial unilateral ureteral obstruction and decreased renal blood flow only in severe partial unilateral ureteral obstruction.[16] The effect on renal blood flow appears to be more severe when obstruction occurs earlier in life.[17] An explanation is based on the various activities of the renin-angiotensin system in young versus adult rats.[18] Chevalier and Thornhill also showed that de-innervation prevented contralateral vasodilatation after ipsilateral obstruction.[19] We do not believe that renal de-innervation is a confounding factor in our preparation since the ureter was buried away from the hilar region. Irrespective of the cause we believe that our data show that ischemia is not the cause of decreased renal function.

Studies of the effect of high versus low salt diets have primarily used a preparation of renal hypertrophy driven by contralateral or five-sixths nephrectomy consisting of contralateral nephrectomy and ischemia of two-thirds of the remnant kidney. Daniels and Hostetter showed in a five-sixths nephrectomy model that serum creatinine was the same in rats fed a low or normal salt diet but those on a low salt diet had less proteinuria despite a high renin level.[20] Benstein et al observed in spontaneously hypertensive rats that underwent nephrectomy that a low salt diet resulted in less proteinuria and glomerulosclerosis, presumably by preventing hypertrophy.[21] This effect was believed to be independent of the renin level because micropuncture studies showed similarly elevated glomerular pressure despite a normal or low salt diet, or hydrochlorothiazide administration. Also, Lax et al reported that rats with five-sixths nephrectomy had stable glomerular size when maintained on a low salt diet, whereas glomerular size increased markedly when they were placed on a high salt diet.[22]

These 3 studies suggest that salt restriction prevents proteinuria in the setting of renal hypertrophy by limiting the degree of compensatory growth, and the renin level and glomerular pressure are not affected by dietary salt. To make these data applicable to the partial obstruction model one must ask whether limiting salt intake has the same effect when there is no stimulus for hypertrophy or when pathological changes are due to apoptosis.[23] In fact, Chevalier et al suggested that limiting salt intake may worsen apoptotic loss in adult animals with complete ureteral obstruction.[24] Because infants require a positive sodium balance for somatic growth, it is possible that a low salt diet may limit renal growth.[25] It is difficult to draw conclusions on glomerular pressure in the partial obstruction model. Therefore, based on previous studies it is not clear whether limiting salt intake in the setting of partial obstruction would be beneficial by maintaining low urine flow and decreasing renal pelvic pressure, or detrimental by preventing appropriate renal growth or promoting apoptosis in an obstructed kidney.

Since we demonstrated the benefits of maintaining normal

urine flow in young rats with partial unilateral ureteral obstruction, we wondered about the clinical correlates. Infants generally ingest fluid in excess of their nutritional requirement but their urine concentrates only to 900 to 1,100 mOsm./l. The renal solute load is calculated based on electrolytes and protein breakdown products in a given diet, which is a more accurate way of estimating what the kidney processes rather than osmolality only.[26] Human breast milk has a lower solute load than milk or soy based formulas and it is much more dilute than whole milk. Therefore, the minimum volume that an infant must consume to excrete the solute load would likely be the least when human breast milk is given. Therefore, it is theoretically possible that breast fed patients with prenatal hydronephrosis have less postnatal hydronephrosis than those given formula. Between ages 1 and 4 months breast fed infants gain 2.5 gm./kg. daily compared to those fed formula, who gain 3.5 gm./kg. daily. This difference is not maintained indefinitely and as the child grows it may reflect the importance of other factors, such as the introduction of solid foods.[27] Whole and evaporated milk may cause hypernatremia in infants due to the high solute load and they should be avoided even when a child has normal kidneys. Muchant et al suggested that blunted natriuresis and diuresis with a more activated renin-angiotensin system in neonates may be a developmental adaptation to breast milk, which is more dilute.[25]

The response of the partially obstructed kidney to challenges of fluid and solute loads has been shown to be normal in weanling rats with short-term obstruction[28] and markedly worse in weanling rats after 1 year of obstruction (40% decrease in concentrating ability).[13] In human infants with unobstructed kidneys the fractional excretion of sodium decreases from 5% prenatally to less than 0.2% postnatally.[29] Since the glomerular filtration rate also doubles in week 1 of life and concentrating ability increases,[18] one may postulate that urine flow decreases as urine becomes more concentrated postnatally. This hypothesis may be another explanation for the fact that most cases of prenatally detected hydronephrosis resolve spontaneously. On the other hand, if an infant starts with high urine flow due to tubular dysfunction from obstruction or reflux, that condition not only may worsen renal pelvic pressure, but also interfere further with normal maturation of kidney concentrating ability, leading to persistent high urine flow and worsening hydronephrosis. Moreover, although these findings are true in intrinsic obstruction, they may have a significantly greater effect in extrinsic obstruction. The effect of diet on the natural history of hydronephrosis may partially explain why the condition of some patients improves while that of others deteriorates.

CONCLUSIONS

Chronic high urine flow caused loss of renal function in partially obstructed weanling rat kidneys. Research should be done to determine whether human infants with hydronephrosis and partial ureteral obstruction would benefit from the prevention of excessive fluid and salt intake.

Dr. Frank Hinman, Jr. encouraged us to study the effect of diet on hydronephrosis.

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