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# Renal Oxygen Consumption and Sodium Reabsorption during Isotonic Volume Expansion in the Developing Rat

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## Summary

**The aim of the present study was to obtain more information on oxygen consumption in relation to Na reabsorption in the immature kidney during hydropenia (HP) and isotonic volume expansion (VE) corresponding to 5% of the body weight. The study was performed in 24 male Sprague Dawley rats, which were divided into four groups containing six rats each: (1) 22- to 24-day-old rats during HP; (2) 22- to 24-day-old rats during VE; (3) 40- to 42-day-old rats during HP; and (4) 40- to 42-day-old rats during VE. Blood from the renal vein and from the artery was sampled for estimation of oxygen consumption. Glomerular filtration rate (GFR) was estimated by inulin clearance and renal plasma flow by renal extraction of inulin.**

**Hemoglobin conentration and hematocrit are lower in younger rats than in older ones. VE induces a significant and equivalent fall in both age groups. During HP, renal blood flow (RBF) and GFR in relation to body weight are almost 50% lower in younger than in older rats ($P < 0.001$). During VE, RBF and GFR increase by almost 100% in younger rats ($P < 0.001$) and reach the same values as in older rats ($P > 0.05$). The RBF and GFR increased by about 15% in the older rats during the transition from HP and VE ($P > 0.05$). The amount of Na reabsorbed in 24-day-old HP rats was only 56% of the amount of Na reabsorbed in 40-day-old rats ($P < 0.001$). After VE, Na reabsorption increased more in 24- than in 40-day-old rats (by 52% and 12%, respectively). The fractional sodium excretion ($C_{Na}/C_{in}$) was lower in the 24- than in the 40-day-old VE rats. Renal oxygen consumption (ROC) in the 24-day-old HP rats (including basal oxygen consumption of 1 μmole/g kidney weight) was 53% of the ROC in 40-day-old HP rats. During VE, ROC increased more in the 24-day-old rats (34% ) ($P < 0.01$) than in the 40-day-old rats (4%) ($P > 0.05$).**

**The renal $Na/O_2$ ratio was the same in the 24-day-old HP, the 40-day-old HP and the 40-day-old VE rats. The 24-day-old VE rats had a significantly higher $Na/O_2$ ratio than the other groups studied ($P < 0.01$).**

**It is concluded that the energy demands of the immature kidney are related to the GFR and the amount of reabsorbed Na. If the GFR and the amount of reabsorbed Na increase, the oxygen consumption also increases. The amount of the increase, however, is less in the younger rats during VE than it would be if the renal $Na/O_2$ ratio was fixed.**

## Speculation

**Equivalent volume expansion induces a considerably larger increase in glomerular filtration rate and a concurrent increase in Na reabsorption in the neonatal period. Because the increased Na reabsorption during VE is partly due to increased $O_2$-demanding Na transport, VE may be hazardous, at least in the neonatal period when the oxygen and energy resources are limited.**

Under basal conditions the immature kidney is able to conserve water and electrolytes by means of a low renal blood flow (RBF) and a low glomerular filtration rate (GFR) (4, 11, 12, 15). The resulting small filtered load should lower the need for energy-demanding tubular reabsorption.

In the mature kidney, renal oxygen consumption (ROC) depends mainly on the amount of reabsorbed sodium (5, 9), which in turn is largely determined by the amount of filtered Na. In the adult animal, ROC during hydropenia (HP) corresponds to 8–10% of the total body oxygen consumption (16). Volume expansion (VE) amounting to 10% of the body weight (bw) increases the amount of filtered and reabsorbed Na and also increases ROC to about 15% of the total body oxygen consumption (22).

The aim of the present study was to obtain more information on oxygen consumption in relation to Na reabsorption in the immature kidney during HP and VE. Earlier studies from this laboratory have shown that during moderate VE, the immature kidney responds with a much more prompt increase in RBF, GFR and Na reabsorption than does the mature kidney (12). If the increased Na reabsorption during VE is due mainly to increased $O_2$-demanding active Na transport, VE could increase the oxygen consumption considerably. This could have clinical implications, at least in the neonatal period when the oxygen resources are limited.

## MATERIALS AND METHODS

The studies were performed on 24 male Sprague Dawley rats. The bw of the younger and the older rats averaged 66.2 ± 2.6 g (mean ± S.D.) and 151.2 ± 18.1 g (mean ± S.D.), respectively. The rats were weaned up to 17 days of age. Before the study, all animals were given water *ad libitum* and an ordinary laboratory diet. The rats were divided into four groups containing six rats each: (1) 22- to 24-day-old rats during HP (24-d HP); (2) 22- to 24-day-old rats during VE (24-d VE); (3) 40- to 42-day-old rats during HP (40-d HP); and (4) 40- to 42-day-old rats during VE (40-d VE).

*Preparations.* The rats were anesthetized with 80 mg/kg Inactin (Promonta, West Germany) intraperitoneally and intubated with a polyethylene catheter (pp-205). The rats were kept warm with an infrared lamp. The rectal temperature ranged between 37°–37.5°C. Cannulae were placed in one or both jugular veins and one carotid artery. The left renal vein and ureter were approached by means of a subcostal incision in the left flank. Urine was obtained from a cannula positioned in the ureter 8–10 mm distal to the pelvis. The renal vein was cannulated by a sharp needle (0.3 mm) anaerobically connected to a 1 ml heparinized syringe (Braunswick). The piston was exposed to a continuous mechanical traction, which resulted in suction of 0.5 ml renal vein blood into the syringe at a rate of 0.3 ml/min in younger rats and 0.6 ml/min in older rats. The position of the needle was at least 5 mm from

the renal vein's entrance into the inferior vena cava. Arterial blood was sampled from the carotid artery. A continuous infusion of inulin (6.25%) in Ringer's solution was given at a rate of 0.015 ml/100 g bw/min. The infusion was started 1 h before the study and was preceded by a prime dose of 1 ml/100 g bw of the same solution. The fluid used for expansion in group 24-d VE and group 40-d VE consisted of isotonic sodium chloride given in the other jugular vein 30 min before sampling. The infusion rate was 0.25 ml/100 g bw/min the first 20 min and 0.07 ml/100 g bw/min during the rest of the study. Urinary sampling was started 70–80 min after the prime dose of inulin and terminated 20–30 min later. The blood samples were immediately sealed and put into ice water. The arterial blood pressure was determined periodically by connecting the carotid artery catheter to a Hewlett-Packard pressure recorder (No. 7744A).

*Analytical procedures.* The concentrations of hemoglobin (Hb), pH and $Po_2$ in 0.1 ml of either arterial or venous blood were recorded in a blood-gas analyzer (ABL 2, Radiometer, Copenhagen). The oxygen saturation was calculated from an oxygen-saturation curve estimated earlier from Sprague Dawley rats, to pH = 7.35–7.45 (11).

The accuracy of the Hb measurements, using ABL 2, was checked in 20 double samples by means of conventional cyan-meth-hemoglobin methods used routinely in our laboratory. No significant difference was found between the methods.

*Calculations.* In the left experimental kidney, GFR was estimated with inulin clearance, renal plasma flow (RPF) by renal extraction of inulin according to the formula: $RPF = \dot{V}(U_{in} - V_{in})/(A_{in} - V_{in})$. $\dot{V}$ is the rate of urinary flow, $U_{in}$, $V_{in}$ and $A_{in}$ are the concentration of inulin in urine, renal vein plasma and artery plasma, respectively. Oxygen consumption was estimated by multiplying RBF (RPF/1−Hct) by the arteriovenous difference of the blood oxygen content.

Basal oxygen consumption was calculated from the regression line, when the sodium reabsorption was zero. This value was found to be 0.5 μmole/min/100 g bw, a value in accordance with earlier findings of 1 μmole/g kidney weight/min (5, 16). The $Na/O_2$ ratio was calculated by dividing the amount of Na reabsorbed [(GFR × serum $Na^+$) − ($C_{Na}$ × serum $Na^+$)] by the amount of oxygen consumed (actual mean value for oxygen consumption subtracted from the basal oxygen consumption).

*Statistical analysis.* The sources of variations were analyzed by analyses of variance. The distribution table for F used in the study was $P = 0.975$.

## RESULTS

The hemoglobin and hematocrit are lower in younger than in older rats. VE induces a significant and equivalent fall in both age groups. The values for serum Na are constant in all four groups (Table 1).

During HP, RBF and GFR in relation to body weight are almost 50% lower in younger than in older rats (Table 2). During VE, RBF and GFR increased by almost 100% in younger rats and reached the same values as those recorded in older VE rats. The older rats increased their RBF and GFR by about 15% during the transition from HP to VE.

The reliability of the extraction technique (21, 22) for determination of RBF is shown in Table 3. The values obtained with the extraction technique have been compared with those obtained previously with the microsphere technique (12) under similar experimental conditions. There is a good correlation between the values obtained with the microsphere technique and the values obtained with the extraction technique, although the extraction technique gives 10–15% higher values than the microsphere technique.

The amount of Na reabsorbed in 24-d HP rats was only 56% of the amount of Na reabosrbed in 40-d HP rats. After VE, Na reabsorption increased more in 24- than in 40-d rats (by 52% and 12%, respectively). Fractional excretion of Na was lower in 24- than in 40-d VE rats. ROC in 24-d HP rats (including basal oxygen consumption of 1 μmole/g kidney weight) was 53% of ROC in 40-d HP rats. During VE, ROC increased more in 24-d rats (34%) than in 40-d rats (4%) (Table 2).

The renal $Na/O_2$ ratio was the same in 24-d HP, 40-d HP and 40-d VE rats. The 24-d VE rats had a significantly higher $Na/O_2$ ratio than the other groups studied.

## DISCUSSION

The measurement of renal oxygen consumption entails certain methodological problems. The value is dependent on eight different primary values, and analytical as well as measurement errors may invalidate the results. The methods used to minimize errors in hemoglobin determinations are discussed in the section "Materials and Methods." The extraction method for RBF determination appears to be as reliable as the microsphere method (12), when comparing RBF in different groups of rats (Table 3). The recent criticism of the microspheres method (6) is valid only for measuring regional RBF, not for whole kidney RBF. The $Po_2$ and pH may vary considerably if the samples are not cooled immediately and sealed properly. The low deviation of the mean values

Table 1. *Hemoglobin, hematocrit and serum-Na in 24- and 40-day-old hydropenic (HP) and volume-expanded (VE) rats*[1]

| Age and state of hydration | Hemoglobin concentration (g/ml) | Hematocrit | Serum $Na^+$ (μmole/ml) |
|---|---|---|---|
| 24-day HP | 0.116 ± 0.090[3, 4] | 0.35 ± 0.04[3, 4] | 144.0 ± 4.1 |
| 24-day VE | 0.097 ± 0.010[2, 4, 5] | 0.30 ± 0.03[2, 4, 5] | 144.0 ± 4.0 |
| 40-day HP | 0.144 ± 0.014[2, 3, 5] | 0.42 ± 0.04[2, 3, 5] | 148.0 ± 1.6 |
| 40-day VE | 0.122 ± 0.023[3, 4] | 0.36 ± 0.06[3, 4] | 147.7 ± 2.8 |

[1] Values are mean ± 1 S.D.
[2] Significantly different from 24-day HP.
[3] Significantly different from 24-day VE.
[4] Significantly different from 40-day HP.
[5] Significantly different from 40-day VE.

Table 2. *Renal blood flow, glomerular filtration rate, fractional sodium excretion, $O_2$-consumption and $Na/O_2$ ratio in both age groups during hydropenia and isotonic volume expansion*[1]

| Age and state of hydration | RBF (ml/min/100 g bw) | GFR (ml/min/100 g bw) | $C_{Na}/C_{In}$ | $O_2$ consumption (μmole/min/100 g bw) | $Na/O_2$ ratio μmole/μmole |
|---|---|---|---|---|---|
| 24-day HP | 1.33 ± 0.94[3, 4, 5] | 0.27 ± 0.10[3, 4, 5] | 0.009 ± 0.004[5] | 3.21 ± 0.94[3, 4, 5] | 14.5 ± 1.7[3] |
| 24-day VE | 2.65 ± 0.69[2] | 0.52 ± 0.15[2] | 0.011 ± 0.005[5] | 4.31 ± 0.84[2] | 19.6 ± 3.1[2, 4, 5] |
| 40-day HP | 2.50 ± 0.60[2] | 0.47 ± 0.11[2] | 0.012 ± 0.008[5] | 4.90 ± 0.90[2] | 15.3 ± 2.3[3] |
| 40-day VE | 2.80 ± 0.71[2] | 0.54 ± 0.17[2] | 0.027 ± 0.012[2] | 5.11 ± 0.81[2] | 16.7 ± 1.5[3] |

[1] Values are mean ± 1 S.D.
[2] Significantly different from 24-day HP.
[3] Significantly different from 24-day VE.
[4] Significantly different from 40-day HP.
[5] Significantly different from 40-day VE.

Table 3. *Renal blood flow measured with microspheres and with inulin extraction*[1]

| | Renal blood flow (ml/min) | |
|---|---|---|
| | Microspheres | Inulin extraction |
| 24-day HP | 0.72 ± 0.22 ($n$ = 6) | 0.88 ± 0.62 ($n$ = 6) |
| 24-day VE | 1.30 ± 0.03 ($n$ = 8) | 1.47 ± 0.46 ($n$ = 6) |
| 40-day HP | 3.42 ± 0.83 ($n$ = 6) | 3.77 ± 0.71 ($n$ = 6) |
| 40-day VE | 3.95 ± 0.87 ($n$ = 7) | 4.22 ± 1.07 ($n$ = 6) |

[1] Mean values ± 1 S.D. with number of rats per group in parentheses.

and the close resemblance of the oxygen consumption values earlier reported in rats speak against systematic as well as analytical errors (21).

During hydropenia, the renal $Na/O_2$ ratio was approximately the same in 24- and 40-d rats. This could imply that the energy demand for Na transport is similar in the immature and mature kidney. It should, however, be pointed out that the present study was performed on a whole kidney preparation and that there is a maturational gradient in the kidney of 24-d rats between the well developed juxtamedullary nephrons and the more newly formed superficial nephrons (17). The results of the present study, therefore, do not rule out that very immature nephrons differ in the energy sources for Na transport.

It is well documented that the oxygen consumption of the mature kidney is related to the amount of reabsorbed Na (5, 9, 16, 18). The results of the present study show that this relationship is also true for the immature kidney. The large increase in GFR and the corresponding large amount of reabsorbed Na that was induced by VE in young rats was followed by a large increase in oxygen consumption.

The 24-d VE rats did, however, have a significantly higher renal $Na/O_2$ ratio than the rats in the other three groups. By increasing the renal $Na/O_2$ ratio, the immature kidney was able to conserve some oxygen during VE. This ability of the immature kidney might be explained by two mechanisms: (1) increased passive reabsorption of Na and/or (2) a change from aerobic to anaerobic energy sources. The proximal tubule of the immature nephron has a relatively high hydraulic conductivity (14) and the transtubular pressure gradients during VE (12) could induce a passive paracellular reabsorption of fluid and Na. In the distal tubule, evidence of a higher proportion of anaerobic energy production compared to that of the proximal tubule is found (20). Immature nephrons reabsorb a higher proportion of filtered Na in the distal tubule than mature nephrons (3). During VE, this high fractional reabsorption in the immature compared to the mature distal nephrons is even more pronounced (3). The immature kidney shows a higher degree of anaerobic energy sources than the more mature (10). The increase in renal $Na/O_2$ ratio in response to VE, however, is not necessarily due to ontogenic changes in energy sources. The degree of VE used in the present study (5–6% of the bw) only increased the GFR and the amount of reabsorbed Na significantly in the young rats. When adult rats are volume expanded by 10% of the bw, they have a 20% increase in the amount of filtered Na and there is a parallel increase in the renal $Na/O_2$ ratio (22).

The findings might have several clinical implications. GFR is low in the newborn human infant (1, 7, 19). GFR increases rapidly in the full term infant and more slowly in the preterm infant during the first wk of life (1). This implies that the renal oxygen demand is low immediately after birth and lower in preterm than in full term infants. It is also of interest that infants with low oxygen uptake due to severe RDS also have a very low GFR (8, 13).

A large increase in GFR following VE has also been observed in the human infant (19). This increase in GFR will result in a large increase in renal Na reabsorption since human infants also have a blunted natriuretic response to VE (2). We therefore suggest that the possibility of an increase in renal oxygen demand should be considered before increasing the fluid and salt intake of newborn infants.

In summary, this study shows that the energy demands of the immature kidney are related to the GFR and the amount of reabsorbed Na. If the GFR and the amount of reabsorbed Na increase, the oxygen consumption also increases. The amount of increase, however, is less than it would be if the renal $Na/O_2$ ratio was fixed.

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