

# Glomerular Hyperfiltration in Experimental Diabetes Mellitus: Potential Role of Tubular Reabsorption

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**Abstract.** An increase in Na<sup>+</sup>/glucose cotransport upstream to the macula densa might contribute to the increase in single nephron GFR (SNGFR) in early diabetes mellitus by lowering the signal of the tubuloglomerular feedback, *i.e.*, the luminal Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> concentration sensed by the macula densa. To examine this issue, micropuncture experiments were performed in nephrons with superficial glomeruli of streptozotocin-induced diabetes mellitus in rats. First, in nondiabetic control rats, ambient early distal tubular concentrations of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> were about 21, 20, and 1.2 mM, respectively, suggesting collection sites relatively close to the macula densa. Second, glomerular hyperfiltration in diabetic rats was associated with a reduction in ambient early distal tubular concentrations of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> by 20 to 28%, reflecting an

increase in fractional reabsorption of these ions up to the early distal tubule. Third, in diabetic rats, early proximal tubular application of phlorizin, an inhibitor of Na<sup>+</sup>/glucose cotransport, elicited (1) a greater reduction in absolute and fractional reabsorption of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> up to the early distal tubule, and (2) a greater increase in early distal tubular concentration of these ions, which was associated with a more pronounced reduction in SNGFR. These findings support the concept that stimulation of tubular Na<sup>+</sup>/glucose cotransport by reducing the tubuloglomerular feedback signal at the macula densa may contribute to glomerular hyperfiltration in diabetic rats. Glomerular hyperfiltration in diabetic rats serves to compensate for the rise in fractional tubular reabsorption to partly restore the electrolyte load to the distal nephron.

Diabetes mellitus is a leading cause of end-stage renal disease. The pathogenesis of diabetic nephropathy is poorly understood, but glomerular injury has been ascribed at least in part to glomerular hyperfiltration, which occurs early in the course of diabetes mellitus (1,2). The path leading from diabetes to changes in glomerular hemodynamics has not been delineated, but the proximate cause of glomerular hyperfiltration is a reduced vascular resistance of the afferent arteriole (3).

The afferent arteriolar tone is controlled by pressure-induced (myogenic) vasomotion and by tubuloglomerular feedback (TGF). TGF refers to a series of events whereby changes in the concentration of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> in the tubular fluid are sensed by the macula densa, which then causes reciprocal changes in single nephron GFR (SNGFR) (4–6). The net effect of this negative feedback mechanism is a relative constancy of fluid and electrolyte delivery to the distal nephron, which in these nephron segments allows fine adjustment of reabsorption and excretion according to hormonal stimulation reflecting body needs.

There is evidence to suggest that fractional reabsorption of fluid and electrolyte in the proximal tubule is increased in early insulin-dependent diabetes mellitus in humans (7), as well as in rats with streptozotocin-induced experimental diabetes mellitus (8,9). These changes may predominantly relate to increased Na<sup>+</sup>/glucose cotransport (8). A primary increase in fractional tubular reabsorption upstream to the macula densa, however, could lower the electrolyte concentration at the macula densa. The latter would be expected to elicit a TGF-dependent increase in SNGFR to restore the fluid and electrolyte load to the distal tubule.

To assess a role of tubular reabsorption in glomerular hyperfiltration in diabetes mellitus, micropuncture experiments were performed in rats with streptozotocin-induced diabetes mellitus. First, we determined SNGFR, the reabsorption of fluid, and Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> upstream to the early distal tubule, as well as the concentration of these ions in early distal tubular fluid as a measure of the luminal TGF signal. To get relatively close to the macula densa segment, all experiments were performed in nephrons with superficial glomeruli. These experiments were complemented by assessment of hydrostatic pressures in glomerular capillaries, Bowman space ( $P_{\text{Bow}}$ ), and efferent arterioles. To establish a role of Na<sup>+</sup>/glucose cotransport, we subsequently determined the response in tubular reabsorption, the early distal tubular electrolyte concentrations,  $P_{\text{Bow}}$ , as well as SNGFR in control and diabetic rats to early proximal tubular application of the Na<sup>+</sup>/glucose cotransport inhibitor phlorizin.

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## Materials and Methods

All animal experimentation was conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Munich-Wistar-Frömter rats weighing 250 to 280 g were made diabetic by intraperitoneal injection of streptozotocin (STZ; Sigma, St. Louis, MO; 65 mg/kg dissolved in sodium citrate buffer). Two days later, the glucose concentration was determined in tail blood samples, and only those animals with blood glucose levels >300 mg/dl were included in additional experiments. Diabetic rats were treated daily with a long-acting insulin (Ultratard® HM; Novo Nordisc Pharma, Mainz, Germany; 0.5 to 1.5 IU subcutaneously in late afternoon) to adjust blood glucose levels at approximately 300 mg/dl. The animals were allowed free access to a regular rat pellet diet and tap water. Three to four weeks after onset of diabetes mellitus, nonfasted rats were prepared for micropuncture studies. Nondiabetic rats initially receiving application of STZ-vehicle intraperitoneally and being fed the same diet served as controls.

### Preparation for Micropuncture Studies

The preparation for micropuncture was carried out according to standard protocols described previously (9). Briefly, animals were anesthetized with thiobutabarbital (120 mg/kg body wt, intraperitoneally; Research Biochemicals International, Natick, MA), and body temperature was maintained at 37°C. A tracheostomy was performed to facilitate free breathing. The left femoral artery was cannulated to obtain blood samples and monitor arterial BP (P23dB Gould-Statham pressure transducer; Oxnard, CA). The right jugular vein was cannulated for infusion of Ringer's saline (in mM): 30 NaHCO<sub>3</sub>, 4.7 KCl, 111 NaCl) at a rate of 1.5 ml/h. For assessment of left kidney GFR (GFR<sub>LK</sub>) and SNGFR, [<sup>3</sup>H]-inulin was added to Ringer's saline to deliver 100 μCi/h. Additional Ringer's saline was infused to match urinary flow rate and to maintain arterial hematocrit constant (additional infusion rates: control rats, 16 μl/min; diabetic rats, 40 μl/min). After cannulating the bladder, the left kidney was exposed by flank incision, immobilized in a Lucite cup with warmed agar (3%), covered with warm paraffin oil, and the left ureter was cannulated. The rats were allowed 60 min to stabilize before micropuncture experiments were started.

### Series 1: SNGFR, Tubular Reabsorption, and Early Distal Tubular Flow Rate and Electrolyte Concentration

Distal tubular configuration of nephrons with superficial glomeruli was identified by inserting a micropipette (1- to 3-μm tip) into Bowman space to inject a small bolus of stained artificial tubular fluid (ATF) (in mM): 113 NaCl, 25 NaHCO<sub>3</sub>, 4 KCl, 1 MgSO<sub>4</sub>, 2 CaCl<sub>2</sub>, 1 Na<sub>2</sub>HPO<sub>4</sub>, 5 glucose, 5 urea, and 0.075% FD&C green, pH 7.4). After clearing of the dye from the distal tubule, a collecting pipette (7- to 9-μm tip) was inserted into the first accessible distal tubular loop to perform a timed collection of tubular fluid (3 to 4 min in duration) under free-flow conditions, using a short mineral oil block. Timed early distal tubular fluid collections were analyzed for early distal tubular flow rate ( $V_{ED}$ ) and sodium, potassium, and chloride ion concentration ( $[Na^+]_{ED}$ ,  $[K^+]_{ED}$ ,  $[Cl^-]_{ED}$ ) as well as <sup>3</sup>H-inulin concentration to determine SNGFR (see Analytical Methods). Absolute and fractional reabsorption of fluid (AR-fluid, FR-fluid) or Na<sup>+</sup>, K<sup>+</sup>, or Cl<sup>-</sup> (AR-Na<sup>+</sup>, FR-Na<sup>+</sup>, AR-K<sup>+</sup>, FR-K<sup>+</sup>, AR-Cl<sup>-</sup>, or FR-Cl<sup>-</sup>)

between the glomerulus and the early distal tubule were calculated as follows:

$$AR\text{-fluid} = SNGFR - V_{ED}$$

$$FR\text{-fluid} = AR\text{-fluid}/SNGFR$$

$$AR\text{-Na}^+ = SNGFR \times [Na^+]_{plasma} - V_{ED} \times [Na^+]_{ED}$$

$$FR\text{-Na}^+ = AR\text{-Na}^+ / (SNGFR \times [Na^+]_{plasma})$$

with  $[Na^+]_{plasma}$  being the concentration of Na<sup>+</sup> in plasma. Calculations for reabsorption of K<sup>+</sup> or Cl<sup>-</sup> were performed accordingly.

### Series 2: Hydrostatic Pressures

Hydrostatic pressures were directly measured in Bowman space ( $P_{Bow}$ ) and efferent arterioles ( $P_E$ ) with a servo-nulling pressure device (World Precision Instruments, New Haven, CT), using a micropipette filled with hypertonic saline (1.5 M). Stop-flow pressure ( $P_{SF}$ ) was determined by placing the pressure pipette into Bowman space and using an obstructing oil block into the first superficial loop of the proximal tubule. Systemic arterial blood was sampled from the femoral artery catheter, and the plasma protein concentration was measured by the Lowry technique (10). The results of the latter were used to calculate plasma oncotic pressure ( $\pi_A$ ) from the Landis-Pappenheimer equation (11). Glomerular capillary pressure ( $P_{GC}$ ) was calculated as  $P_{GC} = P_{SF} + \pi_A$ . The effective afferent filtration pressure ( $EFP_A$ ) was calculated for the single nephron as  $EFP_A = P_{GC} - \pi_A - P_{Bow}$ .  $P_{GC}$  was calculated as described instead of being directly measured to increase the number of measurements in each rat. In addition, we recently observed that there is no significant difference in directly measured  $P_{GC}$  values and those calculated from SFP measurements in control or diabetic rats (12). These results were confirmed in an initial series of experiments in the rats used in the present study in which the following values were obtained for directly measured  $P_{GC}$  versus calculated  $P_{GC}$ : control ( $n = 7$ )  $50.4 \pm 2.6$  versus  $51.0 \pm 2.9$  mmHg, NS by paired  $t$  test; diabetes ( $n = 8$ )  $49.9 \pm 2.8$  versus  $50.6 \pm 3.0$  mmHg, NS.

This finding at first might seem unexpected because to measure SFP, an oil block is placed into the proximal tubule, which will lower the luminal TGF signal at the macula densa and increase SNGFR, which could involve a rise in  $P_{GC}$ , and therefore SFP. Thus, one might expect calculated  $P_{GC}$  which considers values for SFP to be higher than directly measured  $P_{GC}$ . In accordance with the present results, however, the literature strongly supports the fact that  $P_{GC}$  does not, in general, rise in any persistent, steady-state manner with acute reductions in distal tubular NaCl delivery (13–15). The increase in SNGFR in response to a reduction in the ambient luminal signal at the macula densa is the result of a reduction in vascular tone of both the afferent and the efferent arteriole, which increases plasma flow and thus SNGFR without a significant change in  $P_{GC}$  (13–15).

### Series 3: Effect of Phlorizin, an Inhibitor of Na<sup>+</sup>/Glucose Cotransport, on Tubular Reabsorption, Early Distal Tubular Electrolyte Concentration, and SNGFR

After identification of distal tubular configuration, a collecting pipette (7- to 9-μm tip) was inserted into the first accessible distal tubular loop to perform a timed collection of tubular fluid (3 to 4 min in duration) under free-flow conditions using a short mineral oil block. Subsequently, a perfusion pipette connected to a microperfusion pump and filled with ATF (see above) containing phlorizin (100 μM) was inserted into the first surface loop of the proximal convoluted tubule to deliver 5 nl/min. To dissolve phlorizin, 1% of ethanol was added to

ATF. After 5 min, the early distal tubule was punctured for recollection. Timed early distal tubular fluid collections were analyzed for the parameters described in Series 1. During application of 5 nl/min into the first surface loop of the proximal tubule, fractional reabsorption of fluid (FR-fluid) or Na<sup>+</sup>, K<sup>+</sup>, or Cl<sup>-</sup> (FR-Na<sup>+</sup>, FR-K<sup>+</sup> or FR-Cl<sup>-</sup>) between the glomerulus and the early distal tubule were calculated as follows:

$$\text{FR-fluid} = (\text{SNGFR} + 5\text{nl/min} - V_{\text{ED}}) / (\text{SNGFR} + 5\text{nl/min})$$

$$\text{FR-Na}^+ = (\text{SNGFR} \times [\text{Na}^+]_{\text{plasma}} + 5\text{nl/min} \times 140\text{mM} - V_{\text{ED}} \times [\text{Na}^+]_{\text{ED}}) / (\text{SNGFR} \times [\text{Na}^+]_{\text{plasma}} + 5\text{nl/min} \times 140\text{mM})$$

Calculations for FR-K<sup>+</sup> or FR-Cl<sup>-</sup> were performed accordingly with concentrations of K<sup>+</sup> and Cl<sup>-</sup> in ATF being 4 and 121 mM, respectively.

#### Series 4: Effect of Phlorizin on Hydrostatic Pressure in Bowman Space

After identification of proximal tubular configuration,  $P_{\text{Bow}}$  was measured under free-flow conditions as described above. Subsequently, a perfusion pipette connected to a microperfusion pump (Hampel, Neu-Isenburg, Germany) and filled with ATF containing phlorizin (100 μM) was inserted into the first surface loop of the proximal convoluted tubule to deliver 5 nl/min.  $P_{\text{Bow}}$  was recorded continuously for the next 5 min.

#### Analytical Methods

Urinary flow rate was determined gravimetrically. Plasma concentrations of sodium and potassium ion were measured by flame photometry (ELEX 6361; Eppendorf, Hamburg). Plasma concentration of chloride was measured by electrometric titration (Chloridometer 6610; Eppendorf). GFR<sub>LK</sub> and SNGFR were determined by <sup>3</sup>H-inulin clearance. Early distal tubular flow rate ( $V_{\text{ED}}$ ) was determined after transferring the tubular fluid collections to a constant bore capillary.  $[\text{Na}^+]_{\text{ED}}$  and  $[\text{K}^+]_{\text{ED}}$  were determined as described previously (6), using a micro flame photometer that was developed and built by Rolf Englert and Klaus Stieler (Department of Pharmacology, University of Tübingen) based on the original concept by Hampel (Frankfurt/Main, Germany).  $[\text{Cl}^-]_{\text{ED}}$  was measured with a micro adaptation of the electrometric titration method. The equipment for the latter (Microtitre ET-1; World Precision Instruments, Sarasota, FL) was kindly placed at our disposal by D. Häberle (Department of Physiology, University of Munich). Concentration of <sup>3</sup>H-inulin in plasma, tubular collections, and urine was measured by liquid phase scintillation counting.

#### Statistical Analyses

Unpaired *t* test was performed to analyze for statistical differences in diabetic compared with control rats. After establishing by ANOVA analysis that there were no significant differences between rats within an experimental group (control or diabetic), data of single nephrons of experimental groups were pooled and subsequently compared for significant differences between experimental groups. *P* < 0.05 was considered statistically significant.

### Results

#### Systemic and Whole Kidney Data

As depicted in Table 1, diabetic rats exhibited moderate hyperglycemia and a lower body weight without a significant change in mean arterial BP or arterial hematocrit compared

with control rats. Plasma concentrations of sodium or chloride ion were not significantly altered, whereas plasma potassium ion concentration was slightly lower in diabetic animals. Diabetic rats exhibited an increase in GFR (by approximately 27%), urinary flow rate, and wet weight of the left kidney.

#### Series 1: SNGFR, Tubular Reabsorption, and Early Distal Tubular Electrolyte Concentration

SNGFR was increased in diabetic compared with control rats by approximately 19% (Figure 1). Absolute and fractional tubular reabsorption of fluid up to the early distal tubule (AR-fluid, FR-fluid) were increased in diabetic compared with control rats (Table 2, Figure 1) such that early distal tubular flow rate ( $V_{\text{ED}}$ ) was not significantly different between diabetic and control rats (10.3 ± 0.7 versus 11.3 ± 0.7 nl/min, NS). Besides the increase in fluid reabsorption, diabetic rats also exhibited a significant rise in absolute and fractional sodium, chloride, and potassium ion reabsorption in the tubular segments upstream to the early distal tubule (Table 2, Figure 1) such that the concentrations in early distal tubular fluid (Figure 1) as well as the total distal tubular delivery of all three ions (Table 2) were significantly lower in diabetic compared with control rats.

#### Series 2: Hydrostatic Pressures

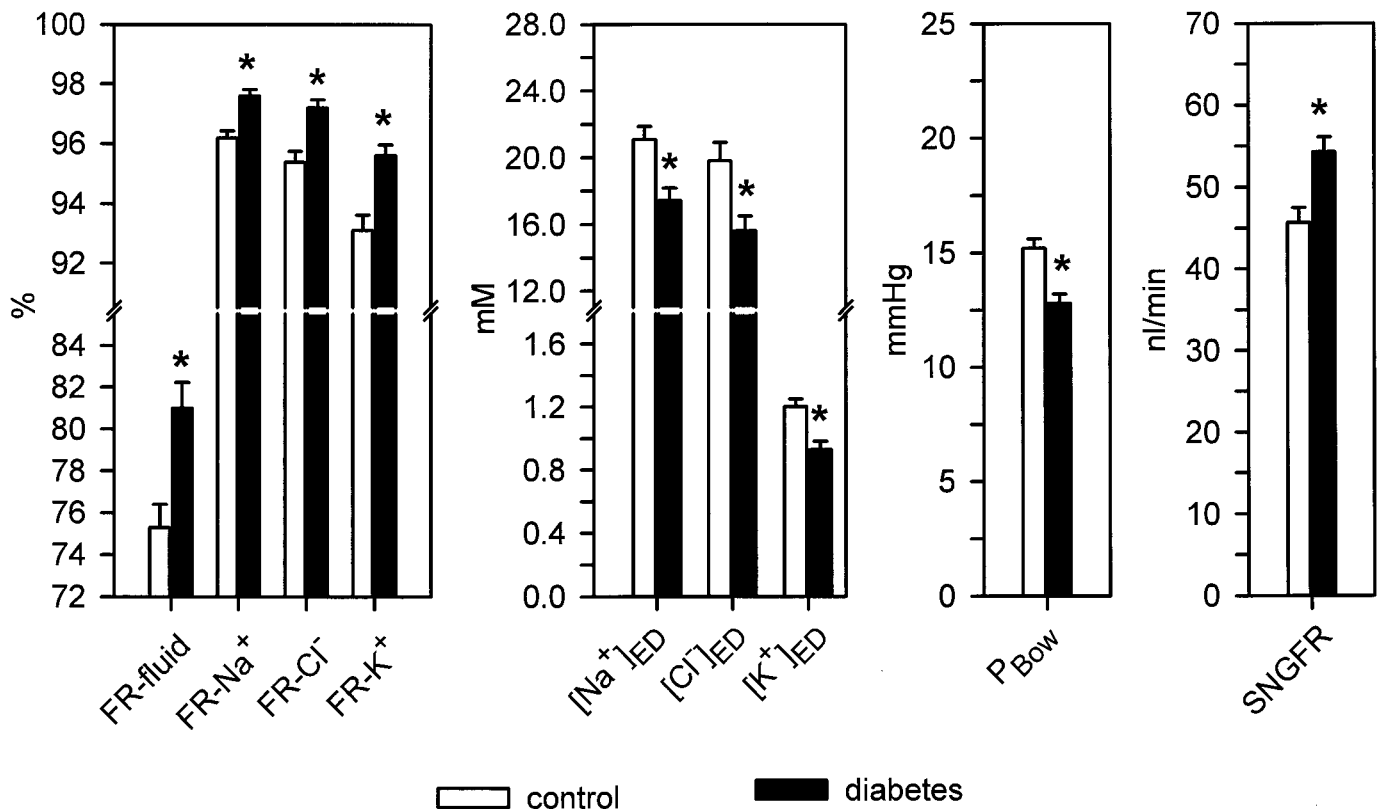
Measurements were obtained in 17 to 20 tubules or peritubular capillaries of five control or six diabetic rats. No significant differences were observed in glomerular capillary pressure ( $P_{\text{GC}}$ ) or efferent arterioles ( $P_{\text{E}}$ ) between diabetic and control rats ( $P_{\text{GC}}$ : 50 ± 1 versus 51 ± 1 mmHg, NS;  $P_{\text{E}}$ : 13.1 ± 0.4 versus 13.4 ± 0.3 mmHg, NS). Diabetic rats exhibited an increase in effective afferent filtration pressure

Table 1. Systemic and whole kidney data in control and diabetic rats<sup>a</sup>

Parameter	Control (n = 11)	Diabetes Mellitus (n = 10)
Body weight (g)	347 ± 10	308 ± 7 <sup>b</sup>
Blood glucose level (mg/dl)	101 ± 7	335 ± 11 <sup>b</sup>
Mean arterial pressure (mmHg)	123 ± 4	111 ± 4
Arterial hematocrit (%)	52 ± 1	50 ± 1
$[\text{Na}^+]_{\text{plasma}}$ (mM)	141 ± 1	140 ± 1
$[\text{Cl}^-]_{\text{plasma}}$ (mM)	106 ± 2	103 ± 2
$[\text{K}^+]_{\text{plasma}}$ (mM)	4.5 ± 0.1	4.2 ± 0.1 <sup>b</sup>
GFR left kidney (ml/min)	1.11 ± 0.05	1.41 ± 0.06 <sup>b</sup>
Urinary flow rate (μl/min)	2.7 ± 0.2	13.2 ± 2.5 <sup>b</sup>
Left kidney weight (g)	1.06 ± 0.03	1.16 ± 0.02 <sup>b</sup>

<sup>a</sup> Data are mean ± SEM. *n* = number of rats.

<sup>b</sup> *P* < 0.05 versus control.



**Figure 1.** Fractional reabsorption between the glomerulus and the early distal tubule of fluid and electrolyte (FR-fluid, FR-Na<sup>+</sup>, FR-Cl<sup>-</sup>, FR-K<sup>+</sup>), early distal tubular electrolyte concentration ([Na<sup>+</sup>]<sub>ED</sub>, [Cl<sup>-</sup>]<sub>ED</sub>, [K<sup>+</sup>]<sub>ED</sub>), hydrostatic pressure in Bowman space ( $P_{Bow}$ ), and single nephron GFR (SNGFR) in control and diabetic rats. Data are mean  $\pm$  SEM.  $n = 20$  tubules in five control and six diabetic rats for  $P_{Bow}$ ;  $n = 30$  nephrons in five control rats and 31 nephrons in six diabetic rats for the other parameters. \* $P < 0.05$  versus control. Notice that the glomerular hyperfiltration in diabetic rats was associated with an increased fractional fluid and electrolyte reabsorption, a reduced early distal tubular electrolyte concentration, and a reduction in  $P_{Bow}$ .

**Table 2.** Absolute reabsorption (AR) upstream to the early distal tubule as well as delivery (D) to the early distal tubule of fluid and electrolyte in control and diabetic rats<sup>a</sup>

Variable	Control ( $n = 30/5$ )	Diabetes Mellitus ( $n = 31/6$ )
AR-fluid (nl/min)	$34 \pm 1$	$44 \pm 2^b$
AR-Na <sup>+</sup> (nmol/min)	$6.1 \pm 0.3$	$7.3 \pm 0.2^b$
AR-Cl <sup>-</sup> (nmol/min)	$4.7 \pm 0.2$	$5.6 \pm 0.2^b$
AR-K <sup>+</sup> (pmol/min)	$186 \pm 7$	$206 \pm 7^b$
D-Na <sup>+</sup> (pmol/min)	$241 \pm 18$	$183 \pm 17^b$
D-Cl <sup>-</sup> (pmol/min)	$228 \pm 20$	$162 \pm 16^b$
D-K <sup>+</sup> (pmol/min)	$14 \pm 1$	$10 \pm 1^b$

<sup>a</sup> Data are mean  $\pm$  SEM.  $n =$  number of nephrons/number of rats.

<sup>b</sup>  $P < 0.05$  versus control.

(EFP<sub>A</sub>:  $23.6 \pm 0.9$  versus  $20.2 \pm 0.7$  mmHg,  $P < 0.05$ ) that was associated with a fall in the hydrostatic pressure in Bowman space ( $P_{Bow}$ ) (Figure 1).

### Series 3: Effect of Phlorizin on Tubular Reabsorption, Early Distal Tubular Electrolyte Concentration, and SNGFR

As in Series 1, in this set of experiments, diabetic rats during basal measurements exhibited a significant increase in fractional reabsorption of fluid and electrolyte, a reduction in early distal tubular electrolyte concentration, as well as an increase in SNGFR compared with control rats (Table 3). In diabetic rats, application of phlorizin into the first superficial loop of the proximal tubule under free-flow conditions elicited (1) a greater reduction in absolute and fractional reabsorption of fluid and Na<sup>+</sup>, Cl<sup>-</sup>, or K<sup>+</sup> up to the early distal tubule, and (2) a greater increase in early distal tubular concentrations of all three ions, which was associated with a more pronounced fall in SNGFR (Figure 2, Table 3).

### Series 4: Effect of Phlorizin on the Hydrostatic Pressure in Proximal Tubule

As in Series 2, in this set of experiments diabetic rats during basal measurements exhibited a significant reduction in  $P_{Bow}$  ( $13.0 \pm 0.3$  versus  $14.8 \pm 0.5$  mmHg,  $P < 0.05$ ). Application of phlorizin into the first superficial loop of the proximal tubule under free-flow conditions elicited a larger increase in  $P_{Bow}$  in diabetic compared with control rats (Figure 2). Intratubular

**Table 3.** Effect of phlorizin on fractional and absolute reabsorption (FR, AR) of fluid and electrolyte upstream to the early distal tubule, early distal tubular concentrations of electrolyte ( $[ ]_{ED}$ ), early distal tubular delivery of fluid ( $V_{ED}$ ) and electrolyte (D), as well as SNGFR in control and diabetic rats<sup>a</sup>

Variable	Control ( <i>n</i> = 12/4)		Diabetes Mellitus ( <i>n</i> = 15/4)	
	Basal/+ Phlorizin	Δ	Basal/+ Phlorizin	Δ
FR-fluid (%)	79 ± 2/68 ± 2	-11 ± 2	84 ± 2 <sup>b</sup> /64 ± 4	-21 ± 2 <sup>c</sup>
FR-Na <sup>+</sup> (%)	97 ± 1/95 ± 1	-2 ± 1	99 ± 1 <sup>b</sup> /94 ± 1	-5 ± 1 <sup>c</sup>
FR-Cl <sup>-</sup> (%)	96 ± 1/92 ± 1	-4 ± 1	98 ± 1 <sup>b</sup> /92 ± 1	-7 ± 1 <sup>c</sup>
FR-K <sup>+</sup> (%)	95 ± 1/94 ± 1	-1 ± 1	97 ± 1 <sup>b</sup> /91 ± 2	-6 ± 1 <sup>c</sup>
AR-fluid (nl/min)	33 ± 1/30 ± 2	-3 ± 1	44 ± 2 <sup>b</sup> /26 ± 2	-18 ± 3 <sup>c</sup>
AR-Na <sup>+</sup> (nmol/min)	5.9 ± 0.3/6.1 ± 0.3	0.1 ± 0.2	7.1 ± 0.2 <sup>b</sup> /5.3 ± 0.3	-1.8 ± 0.4 <sup>c</sup>
AR-Cl <sup>-</sup> (nmol/min)	4.2 ± 0.2/4.3 ± 0.2	0.1 ± 0.1	5.3 ± 0.2 <sup>b</sup> /4.0 ± 0.2	-1.4 ± 0.3 <sup>c</sup>
AR-K <sup>+</sup> (pmol/min)	192 ± 11/195 ± 10	2 ± 6	216 ± 7 <sup>b</sup> /155 ± 9	-60 ± 12 <sup>c</sup>
$V_{ED}$ (nl/min)	9 ± 1/14 ± 1	5 ± 1	8 ± 1/15 ± 1	7 ± 1 <sup>c</sup>
$[Na^+]_{ED}$ (mM)	21 ± 1/23 ± 2	2 ± 1	15 ± 2 <sup>b</sup> /25 ± 2	10 ± 2 <sup>c</sup>
$[Cl^-]_{ED}$ (mM)	20 ± 1/26 ± 1	6 ± 1	16 ± 3 <sup>b</sup> /25 ± 3	9 ± 2 <sup>c</sup>
$[K^+]_{ED}$ (mM)	1.2 ± 0.1/1.0 ± 0.1	-0.2 ± 0.1	1.0 ± 0.1 <sup>b</sup> /1.1 ± 0.1	0.1 ± 0.1 <sup>c</sup>
D-Na <sup>+</sup> (pmol/min)	193 ± 24/332 ± 40	139 ± 26	113 ± 21 <sup>b</sup> /361 ± 35	247 ± 31 <sup>c</sup>
D-Cl <sup>-</sup> (pmol/min)	184 ± 17/383 ± 37	199 ± 19	106 ± 13 <sup>b</sup> /353 ± 35	246 ± 21 <sup>c</sup>
D-K <sup>+</sup> (pmol/min)	11 ± 1/14 ± 1	3 ± 1	8 ± 1 <sup>b</sup> /17 ± 3	9 ± 2 <sup>c</sup>
SNGFR (nl/min)	43 ± 2/39 ± 2	-4 ± 1	51 ± 2 <sup>b</sup> /35 ± 2	-17 ± 3 <sup>c</sup>

<sup>a</sup> Data are mean ± SEM. *n* = number of nephrons/number of rats. Δ, absolute change.

<sup>b</sup> *P* < 0.05 versus control under basal conditions.

<sup>c</sup> *P* < 0.05 versus control.

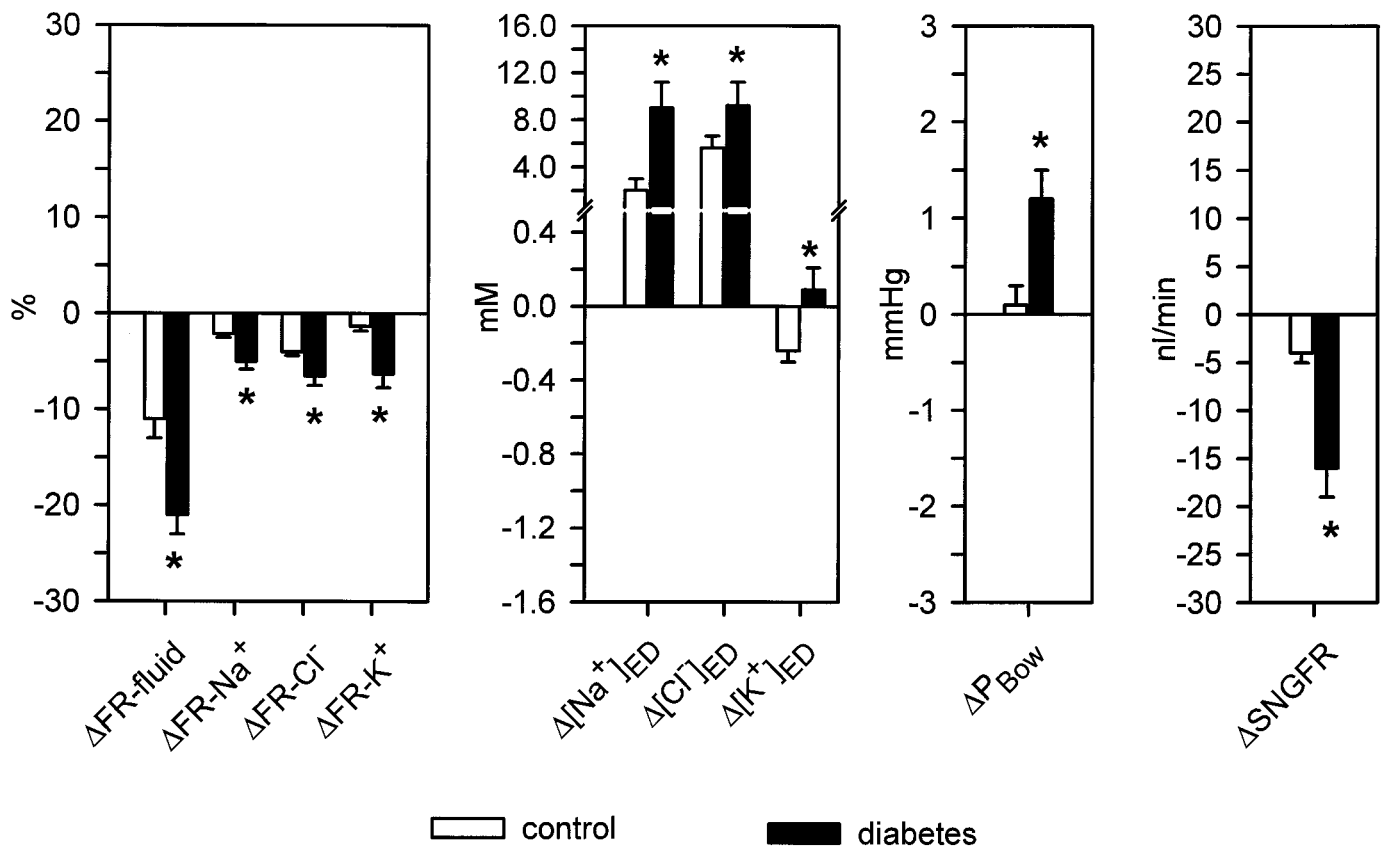
application of vehicle did not significantly alter  $P_{Bow}$  in control or diabetic rats (not shown).

## Discussion

The results of these experiments show that the fractional tubular reabsorption of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> is significantly increased upstream to the early distal tubule in diabetic rats, such that the early distal tubular concentration as well as delivery of all three ions are reduced in diabetic compared with control rats. The finding in diabetic rats of a reduction in the early distal tubular electrolyte concentration in association with glomerular hyperfiltration may implicate a role of the TGF under these conditions. The TGF, which refers to the inverse relationship between the luminal concentration of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> at the macula densa and the SNGFR (4–6), serves to stabilize the electrolyte delivery to the distal tubule. In this regard, the present findings would be in accordance with the following concept: A primary increase in fractional tubular reabsorption lowers the concentration of Na<sup>+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> at the macula densa and elicits a TGF-dependent increase in SNGFR in diabetic rats. The latter compensates at least in part for the increased fractional reabsorption to stabilize the electrolyte delivery to the distal nephron (Figure 3). This concept is further supported by previous studies showing that fractional Na<sup>+</sup> reabsorption in the proximal tubule (1) is increased in insulin-dependent diabetic patients, and (2) is positively correlated with GFR in healthy subjects as well as in insulin-dependent diabetic patients (7). A substantial role of TGF in

glomerular hyperfiltration in diabetes mellitus is also in accordance with studies showing that acute hyperglycemia in dogs causes an increase in GFR under conditions with intact TGF, whereas nonfiltering kidneys in which TGF is blocked still respond with vasodilation to application of acetylcholine, but do not exhibit glomerular hyperfiltration in response to hyperglycemia (16). Similar findings, namely a reduction in early distal tubular electrolyte concentration in association with glomerular hyperfiltration, have been observed in rats fed a high protein diet. Also, under those conditions, a TGF-dependent increase in SNGFR was proposed (17).

The reduced electrolyte concentration in early distal tubular fluid in diabetic rats was caused by an increase in fractional reabsorption upstream to this nephron site. Previous studies have also suggested that fractional tubular reabsorption of fluid and electrolyte is increased in early insulin-dependent diabetes mellitus in humans (7), as well as in rats with streptozotocin-induced diabetes mellitus (8,9). Furthermore, microperfusion studies by Bank and Aynedjian proposed that the stimulation of Na<sup>+</sup> absorption by glucose in the proximal tubule is mediated by the Na<sup>+</sup>/glucose cotransporter in the brush border (8). To test for a role of the Na<sup>+</sup>/glucose cotransporter in the increased tubular reabsorption, the reduced electrolyte concentration in early distal tubular fluid, and glomerular hyperfiltration in diabetic rats, we applied phlorizin, an inhibitor of Na<sup>+</sup>/glucose cotransport, into the early proximal tubule of control and diabetic rats. It was observed that in diabetic rats, application of phlorizin elicited (1) a greater reduction in absolute and

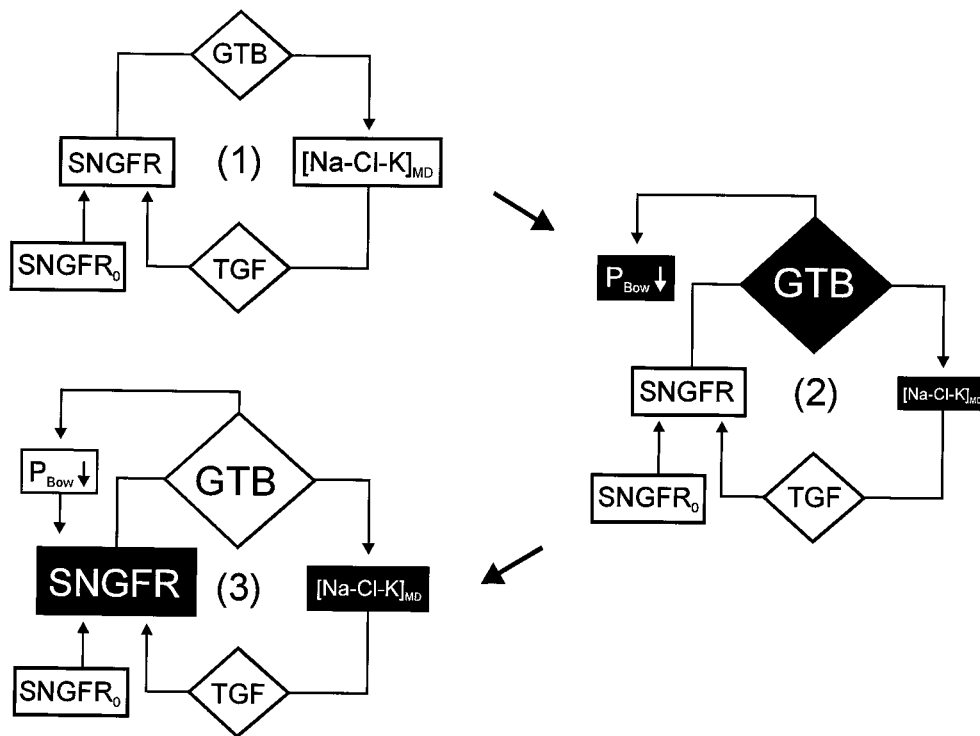


**Figure 2.** Change in fractional reabsorption between the glomerulus and the early distal tubule of fluid and electrolyte ( $\Delta\text{FR-fluid}$ ,  $\Delta\text{FR-Na}^+$ ,  $\Delta\text{FR-Cl}^-$ ,  $\Delta\text{FR-K}^+$ ), early distal tubular electrolyte concentration ( $\Delta[\text{Na}^+]_{\text{ED}}$ ,  $\Delta[\text{Cl}^-]_{\text{ED}}$ ,  $\Delta[\text{K}^+]_{\text{ED}}$ ), hydrostatic pressure in Bowman space ( $\Delta P_{\text{Bow}}$ ), and SNGFR ( $\Delta\text{SNGFR}$ ) in control and diabetic rats in response to early proximal tubular application of phlorizin. Data are mean  $\pm$  SEM.  $n = 6$  nephrons in three rats each group for  $P_{\text{Bow}}$ ;  $n = 12$  nephrons in four control rats and 15 nephrons in four diabetic rats for the remaining parameters; \* $P < 0.05$  versus control. Notice that the more pronounced fall in SNGFR in diabetic rats in response to application of phlorizin was associated with a greater reduction in fractional fluid and electrolyte reabsorption and a greater rise in early distal tubular electrolyte concentration as well as  $P_{\text{Bow}}$ .

fractional reabsorption of fluid and  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{K}^+$  up to the early distal tubule, and (2) a larger increase in early distal tubular concentrations of these three ions. This response was associated with a much more pronounced fall in SNGFR in diabetic compared with control rats. The significant fall in SNGFR in response to phlorizin in diabetic rats most likely reflected a more pronounced acute TGF activation. The latter resulted from a greater increase in early distal tubular electrolyte concentration due to a greater dependence of proximal tubular reabsorption on  $\text{Na}^+$ /glucose cotransport in these rats. An increased TGF sensitivity is less likely to be involved in the above response because, as shown previously, the slope of the TGF function at the operating point may actually be somewhat reduced in rats with established STZ-diabetes mellitus (9). These findings support the concept that the increase in fractional tubular reabsorption upstream to the early distal tubule in diabetic rats involved stimulation of  $\text{Na}^+$ /glucose cotransport and that the resulting reduction in the TGF signal at the macula densa may contribute to the rise in SNGFR in these rats (Figure 3).

Although the predominant reason for glomerular hyperfiltration in diabetes mellitus most likely is an alteration in the

control of vascular tone, the present experiments propose that a reduction in the hydrostatic pressure in Bowman space ( $P_{\text{Bow}}$ ) could, probably to a minor degree, also contribute to glomerular hyperfiltration in diabetic rats by increasing the effective filtration pressure. Evidence to suggest that  $P_{\text{Bow}}$  or the hydrostatic pressure in proximal tubule is reduced in experimental diabetes mellitus has been published before (18–21). Furthermore, we observed that acute early proximal tubular application of phlorizin elicited a more pronounced increase in  $P_{\text{Bow}}$  in diabetic rats compared with control rats. Like the above-mentioned response in SNGFR, the rise in  $P_{\text{Bow}}$  in diabetic rats in response to phlorizin may reflect the greater dependence of proximal tubular reabsorption on  $\text{Na}^+$ /glucose cotransport in these rats. The resulting increased fluid and electrolyte load in diabetic rats to the further distal nephron segments, which have a high flow resistance, is expected to increase  $P_{\text{Bow}}$  as indicated by studies of Leyssac (22,23). Conversely, an increase in fractional tubular reabsorption may have contributed to the initial fall in  $P_{\text{Bow}}$  in diabetic rats by lowering distal tubular fluid delivery. As observed in the present study, however, the increase in SNGFR in diabetic rats normalized the fluid delivery to the distal nephron. Because an



**Figure 3.** Proposed role of tubular reabsorption in glomerular hyperfiltration in diabetes mellitus. As illustrated in (1), the tubuloglomerular feedback (TGF) refers to the inverse dependency of SNGFR on the luminal  $Na^+$ ,  $Cl^-$ , and  $K^+$  concentration at the macula densa (MD). The glomerulotubular balance (GTB) refers to the flow dependence of tubular reabsorption upstream to the macula densa.  $SNGFR_0$  is the input to SNGFR independent of TGF. A primary increase in fractional tubular reabsorption (GTB) in diabetes mellitus elicits a reduction in the TGF signal at the macula densa (2), which increases SNGFR (3). The increase in fractional tubular reabsorption may in addition reduce the hydrostatic pressure in Bowman space ( $P_{Bow}$ ) (2). By increasing the effective filtration pressure, the latter changes may also increase SNGFR, although probably to a minor degree (3). The resulting increase in SNGFR serves to partly restore the fluid and electrolyte load to the distal nephron (3). The concomitant prolonged glomerular hyperperfusion, however, could contribute to the development of diabetic glomerulosclerosis.

increased fractional tubular reabsorption is thought to lower  $P_{Bow}$  by reducing the fluid load to the distal nephron (22,23), other mechanisms, which remain to be determined, have to contribute to the reduced  $P_{Bow}$  in diabetic rats with glomerular hyperfiltration and reestablished distal tubular flow rate.

A critical role of tubular reabsorption in the control of renal hemodynamics in diabetes mellitus could also be reflected by the paradoxical renal response to variation in NaCl intake under these conditions: whereas NaCl restriction has been shown to elicit renal vasodilation and a further increase in GFR in experimental diabetes mellitus (24) as well as in young patients with uncomplicated early insulin-dependent diabetes mellitus (25), chronic NaCl loading was shown to normalize GFR in early experimental diabetes mellitus (26). Thus, dietary NaCl restriction may paradoxically aggravate glomerular hyperfiltration in early diabetes mellitus because this maneuver refers to a basic mechanism of diabetic glomerular hyperfiltration, *i.e.*, increased tubular reabsorption.

In summary, the present findings suggest that the increase in fractional tubular reabsorption upstream to the early distal tubule as observed in diabetic rats involves stimulation of  $Na^+$ /glucose cotransport. Evidence is provided that the resulting reduction in the TGF signal at the macula densa may

contribute to the rise in SNGFR in diabetic rats. The increase in SNGFR serves to compensate for the increase in fractional tubular reabsorption to partly restore the fluid and electrolyte load to the distal nephron.

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