Regulation of renal NaPi-2 expression and tubular phosphate reabsorption by growth hormone in the juvenile rat

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Woda, Craig B., Nabil Halaihel, Paul V. Wilson, Aviad Haramati, Moshe Levi, and Susan E. Mulroney. Regulation of renal NaPi-2 expression and tubular phosphate reabsorption by growth hormone in the juvenile rat. Am J Physiol Renal Physiol 287: F117–F123, 2004. First published March 2, 2004; 10.1152/ajprenal.00357.2002.—Growth hormone (GH) is an important factor in the developmental adaptation to enhance Pi reabsorption; however, the nephron sites and mechanisms by which GH regulates renal Pi uptake remain unclear and are the focus of the present study. Micropuncture experiments were performed after acute thyroparathyroidectomy in the presence and absence of parathyroid hormone (PTH) in adult (14- to 17-wk old), juvenile (4-wk old), and GH-suppressed juvenile male rats. While the phosphaturic effect of PTH was blunted in the juvenile rat compared with the adult, suppression of GH in the juvenile restored fractional Pi excretion to adult levels. In the presence or absence of PTH, GH suppression in the juvenile rat caused a significant increase in the fractional Pi delivery to the late proximal convoluted (PCT) and early distal tubule, so that delivery was not different from that in adults. These data were confirmed by Pi uptake studies into brush-border membrane (BBM) vesicles. Immunofluorescence studies indicate increased BBM type IIa NaPi cotransporter (NaPi-2) expression in the juvenile compared with adult rat, and GH suppression reduced NaPi-2 expression to levels observed in the adult. GH replacement in the [N-acetyl-Tyr1-D-Arg2]-GRF-(1-29)-NH2-treated juveniles restored high NaPi-2 expression and Pi uptake. Together, these novel results demonstrate that the presence of GH in the juvenile animal is crucial for the early developmental upregulation of BBM NaPi-2 and, most importantly, describe the enhanced Pi reabsorption along the PCT and proximal straight nephron segments in the juvenile rat.

development: sodium-phosphate cotransporters; parathyroid hormone; antagonist to growth hormone-releasing factor; brush-border membrane vesicles

DURING STATES OF HIGH Pi demand, such as growth, the kidneys play a central role in maintaining a positive phosphate (Pi) balance. This occurs through enhanced tubular Pi reabsorption (20, 21, 29) and a blunted response to the phosphaturic effect of parathyroid hormone (PTH) (8, 21, 36). In the adult animal, renal Pi uptake has been shown to depend mainly on type IIa NaPi cotransporters (NaPi-2) (12, 24–26), which are regulated by PTH (23), dietary Pi content (23, 25, 31), vitamin D (26, 33), and thyroid hormone (1), factors that are traditionally known to alter Pi reabsorption.

However, whether regulation of type IIa NaPi transporters is an important mechanism in the juvenile animal is unknown. Recently, we reported that enhanced renal Pi reabsorption in the proximal convoluted and proximal straight nephron segments was responsible for reclaiming the bulk of filtered Pi in the juvenile rat and also accounted for the attenuated response to the phosphaturic effects of PTH (37). In addition, this enhanced proximal tubular Pi uptake in the juvenile rat was associated with a significant increase in type IIa NaPi protein expression in the proximal tubular apical brush-border membrane (BBM) (37). However, the factor(s) responsible for the increases in type IIa NaPi protein expression and Pi reabsorption in the juvenile rat remains unclear.

Growth hormone (GH) plays a central role during the growth process and has been shown to be a factor that increases the renal uptake of Pi. When GH is elevated, or administered on a chronic basis to adult humans or adult animals, there is a reduction in urinary Pi excretion and elevation in plasma Pi levels (3, 9–11, 16). Conversely, removal of GH in the adult rat (through hypophysectomy) causes a significant decline in the maximum capacity to reabsorb Pi (TmPi) by the whole kidney and results in increased phosphaturia (4). However, this effect could not be attributed solely to GH because all pituitary hormones were removed. Furthermore, Hammerman et al. (16) reported that the effects of pharmacological doses of GH result in a selective stimulation of proximal tubular BBM NaPi transport systems.

Mulroney et al. (27, 28), using a peptidic antagonist to GH-releasing factor, [N-acetyl-Tyr1-D-Arg2]-GRF-(1-29)-NH2 (GRF-AN), to suppress the pulsatile release of GH from the anterior pituitary, determined the physiological role of GH in Pi homeostasis. Administration of GRF-AN to juvenile animals for 2 days led to doubling of the urinary excretion of Pi and attenuation of somatic body growth (19). These effects were attributed to a decrease in the TmPi (19, 27) and highlighted an important interrelationship among GH, growth, and the renal reabsorption of Pi. Interestingly, short-term GRF-AN treatment of adult rats had no effect on lowering the TmPi and increasing urinary Pi excretion (19, 27). In addition, our laboratory has demonstrated that GRF-AN treatment for 48 h is associated with a 30% reduction in the Vmax of Pi transport in proximal tubular BBM vesicles prepared from weanling rats, implicating GH.

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in proximal tubule Pi uptake. However, the nephron sites of GH action and the effect of GH on type IIA NaPi transport expression in the proximal convoluted tubule (PCT) and proximal straight segments in the juvenile rat are unknown.

The present micropuncture and renal cortical BBM Na gradient-dependent Pi (NaPi) uptake studies were performed to determine the nephron sites of action of GH on renal tubular Pi reabsorption seen in juvenile rats. In addition, using Western blot analysis and immunofluorescence microscopy, we assessed the role of GH in regulating the expression of proximal tubular BBM type IIA NaPi transporters in the juvenile rat.

MATERIALS AND METHODS

Animal models. To explore the role of GH on the nephron sites responsible for the increased renal phosphate reabsorption seen during growth, micropuncture studies were performed in adult (14- to 17-wk old) and juvenile (4-wk old) male Wistar rats. The rats were fed a normal-phosphate diet (0.7% Pi) and allowed access to diet and water ad libitum. In juvenile rats, suppression of the pulsatile release of GH was achieved through intravenous administration of the GH-releasing hormone antagonist GRF-AN (Bachem, Torrance, CA) at a dose of 100 μg/kg twice daily. Previous studies in this laboratory have demonstrated this dosage to be effective in completely blocking pulsatile GH release (19, 27, 28).

Briefly, juvenile rats were anesthetized with Nembutal (0.1 ml/100 g ip), and a Silastic catheter (inner diameter, 0.020 in.; outer diameter, 0.037 in.; Dow Corning, Medfield, MA) filled with 500 U/ml heparinized saline was placed into the left jugular vein. Patency of the catheter was maintained with 200 μl of 500 U/ml heparinized saline daily. The animals were housed in separate cages and injected twice daily at 0900 and 1300 for 2 days with saline or GRF-AN. Body weight was measured throughout the experimental protocol, and the reduced growth rate was used as an indicator of the suppression of pulsatile GH release.

The acquisition of the data for the three experimental groups was performed in parallel. The data for the adult and juvenile rats have been previously published (37). This manuscript includes parts of the previously published data as controls to contrast the effects of GH suppression and replacement on the regulation of renal tubular reabsorption of Pi in the juvenile rat.

In vivo micropuncture studies. On the day of the micropuncture experiments, the animals were anesthetized with an intraperitoneal injection of Inactin (80 mg/kg; Promonta, Hamburg, Germany) and placed on a heated table. Body temperature was maintained at 37°C. The animals were anesthetized with Nembutal (0.1 ml/100 g ip), and a Silastic catheter (inner diameter, 0.020 in.; outer diameter, 0.037 in.; Dow Corning, Medfield, MA) filled with 500 U/ml heparinized saline was placed into the left jugular vein. Patency of the catheter was maintained with 200 μl of 500 U/ml heparinized saline daily. The animals were housed in separate cages and injected twice daily at 0900 and 1300 for 2 days with saline or GRF-AN. Body weight was measured throughout the experimental protocol, and the reduced growth rate was used as an indicator of the suppression of pulsatile GH release.

GBM isolation. Intact and GRF-AN-treated juvenile rats were anesthetized via an injection of pentothal sodium (100 mg/kg ip), and the kidneys were removed for GBM isolation. The superficial cortex (SC) and outer juxtamedullary cortex (JMC) were dissected and homogenized in 15 ml of an isolation buffer consisting of (in mM) 300 mannitol, 5 EGTA, 1 PMSF, 16 HEPES, and 10 Tris, pH 7.5. GBM from both regions were isolated from the homogenate by Mg2+ precipitation and differential centrifugation as described previously (24, 26). The resulting GBM pellet was resuspended in a buffer of (in mM) 300 mannitol, 16 HEPES, and 10 Tris, pH 7.5, and aliquoted for NaPi transport measurements and Western blotting.

GBM phosphate transport activity measurements. Phosphate transport activity measurements were performed in freshly isolated SC-GBM and JMC-GBM vesicles by radiotracer uptake followed by rapid Millipore filtration. To measure Na gradient-dependent 32Pi uptake (NaPi cotransport), 10 μl of BBM preloaded in an intravesicular buffer comprising (in mM) 300 mannitol, 16 HEPES, and 10 Tris, pH 7.5, were vortexed at 25°C with 40 μl of an extravesicular transport buffer consisting of (in mM) 150 NaCl, 16 HEPES, and 10 Tris, as well as 100 μM K2HPO4, pH 7.5. The final concentration of the buffer plus vesicles was (in mM) 120 NaCl, 80 P, 12.8 HEPES, and 8 Tris, as well as 80 μM K2HPO4, pH 7.5. All BBM vesicles were handled in the same manner. Uptake after 10 s (representing the initial linear rate) was terminated by an ice-cold stop solution. All uptake measurements were performed in triplicate, and uptake was calculated on the basis of specific activity determined in each experiment and expressed as picomoles 32P per 10 seconds per milligram GBM protein.

SDS-PAGE and Western blot analysis. Samples of SC-GBM and JMC-GBM were denatured for 2 min at 95°C in 2% SDS, 10% glycerol, 0.5 mM EDTA, and 95 mM Tris-HCl, pH 6.8 (final concentrations). Ten micrograms of GBM protein/lane were separated on 9% polyacrylamide gels and electrotransferred onto nitrocellulose paper. After blockage with 5% nonfat milk powder and the kidneys were removed for BBM isolation. The supernatant was filtered in a retrograde fashion into the centrifugation tube. To measure Na gradient-dependent 32P uptake (NaPi cotransport), 10 μl of BBM preloaded in an intravesicular buffer comprising (in mM) 300 mannitol, 16 HEPES, and 10 Tris, pH 7.5, were vortexed at 25°C with 40 μl of an extravesicular transport buffer consisting of (in mM) 150 NaCl, 16 HEPES, and 10 Tris, as well as 100 μM K2HPO4, pH 7.5. The final concentration of the buffer plus vesicles was (in mM) 120 NaCl, 80 P, 12.8 HEPES, and 8 Tris, as well as 80 μM K2HPO4, pH 7.5. All BBM vesicles were handled in the same manner. Uptake after 10 s (representing the initial linear rate) was terminated by an ice-cold stop solution. All uptake measurements were performed in triplicate, and uptake was calculated on the basis of specific activity determined in each experiment and expressed as picomoles 32P per 10 seconds per milligram GBM protein.

Immunofluorescence microscopy. To assess the role of GH in the regulation of renal type IIA NaPi transporter protein, immunofluorescence microscopy was performed for adult, juvenile, and GRF-AN-treated juvenile male Wistar rats fed a normal-phosphate diet. In a separate group of chronically GH-suppressed juvenile rats, pulses of rat GH were exogenously administered at 1000, 1400, and 1800 daily to mimic three GH pulses. The animals were anesthetized using thiopental (pentothal sodium, 100 mg/kg BW ip), and a catheter (PE-190 in adults and PE-60 in juveniles) was inserted into the abdominal aorta below the renal arteries. The kidneys were fixed in vivo by perfusion with a fixative buffer in a retrograde fashion into the renal arteries. The fixative buffer consisted of 0.1% glutaraldehyde,
7.4, 300 mosmol/kg H2O). The kidneys were removed, sliced, and mounted on chromalum-gelatin-

Table 1. Parameters of renal function in GH-suppressed juvenile rats in the presence of PTH

<table>
<thead>
<tr>
<th>Designation</th>
<th>n</th>
<th>BW, g</th>
<th>MAP, mmHg</th>
<th>Plasma Pi, mM</th>
<th>GFR, ml/min</th>
<th>FE Pi, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-NPD</td>
<td>6</td>
<td>438 ± 24</td>
<td>119 ± 8</td>
<td>2.45 ± 0.1</td>
<td>4.26 ± 0.5</td>
<td>39.1 ± 2.5</td>
</tr>
<tr>
<td>J-NPD</td>
<td>5</td>
<td>128 ± 5*</td>
<td>93 ± 4*</td>
<td>2.93 ± 0.2*</td>
<td>1.26 ± 0.1*</td>
<td>23.27 ± 2.5*</td>
</tr>
<tr>
<td>J-NPD + GRF-AN</td>
<td>7</td>
<td>134 ± 4*</td>
<td>98 ± 4</td>
<td>2.04 ± 0.1*†</td>
<td>1.43 ± 0.1*</td>
<td>40.5 ± 4.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, no. of rats; GH, growth hormone; PTH, parathyroid hormone; BW, body wt; MAP, mean arterial pressure; GFR, glomerular filtration rate; FE Pi, fraction Pi excretion; NPD, normal-Pi diet; J-NPD and A-NPD, juvenile and adult rats on NPD, respectively; GRF-AN, [N-acetyl-Tyr1-D-Arg2]-GRF-(1-29)-NH2. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.
controls (Table 2). This indicates a role for the normal pulsatile circulating GH in the enhanced reabsorption of P\textsubscript{i} uptake in the juvenile rat. Moreover, while there was a significant decline in the delivery of P\textsubscript{i} to the eDT in the juvenile control rat, GH suppression in the juvenile rats blocked this effect. Interestingly, the delivery of P\textsubscript{i} to the eDT in the GRF-AN-treated juvenile rats was similar to that seen in the adult animals. This finding strongly supports a role for GH in the regulation of P\textsubscript{i} reabsorption along the proximal straight tubule, which contributes to the attenuation of the phosphaturic effect of PTH in the juvenile animal.

In vivo experiments in the absence of endogenous PTH. To assess the intrinsic P\textsubscript{i} transport in the GH-suppressed juvenile rat, micropuncture experiments were performed after acute TPTX. Table 3 provides values of various functional parameters in the absence of PTH. MAP was significantly higher in the adult rats compared with intact juveniles but was not significantly different vs. the GRF-AN-treated juvenile animals. Although the plasma P\textsubscript{i} concentration was significantly higher in the juvenile rats, there was no difference between the adult and GH-suppressed juvenile animals. As expected, after TPTX fractional P\textsubscript{i} excretion fell to \( \leq 1\% \) and was not significantly different between the groups.

Table 4 contains the results of micropuncture experiments from the late PCT and eDT. Although adult rats had a significantly higher SNGFR compared with the juvenile groups, the tubular fluid-to-plasma inulin ratio values from both the PCT and eDT were consistent between the groups, indicating similar fractional water delivery. The significantly lower fractional delivery of P\textsubscript{i} to the late PCT of juvenile controls compared with adult animals was completely inhibited by GH suppression in the juvenile rats (Table 4). This provides further evidence that pulsatile GH secretion modulates P\textsubscript{i} reabsorption in the PCT. In addition, while there was a significant decline in the delivery of P\textsubscript{i} to the eDT in the juvenile rats compared with adults, there was no change in the delivery of P\textsubscript{i} between the PCT and eDT of GH-deprived juvenile rats. Again, this finding supports the role of GH in increasing P\textsubscript{i} reabsorption in the proximal straight tubules of the juvenile rat.

**Table 2. Micropuncture data obtained from the late proximal convoluted tubule and early distal tubule in GH-suppressed juvenile rats in the presence of PTH**

<table>
<thead>
<tr>
<th></th>
<th>Late Proximal Convoluted Tubule</th>
<th>Early Distal Convoluted Tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SNGFR, nl/min</td>
<td>TF/P (inulin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-NPD</td>
<td>50.4 ± 5.3</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>J-NPD</td>
<td>17.8 ± 1.9*</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>J-NPD + GRF-AN</td>
<td>20.7 ± 1.1*</td>
<td>1.6 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. SNGFR, single-nephron GFR; TF/P, tubular fluid-to-plasma ratio; FDP, fractional P delivery. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.

**DISCUSSION**

The present study demonstrates that pulsatile GH release is a key factor in the enhanced proximal tubular phosphate reabsorption observed in the juvenile rat. The micropuncture studies indicate for the first time that GH is responsible, independently of PTH, for the enhanced P\textsubscript{i} uptake in both the PCT and straight tubules of juvenile rats on a normal-P\textsubscript{i} diet. In addition, circulating GH contributes significantly to the blunted phosphaturic response to PTH in the juvenile rat. The mecha-

**Table 3. Parameters of renal function in GH-suppressed juvenile rats in the absence of PTH**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW, g</th>
<th>MAP, mmHg</th>
<th>Plasma P\textsubscript{i}, mM</th>
<th>GFR, ml/min</th>
<th>FE\textsubscript{P}, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-NPD</td>
<td>7</td>
<td>412 ± 17</td>
<td>127 ± 5</td>
<td>2.83 ± 0.2</td>
<td>3.83 ± 0.4</td>
<td>0.56 ± 0.5</td>
</tr>
<tr>
<td>J-NPD</td>
<td>9</td>
<td>125 ± 7*</td>
<td>98 ± 4*</td>
<td>2.38 ± 0.1*</td>
<td>1.24 ± 0.1*</td>
<td>0.41 ± 0.2</td>
</tr>
<tr>
<td>J-NPD + GRF-AN</td>
<td>9</td>
<td>131 ± 3*</td>
<td>114 ± 12</td>
<td>2.53 ± 0.3†</td>
<td>1.52 ± 0.2*</td>
<td>1.0 ± 0.42</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.
nism for the enhanced NaPi cotransport activity appears to be through the action of GH on the expression of proximal tubular BBM type IIa NaPi transporter protein in the juvenile rat, because suppression of the pulsatile release of GH in the juvenile rat significantly decreased BBM NaPi protein expression. The present findings indicate that GH plays a central role in the renal adaptation to reabsorb Pi seen during growth, by enhancing PCT and proximal straight tubule Pi reabsorption, significantly extending previous findings by Mulroney et al. (27, 28).

Rapidly growing neonatal and juvenile animals have enhanced proximal tubular Pi reabsorption, and it is postulated that the proximal straight tubule may contribute to the reclamation of filtered Pi (22, 27, 28). The present study confirms that Pi uptake in both the PCT and the proximal straight tubule is enhanced in the juvenile rat, and moreover, that the significant uptake in these segments is stimulated by circulating GH. This is in contrast to that observed in the adult animal, where these sites are only upregulated when Pi is conserved, such as with TPTX, dietary Pi deprivation, and respiratory alkalosis. Thus GH appears to selectively stimulate Pi reabsorption in these segments in the young. While it is clear that this effect of GH on Pi reabsorption is crucial for the proper growth and development of the young animal (19, 27, 28), the mechanism enhancing the sensitivity of the kidney to GH is unknown. GH receptor mRNA has been localized to the proximal straight tubule (7), but developmental differences in GH receptor expression have not been reported. Thus it is unclear whether

### Table 4. Micro puncture data obtained from the late proximal convoluted and early distal convoluted tubule in GH-suppressed juvenile rats in the absence of PTH

<table>
<thead>
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<th>Early Distal Convoluted Tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SNGFR (nl/min)</td>
<td>TF/P (inulin)</td>
</tr>
<tr>
<td>A-NPD</td>
<td>48.7±1.9</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>J-NPD</td>
<td>13.9±1.3*</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>J-NPD+GRF-AN</td>
<td>18.8±1.2*</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data for A-NPD and J-NPD were previously published in Ref. 37. *P < 0.05 vs. A-NPD. †P < 0.05 vs. J-NPD.
The upregulation of GH receptors is responsible for the enhanced sensitivity of the juvenile kidney to the effects of GH. The age-related differences in Pi reabsorption within the proximal convoluted and proximal straight nephron segments are also consistent with previous micropuncture and BBM vesicle NaPi cotransport activity studies in our laboratory (37). In addition, the findings in the GH-suppressed juvenile rat confirm previous data showing that proximal tubular BBMV prepared from GRF-AN-treated juvenile rats had a significant reduction in $V_{\text{max}}$ of Na-dependent Pi transport compared with juvenile controls. Na-proline uptake in these studies was unaffected by GRF-AN administration, highlighting the apparent specificity to NaPi cotransporters. Because both GH and IGF-I receptor mRNA have been localized on the apical surface of proximal tubular cells, this provides evidence for a direct and/or indirect action of the GH/IGF-I axis on Pi uptake in the proximal segments (7, 15, 17, 32).

The fact that chronic suppression of pulsatile GH release in the juvenile rats significantly decreases Pi reabsorption in the proximal tubule does not address whether GH acts directly or indirectly on the tubules. It is clear that both GH and IGF-I can enhance P$_i$ reabsorption, and it may be that GH acts both directly and indirectly through IGF-I to modulate renal P$_i$ reabsorption in the juvenile rat. In rabbit proximal convoluted tubular segments, Quigley et al. (24) found that IGF-I, but not GH, stimulated P$_i$ uptake. Furthermore, administration of IGF-I to the media of opposum kidney cells (2) or proximal tubular BBMV prepared from IGF-I-treated hypophysectomized rats (5) showed that this was associated with an increase in $V_{\text{max}}$ for Na-dependent Pi transport. We have also reported that both GH and IGF-I were able to prevent the rapid decrease in TmPi observed in GRF-AN-treated juvenile rats. The notion that suppression of GH causes a rapid (within 3 h) reduction in P$_i$ transport suggests a direct action of GH on NaPi transporters, because an indirect route through reductions in renal IGF-I would probably take longer. Also, GH receptor mRNA has been localized to the proximal straight tubule (7), which may again point to a direct effect of GH on NaPi protein expression. The evidence from micropuncture, BBMV NaPi transport activity studies, and NaPi protein abundance clearly links circulating GH with the enhanced renal Pi reabsorption in the growing animal and indicates that NaPi may be a target for GH. However, the intracellular mechanism by which GH/IGF-I stimulates NaPi in proximal tubular cells remains unknown.

In summary, phosphate is crucial to the proper development of the rapidly growing juvenile rat. The enhanced renal Pi reabsorption seen during this state of high-P$_i$ conservation occurs predominately in the proximal convoluted and proximal straight tubule. Our findings indicate that pulsatile GH release plays a key role in the enhanced Pi reabsorption at these segments through modulation of BBM type IIa NaPi transporter expression.

**Perspectives.** It is clear that GH is a key regulator of P$_i$ homeostasis in the juvenile animal by facilitating the avid reabsorption of P$_i$. This is crucial for the rapidly developing animal, because limiting P$_i$ supply (via dietary deprivation) or transporter activity severely attenuates growth. Defining the sites of action of GH on the renal tubule is an important step in understanding the regulation of NaPi transporters and allows speculation as to the potential for GH to regulate NaPi in adults. Indeed, our findings that P$_i$ transporters and TmPi are dramatically reduced in the senescent animal (20, 24), coincidentally with decreases in circulating GH, suggest that one mechanism for the “anti-aging” effect of GH may be through increasing P$_i$ transporters in the kidney and perhaps other tissues. The current findings provide strong support for future work on the effects of GH on P$_i$ transport.

**GRANTS**

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REFERENCES