On the mechanism of growth hormone-induced stimulation of renal acidification in humans: effect of dietary NaCl

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ABSTRACT

Sustained administration of growth hormone (GH) to human subjects with NH₄Cl-induced chronic metabolic acidosis (CMA) results in a large (4.5±0.5 mmol/l) increase in the plasma HCO₃⁻ concentration, as mediated by a large increase in renal net acid excretion. The renal mechanism(s) responsible for the potent stimulation of renal hydrogen ion secretion by GH remain to be elucidated. Accordingly, we have assessed the Na⁺ dependence of prolonged GH-stimulated renal acidification in four normal NaCl-restricted subjects (Na⁺ intake 0.3 mmol·kg⁻¹·day⁻¹) during CMA (4.2 mmol of NH₄Cl·kg⁻¹·day⁻¹ for 7 days), CMA plus GH (0.1 unit/kg every 12 h for 5 days) and then CMA plus GH plus NaCl (1.7 mmol·kg⁻¹·day⁻¹ for 6 days). During CMA, urine Na⁺ excretion averaged 22±4 mmol/24 h. In response to GH administration, urinary net acid excretion was essentially unchanged, and the accumulated increment over 5 days of GH treatment was not different from zero (14±12 mmol; not significant). The plasma HCO₃⁻ concentration increased only slightly, from 14.2±0.8 to 15.0±1.1 mmol/l (P < 0.05). Despite the constraint on net acid excretion imposed by NaCl restriction, renal ammonia production increased, as suggested by increases in urine pH from 5.58±0.05 to 5.82±0.04 (P < 0.005) and unchanged NH₄⁺ excretion (202±17 to 211±19 mmol/24 h; not significant). In response to dietary NaCl, urine pH decreased to 5.27±0.1 (P < 0.001) and a large increment in net acid excretion accumulated (233±20 mmol; P < 0.05), in association with an increase in plasma HCO₃⁻ to 18.7±1.3 mmol/l (P < 0.001), a plasma HCO₃⁻ value similar to that reported previously in salt-replete, NH₄Cl-fed subjects. These results demonstrate for the first time in any species that the acid excretory effect of GH administration is critically dependent on the availability of a surfeit of Na⁺ for tubular reabsorption. GH and/or insulin-like growth factor-1 affect renal acid excretion proximally (by stimulation of NH₃ production) and by a Na⁺-transport-dependent mechanism in the collecting duct (voltage-driven acidification) in humans. The present results indicate that an isolated increase in renal NH₃ production is insufficient to obligate an increase in net acid excretion.

INTRODUCTION

We have reported recently that sustained administration of growth hormone (GH) to human subjects with NH₄Cl-induced chronic metabolic acidosis (CMA) resulted in a large (4.5±0.5 mmol/l) and significant increase in the plasma bicarbonate concentration, mediated by a marked increase in renal net acid excretion [1]. The renal mechanism(s) responsible for the potent stimulation of renal hydrogen ion secretion by GH remain to be elucidated. In the case of the other four hormone classes known to stimulate renal acidification...
and thereby to raise the plasma bicarbonate concentration [mineralocorticoid, glucocorticoid, parathyroid hormone (PTH), 1,25-dihydroxyvitamin D], many of the underlying mechanisms have been reported, and knowledge of such mechanisms has contributed to improved management of the associated disease states [2–6].

GH and insulin-like growth factor (IGF)-1 may cause an increase in net acid excretion by several mechanisms. First, our previous report demonstrated that the observed increase in net acid excretion was accompanied by sustained increases in both urine pH and urine NH₄⁺ concentration, suggesting an increase in renal ammonia production [1]. Thus a GH-mediated increment in the supply of distal nephron luminal buffer (NH₄⁺) might have augmented renal hydrogen ion secretory capacity. Secondly, although neither luminal nor basolateral GH or IGF-1 have been found to increase proximal bicarbonate or Na⁺ transport in the isolated rabbit proximal tubule [7], data from human cell culture models of the proximal tubule have demonstrated that GH/IGF-1 stimulate the luminal Na/H antipporter (NHE3), which is responsible for mediating the bulk of proximal bicarbonate reabsorption [8]. Thirdly, IGF-1 has been reported to increase Na⁺–K⁺-ATPase activity in the canine proximal tubule [9]. Although an increase in Na⁺–K⁺-ATPase activity in either proximal or distal nephron segments could theoretically stimulate hydrogen ion secretory capacity, effects on distal segmental enzyme activity or on transepithelial electrolyte transport have not been reported.

A fourth possibility is that GH/IGF-1 might increase renal hydrogen ion secretion as a secondary consequence of an effect (proximal or distal) to increase renal Na⁺ transport. A sustained stimulation of the proximal tubule Na/H antipporter [8,10] might increase bicarbonate reabsorption in this segment and decrease bicarbonate delivery to the distal nephron. Consequently, even an unchanged distal H⁺ secretory capacity would lead to augmented excretion of ammonium and titratable acid and, thereby, to an increase in plasma bicarbonate concentration. For example, a chronic PTH excess might exert its effect of stimulating renal acid excretion by this mechanism. PTH was shown to chronically increase proximal tubule cAMP levels [5,6], which have been mechanisms [12] and Na⁺-dependent mechanisms [13]. However, at least for mineralocorticoid administration in humans and dogs, it has been established that the increases in both renal net acid excretion and plasma bicarbonate concentration are completely dependent on the provision of sufficient distal Na⁺ delivery, as provided by a normal NaCl intake [2,14]. Since IGF-1 has been shown to activate Na⁺ channels in A-6 cells, a distal-tubule-derived cell line [15] and to stimulate transepithelial Na⁺ transport in the toad bladder, a model of mammalian collecting duct H⁺ and Na⁺ transport [16], it is reasonable to predict that Na⁺-dependent collecting duct H⁺ secretion might modulate the effect of GH to increase net acid excretion in humans. The present studies in normal subjects were designed to assess the possibility that, like chronic mineralocorticoid administration, chronic GH administration stimulates distal nephron H⁺ secretion largely by a Na⁺-dependent mechanism. Accordingly, the effects of wide variations in dietary NaCl intake on the GH-modulated stimulation of renal net acid excretion and increased plasma bicarbonate concentration were investigated.

**METHODS**

For this study, four normal volunteers [body weight 67 ± 9 kg (mean ± S.D.); 22.5 ± 2.9 years; two male and two female subjects] were examined under metabolic ward conditions. None were smokers or were taking any drugs before or during the study. Each subject ingested a constant low-Na⁺ metabolic diet during the three study periods. The diet provided, per day, 18.48 mmol of Na⁺, 87.62 mmol of potassium, 1600 ml of water and 9000 kJ (2151 kcal) of energy.

CMA was induced by oral administration of NH₄Cl (4.2 mmol·kg⁻¹·day⁻¹) in gelatin capsules in six divided doses during all study periods. After a metabolic steady state had been reached in this control CMA period [definition of steady state: plasma values obtained on 3 consecutive days varied by no more than 1.5 mmol/l for bicarbonate and by no more than 3 mmHg (0.4 kPa) for arterial partial pressure of CO₂], the subjects received recombinant human GH (0.1 unit·kg⁻¹·12 h⁻¹) subcutaneously; Genotropin; Kabi Pharmacia, Uppsala, Sweden) until a new metabolic steady state was established (acidosis + GH period). During the third study period, 120 mmol of Na⁺ was administered as NaCl in gelatin capsules while NH₄Cl feeding was continued. GH was also continued in the same dose and route of administration during this period (acidosis + GH + NaCl period).

Daily fasting arterialized venous blood samples were obtained from a heated hand or forearm vein [17]. Blood samples were accepted only if the partial pressure of oxygen exceeded 70 mmHg (9.3 kPa). Blood samples for plasma and whole-blood (pH) analyses were obtained in heparinized syringes. In addition, 24-h urine was collected daily, as previously described [18].

**Analytical procedures**

All determinations were performed in duplicate. Acid–base and electrolyte values in blood or plasma and
Table 1  Steady-state plasma acid–base and electrolyte composition during CMA, CMA with GH administration and CMA with GH and NaCl administration

<table>
<thead>
<tr>
<th>Study period</th>
<th>H+ (mmol)</th>
<th>NaCl (mmol/l)</th>
<th>HCO3− (mmol/l)</th>
<th>Na+ (mmol/l)</th>
<th>K+ (mmol/l)</th>
<th>Cl− (mmol/l)</th>
<th>PO4− (mmol)</th>
<th>Creatinine clearance (ml/s)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMA (control)</td>
<td>51.5 ± 2.6</td>
<td>30.4 ± 1.1</td>
<td>14.2 ± 0.8</td>
<td>13.3 ± 1.0</td>
<td>134.8 ± 0.9</td>
<td>4.0 ± 0.1</td>
<td>111.0 ± 0.8</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>CMA + GH</td>
<td>49.7 ± 2.3</td>
<td>30.8 ± 1.3</td>
<td>15.0 ± 1.1*</td>
<td>13.5 ± 2.0</td>
<td>135.0 ± 0.9</td>
<td>3.8 ± 0.1***</td>
<td>110.0 ± 1.0</td>
<td>1.2 ± 1.0</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>CMA + GH + NaCl</td>
<td>45.0 ± 2.2***</td>
<td>34.7 ± 1.3***</td>
<td>18.7 ± 1.3***</td>
<td>15.7 ± 4.8</td>
<td>140.4 ± 0.7***</td>
<td>3.5 ± 0.1***</td>
<td>111.4 ± 2.7</td>
<td>1.3 ± 0.4</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

Figure 1  Effects of CMA and of GH and Na+ (NaCl) administration during acidosis and provision of a low-NaCl diet, on urinary Na+ excretion and body weight

Urinary Δ[Na+] excretion denotes the daily change in excretion in comparison with the previous steady-state period (i.e. control and + GH respectively). The Σ Δ[Na+] excretion values denote the sum of daily changes in urinary excretion compared with the mean excretion values in the previous steady-state period. To highlight the effect of Na+ repletion, both daily changes and cumulative excretion are depicted for both the measured values and the calculated values corrected for the dietary provision of Na+ during the third period. Since Na+ intake increased by 120 mmol/day in period three, the figure also shows the daily and cumulative change in Na+ excretion as corrected for this intake ([Na+] = 120). Significance of differences: × P < 0.05 compared with previous steady-state period.

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Table 1  Steady-state plasma acid–base and electrolyte composition during CMA, CMA with GH administration and CMA with GH and NaCl administration

Values are means ± S.E.M. PaCO2, arterial pressure of carbon dioxide. Unmeasured anions were calculated as (Na+ + K+) - (Cl− + HCO3−). The decrease in mean body weight from study enrolment values to the end of the CMA control period are characteristic of prolonged CMA and reflect large NH4Cl-induced, dose-dependent initial sodium losses in this condition [18,40]. Significance of differences: * P < 0.05, ** P < 0.005, *** P < 0.001 compared with previous steady-state period; ††† P < 0.005, †††† P < 0.001 compared with CMA (control) period.

Ionized Ca2+ was measured with an ion-selective electrode (Radiometer Copenhagen EML 105). Titratable acidity in urine was calculated from urinary phosphate excretion, urine pH and blood pH, with the pK+ of phosphate corrected for ionic strength by the method of Schwartz et al. [21]. Net acid excretion was calculated as ammonium plus titratable acid minus bicarbonate.
excretion. The urinary anion gap was calculated as 

\[
(Na^+ + K^+ + NH_4^+ + Ca^{2+} + Mg^{2+} - (HCO_3^- + Cl^- + phosphate anion)).
\]

Phosphate anion equivalency was calculated from the corrected pK and urinary phosphate concentration.

Results are reported as means ± S.E.M. unless stated otherwise. Statistical significance was determined by ANOVA for repeated measurements.

The study protocol was approved by the ethics committee of the Kantonsspital St. Gallen, Switzerland. All subjects volunteered for the study, gave informed consent and were paid for their participation.

RESULTS

All subjects tolerated the protocol well. By using a widely spaced dosing pattern and intake of NH₄Cl-containing capsules with meals and fluid, no nausea or vomiting were observed.

As shown in Table 1 and Figure 1, mean body weight decreased significantly by 0.8 ± 0.3 kg during acidosis and GH administration \((P < 0.05)\), whereas supplementation with NaCl induced a significant gain in weight of 1.1 ± 0.2 kg \((P < 0.005)\). In comparison with the control period (acidosis alone) neither GH nor GH plus NaCl administration affected blood pressure significantly.

None of the subjects developed oedema during GH administration. In Figure 1, the daily and cumulative changes in Na⁺ excretion are depicted for the measured values as well as for the calculated values that would be predicted if no surplus of Na⁺ had been provided (subtraction of daily provision of 120 mmol of Na⁺ during the period of NaCl repletion).

Table 1 and Figure 2 depict the changes in plasma acid–base composition during the control CMA period (acidosis and low-NaCl diet), during GH administration with the low-NaCl diet and acidosis (GH period) and during the provision of NaCl (120 mmol/day; GH + NaCl period). In contrast with our earlier observations in salt-replete subjects [22], GH did not significantly decrease the blood hydrogen ion concentration, and the increase in plasma bicarbonate concentration was very small \((0.8 ± 3.0 \text{ mmol/l}; P < 0.05)\) over an observation period of 5 days. However, supplementation of NaCl resulted in a significant decrease in blood hydrogen ion concentration from 49.7 ± 2.3 to 45.0 ± 2.2 \text{ mmol/l} \((P < 0.005)\) and a large and significant increase in plasma bicarbonate concentration from 15.0 ± 1.1 to 18.7 ± 1.3 \text{ mmol/l} \((P < 0.001)\) over a period of 6 days. This increase is very similar to our earlier observation in salt-replete subjects [1]. Plasma unmeasured anion concentration did not change significantly in response to GH during both Na⁺ restriction and repletion periods.

![Figure 2](image-url)  
Figure 2: Effects of CMA and of GH and Na⁺ (NaCl) administration during acidosis and provision of a low-NaCl diet, on the plasma acid–base composition and plasma unmeasured anion concentration.  
Significance of differences: \(\times P < 0.05\) compared with previous steady-state period.
Table 2  Urinary acid–base and electrolyte composition during CMA, CMA with GH administration and CMA with GH and NaCl administration

Values are means ± S.E.M. Net acid excretion was calculated as \( (\text{NH}_4^+ + \text{titratable acidity} - \text{HCO}_3^-) \). Unmeasured anions were calculated as \( (\text{Na}^+ + K^+ + \text{NH}_4^+) - (\text{Pi} + \text{Cl}^- + \text{HCO}_3^-) \). ‘Sum’ values represent the accumulated changes in excretion from the previous steady-state excretion value. Significance of differences: * \( P < 0.05 \), ** \( P < 0.005 \), *** \( P < 0.001 \) compared with previous steady-state value; † \( P < 0.05 \), †† \( P < 0.005 \), ††† \( P < 0.001 \) compared with CMA (control) period; ‡ \( P < 0.05 \), significantly different from zero.

<table>
<thead>
<tr>
<th>Study period</th>
<th>pH</th>
<th>( \text{NH}_4^+ )</th>
<th>( \text{HCO}_3^- )</th>
<th>Net acid</th>
<th>( \text{Na}^+ )</th>
<th>( \text{K}^+ )</th>
<th>( \text{Cl}^- )</th>
<th>( \text{Pi} )</th>
<th>Unmeasured anions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMA (control)</td>
<td>5.58 ± 0.05</td>
<td>202 ± 17</td>
<td>22 ± 2</td>
<td>2.7 ± 0.5</td>
<td>221 ± 18</td>
<td>22 ± 4</td>
<td>86 ± 8</td>
<td>255 ± 22.4</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>CMA + GH</td>
<td>5.82 ± 0.04</td>
<td>211 ± 19</td>
<td>13 ± 2***</td>
<td>4.4 ± 1.2</td>
<td>220 ± 20</td>
<td>6 ± 2***</td>
<td>41 ± 3***</td>
<td>240 ± 21.3</td>
<td>22 ± 3***</td>
</tr>
<tr>
<td>Sum (day 5)</td>
<td></td>
<td>51</td>
<td>-36†</td>
<td>14</td>
<td>-57†</td>
<td>-194†</td>
<td>-28</td>
<td>-47†</td>
<td>-20†</td>
</tr>
<tr>
<td>CMA + GH + NaCl</td>
<td>5.27 ± 0.10*** † † †</td>
<td>219 ± 19</td>
<td>16 ± 2***</td>
<td>2.4 ± 0.2</td>
<td>233 ± 20</td>
<td>88 ± 16***</td>
<td>51 ± 3***</td>
<td>340 ± 19.6***</td>
<td>22 ± 3***</td>
</tr>
<tr>
<td>Sum (day 6)</td>
<td></td>
<td>34†</td>
<td>9†</td>
<td>-7</td>
<td>60†</td>
<td>130†</td>
<td>88†</td>
<td>249†</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 3  Effects of CMA and of GH and Na\(^+\) (NaCl) administration during acidosis and provision of a low-NaCl diet, on urine pH, ammonium (NH\(_4^+\)), titratable acid (TA), and net acid excretion

The \( \Delta \) excretion values denote the daily changes in excretion in comparison with the previous steady-state period (i.e. control and + GH respectively). The \( \Sigma \Delta \) excretion values denote the sum of daily changes in urinary excretion compared with the mean excretion values in the previous steady-state period. Significance of differences: \( \times P < 0.05 \) compared with previous steady-state period.

The effects of GH administration on urinary acid–base composition are shown in Table 2 and illustrated in Figure 3. Administration of GH during the low-Na\(^+\) diet increased urinary pH significantly from 5.58 ± 0.05 to 5.82 ± 0.04 (\( P < 0.005 \)); supplementation of NaCl decreased the urinary pH significantly to a value even lower than that observed in the control period, 5.27 ± 0.10 (\( P < 0.001 \)) for the comparison with the acidosis + GH period.
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Figure 4 Effects of CMA and of GH and Na\(^+\) (NaCl) administration during acidosis and provision of a low-NaCl diet, on plasma potassium concentration and urinary potassium excretion

The $\Delta$ excretion values denote the daily changes in potassium excretion in comparison with the previous steady-state period (i.e. control and $+\ GH$ respectively). The $\Sigma \Delta$ excretion values denote the sum of daily changes in urinary potassium excretion compared with the mean excretion values in the previous steady-state period.

Significance of differences: $\times P < 0.05$ compared with previous steady-state period.

$P < 0.005$ for the comparison with the control period). In comparison with the control CMA period, GH administration during the low-Na\(^+\) diet tended to increase cumulative urinary NH\(_4\)\(^+\) excretion and to decrease urinary excretion of titratable acidity, resulting in no significant change in net acid excretion. NaCl administration significantly increased cumulative urinary NH\(_4\)\(^+\) excretion by 34 mmol for the 6-day GH + NaCl period ($P < 0.05$), while excretion of titratable acidity increased non-significantly. As a result, the cumulative change in net acid excretion increased significantly by 60 mmol for the 6-day GH + NaCl period ($P < 0.05$).

Steady-state urinary unmeasured anion excretion, an index of organic anion excretion [2], decreased slightly during GH and GH + NaCl administration in comparison with the control CMA period (acidosis).

Tables 1 and 2 and Figure 4 illustrate and confirm our previous findings that CMA induces renal hypokalaemia in humans [18]. As depicted in Figure 4 and Tables 1 and 2, GH administration during NaCl restriction resulted in a significant decrease in the plasma potassium concentration and in a large and significant sustained decrease in urinary potassium excretion. During supplementation with NaCl, a further significant decrease in plasma potassium concentration occurred, from 3.8 ± 0.1 to 3.5 ± 0.1 mmol/l ($P < 0.001$), in association with a significant increase in urinary potassium excretion from 41.1 ± 2.9 to 51.1 ± 2.8 mmol/24 h ($P < 0.05$). The cumulative decrease in urinary potassium excretion during GH administration with Na\(^+\) restriction averaged 193 mmol ($P < 0.05$), whereas during GH + NaCl administration potassium excretion increased cumulatively by 88 mmol ($P < 0.05$).

Figure 5 and Tables 1 and 2 depict the effects of GH administration during Na\(^+\) restriction and during Na\(^+\) repletion on the plasma phosphate concentration and urinary phosphate excretion. The plasma phosphate concentration did not change significantly in response to GH administration (Table 1, Figure 5). However, urinary phosphate excretion decreased significantly, from 31.8 ± 7 to 19.6 ± 5 mmol/24 h ($P < 0.001$), with a cumulative urinary phosphate retention of 32 mmol ($P < 0.001$). Provision of NaCl did not affect GH-induced phosphate retention significantly (Table 1, Figure 5).

These results are in accordance with our previous observation that GH/IGF-1 corrects acidosis-induced renal phosphate wasting [23]. This effect may be explained by IGF-1-induced stimulation of proximal tubule phosphate reabsorption via the Na–P\(_i\) co-transporter [24,25]. In addition, part of the decrease in renal phosphate excretion might have been a consequence of the anabolic effect of GH/IGF-1.
DISCUSSION

The results of the present study demonstrate that the ability of GH to stimulate renal net acid excretion in humans, and thereby to increase the plasma bicarbonate concentration, is dependent on a surfeit of dietary Na\textsuperscript{+}. The imposition of the constraint of a low-Na\textsuperscript{+} diet prevented the previously demonstrated GH-induced stimulation of net acid excretion [1]. Subsequent removal of this constraint by provision of surplus dietary Na\textsuperscript{+} resulted in full restitution of the acid excretory response to GH.

Since Na\textsuperscript{+}-dependent acidification processes occur at both proximal and distal nephron sites, and since GH and IGF-1 are reported to influence processes that might conceivably augment proximal [8,10,26] as well as distal acidification, possible proximal mechanisms require consideration. The finding that GH/IGF-1 can augment NH\textsubscript{3} production in canine proximal tubules in vitro [9] cannot account for the present results, since augmented NaCl intake and/or distal Na\textsuperscript{+} delivery dissociated the effect of GH/IGF-1 to increase ammoniagenesis from that of stimulating net acid excretion (Figure 3). The finding that IGF-1 stimulated Na/H exchange in pig LLC-PK1 cells of proximal origin [10] and in primary cultures of human proximal tubule cells [8] does not provide a mechanism for the selective stimulation of proximal bicarbonate reabsorption, since Na/H exchange was stimulated on both apical and basolateral membranes in human cells, whereas this exchange is known to be confined to the basolateral membrane in the pig cell line [26]. In addition, since stimulation of apical Na/H exchange in the proximal tubule can augment Na\textsuperscript{+} reabsorption with chloride as an alternative to augmentation of Na\textsuperscript{+} reabsorption with bicarbonate, demonstration of increased apical Na/H exchange, even if offsetting effects on basolateral Na/H exchange had been absent, would not predict a stimulation of transepithelial bicarbonate transport. Most importantly, however, physiologically relevant effects of GH/IGF-1 are essentially excluded, as neither GH nor IGF-1 had any significant effect on bicarbonate transport in isolated perfused rabbit proximal convoluted tubules [7]. Thus the lack of a plausible mechanism for the stimulation of proximal acidification, the perfused tubule studies [7] and the present finding of prevention of the GH/IGF-1-induced renal acid excretory response by a Na\textsuperscript{+}-dependent mechanism provide strong evidence that GH and IGF-1 exert their effects via stimulation of distal Na\textsuperscript{+}-dependent acidification.

The inverse relationship between urinary pH and urinary ammonium excretion during Na\textsuperscript{+} repletion in the
present study provides additional evidence for a distally localized Na\(^+\)-dependent mechanism. Such a relationship is observed with increased trapping of NH\(_3\) in an increasingly acidic tubular lumen, rather than increased NH\(_3\) buffer availability. The significant decrease in urinary pH (Figure 3, Table 2) during salt repletion despite a high rate of NH\(_3\) production and supply is strong evidence for the distal localization of stimulated Na\(^+\)-dependent acidification. Taken together, prolonged GH administration, like mineralocorticoid hormone administration in humans and dogs [2,14], requires the stimulation of a distal Na\(^+\)-dependent hydrogen ion secretory mechanism for expression of the acid excretory response in the intact organism.

The possibility that a correction of acidosis would have occurred with prolonged acid feeling while ingesting a low-NaCl diet was dispelled by the finding of a rigorously demonstrated unchanged steady-state plasma bicarbonate concentration under such conditions in HCl-treated dogs over an interval of 21 days [29]. The additional possibility that a subject ingesting a high-salt diet might exhibit a higher plasma bicarbonate concentration than during NaCl restriction is excluded by findings in both human subjects [14] and dogs [27], where mean values for plasma bicarbonate concentration under the two conditions were not detectably different when studied robustly.

Our results, i.e. the observation that no significant acid excrelory response occurred in response to GH (Figure 3), confirm these previous observations [14,27]. However, we observed a very small increase in the plasma bicarbonate concentration of 0.8 mmol/l. This could reflect a small, but undetected, GH-induced, Na\(^+\)-dependent increase in net acid excretion, inasmuch our whole-food diet did not permit urinary sodium to reach zero prior to GH treatment (mean value 22 mmol/24 h). This small systemic daily sodium load permitted, but significant, GH-induced sodium retention (averaging 57 mmol over 5 days; Figure 1). Based on a distribution space of bicarbonate of around 50% of body weight, detection of the requisite small sodium-dependent increment in renal acid excretion of only about 25 mmol would be difficult or impossible when basal excretion values are elevated to more than 200 mmol/day as a result of the imposed acid load. Alternatively, the small GH-induced decrease in urinary unmeasured anion excretion (Table 2) could have contributed to a small extrarenal increase in bicarbonate if this decrease reflected a decrease in endogenous acid production [24]. Irrespective of whether the minute GH-induced increase in bicarbonate levels during sodium restriction was of renal or extrarenal origin, the observed NaCl-induced increments in both renal net acid excretion and plasma bicarbonate concentration revealed a GH-induced, Na\(^+\)-dependent net acid-base response of substantially greater magnitude.

GH might enhance renal acidification in humans by actions mediated through a renal GH receptor or by acting via increased IGF-1 levels (generated systemically or intrarenally) at renal IGF-1 receptors [4,28,29]. GH-receptor mRNA has been localized to several nephron segments, including the proximal tubule, the thick ascending limb, cortical distal segments and the medullary collecting tubule [28,29a]. Using anti-IGF-1 antibody, immunostainable IGF-1 in rat kidney was localized to principal cells, thought to be responsible for Na\(^+\) and K\(^+\) transport within cortical and medullary collecting ducts, and such immunostaining was increased by chronic GH administration [30]. Moreover, incubation of isolated collecting ducts with GH has been reported to result in a large increase in IGF-1 generation in vivo, and the signal transduction of GH action is mediated by activation of phospholipase C [31]. Although normal human kidney IGF-1 mRNA expression has not been assessed, none was found by in situ hybridization in tumour-bearing nephrectomy specimens [32,33].

Regardless of whether GH acts directly on the kidney or via systemic delivery of increased circulating levels of IGF-1, both human [32] and rat [34] kidney demonstrate a consistent pattern of type-1 IGF receptor expression by in situ hybridization: high levels of expression are seen in the glomerulus, thick ascending limb, and cortical and medullary collecting ducts. Whether GH could also act via increases in IGF-2 (IGF-2 mRNA has been demonstrated in vascular and stromal tissue, but not epithelial cells [32]) is not known. mRNAs for the additional critical components of the GH/IGF system, i.e. the IGF binding proteins (IGFBPs) 1–5, were demonstrated by in situ hybridization in human tumour-bearing nephrectomy specimens, with IGFBP-2 most abundant in the collecting duct epithelium [35].

The effects of GH are known to not all be mediated by GH itself or IGF-1. A GH-induced increase in insulin levels (not measured) could have mediated part of the sodium-retaining effect of GH [36,37]. In previous studies, we were unable to show significant changes in insulin levels after sustained GH administration.

The localization of IGF-1 protein to collecting duct principal cells in the rat kidney [31] is of interest to the interpretation of the present studies, despite the reported absence of IGF-1 mRNA in human kidney [32], since the reported accumulation of IGFBPs in human collecting duct cells might provide a high content of IGF-1 or IGF-2 protein at that site, regardless of where it is produced. Indeed, it has been shown in epithelial models of the collecting duct principal cell that IGF-1 can activate apical Na\(^+\) channels in A-6 cells [15] and stimulate transepithelial Na\(^+\) transport in the isolated toad bladder [16]. Substantial electrophysiological and transport evidence supports the view that collecting duct principal cell Na\(^+\) reabsorption, as stimulated by mineralocorticoid, determines the rate of Na\(^+\)-dependent hydrogen ion
secretion in the cortical collecting duct by enhancing the lumen negative potential difference [13, 38, 39].

Provision of dietary NaCl increases the rate of delivery of Na\(^+\) to the distal nephron, where a stimulus to distal Na\(^+\) reabsorption can only be expressed if Na\(^+\) delivery to that site is adequate or not constrained. Under the stimulus of GH, which augments renal ammoniagenesis and thereby increases the luminal NH\(_4\)\(^+\) concentration in the loop of Henle, it is possible that the reported effect of an increased luminal NH\(_4\)\(^+\) concentration to inhibit the critical apical K\(^+\) channels needed for normal Na\(^+\) reabsorption via the apical Na\(^+\)/K\(^+\)/2Cl\(^-\)-co-transporter would amplify the effect of NaCl intake to augment distal delivery in the present study [39].

Although active Na\(^+\)-independent hydrogen ion secretion is demonstrable in vitro in rabbit cortical and outer medullary collecting duct [12, 13], the mineralocorticoid-stimulated Na\(^+\)-independent mechanism is of undetectable potency in vivo following prolonged mineralocorticoid administration and a salt-restricted diet in vivo in humans [14] and dogs [2]. That is, as in the present study with GH administration, co-administration of a low-salt diet with mineralocorticoid prevented the large increases in net acid and plasma bicarbonate concentrations demonstrable on a normal-salt diet.

The effects of GH in the present study to cause simultaneous increased renal Na\(^+\) retention resulting in weight gain, increased renal potassium excretion and increased Na\(^+\)-dependent net acid excretion following provision of a surfeit of dietary Na\(^+\) are essentially identical to the pattern of urinary acid-base and electrolyte responses to mineralocorticoid on a similar high intake of dietary Na\(^+\) [2, 13]. It is thus tempting to speculate that the reported effects of IGF-1 to increase electrogenic Na\(^+\) transport in models of the mammalian collecting duct [15, 16] might also operate in the human collecting duct, whereby such augmented electrogenic Na\(^+\) reabsorption might stimulate Na\(^+\)-dependent hydrogen ion secretion, as observed in the present study.

As reported previously [1, 23], some of the effects of GH on phosphate and potassium metabolism reflect the anabolic effects of GH/IGF-1. Potassium excretion decreased during NaCl restriction as anabolism occurred (Figure 2, Table 2) and then increased greatly when NaCl intake was increased.

The effect of prolonged GH administration during salt restriction in the present study to significantly increase urine pH as ammonium excretion tended to increase is of interest for assessment of the putative effect of a primary increase in renal ammonia production to increase net acid excretion. Although there are examples where ammonia production and net acid excretion increase simultaneously (e.g. chronic mineralocorticoid treatment and high-salt diet with ongoing potassium depletion [34]), the present results appear to provide the first reported example in any species whereby augmented ammonia production, as shown by an increase in urine pH with no change in net acid excretion during salt restriction and GH treatment, is dissociated from an acid excretory response. Thus the present results provide evidence that a primary increase in renal ammoniagenesis (buffer supply to the distal nephron) is insufficient to stimulate an acid excretory response in the absence of a co-stimulus to distal hydrogen ion secretion.

In conclusion, our results demonstrate for the first time in any species that the acid excretory effect of GH administration is critically dependent on the availability of a surfeit of Na\(^+\) for tubular reabsorption. Together with the findings of our previous report [1], these studies provide evidence that GH/IGF-1 regulate renal NH\(_4\)\(^+\) excretion in humans by a proximal tubule effect (by increasing renal NH\(_4\)\(^+\) production), and distally by stimulating a Na\(^+\)-transport-dependent mechanism in the collecting duct (voltage-driven acidification). Whether a distal effect on H\(^+\) secretion is sufficient for a GH/IGF-1-induced increase in net acid excretion in the absence of augmented NH\(_4\)\(^+\) production will require additional studies. Additional, but small, effects on Na\(^+\)-dependent proximal acidification and Na\(^+\)-independent distal acidification are not excluded by our results.

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