Chronic Uremia Attenuates Growth Hormone–Induced Signal Transduction in Skeletal Muscle

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Abstract. Malnutrition and muscle wasting are common in chronic renal failure (CRF) and adversely affect morbidity and mortality. Contributing to the muscle wasting is resistance to growth hormone (GH). For testing whether impaired GH signaling is a cause of the skeletal muscle GH resistance and for elucidating its mechanisms, muscle GH signaling and action were studied in GH-deficient rats with surgically induced CRF and sham-operated pairfed control rats. GH treatment increased gastrocnemius muscle IGF-1 mRNA levels significantly in control but not in CRF rats. GH-activated Janus-associated kinase 2 (JAK2)-signal transducers and activators of transcription 5 (STAT5) signaling was impaired in CRF rats, despite normal GH receptor (GHR), JAK2, and STAT5 protein levels. Phosphorylation of the GHR, JAK2, and STAT5 in response to GH was depressed by nearly half in CRF (P < 0.05), and nuclear phospho-STAT5 levels were depressed by approximately one third (P < 0.01). GH-stimulated suppressors of cytokine signaling 2 mRNA levels were significantly higher in CRF. This may be related to inflammatory cytokine activity because C-reactive protein levels were elevated. Muscle protein-tyrosine phosphatase activity was also increased significantly by twofold. In conclusion, rats with CRF acquire skeletal muscle resistance to GH that is caused at least in part by impaired JAK2-GHR-STAT5 phosphorylation and nuclear STAT5 translocation. Furthermore, it seems that the attenuated JAK2-STAT5 phosphorylation may be caused by at least two different processes. One involves depressed phosphorylation of the signaling proteins because of increased suppressors of cytokine signaling 2 expression that may be linked to low-grade inflammation. The other may involve increased signaling protein dephosphorylation because of heightened protein-tyrosine phosphatase activity.

Advanced chronic renal failure (CRF) is often complicated by the appearance of muscle wasting and malnutrition, conditions that have a distinct adverse effect on morbidity and mortality (1,2). Several factors contribute to the development and perpetuation of the wasted state, including anorexia with reduced calorie and protein intake; acidosis; inadequate dialysis with accumulation of toxic products; inflammatory cytokines; diabetes; cardiac failure; loss of amino acids in the dialysate; and resistance to anabolic hormones such as insulin, growth hormone (GH), and IGF-1 which is worsened by malnutrition and inflammation (3–5). To some extent, insensitivity to GH can be overcome by pharmacologic doses of recombinant GH, but this does increase the risk of adverse events. Several small studies have demonstrated that GH treatment of adult ESRD patients produces a salutary effect on urea kinetics, protein turnover, and lean body mass (6,7). In children with advanced CRF, GH resistance is a major cause of impaired body growth, and this can largely be corrected by GH treatment (8–10).

The mechanisms that account for the development of GH resistance in uremia are not well understood (10–12) and include insensitivity to IGF-1, the mediator of most of the actions of GH, decreased IGF-I expression, possibly although controversial depressed GH receptor (GHR) expression, and impaired GH signal transduction through the Janus-associated kinase 2 (JAK2) and signal transducers and activators of transcription (STAT) pathway that we recently uncovered in the liver of uremic rats (11). Whether a similar defect is present in skeletal muscle is the subject of this study. When GH binds to its receptor, it causes receptor dimerization followed by auto-phosphorylation of JAK2, a tyrosine kinase associated with the intracellular domain of the receptor (13). The activated JAK2 in turn phosphorylates the GH, creating binding sites for selective members of the STAT family of proteins, namely STAT1, STAT3, and STAT5a, and STAT5b, which are then phosphorylated by JAK2 (14,15). The phosphorylated STAT form dimers that translocate into the nucleus, bind to specific DNA sequences, and modulate gene transcription. Male mice with STAT5b deficiency and female mice with a combined deletion of STAT5a and STAT5b, homologues with >90% sequence homology, are severely growth retarded (16), whereas in humans, a STAT5b mutation causes growth retardation (17). It turns out that STAT5b is important for mediating GH stimulation of IGF-1 gene expression (18,19). Regulation of GH-mediated signal transduction is a complex process that involves several physiologic mechanisms, including pro-
tein-tyrosine phosphatases (PTPase) that dephosphorylate signaling proteins and a family of suppressors of cytokine signaling (SOCS) that inhibit protein phosphorylation (20). In this study, we tested the postulate that in uremia, there is acquired resistance to the induction of IGF-1 expression in skeletal muscle that is caused by a defect in GH-stimulated JAK2-STAT5 signal transduction and then explored the pathomechanism of the defect. We found that GH-induced IGF-1 expression is indeed impaired in skeletal muscle of uremic rats and that this seems to be caused, at least in part, by upregulated SOCS expression and increased PTPase activity.

Materials and Methods

Experimental Animals and Protocols

GH-deficient dwarf (dw/dw) male rats were used as they are far more sensitive to the action of exogenous GH than hormone-replete animals and avoid any confounding effects that may arise in pituitary-intact rats because of the frequent spontaneous GH secretory activity. CRF was created by a two-step 5/6 nephrectomy procedure with ketamine (80 mg/kg) and xylazine (10 mg/kg) anesthesia as before (11). Sham operations were performed in control animals that were pairfed (PF) with the CRF rats.

GH-Stimulated Gene Expression

Thirteen days after the final surgery was completed, the CRF and control animals were each divided into two groups that were treated for 8 d with either vehicle (V) or recombinant bovine GH (bGH; gift from Monsanto Corp., St. Louis, MO). GH was given at a dose of 25 μg/100 g body wt in the morning and in the evening for 4.5 d and then at 12.5 μg/100 g in the morning and in the evening for 3.5 d. On the last day, the rats received two doses of GH, 12.5 μg/100 g each, or V 5 h apart and killed 1 h after the last injection to detect GH-stimulated IGF-1 and SOCS expression, respectively. Gastrocnemius muscle was excised and stored at −80°C. Serum was collected and frozen. A Western Immunoblotting and Immunoprecipitation

After 16 d of CRF and after an overnight fast, the control and CRF rats were anesthetized and via a midline incision, a submaximal dose of GH (3 μg/100 g) or V was injected into the inferior vena cava. Fifteen minutes thereafter, the gastrocnemius muscle was excised, and stored at −80°C. Serum was collected and frozen. A prolonged course of GH was given to ensure stimulation of IGF-1 expression. The doses used were based on pilot studies of GH-induced IGF-1 expression.

GH-Activated JAK2/STAT5 Signal Transduction

After 16 d of CRF and after an overnight fast, the control and CRF rats were anesthetized and via a midline incision, a submaximal dose of GH (3 μg/100 g) or V was injected into the inferior vena cava. Fifteen minutes thereafter, the gastrocnemius muscle was excised, and stored at −80°C. Serum was collected and frozen. A prolonged course of GH was given to ensure stimulation of IGF-1 expression. The doses used were based on pilot studies of GH-induced IGF-1 expression.

Table 1. Primer sequences for quantitative real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5′–3′)</th>
<th>Reverse (5′–3′)</th>
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<tr>
<td>GAPDH</td>
<td>AAGAGAGGGCCTCAGTTGCT</td>
<td>TTGTGAAGGAGATGCTAGTGT</td>
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<tr>
<td>L-7</td>
<td>GAAATGGAGGAGGAACTCATTT</td>
<td>AATCTCAGTGCGGTACACTGCT</td>
</tr>
<tr>
<td>CIS</td>
<td>GAGAATGAAACCGAGTCTGA</td>
<td>TGTAAATGGAACCAGTACCA</td>
</tr>
<tr>
<td>SOCS1</td>
<td>TTCTTGTGGCAGCACTAGTC</td>
<td>GAAATGCAACAGATGTGCC</td>
</tr>
<tr>
<td>SOCS2</td>
<td>TGGACTCGCTGAAACAGTGGTAC</td>
<td>ATAGGTAGTCGTAGTGGGACGTATC</td>
</tr>
<tr>
<td>SOCS3</td>
<td>CAGCTCCAGAAGCGGACTACA</td>
<td>CGGTACGCGACCTCCGATAGA</td>
</tr>
<tr>
<td>IGF-1</td>
<td>CGCTCGAACGCTTACAAAGT</td>
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</table>

* GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SOCS, suppressors of cytokine signalling.
membranes and then immunodetected with appropriate antibodies and visualized by enhanced chemiluminescence as before (11). Protein expression was quantified with a Fluor-S digital image analyzer and Multianalyst software (Bio-Rad, Hercules, CA). Relative density units refer to mean pixel density with local background subtraction.

**Biochemistry**

Muscle tissue protein content by the Bradford method (Protein Assay Kit; Bio-Rad) and serum creatinine and CO₂ (mmol/L) were measured with a Beckman LX 20 Analyzer (Beckman Coulter, Inc., Fullerton, CA). Serum c-reactive protein (CRP) levels were measured with a highly sensitive Rat CRP ELISA Kit (Alpha Diagnostic International, Inc., San Antonio, TX) according to the company’s instruction.

**Protein-Tyrosine Phosphatase Assay**

This was performed with the Promega Tyrosine Phosphatase Assay System according to the manufacturer’s instructions (Promega Corporation, Madison, WI). The assay system determines the amount of free phosphate generated from a phosphopeptide substrate in a reaction by measuring the absorbance of a molybdate:malachite green: phosphate complex that is formed. Two synthesized phosphopeptides, END(pY)INASL and DADE(pY)LIPQQG, acted upon by a broad array of tyrosine phosphatases, served as substrates (23). Frozen gastrocnemius muscle was homogenized in cold buffer A (1 mM DTT, 4 mM EDTA, 2.5 mM benzamide, 1 μM leupeptin, 1 μM pepstatin, 0.15 μM aprotinin, and 2 mM PMSF in 25 mM HEPES [pH 7.4]) and centrifuged at 350,000 × g for 30 min, and the supernatant was removed. The pellet was solubilized by incubation in 250 μl of buffer B (Buffer A containing 1% Triton X-100 and 0.6 M KCl) on ice for 30 min and recentrifuged at 150,000 × g for 1 h. The supernatant was collected as the solubilized “particulate” fraction, which contains 90% of the PTPase activity in muscle (24), and this fraction was assayed and corrected for endogenous phosphate.

**Statistical Analyses**

Specific mRNA were normalized for the internal control gene and are expressed as transcript/housekeeping ratios. Tyrosine-phosphorylated protein levels were normalized for the respective protein levels. The control V-treated PF group mean was given a value of 100, and individual values are expressed relative to this value. Data are given as mean ± SEM. Two-tailed unpaired t tests were applied for comparison of two normally distributed groups; comparisons between more than two normally distributed groups were made by one-way ANOVA followed by pairwise multiple comparison with the Holms t test (25). For more than two nonnormally distributed groups, the Kruskal-Wallis statistic was applied followed by the Student Newman Keuls test to distinguish between groups (25). P < 0.05 was considered statistically significant.

**Results**

**Serum Biochemistry**

The serum creatinine and urea nitrogen levels were increased significantly in the CRF rats (1.18 ± 0.2 and 71.7 ± 15.5 mg/dl) compared with the PF controls (0.4 ± 0.02 and 17.2 ± 0.6 mg/dl). However, serum total CO₂ levels in the CRF rats were similar to those in the controls (22.1 ± 0.7 versus 20.6 ± 0.5 mmol/L; P > 0.05). There was a significant (19%) increase in the serum CRP levels in the CRF rats compared with the PF controls (204 ± 7 versus 171 ± 5 pmol/L; P < 0.05).

**IGF-1 and Suppressor of Cytokine Signaling mRNA Levels**

The results of the real-time PCR assays for IGF-1 are shown in Figure 1. Basal IGF-1 mRNA levels measured in gastrocnemius muscle of the V-treated CRF and control rats were similar. However, whereas GH treatment induced a significant (42%) increase in IGF-1 expression in the controls, the response to GH was muted and insignificant in the CRF group, reflecting resistance to the hormone (Figure 1). SOCS1 and SOCS3 mRNA levels did not differ significantly between the CRF and control groups (Table 2). The basal expression of SOCS2 and CIS was increased by 55% in CRF muscle, but this did not reach statistical significance. GH treatment caused a significant two- to threefold increase in SOCS2, SOCS3, and CIS mRNA levels in both groups with a notably greater increase in SOCS2 expression in CRF compared with the PF control group (465 ± 104 versus 266 ± 35 relative arbitrary units; P < 0.05).

**GH-Mediated JAK2/STAT5 Signal Transduction**

The GHR, JAK2, and STAT5 protein levels were similar in the gastrocnemius muscle of CRF and PF control groups (Figure 2A). In the V-treated rats, tyrosine phosphorylation of these proteins was negligible or absent, whereas 15 min after an intravenous bolus of bGH, 3 μg/100 g body wt, tyrosine

![Figure 1](Image)
phosphorylation of these proteins was marked (Figure 2A). Of note, however, in the CRF group, phosphorylation of the GHR, JAK2, and STAT5 proteins was depressed significantly and to a similar extent (40% to 48%), compared with the control values (Figure 2, B and C). We then set out to determine whether the translocation of phospho-STAT5 into the nucleus was also reduced. Phospho-STAT5 levels were measured in nuclear extracts obtained 15 min after GH administration. As shown in Figure 3, the nuclear phospho-STAT5 levels were significantly lower in the CRF group (68 ± 11 versus 100 ± 8 relative arbitrary units; P < 0.01). It is interesting that there was a significant (P < 0.05) linear relationship between the serum creatinine and the relative phospho-STAT5 levels in muscle and the relative phospho-JAK2 and relative phospho-STAT5 and phospho-GHR levels (Figure 4). This illustrates the interrelationship between the different signaling molecules and suggests that the signaling defect arises at the level of JAK2 phosphorylation and further that it is influenced by the degree of renal failure. Finally, to determine whether the resistance to GH can be overcome by a supramaximal dose of GH, we measured STAT5 phosphorylation in gastrocnemius muscle collected 10 min after an intravenous bolus of bGH 25 µg/100 g. At this GH dose, relative phospho-STAT5 levels were similar in the CRF and control groups (107 ± 4 versus 100 ± 5 relative arbitrary units, respectively; Figure 5).

### Discussion

Skeletal muscle wasting and weakness are common in patients with advanced renal failure, and as many of these patients are elderly and inactive, the changes induced by renal failure can be especially disabling (26,27). Contributing to the muscle wasting is resistance to the action of GH (28), and in this study, we set out to define the mechanisms that account for the development of GH resistance in renal failure. We confirmed that in rats with surgically induced CRF, there is indeed skeletal muscle resistance to the action of GH, for GH-induced IGF-1 gene expression was depressed significantly. Because most of the actions of GH are mediated through IGF-1, a potent anabolic hormone essential for maintaining normal muscle mass (29), attenuated GH-induced skeletal muscle IGF-1 expression may be one way whereby GH resistance contributes to uremic muscle wasting. We then studied the effect of uremia on GH-mediated JAK2-STAT5 signal transduction as this pathway mediates IGF-1 transcription and body growth (16–19). We found that despite normal GHR, JAK2, and STAT5 protein levels, activation of these proteins, as measured by the level of protein tyrosine phosphorylation, was impaired by nearly half. Nuclear translocation of the phosphorylated STAT5 was also depressed significantly, and as this would be expected to reduce gene transcription, it provides an explanation for the failure of GH to stimulate IGF-1 gene expression.

Two potential mechanisms that account for the attenuated GH-mediated JAK2-STAT5 signal transduction were uncovered in this study. First, the basal mRNA level of the intracellular feedback regulatory protein SOCS2 and CIS were elevated by 55% in skeletal muscle of CRF animals, which did not reach statistical significance, whereas after treatment with GH, there was an exaggerated increase in SOCS2 mRNA level. SOCS proteins are a family of cytosolic proteins that are induced by cytokines and serve as negative feedback regulators of the JAK-STAT pathway by binding to cytokine receptor-JAK signaling complexes (20,30). Among the members of the SOCS family of proteins, GH stimulates SOCS-1, -2, and -3 and CIS expression. These GH-inducible proteins partially or completely inhibit GH-dependent JAK2 activation. In SOCS2-

### Table 2. Effect of 8-day GH treatment and CRF on skeletal muscle CIS, SOCS-1, SOCS-2, and SOCS-3 mRNA levels

<table>
<thead>
<tr>
<th></th>
<th>CRF Effect</th>
<th>GH Effect</th>
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<tr>
<td>Vehicle</td>
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<tr>
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</tr>
<tr>
<td>GH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIS</td>
<td>155 ± 60b</td>
<td>326 ± 54c</td>
</tr>
<tr>
<td>SOCS1</td>
<td>73 ± 9b</td>
<td>132 ± 28b</td>
</tr>
<tr>
<td>SOCS2</td>
<td>155 ± 30b</td>
<td>465 ± 104d</td>
</tr>
<tr>
<td>SOCS3</td>
<td>115 ± 8b</td>
<td>282 ± 52c</td>
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</table>

**a** GH, growth hormone; CRF, chronic renal failure. Rats with CRF or pairfed controls were treated with GH or vehicle for 8 d. On the final day, GH or vehicle was administered 6 h and then again 1 h before the rats were killed. mRNA levels were measured by quantitative real-time PCR and adjusted for the internal housekeeping genes GAPDH or ribosomal L7. The results, mean ± SEM of 6 to 8 rats/group, are expressed relative to the pairfed vehicle-treated control group assigned a mean value of 100. Dissimilar superscript letters indicate significant difference between groups in each row (P < 0.05). Groups with common superscript letters are similar.
deleted mice, GH-mediated STAT5 activation is enhanced and the animals grow larger than the wild-type mice (31). Proinflammatory cytokines also stimulate SOCS expression and in this way can inhibit GH action (32,33). Because the serum CRP levels were elevated significantly in the CRF animals, the increase in SOCS2 and CIS expression may have arisen in part because of heightened inflammatory cytokine activity (20). This may be relevant to the patient with ESRD (34), for there is evidence that chronic inflammation is present in 30 to 60% of all ESRD patients (5). However, it is unclear how GH causes an exaggerated increase in SOCS2 expression in CRF when GH-activated JAK2-STAT5 signal transduction is impaired. One possibility is that this response is induced through a non-STAT–mediated signaling pathway.

The other key step in the regulation of JAK2/STAT5 that may be altered in the skeletal muscle of uremic rats is dephosphorylation of the signaling molecules by protein tyrosine phosphatases; skeletal PTPase activity was increased significantly by twofold in the uremic rats. If this general increase of PTPase activity includes specific PTPases responsible for the dephosphorylation of proteins in the JAK2-STAT5 signaling pathway, then this could potentially lead to more rapid dephosphorylation and thus inactivation of the signaling proteins (20).

Several PTPases, including SHP-1, SHP-2, and PTP1B, are involved in the regulation of GH-stimulated JAK-STAT signal transduction, and we are currently attempting to identify whether the GH receptor (GHR), Janus-associated kinase 2 (JAK2), and signal transducers and activators of transcription 5 (STAT5) protein levels are unchanged in skeletal muscle of CRF rats, but GH-induced protein tyrosine phosphorylation of these proteins is attenuated. (A) Western immunoblots of GHR, JAK2, and STAT5 protein levels in lysates prepared from the gastrocnemius muscle of CRF and control pairfed (Con) rats obtained 15 min after an intravenous bolus of GH (3 μg/100 g) or vehicle. The samples from vehicle-treated animals were pooled for this immunoblot. (B) Tyrosine-phosphorylated GHR, JAK2, and STAT5 levels in the same samples as shown in A. The phospho-GHR and phospho-JAK2 were detected in immunoblots of GHR and JAK2 immunoprecipitates with antiphosphotyrosine antibody (clone 4G10). Phospho-STAT5 was detected without immunoprecipitation using a phospho-STAT5 specific antibody (n = 5 to 8 rats/group). (C) Relative phosphorylation of GHR, JAK2, and STAT5 proteins 15 min after bGH bolus in CRF and control animals. *P < 0.05. Phospho-protein signals were corrected for the specific protein levels, and the ratios were normalized to the pairfed control mean, which was assigned a value of 100. Bars indicate mean ± SEM.

Figure 2. GH receptor (GHR), Janus-associated kinase 2 (JAK2), and signal transducers and activators of transcription 5 (STAT5) protein levels are unchanged in skeletal muscle of CRF rats, but GH-induced protein tyrosine phosphorylation of these proteins is attenuated. (A) Western immunoblots of GHR, JAK2, and STAT5 protein levels in lysates prepared from the gastrocnemius muscle of CRF and control pairfed (Con) rats obtained 15 min after an intravenous bolus of GH (3 μg/100 g) or vehicle. The samples from vehicle-treated animals were pooled for this immunoblot. (B) Tyrosine-phosphorylated GHR, JAK2, and STAT5 levels in the same samples as shown in A. The phospho-GHR and phospho-JAK2 were detected in immunoblots of GHR and JAK2 immunoprecipitates with antiphosphotyrosine antibody (clone 4G10). Phospho-STAT5 was detected without immunoprecipitation using a phospho-STAT5 specific antibody (n = 5 to 8 rats/group). (C) Relative phosphorylation of GHR, JAK2, and STAT5 proteins 15 min after bGH bolus in CRF and control animals. *P < 0.05. Phospho-protein signals were corrected for the specific protein levels, and the ratios were normalized to the pairfed control mean, which was assigned a value of 100. Bars indicate mean ± SEM.

Figure 3. Decreased nuclear accumulation of phosphorylated STAT5 in skeletal muscle of rats with CRF 15 min after GH administration. bGH (3 μg/100 g) was given intravenously as a bolus. Western immunoblots of nuclear protein extracts were performed with phospho-tyrosine-specific antibodies against phospho-STAT5.

Figure 4. (A) Relationship between relative phospho-STAT5 and the serum creatinine level. (B) Relationship between relative phospho-STAT5 and relative phospho-JAK2 levels. (C) Relationship between relative phospho-GHR level and relative phospho-JAK2 levels.

increase in SOCS2 and CIS expression may have arisen in part because of heightened inflammatory cytokine activity (20). This may be relevant to the patient with ESRD (34), for there is evidence that chronic inflammation is present in 30 to 60% of all ESRD patients (5). However, it is unclear how GH causes an exaggerated increase in SOCS2 expression in CRF when GH-activated JAK2-STAT5 signal transduction is impaired. One possibility is that this response is induced through a non-STAT–mediated signaling pathway.

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any of these PTPases could be a cause of the increased activity in uremic skeletal muscle. In this context, it is interesting to note that PTPase overactivity has been identified as a cause of insulin resistance in obesity and type 2 diabetes (24). Other regulatory processes may also be altered in CRF, for the regulation of GH-activated JAK2-STAT signal transduction involves multiple biochemical events that are currently not well defined. These include ubiquitination of the dephosphorylated GHR leading to receptor internalization and degradation, reduced affinity of activated STATS for their DNA binding sequences through the action of protein inhibitors of activated STATS (PIAS), and cross-talk between other cytokines and GH (20,35,36). It is of interest to note that when a supramaximal dose of GH was administered to the uremic rats, the defect in signal transduction was overcome and the phospho-STAT5 levels increased to a similar extent in CRF and control rats. This may explain the effectiveness of the clinical use of GH in the treatment of growth retardation in uremic children and muscle wasting in adults with ESRD (9,28).

The changes in the GH signal transduction that we observed in skeletal muscle are consistent with our previous description of impaired GH-mediated JAK2-STAT signal transduction in the liver of the uremic rat (11). In that study, JAK2 and STAT5 phosphorylation was impaired, and there was upregulation of GH-stimulated SOCS2 expression, but unlike the current findings in muscle, basal SOCS3 mRNA levels were elevated. GHR phosphorylation and hepatic PTPase activity were not examined. We have also found that GH-mediated JAK2-STAT5 signaling is impaired in the heart of uremic rats (37). In contrast to the changes observed in skeletal muscle and liver of uremic rats, a somewhat different response occurs in sepsis, a condition that also causes GH resistance. In the septic rat, GH-mediated JAK2-STAT5 signaling is impaired in liver, whereas in skeletal muscle, the signaling is intact (38).

In conclusion, it seems that skeletal muscle resistance to GH arises in uremia because of impaired JAK2-STAT5 signal transduction. This may be caused by at least two exaggerated regulatory processes. One is an increase in protein tyrosine phosphatase activity that could potentially increase the rate of dephosphorylation and thus inactivation of the signaling proteins. The other is overexpression of the negative regulator of GH signaling, SOCS2, and this may in part reflect a response to chronic inflammation. In turn, the attenuated GH-activated JAK2-STAT5 signal transduction pathway leads to impaired GH-stimulated IGF-1 expression, which likely contributes to the muscle wasting of uremia.

Acknowledgments
This study was supported by a Merit Review Grant from the Research Service of the Department of Veterans Affairs.

References
3. Kalantar-Zadeh K, Kopple JD, Block G, Humphreys MH: A malnutrition-inflammation score is correlated with morbidity and