Skeletal muscle mRNA for IGF-IEa, IGF-II, and IGF-I receptor is decreased in sedentary chronic hemodialysis patients

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Skeletal muscle mRNA for IGF-IEa, IGF-II, and IGF-I receptor is decreased in sedentary chronic hemodialysis patients.

Background. Maintenance hemodialysis patients often display evidence for protein-energy malnutrition, inflammation, and sarcopenia. We therefore investigated whether sedentary maintenance hemodialysis patients have decreased skeletal muscle mRNA levels and muscle and serum protein concentrations of certain growth factors.

Methods. Fifty-one clinically stable maintenance hemodialysis patients (32 men and 19 women), and 21 normal adults (16 men and five women) of similar age, gender mix, racial/ethnic backgrounds, serum albumin, body composition, and level of sedentary activity were studied. Individuals underwent biopsy of the right vastus lateralis muscle, and real-time polymerase chain reaction (PCR) amplification of mRNAs for insulin-like growth factor-I (IGF-I), IGF-II, IGF-I receptor (IGF-IR), IGF-IIR, and myostatin (44 patients) was performed. Serum and muscle IGF-I and IGF-II, serum proinflammatory cytokines, and leg muscle strength, power, and fatigability were measured.

Results. Maintenance hemodialysis patients displayed significantly reduced mRNA levels for IGF-IEa mRNA ($P < 0.05$), IGF-II ($P < 0.001$), and IGF-IR ($P < 0.001$), and no difference in mRNAs for IGF-IEc, IGF-IIR, or myostatin as compared to normal controls. Muscle mRNA levels, in general, followed the same pattern in male and female maintenance hemodialysis patients considered separately. In the maintenance hemodialysis patients, muscle IGF-I protein, serum IGF-II and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) were each increased, whereas serum C-reactive protein (CRP) and interleukin-6 (IL-6) were normal. Muscle strength and power, but not fatigability, were reduced in the maintenance hemodialysis patients.

Conclusion. In sedentary, clinically stable maintenance hemodialysis patients as compared to sedentary normal individuals, the mRNA levels for IGF-IEa, IGF-II, and the IGF-I receptor are decreased in vastus lateralis muscle. Protein levels for muscle IGF-I and serum IGF-II are increased.

Individuals with end-stage renal disease (ESRD), including those undergoing maintenance dialysis, often have protein-energy malnutrition [1] and an inflammatory state [2]. These conditions may promote a catabolic or anabolic state leading to a reduction in skeletal muscle protein and sarcopenia. Indeed, reduced noncollagenous alkali-soluble protein has been described in skeletal muscle of both adults and children with advanced chronic renal failure (CRF) [3, 4], and maintenance hemodialysis patients are often sarcopenic [5–7]. The quantity of growth factors expressed in muscle tissue is a determinant of protein synthesis and degradation and hypertrophy of skeletal muscle [8–10]. The degree of expression of such proteins, in turn, is dependent on the magnitude of gene expression or transcription of the genes encoding these proteins.

As part of an investigation of the potential causes of sarcopenia in individuals with ESRD, we examined the mRNA levels for such growth factors as insulin-like growth factor-I (IGF-I), both the splice variants, IGF-IEa and IGF-IEc, and IGF-II, their receptors (IGF-IR and IGF-IIR), and myostatin in skeletal muscle of clinically stable, sedentary maintenance hemodialysis patients. Serum and skeletal muscle levels of the IGF-I and IGF-II proteins as well as serum levels of certain proinflammatory cytokines, and leg muscle function were also measured. Normal sedentary individuals of similar age, gender distribution, and racial/ethnic backgrounds served as controls.

METHODS

This study was carried out in 51 patients, 32 men and 19 women, who had been undergoing maintenance hemodialysis for a mean of 54.7 months (range 6 to 297
months) and 21 normal control subjects, 16 men and five women. The maintenance hemodialysis patients were participants in a clinical trial to examine the physiologic and metabolic responses to exercise training. The muscle biopsies on which this study is based were performed before the initiation of the exercise training intervention. Normal controls were selected on the basis of having no chronic illnesses or acute inflammatory processes. An inclusion requirement for participation in this study was that each maintenance hemodialysis patient and normal control give a history of not engaging in recent manual labor or exercise training. Although it could not be determined with certainty that the normal individuals were as physically inactive as the maintenance hemodialysis patients, none of the maintenance hemodialysis patients or normal controls described participation in regular physical exercise or frequent sports activities.

Maintenance hemodialysis patients frequently sustain intercurrent illnesses or suffer from severe comorbidity [11] which could affect the results of this study. Hence, in order to examine the status of ESRD patients undergoing maintenance hemodialysis rather than the potential effects of complicating illnesses, only clinically stable maintenance hemodialysis patients who had volunteered and been accepted for participation in an exercise training program were evaluated. The maintenance hemodialysis patients did not have evidence of acute or chronic inflammatory diseases, severe heart, lung or liver failure, muscle diseases, any joint infirmity that would prevent them from exercising their lower extremities, or abuse of alcohol or other drugs. One woman and four men receiving maintenance hemodialysis had noninsulin-requiring diabetes mellitus.

The normal subjects had normal serum creatinine concentrations, and none had a history of kidney disease or hypertension. None of the maintenance hemodialysis patients or normal individuals manifested evidence of coronary artery ischemia during an exercise electrocardiogram. The dorsal pedal artery:brachial artery blood pressure ratio was 0.90 or greater in all maintenance hemodialysis patients and normal subjects. This study was approved by the Human Subjects Committee at Harbor-UCLA Medical Center, and informed written consent was obtained from all subjects.

Skeletal muscle biopsy

Biopsies were performed on the right vastus lateralis muscle about 10 cm cephalad to the superior border of the patella, 1 to 2 cm anterior to the midlateral line of the right leg. A Bergstrom or U.C.H. skeletal muscle biopsy needle (Popper & Sons, Inc., New Hyde Park, NY, USA) was used. All subjects were fasted from 10:00 p.m. the night before biopsy, except occasionally for a small glass of water imbibed the morning of the biopsy. Maintenance hemodialysis patients were biopsied in the midweek, 1 day after a hemodialysis treatment.

RNA extraction

Blood was blotted off the tissue, and fat and connective tissue were quickly dissected away from the skeletal muscle which was then placed immediately in liquid nitrogen. At the time of analysis, the frozen tissue was homogenized in TRIzol Reagent using a power homogenizer (PowerGen) (Fisher Scientific, Pittsburgh, PA, USA) as previously described [12]. The homogenate was incubated for 5 minutes at room temperature; chloroform was added; the sample was shaken vigorously for 15 seconds, and incubated at 15 to 30°C for 2 to 3 minutes. The mixture was separated into two phases by centrifuging at 12,000 rpm for 15 minutes at 4°C. RNA was precipitated from the aqueous phase by mixing with isopropanol. The sample was incubated at 15 to 30°C and centrifuged at 12,000 rpm at 4°C. The pellet was washed twice with 75% ethanol and centrifuged at no more than 7500 × g for 5 minutes at 4°C. Any remaining ethanol was removed. The RNA was dissolved in RNase free water and incubated for 10 minutes at 55 to 60°C. The concentration of RNA was measured with a spectrophotometer (Beckman DU 640, Fullerton, CA, USA).

Reverse-transcriptase (RT) reaction

cDNA synthesis was accomplished with 1 μg RNA; the final reaction mixture contained 1 μg total RNA, 10 units RNase inhibitor, 1 mmol/L each deoxynucleoside triphosphate (dNTP), 3.2 μg random primer [dN], avian myeloblastosis virus (AMV) Reverse Transcriptase 20 U and RNase free water, in a final volume of 20 μL of buffer as described in the manufacturer’s protocol (Roche Applied Science, Indianapolis, IN, USA). The reaction was allowed to proceed for 10 minutes at 25°C, 1 hour at 42°C, and was then terminated after 5 minutes at 99°C.

Real-time polymerase chain reaction (PCR) amplification of mRNAs for IGF-I Ea and IGF-I Ec, IGF-II, IGF-1R, IGF-II R, and myostatin

For real-time PCR, the ABI Prism Sequence Detection System 7000 was used (Applied Biosystems Inc., Foster City, CA, USA). Specific primers were designed using the m-fold and Primer-3 Web-based programs, to amplify the mRNA from reverse transcribed cDNA for IGF-I, IGF-II, IGF-1R, IGF-II R, and myostatin with the following oligonucleotides: IGF-IEa forward, 5′-CTTCCGAGCTGTGATCTA-3′, and reverse, 5′-TGCGTTCCTCAATGTACTTC-3′; IGF-I Ec forward, 5′-CGAAGTCTCAGAGAAGGAAAGG-3′, and reverse, 5′-ACAGGTAACCTCGTGCAAGGC-3′; IGF-II forward, 5′-CCTGGAGACGTACTGTGCTA-3′, and...
reverse 5′-GGACTGCTTCCAGGTGTC-3′; IGF-IR forward, 5′-CTGCTGATGTTGACGTTCT-3′, and reverse, 5′-TCAAGTTGATCTTCACCA-3′; IGF-II forward, 5′-GGTTTGTATGTCAGACTT-3′, and reverse, 5′-TGCTCTGAGACTGTAGTT-3′; myostatin forward, 5′-GGAAACACATCATACCATGC-3′ and reverse, 5′-ATCCATAGTTGGCCCTTTC-3′; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward, 5′-ACTACTGACCACAGAGACT-3′, and reverse, 5′-CATGCACTGAGCTCCCGTT-3′.

Primers were designed to include an intron in the PCR product to distinguish between genomic DNA contamination and cDNA. The primer concentration was chosen without primer dimer formation. The primers for IGF-I mRNA specifically replicated the mRNA isoform for IGF-I or IGF-II [13] or IGF-II [14]. Fifty microliters of reaction were set up containing 2 μL of cDNA, 25 μL 2 × SYBR Green PCR Master Mix (Qiagen, Inc., Valencia, CA, USA), and 0.5 μmol/L of each specific sense and antisense primer. PCR parameters for amplification were as follows: incubation for 2 minutes at 50°C, initial denaturation for 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C, and 1 minute at 60°C. Each sample was analyzed in duplicate. Quality control was performed with melting curve analysis and amplified products were electrophoresed on 2% agarose gel and stained with ethidium bromide to obtain only one specific band with correct size.

Gene expression for GAPDH was assayed as an internal control (done in parallel for each experiment, not in the same sample well). For quantification we used Relative Quantification with External Standards (Roche Applied Science). Standard curves representing five-point serial dilutions of known amounts of cDNA were analyzed in each assay. A known amount of cDNA was used as a calibrator in each assay. Standard curves were used in the relative quantification of the product generated in the early exponential phase of the amplification curve. The r² was greater than 0.99 for all standard curves, and amplification efficiency varied between 85% and 100%. Sample quantification was calculated from the standard curve. Duplicates were averaged and normalized by dividing by the internal control GAPDH mRNA in all of the experiments.

**Protein assay for muscle IGF-I and IGF-II**

The muscle sample was weighed and homogenized in acid/ethanol solution (12.5% N HCl/87.5% ethanol) at a concentration of 20 μL per mg muscle (PowerGen Homogenizer 125) (Fisher Scientific). The acid/ethanol extraction was used to separate IGF-I and IGF-II from their binding proteins [15]. The homogenate was then incubated for 1 hour at 4°C followed by centrifugation at 3000g at 4°C for 30 minutes. The resultant supernatant was decanted and neutralized with 0.855 mol/L Tris base. The neutralized supernatant was incubated overnight at −20°C and then centrifuged at 3000g at 4°C for 30 minutes. The final supernatant was stored at −80°C for determination of IGF-I and IGF-II by radioimmunoassay (RIA).

Muscle IGF-I and IGF-II were measured as follows. The DSL-5600 immunoradiometric assay (IRMA) Kit (Diagnostic System Laboratories, Inc. Webster, TX, USA) was used for the IGF-I assay. Fifty microliters of reconstituted standards, controls, and supernatants extracted from the muscle of the subjects were added to the appropriate tubes; then 200 μL of anti-IGF-I (125I-labeled) were added to all tubes. Duplicate tubes were prepared for each specimen, including standards and controls. All tubes were then incubated at room temperature for 3 hours on a shaker set at 180 rpm. Except for the total count tubes, all of the tubes were decanted and then washed three times. All tubes were counted for radioactivity in a gamma counter for 1 minute. Net counts per minute for standards, unknowns, and controls in duplicate tubes were averaged, and nonspecific binding was subtracted. A linear regression analysis of the net counts per minute from five IGF-I standards was used to calculate the unknown IGF-I concentration. The IGF-II assay followed a similar procedure as specified in the DSL-2600 Kit.

**Protein assay for serum IGF-I and IGF-II**

Nine hundred microliters of acid-ethanol solution (12.5%/87.5% vol/vol) were added to 100 μL of the sample sera. The mixtures were vortexed and incubated at room temperature for 30 minutes and then centrifuged at 3000g at 4°C for 30 minutes. After centrifugation, 200 μL of supernatants were transferred to the corresponding tubes; then 0.1 mL of Tris base solutions were added to the supernatant. The mixtures were vortexed thoroughly and incubated for 30 minutes at room temperature, then centrifuged at 3000g at 4°C for 30 minutes. After centrifugation, 100 μL supernatants were transferred to the tubes containing 1.4 mL phosphate dilution buffer, the final dilution of the sample was 1 to 225. The samples were mixed thoroughly and underwent the RIA. Extraction for the serum IGF-II assay followed a similar procedure as specified in the DSL-2600 Kit.

Serum IGF-I and IGF-II were measured as follows. Nichols Institute Diagnostics IGF-I 100T Kit (San Clemente, CA, USA) was used for the serum IGF-I assay. Except for the total count tubes, 150 μL of standards, extracted controls and samples were added to the corresponding tubes; then 250 μL of phosphate buffer were added to nonspecific binding tubes and 150 μL of phosphate buffer were added to the other tubes. Except for the total count and nonspecific binding tubes, 100 μL IGF-I antibodies from rabbit were added to all tubes; the mixtures was vortexed and then incubated at room
temperature for 1 hour. One hundred microliters of IGF-I antibodies (125I-labeled) were added to all tubes. Except for the total count tubes, all tubes were vortexed and incubated at 4°C for 18 hours; 500 μL of cold and gently mixed antirabbit precipitants was added. Then 50 μL of normal rabbit serum were added to the tubes. All tubes were vortexed and incubated for 20 minutes at room temperature. All tubes, except for the total column tubes, were centrifuged at 3000 g for 18 hours; 500 μL of cold and gently mixed antirabbit precipitants was added. Then 50 μL of normal rabbit serum were added to the tubes. All tubes were vortexed and incubated for 20 minutes at room temperature. All tubes, except for the total column tubes, were centrifuged at 3000 g for 18 hours and then decanted. All tubes were counted, and results were calculated. The serum IGF-II assay followed the same procedure as specified in the DSL-2600 Kit.

C-reactive protein (CRP) assay

The Wako Diagnostics (Richmond, VA, USA) CRP-UL Kit was used for the CRP assay. It is based on latex immunoassay. The Hitachi 704 analyzer was used for this assay.

Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) assay

The R&D Systems (Minneapolis, MN, USA) IL-6 Kit was used for the IL-6 assay. One hundred microliters of assay diluent and 100 μL of standard or sample were added to each well; the plate was incubated for 2 hours at room temperature on a microplate shaker at 500 rpm. The wells were washed six times. Two hundred microliters of IL-6 conjugate were added to each well, incubated for 2 hours at room temperature on the shaker, and then washed again. Fifty microliters of substrate solution were added to each well and incubated for 30 minutes. Then 50 μL of stop solution were added to each well. The optical density was determined using a microplate reader at 490 nm; 650 nm reading was used for correction. The TNF-α assay followed a similar procedure as specified in R&D Systems TNF-α Kit.

Nutrient intake, clinical laboratory measurements, and anthropometry

Nutrient intake was determined at the time of the muscle biopsy from 3-day dietary diaries and dietary interviews conducted by research dieticians in the Harbor-UCLA General Clinical Research Center. Serum for assays of IGF-I, IGF-II, CRP, IL-6, and TNF-α were obtained at the time of the muscle biopsy. Other serum and whole blood values reported here were measured in blood obtained immediately before the onset of a midweek hemodialysis. Measurements of serum urea nitrogen (SUN), creatinine, bicarbonate and albumin, and the hemoglobin and hematocrit were performed using standard clinical laboratory techniques.

Body weight, height, biceps, triceps, subcapular and mid-calf skinfold thickness, and mid-arm, mid-thigh, and mid-calf circumferences were measured by experienced Harbor-UCLA General Clinical Research Center research nutritionists as previously described [16]. Body mass index and mid-arm muscle circumference were assessed as previously reported [16]. Mid-arm muscle circumference was calculated from the following equation:

\[
\text{Mid-arm muscle circumference} = \text{mid-arm circumference} - [0.31415927 \times \text{triceps skinfold (cm)}]
\]

Mid-thigh and mid-calf circumferences were measured at the point of largest cross-sectional area of the thigh and calf, respectively. Mid-thigh muscle circumference and mid-calf muscle circumference were calculated from the following equations:

\[
\text{Mid thigh muscle circumference} = \text{mid - thigh circumference} - [0.31415927(\pi) \times \text{mid-thigh skinfold (cm)}]
\]

Body density was calculated from the equation:

\[
\text{Body density (kg/m}^3\text{)} = K_1 - [K_2 \times \log_{10} \text{SK4 (mm)}]
\]

where K1 and K2 are constants specific for a given gender and age range of normal people [17] and SK4 refers to the sum of the thicknesses of the biceps, triceps, subcapular, and suprailiac skinfolds.

Leg muscle function

Quadriceps strength was assessed with a seated leg press exercise instrument (Keiser Sport, Fresno, CA, USA) using the five repetition maximum technique [18] rather than the single repetition method. Knee and hip extension power (rate of generating force) was assessed with a validated leg power instrument (University of Nottingham Medical College, Nottingham, UK) [19]. Fatigability (the ability to make repetitive submaximal dynamic contractions) was assessed with the same bilateral leg press exercise used in strength testing.

Statistical analyses

Data are expressed as the mean value ± SD or range of values. Statistical comparisons were performed by the unpaired t test and by linear regression analyses. For
comparison of values between groups, a statistically significant difference was taken as $P < 0.05$. Since many linear regression analyses were performed, only associations with a regression coefficient greater than 0.30 are reported.

**RESULTS**

Characteristics of the maintenance hemodialysis patients and normal controls are shown in Table 1. More men than women were present in both the maintenance hemodialysis patient and normal control groups, and most of the maintenance hemodialysis patients and normals were either African American or non-African American Hispanic. The mean age and serum albumin concentrations were similar in the two groups, whereas serum bicarbonate was slightly higher and the hemoglobin and hematocrit were slightly lower in the maintenance hemodialysis patients.

Dietary energy and protein intakes, expressed in kcal/day and g protein/day, were each decreased in male maintenance hemodialysis patients as compared to normal men (Table 2). Protein intake in male and female maintenance hemodialysis patients combined, expressed as g protein/day, were also lower than in the normal subjects. On the other hand, dietary energy or protein intake in the female maintenance hemodialysis patients or, when expressed as kcal/kg/day or g protein/kg/day, in males or females considered separately or combined were not different from normals (Table 2).

Body composition measurements, obtained by standard manual anthropometric methods, are shown in Table 3. No maintenance hemodialysis patient or normal individual manifested edema. There were no significant differences in anthropometric measurements in the maintenance hemodialysis patients vs. the normal controls when the males or females were considered separately or when the two genders were combined. However, the absolute values for the edema-free body weight of the female maintenance hemodialysis patients were greater than in the normal women; this was due to one obese female maintenance hemodialysis patient who weighed 164.1 kg.

The mRNA levels for IGF-IEa, IGF-IEc, IGF-II, IGF-IR, IGF-IIR, and myostatin in the right vastus lateralis muscle are shown in Table 4. Because of technical difficulties, myostatin mRNA was assayed in only 44 maintenance hemodialysis patients (27 men) and 18 normal controls (13 men). There was a statistically significant reduction in IGF-IEa, IGF-II mRNA, and IGF-IR mRNA in the MHD patients as compared to the normal controls. There were no significant differences in the mRNA levels for IGF-IEc, IGF-IIR, or myostatin between the two groups, although the absolute values for IGF-IEc averaged 32% lower in the maintenance hemodialysis patients vs. normals.

Most of these differences or lack of differences persisted when the analyses were limited to a single gender (Table 4). Thus, in male and female maintenance hemodialysis patients, the mRNA levels for IGF-II and IGF-IR were significantly lower than in the normals of the same gender. IGF-IEa mRNA was lower than the normal values in men, but not in women receiving maintenance hemodialysis, possibly because the sample size of the women was smaller. There was no significant difference in the mRNA for IGF-IEc, IGF-IIR, or myostatin in either men or women receiving maintenance hemodialysis as compared to the respective normal controls of the same gender.

Among the patients undergoing maintenance hemodialysis, the women had greater mRNA levels for IGF-IIR in their vastus lateralis muscle than did men ($P = 0.018$) (Table 4). There were no other significant differences in gene expression for any of the other growth factor proteins assessed in men vs. women in either the maintenance hemodialysis or the normal control groups.

The five maintenance hemodialysis patients who had diabetes mellitus showed the same trend in mRNA levels as the nondiabetic maintenance hemodialysis patients (data not shown) with one exception. The exception was significantly higher muscle IGF-II mRNA in the diabetic maintenance hemodialysis patients (12.7 ± 4.8) as compared to the nondiabetic maintenance hemodialysis patients (6.6 ± 4.5) ($P < 0.05$). When the 46 maintenance hemodialysis patients who did not have diabetes mellitus were analyzed separately, the identical muscle mRNAs were significantly different from the controls as was...
observed for the entire group of 51 diabetic and nondiabetic maintenance hemodialysis patients.

IGF-I protein concentrations in the vastus lateralis muscle were significantly increased in maintenance hemodialysis patients vs. normal controls (Table 5). Muscle IGF-II protein was not different between the two groups. Serum IGF-II and TNF-α were significantly greater in the maintenance hemodialysis patients vs. normal controls (Table 5). In contrast, there were no significant differences in serum IGF-I, CRP, and IL-6 between the two groups, although values for each of these proteins tended to be higher in the maintenance hemodialysis patients (Table 5).

Because a rather large number of variables were measured in this study, only correlations concerning growth factors and cytokines that had a regression coefficient value greater than 0.30 are reported here. In maintenance hemodialysis patients, muscle mRNA levels for IGF-Iα were correlated with IGF-IEc (r = 0.385, P = 0.005). Muscle IGF-II mRNA was correlated with muscle IGF-IR mRNA (r = 0.321, P = 0.022) and with muscle IGF-II protein, expressed per mg of wet muscle (r = 0.424, P < 0.002). Muscle IGF-I protein, expressed per µg of muscle protein, was correlated with IGF-II protein, expressed per µg muscle protein (r = 0.595, P < 0.001). Muscle IGF-II protein, expressed per µg of muscle protein, was correlated with serum IGF-II protein (r = 0.491, P < 0.001) and with serum IGF-I (r = 0.315, P = 0.0244). In serum, IGF-I was correlated with IGF-II (r = 0.456, P < 0.001). In the maintenance hemodialysis patients, serum CRP correlated negatively with muscle IGF-IEc mRNA (r = −0.308, P < 0.05), and serum TNF-α was negatively correlated with serum IGF-II (r = −0.331, P < 0.03).

In the normal individuals, skeletal muscle IGF-I was strongly correlated with muscle IGF-II whether expressed per mg wet muscle weight (r = 0.761, P < 0.001) or per µg muscle protein (r = 0.689, P < 0.001). Muscle IGF-I, expressed per µg of muscle protein, correlated inversely with IL-6 (r = −0.543, P = 0.02), and serum CRP correlated positively with IL-6 (r = 0.55, P = 0.018).

Muscle strength and power were significantly reduced in the maintenance hemodialysis patients as compared to the normal controls (Fig. 1). There was no difference in muscle fatigability between the two groups.

**DISCUSSION**

This study indicates that in vastus lateralis muscle mRNA levels for some growth factors that promote protein synthesis and skeletal muscle mass are significantly decreased in clinically stable, sedentary maintenance hemodialysis patients as compared to sedentary normal

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**Table 2. Food intake**

<table>
<thead>
<tr>
<th></th>
<th>Maintenance hemodialysis patients</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>46</td>
<td>20</td>
</tr>
<tr>
<td>Energy intake kcal/day</td>
<td>1922 ± 830</td>
<td>1601 ± 136</td>
</tr>
<tr>
<td>Energy intake kcal/kg/day</td>
<td>27 ± 14</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Protein intake g/day</td>
<td>74.3 ± 29.4</td>
<td>64.0 ± 22.8</td>
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<tr>
<td>Protein intake g/kg/day</td>
<td>1.11 ± 0.52</td>
<td>1.12 ± 0.60</td>
</tr>
</tbody>
</table>

*aMean ± SD.
*bSignificantly different from normal subjects of the same gender or from the normal males and females combined, P < 0.05.

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**Table 3. Body composition by anthropometry**

<table>
<thead>
<tr>
<th></th>
<th>Maintenance hemodialysis patients</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>51</td>
<td>21</td>
</tr>
<tr>
<td>Height cm</td>
<td>165.0 ± 8.7</td>
<td>169.0 ± 9.7</td>
</tr>
<tr>
<td>Weight kg</td>
<td>72.4 ± 24.4</td>
<td>70.4 ± 11.0</td>
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<tr>
<td>Skinfold thickness</td>
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</tr>
<tr>
<td>Subscapular mm</td>
<td>22.9 ± 14.8</td>
<td></td>
</tr>
<tr>
<td>Triceps mm</td>
<td>21.7 ± 10.5</td>
<td></td>
</tr>
<tr>
<td>Biceps mm</td>
<td>14.0 ± 9.3</td>
<td></td>
</tr>
<tr>
<td>Mid-arm muscle circumference cm</td>
<td>31.4 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Mid-calf muscle circumference cm</td>
<td>34.8 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>Mid-thigh muscle circumference cm</td>
<td>47.9 ± 8.2</td>
<td></td>
</tr>
<tr>
<td>% Body fat†</td>
<td>26.1 ± 8.7</td>
<td></td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
<td>24.0 ± 3.0</td>
<td></td>
</tr>
</tbody>
</table>

*aAnthropometry performed by Harbor-UCLA General Clinical Research Center research dieticians. There were no significant differences between male, female or total maintenance hemodialysis patients vs. the respective normal controls.
*bMean ± SD.
†Total body fat expressed as percent of total body weight.
and also for muscle hypertrophy [10]. Circulating IGF-I appears to have relatively little effect on muscle hypertrophy [20], and evidence suggests that most skeletal muscle IGF-I is synthesized in situ [21]. Hence, alterations in messenger RNA levels for growth factors in skeletal muscle may affect muscle mass.

Three isoforms of IGF-I are recognized in humans: IGF-IEa, IGF-IEb, and IGF-IEc. These isoforms are generated by splice variants of the same IGF-I gene [22]. IGF-IEa and IGF-IEc, the isoforms for which the IGF-I mRNA was measured in the present study, have been identified in skeletal muscle. IGF-IEb is found primarily in liver, and it is not known whether it has any effect on muscle. Thus these reductions in gene expression for IGF-I (i.e., the IGF-IEa and IGF-IEc isoforms), IGF-II and IGF-IR may predispose individuals to the atrophy of skeletal muscle fibers and sarcopenia that have been described in patients with advanced CRF or with ESRD who are undergoing maintenance dialysis therapy [5–7]. In humans, muscle strength is proportional to muscle mass [23]. Hence, sarcopenia, engendered in part by decreased mRNA levels for IGF-IEa, IGF-II and IGF-IR, may be a cause of the decreased strength described in individuals with advanced CRF or ESRD [24]. Although IGF-IEc mRNA was not significantly reduced in the maintenance hemodialysis patients, the same trend toward lower mRNA values was observed for this growth factor as for IGF-IEa mRNA (Table 4).

The causes for decreased mRNA levels for IGF-IEa, IGF-II, and IGF-IR in the maintenance hemodialysis patients are not clear. Growth factors in skeletal muscle may decrease with age [25], but the ages of the maintenance hemodialysis patients and normal volunteers were not different (Table 1). Similarly, the racial/ethnic and gender distribution of the two groups were similar, and, the same trend toward reductions in the mRNA for IGF-I, IGF-II, and IGF-IR was observed in male and female maintenance hemodialysis patients considered separately (Table 4).

States of protein-energy malnutrition (PEM) and inflammation are commonly present in maintenance hemodialysis patients [1, 2]. It is possible that these conditions may have led to a reduction in gene expression or in mRNA levels in skeletal muscle of these patients. The maintenance hemodialysis patients in this study showed a significantly increased serum TNF-α, and the absolute values for serum CRP and IL-6 were somewhat greater in these individuals, although the increase was not statistically significant (Table 5). Moreover, in skeletal muscle of the maintenance hemodialysis patients, serum CRP was negatively correlated with mRNA levels for IGF-IEa, and TNF-α was negatively correlated with serum IGF-II.

Reduced nutritional intake might also lead to alterations in the mRNA levels in skeletal muscle for some individuals of similar age, gender, and racial/ethnic characteristics. This reduction was observed for the mRNA levels for IGF-IEa, IGF-II, and the IGF-IR. In contrast, the mRNA levels for IGF-IEc, IGF-IIR, and myostatin were not significantly different between the maintenance hemodialysis patients and the normal subjects, whether the data were analyzed separately by gender or with the two genders combined.

Skeletal muscle IGF-I appears to be important for the preservation of skeletal muscle architecture and protein and also for muscle hypertrophy [10]. Circulating IGF-I was measured in the present study, have been identified in skeletal muscle. IGF-IEb is found primarily in liver, and it is not known whether it has any effect on muscle. Thus these reductions in gene expression for IGF-I (i.e., the IGF-IEa and IGF-IEc isoforms), IGF-II and IGF-IR may predispose individuals to the atrophy of skeletal muscle fibers and sarcopenia that have been described in patients with advanced CRF or with ESRD who are undergoing maintenance dialysis therapy [5–7]. In humans, muscle strength is proportional to muscle mass [23]. Hence, sarcopenia, engendered in part by decreased mRNA levels for IGF-IEa, IGF-II and IGF-IR, may be a cause of the decreased strength described in individuals with advanced CRF or ESRD [24]. Although IGF-IEc mRNA was not significantly reduced in the maintenance hemodialysis patients, the same trend toward lower mRNA values was observed for this growth factor as for IGF-IEa mRNA (Table 4).

The causes for decreased mRNA levels for IGF-IEa, IGF-II, and IGF-IR in the maintenance hemodialysis patients are not clear. Growth factors in skeletal muscle may decrease with age [25], but the ages of the maintenance hemodialysis patients and normal volunteers were not different (Table 1). Similarly, the racial/ethnic and gender distribution of the two groups were similar, and, the same trend toward reductions in the mRNA for IGF-I, IGF-II, and IGF-IR was observed in male and female maintenance hemodialysis patients considered separately (Table 4).

States of protein-energy malnutrition (PEM) and inflammation are commonly present in maintenance hemodialysis patients [1, 2]. It is possible that these conditions may have led to a reduction in gene expression or in mRNA levels in skeletal muscle of these patients. The maintenance hemodialysis patients in this study showed a significantly increased serum TNF-α, and the absolute values for serum CRP and IL-6 were somewhat greater in these individuals, although the increase was not statistically significant (Table 5). Moreover, in skeletal muscle of the maintenance hemodialysis patients, serum CRP was negatively correlated with mRNA levels for IGF-IEa, and TNF-α was negatively correlated with serum IGF-II.

Reduced nutritional intake might also lead to alterations in the mRNA levels in skeletal muscle for some individuals of similar age, gender, and racial/ethnic characteristics. This reduction was observed for the mRNA levels for IGF-IEa, IGF-II, and the IGF-IR. In contrast, the mRNA levels for IGF-IEc, IGF-IIR, and myostatin were not significantly different between the maintenance hemodialysis patients and the normal subjects, whether the data were analyzed separately by gender or with the two genders combined.

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Fig. 1. Leg press strength, extension power and fatigability in sedentary maintenance hemodialysis (MHD) patients (N = 51) and sedentary normal controls (N = 21) of similar age, gender distribution, and racial/ethnic backgrounds. Brackets indicate standard deviation. Significant difference between maintenance hemodialysis patients and controls. *P = 0.009; †P < 0.001; NS, not significantly different.

growth factors [26, 27], although the literature is somewhat conflictive with regard to the effects of nutrient restriction or fasting on tissue mRNA levels [26–29]. Moreover, estimates of dietary energy and protein intake in our maintenance hemodialysis patients, when adjusted for body size, were not different from the normal levels (Table 2). However, dietary protein requirements are increased in maintenance hemodialysis patients [30], and the estimated intakes indicated that many of the maintenance hemodialysis patients were probably ingesting less energy or protein than is recommended for them by the National Kidney Foundation K/DOQI Clinical Practice Guidelines [31]. Whether the evidence for active inflammation or the reductions in energy and/or protein intake, particularly in the male maintenance hemodialysis patients, were sufficient to contribute to the lower skeletal muscle mRNA levels is not known.

The results of the present study also indicated elevated IGF-I and normal IGF-II levels in skeletal muscle and elevated IGF-II and normal IGF-I concentrations in serum of maintenance hemodialysis patients. It is emphasized that we were not able to measure the IGF-Iα, IGF-Iβ, or IGF-Ic proteins, and the IGF-I protein concentrations reported refer to mature IGF-I [15]. IGF-I and IGF-II are largely bound to various binding proteins in both plasma and solid tissue, including skeletal muscle [32]. The binding proteins may serve several functions, including storage of IGF-I and rendering the bound component of this compound inactive [33]. Therefore, the finding of elevated IGF-I and normal IGF-II in skeletal muscle does not necessarily indicate either increased or normal rates of synthesis or activity of these proteins in skeletal muscle. Hence, these findings are not inconsistent with low mRNA levels for these proteins. Studies of cultured myoblasts indicate that the IGF-II protein may suppress IGF-II mRNA [34]. Whether increased IGF-I will suppress IGF-Iα mRNA in human skeletal muscle is not known.

It is pertinent that Ding et al [12] found decreased skeletal muscle IGF-I mRNA and IGF-I protein in rats with chronic renal insufficiency as compared with paired, sham-operated control rats. Tonshoff et al [35] also found that rats with chronic renal insufficiency displayed reduced hepatic, muscle and lung IGF-I mRNA, and normal plasma IGF-I concentrations. In contrast to the present study, Ding et al [12] observed increased IGF-IR mRNA and IGF-IR number in their rats with chronic renal insufficiency. Species differences, or the comorbidity, inflammation, more advanced renal failure or effects of the hemodialysis procedure in the maintenance hemodialysis patients might account for these disparate findings.

A possible limitation of this study is that the maintenance hemodialysis patients may have been sicker from comorbid illness and/or they may have been more sedentary. The maintenance hemodialysis patients were selected to be clinically stable individuals without active catabolic illness, and only five of these patients had noninsulin-requiring diabetes mellitus. The maintenance hemodialysis patients (but not the normal controls) had all agreed to participate in an exercise training project, and were almost certainly a healthier subset of maintenance hemodialysis patients. This may explain why their nutrient intake and anthropometric measures of body composition did not indicate a more malnourished status than the normal controls (Tables 2 and 3).

Both maintenance hemodialysis patients and normal controls in this study were selected to be sedentary individuals, although it is possible that the maintenance hemodialysis patients might have been less active physically than the normal control subjects. Individuals undergoing regular dialysis therapy often have markedly
limited daily physical activity [36]. Since exercise will increase mRNA levels in vastus lateralis muscle for a number of these growth factors in maintenance hemodialysis patients [16], it remains possible that despite our efforts to select normal control subjects with similar levels of physical activity, a lower level of exercise might have contributed to the lower gene expression for growth factors in the maintenance hemodialysis patients. However, the findings that the body weight, total body fat, fat-free, edema-free mass, and mid-arm muscle circumference, mid-thigh muscle circumference, and mid-calf muscle circumference were similar in the maintenance hemodialysis patients vs. the normal subjects suggest that there were not great differences in physical activity between the two groups.

The possibly healthier physical status of the maintenance hemodialysis patients in this study is consistent with the fact that CRP and IL-6 levels were not different from the normal controls and that the maintenance hemodialysis patients displayed elevated muscle IGF-I and serum IGF-II levels. It is not unlikely that more malnourished and debilitated maintenance hemodialysis patients may have even more profound disturbances in the skeletal muscle mRNA levels for various growth factors.

Finally, whether factors in the uremic milieu of maintenance hemodialysis patients may have lowered the mRNA levels is not known. It should be emphasized that mRNA levels were only assessed in the right vastus lateralis muscle. It is possible that mRNA levels for these growth factors in other muscles may not show the same abnormalities.

In view of the low muscle mRNA levels for IGF-IEa, IGF-II, and the IGF-IR, it might be questioned why body composition was similar in the maintenance hemodialysis patients and normal controls. It is possible that the decreased mRNA levels for growth factors could lead to a reduction in remodeling of certain functional proteins in the muscle cell which may affect muscle structure, metabolism and function but not overall muscle mass. For example, capillary and mitochondria density and the content or activity of many enzymes may rise or fall in myocytes in response to various stimuli [37]. In this regard, muscle strength and power were reduced in our maintenance hemodialysis patients as compared to the normal controls, notwithstanding the similar anthropometric measurements in the two groups. In addition, factors independent of mRNA levels may influence protein synthesis, protein mass, and hence body composition [38]. Also, anthropometry under the best of circumstances is a rather imprecise measure of muscle mass.

Nonetheless, the findings of this study indicate alterations in the molecular processes underlying protein synthesis in skeletal muscle. It should be emphasized that we deliberately chose rather well-nourished and healthy maintenance hemodialysis patients so that some of mechanisms underlying skeletal muscle protein synthesis in CRF patients undergoing maintenance hemodialysis could be examined independently of such complicating adverse conditions as malnutrition, superimposed illness or muscle atrophy.

The current study has several strengths. It is the first comparison in patients with advanced CRF undergoing maintenance hemodialysis vs. normals of the mRNA levels in skeletal muscle for growth factors related to IGF-I, IGF-II, the IGF-IR, IGF-IR, and myostatin. The normal control subjects were selected to have similar characteristics to the maintenance hemodialysis patients with regard to a number of factors extraneous to renal failure or maintenance hemodialysis that conceivably might affect the mRNA levels. In this regard, the similar age, gender distribution, and approximate level of physical activity and the lack of acute intercurrent illness or severe comorbidity in the maintenance hemodialysis patients and normal controls were probably particularly important. Finally, real-time PCR rather than conventional PCR was used; the former method is considered to be more sensitive and accurate [39]. Further studies would be helpful to ascertain the causes of these decreased mRNA levels and whether enhancing these levels would reduce sarcopenia and physical disabilities in maintenance hemodialysis patients.

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REFERENCES


