Reduced synthesis of muscle proteins in chronic renal failure

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Muscle wasting and weakness are common clinical features in people with chronic renal failure. The cachexic state contributes to increased morbidity in chronic renal failure patients. This phenomenon is similar to muscle wasting in patients with chronic renal failure. In patients with chronic renal failure, muscle wasting is related to decreased protein synthesis or a combination of both. Decreased muscle protein synthesis occurs in many chronic muscle-wasting conditions, such as Duchenne muscular dystrophy and myotonic dystrophy. Muscle wasting and weakness also occur in aging as a result of decreased synthesis rates of several muscle proteins and muscle contractile proteins such as myosin heavy chain. Because protein synthesis is dependent on the availability of energy, we also investigated whether synthesis of mitochondrial proteins is altered in chronic renal failure. Reduced synthetic rates of several muscle proteins are the likely biochemical basis of muscle loss and muscle weakness in people with chronic renal failure.

METHODS

Materials

\( [1-^{13}C] \)leucine (99 atom percent excess) and \( ^{13}C \)-labeled sodium bicarbonate were purchased from Cambridge Isotopes Laboratories (Andover, MA). Chemical and isotopic purities were confirmed before use. Various isotopically labeled leucine and sodium bicarbonate solutions were prepared in sterile normal saline. The absence of pyrogens and bacteria was confirmed before human use. All studies were approved by the Mayo Foundation Institutional Review Board, and written informed consent was obtained from participants.

Subjects

Twelve patients with chronic renal failure of varying degrees and ten healthy control subjects were studied. All of the renal failure patients were in a predialysis phase and had never undergone dialysis therapy. Exclusion criteria included diabetes mellitus, proteinuria >2.0 g/24 h, use of glucocorticoids, anti-coagulants, or cytotoxic agents within the last year, chronic antibiotic therapy, dialysis therapy, or renal transplantation. The subjects studied had poly cystic kidney disease \( (n = 6) \), hypertension with nephrosclerosis \( (n = 1) \), or parenchymal renal disease \( (n = 5) \). Glomerular filtration rate (GFR) was determined by the clearance of iothalamate. In patients with chronic renal failure, iothalamate clearance ranged between 8 and 60 ml/min, and in healthy control subjects, it ranged between 75 and 117 ml/min. All control subjects were determined to be healthy after review of their
medical records, physical examination, and review of labora-
tory values.

Diet

All study volunteers followed a similar diet with respect to protein intake (1 g·kg body wt⁻¹·day⁻¹) for the month before study. Food records were kept by the subjects and were reviewed by dietitians on every 10–14 days during this 1-mo period before the study. Adjustments in protein intake were made by the subjects as needed. During the 3 days before the study, all meals were prepared by the metabolic kitchen of the Mayo Clinic General Clinical Research Center. They were comparable in composition with respect to carbohydrate, protein, and fat (55:15:30, respectively), with a protein intake of 1 g/kg body wt. Such standard diets 3 days before the protein turnover measurements in six subjects resulted in <4% coefficient variation in leucine flux, leucine oxidation, and nonoxidative leucine flux measurements in five repeated studies at >2-wk intervals between each study (10).

Study Design

All subjects were studied after an overnight fast. Priming doses of L-[1-¹³C]leucine (1.0 mg/kg) and [¹³C]sodium bicarbonate (0.2 mg/kg) were given intravenously to prime the respective pools and bring about early isotope plateaus (26). Immediately after the priming dose, a continuous infusion of L-[1-¹³C]leucine (1.0 mg·kg⁻¹·h⁻¹) was given for 10 h. Percu-
taneous needle muscle biopsies were performed under sterile conditions at 5 and 10 h after the beginning of the infusion (31, 34). Blood and breath samples were drawn at baseline and every hour from 5 to 10 h. Rest CO₂ production was measured for 60 min by indirect calorimetry at 6–7 h.

Body Composition

All study subjects underwent evaluation of body composition by dual-energy X-ray absorptiometry (DEXA), which provided an assessment of fat mass (21).

Analytical Techniques

Breath and plasma sample measurement. Measurement of [¹³CO₂] was performed with a gas chromatograph-isotope ratio mass spectrometer, as previously described (4). Isotopic enrichment of plasma [¹³C]ketosocaproate (KIC) was measured with a gas chromatograph-mass spectrometer as the quinoxalinol-trimethylsilyl derivative (27) by use of electron impact ionization conditions. Selective mass-to-charge (m/z) fragment ions 233/232 were monitored. Plasma KIC concentra-
tion was measured using ketocaler acid as an internal standard. Muscle tissue fluid was separated as previously described (9), and the amino acids were derivatized as their trifluoroacetyl isopropyl esters (1). Enrichment of [¹³C]leucine was measured in a gas chromatograph-mass spectrometer under chemical ionization conditions with ammonia as a carrier gas.

Muscle proteins. Mixed muscle proteins in the biopsy sample were separated and hydrolyzed as previously de-
scribed (9, 35). The constituent amino acids in the hydroly-
sate were purified by cation exchange chromatography (Dowex 50, H⁺ form, Bio-Rad Laboratories, Hercules, CA), and amino acids were eluted with the use of 4 M ammonium hydroxide, as previously described (9). The purification of muscle mitochondrial proteins (39, 40) and myosin heavy chains (5, 6) was performed as previously described. These proteins were also hydrolyzed, and the constituent amino acids were purified as noted above. Leucine from the purified amino acids of the hydrolasate (both mixed muscle protein and myosin heavy chain) was purified by high-performance liquid chromatography, without derivatization, by use of a reversed-phase C₁₈ column, as previously described (8). Carbon dioxide from the carboxyl group of purified leucine was liberated by the ninhydrin reaction, and isotopic enrichment was measured with a gas chromatograph-isotope ratio mass spectrometer (Delta S, Finnigam MAT, Bremen, Germany) as described (4). The isotopic enrichment of leucine in the mitochondrial hydrolasate was also measured with the same instrument, by use of a combustion system as previously described (4).

Measurement of glucose and amino acids. Serum total CO₂ content was measured using the bicarbonate kit based on phosphoenolpyruvate conversion of bicarbonate to oxaloace-
etate and ultrasound detection of the decline of NADH in the solution (Boehringer Mannheim, Indianapolis, IN). Plasma glucose concentrations were measured enzymatically with an auto analyzer (Beckman Instruments, Fullerton, CA). Plasma concentrations of amino acids were measured as previously reported (19, 32). Hormone levels were measured using established assays. Insulin was measured by chemilumines-
cent sandwich assay (Sanofi Diagnostics, Chaska, MN) (46). Growth hormone was measured using a locally developed two-site chemiluminescent sandwich assay using antibodies from Sanofi Diagnostics. Insulin-like growth factor (IGF)-I, IGF-II, and IGF-binding proteins were measured using a commercially available kit (Diagnostic Systems Laboratories, Webster, TX). The latter analyses included a simple extrac-
tion step in which IGF-I is separated from its binding proteins (22). Plasma concentrations of epinephrine and norepineph-
rine (11) and parathyroid hormone (23) were measured by re-
port method (19, 32). Hormone levels were measured using established assays. Insulin was measured by chemilumines-
cent sandwich assay (Sanofi Diagnostics, Chaska, MN) (46).

Measurement of glomerular filtration. GFR was measured using a short iohexalumine clearance method (47) and stan-
dard 24-h urine creatinine clearance.

Calculations

Leucine kinetics. Leucine flux (Q) is calculated on the basis of a stochastic model, and the underlying assumptions are discussed in detail elsewhere (31). Q in an isotopic steady state represents both leucine appearance rate (Rₐ) or protein breakdown (B) and leucine disappearance rate (Rₐ). Q is calculated as follows

\[ Q = (E₁/Eₚ - 1) \times l_i \]

where \( E₁ \) is the isotopic enrichment of the infusate (99%), \( Eₚ \) is the mean isotope enrichment of [¹³C]KIC (transamination product of leucine) in plasma at plateau, and \( l_i \) is the rate of infusion isotope. Plasma KIC enrichment is used as a surro-
gate measure of intracellular leucine enrichment (27, 42).

The rate of [¹³CO₂] release from isotopically labeled leucine, with the assumption of 81% recovery of [¹³CO₂] released from leucine oxidation, is calculated as

\[ F[¹³CO₂] = (F[CO₂] × Eₚ/FFM) \times 33.037 \]

where \( F[CO₂] \) is the production rate of CO₂, \( Eₚ \) is the enrichment of [¹³CO₂] in expired air, FFM represents fat-free mass, and 33.037 is a constant to account for standard conditions and CO₂ retention (48).

The rate of leucine oxidation (Qₐ) is

\[ Qₐ = F[¹³CO₂]/(1/Eₚ - 1/E₁) \times 100 \]
DEFECTIVE SYNTHESIS OF MUSCLE PROTEINS IN RENAL FAILURE

Fractional synthesis rate of muscle proteins. Fractional synthesis rate (FSR) of a muscle protein (29) is determined as

\[ \text{FSR} = \frac{(E_{10h} - E_{5h}) \times TFE_L}{5 \text{ h}} \times 100 \]

where \( E_{10h} \) and \( E_{5h} \) are the respective enrichment values in the muscle protein (e.g., mixed muscle protein, myosin heavy chain, and mitochondrial protein obtained at biopsy at 10 and 5 h, respectively; 5 h refers to the amount of time elapsed between the first and second biopsies), and \( TFE_L \) represents the mean isotopic enrichment of leucine in muscle tissue fluid at 5 and 10 h. It has been demonstrated that muscle tissue fluid leucine enrichment best represents the isotopic enrichment of leucine acylated to tRNA, the obligate precursor of protein synthesis (24), and it therefore was used in the calculations.

As we previously reported, the coefficient of variation of repeated isotopic enrichment measurements in plasma and tissue proteins is <5% (24).

Statistical analysis

All values are given as means ± SE. First, we performed unpaired analysis to compare the outcome measurements of chronic renal failure patients with healthy control subjects. We performed a one-way ANOVA and tested whether the chronic renal failure group differed from the control group by unpaired analysis to compare the outcome measurements of chronic renal failure patients with healthy control subjects. When a significant difference was found, we also performed linear regression of the outcome measures against GFR to determine whether the differences between the groups were related to the severity of renal failure.

RESULTS

Subject Data

Table 1 gives the subject data. There were no significant differences between the two groups of subjects in their body weight or body composition, although the renal failure patients tended to have higher fat mass. Patients with chronic renal failure had lower GFR than the control group (chronic renal failure: range 8–60 ml/min, normal: range 75–117 ml/min, \( P < 0.01 \)).

Plasma concentrations of hormones are given in Table 2 and show that concentrations of insulin, IGFBP-2, IGFBP-3, and parathyroid hormone (PTH) were significantly higher in patients with chronic renal failure than in the control subjects.

Protein Metabolism

Plasma amino acid concentrations and KIC are given in Table 3. Plasma concentrations of tyrosine and leucine and KIC were lower in people with chronic renal failure. Whole body leucine kinetics: leucine flux, leucine oxidation, and nonoxidative leucine disposal per unit FFM (DEPX), were not different between patients with chronic renal failure and normal control subjects (Table 4).

Muscle

Muscle protein synthesis and muscle mitochondrial enzyme data are summarized in Figs. 1 and 2. Fractional synthesis rates (FSR) of mixed muscle protein (\( P < 0.05 \)), myosin heavy chain (\( P < 0.02 \)) (Fig. 1), and mitochondrial protein (\( P < 0.05 \)) were significantly lower in subjects with chronic renal failure (Fig. 2). In addition, muscle mitochondrial enzyme activity (citrate synthase and cytochrome-c-oxidase) were also significantly lower in patients with chronic renal failure than in the healthy control subjects (\( P < 0.01 \)).

Table 2. Hormone levels

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control</th>
<th>Renal Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, µU/ml</td>
<td>5.1 ± 0.5</td>
<td>9.7 ± 1.5*</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>92 ± 6</td>
<td>135 ± 21</td>
</tr>
<tr>
<td>Cortisol, µg/dl</td>
<td>16.6 ± 1.8</td>
<td>14.7 ± 0.8</td>
</tr>
<tr>
<td>GH, ng/ml</td>
<td>1.45 ± 0.41</td>
<td>1.08 ± 0.39</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td>143 ± 19</td>
<td>149 ± 17</td>
</tr>
<tr>
<td>IGF-II, ng/ml</td>
<td>869 ± 68</td>
<td>1,026 ± 54</td>
</tr>
<tr>
<td>IGF-BP-1, ng/ml</td>
<td>38 ± 6</td>
<td>55 ± 11</td>
</tr>
<tr>
<td>IGF-BP-2, ng/ml</td>
<td>412 ± 62</td>
<td>979 ± 92*</td>
</tr>
<tr>
<td>IGF-BP-3, ng/ml</td>
<td>2,680 ± 141</td>
<td>3,358 ± 213*</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>39 ± 7</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>178 ± 18</td>
<td>230 ± 30</td>
</tr>
<tr>
<td>PTH, pmol/l</td>
<td>5.1 ± 0.5</td>
<td>11.5 ± 2.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. GH, growth hormone; IGF-I and -II, insulin-like growth factors I and II; IGFBP-1, -2, and -3, IGF-binding proteins 1, 2, and 3; PTH, parathyroid hormone. *Significant difference from control group (\( P < 0.05 \)).

Table 1. Subject data

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, yr</th>
<th>Gender, M/F</th>
<th>Body Mass Index, kg/m²</th>
<th>Fat Mass, kg</th>
<th>Fat-Free Mass, kg</th>
<th>GFR, ml/min</th>
<th>Total Serum CO₂, meq/l</th>
<th>Serum Albumin, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>41.3 ± 3.3</td>
<td>5/5</td>
<td>25.5 ± 1.4</td>
<td>19.8 ± 2.3</td>
<td>51.3 ± 4.2</td>
<td>90.5 ± 4.6</td>
<td>27.6 ± 0.9</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Renal failure (n = 12)</td>
<td>43.3 ± 2.7</td>
<td>6/6</td>
<td>26.4 ± 0.87</td>
<td>25.2 ± 2.1</td>
<td>50.6 ± 3.2</td>
<td>33.2 ± 5.1*</td>
<td>24.3 ± 1.2</td>
<td>4.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. GFR, glomerular filtration rate. Total serum CO₂ normal range is 22–29 meq/l. *Significant difference from control groups (\( P < 0.05 \)).
Table 3. Plasma concentrations of amino acids and KIC

<table>
<thead>
<tr>
<th>Amino Acid, µM</th>
<th>Control</th>
<th>Renal Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>70±10</td>
<td>54±8</td>
</tr>
<tr>
<td>Serine</td>
<td>104±8</td>
<td>106±10</td>
</tr>
<tr>
<td>Glutamine</td>
<td>851±44</td>
<td>844±70</td>
</tr>
<tr>
<td>Histidine</td>
<td>87±4</td>
<td>83±5</td>
</tr>
<tr>
<td>Glycine</td>
<td>247±28</td>
<td>274±16</td>
</tr>
<tr>
<td>Threonine</td>
<td>141±13</td>
<td>127±9</td>
</tr>
<tr>
<td>Alanine</td>
<td>424±53</td>
<td>417±46</td>
</tr>
<tr>
<td>Arginine</td>
<td>144±8</td>
<td>141±12</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>71±5</td>
<td>56±4*</td>
</tr>
<tr>
<td>Valine</td>
<td>221±16</td>
<td>222±23</td>
</tr>
<tr>
<td>Methionine</td>
<td>37±2</td>
<td>33±2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>61±4</td>
<td>73±7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>66±4</td>
<td>66±3</td>
</tr>
<tr>
<td>Leucine</td>
<td>160±9</td>
<td>129±7*</td>
</tr>
<tr>
<td>Lysine</td>
<td>162±12</td>
<td>144±11</td>
</tr>
<tr>
<td>KIC</td>
<td>36±2</td>
<td>26±2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. KIC, ketoisocaproate. *Significant difference from control groups (P < 0.05).

Table 4. Whole body leucine kinetics

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Renal Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body kinetics, µmol·kg FFM⁻¹·h⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine flux</td>
<td>136 ± 6</td>
<td>141 ± 4</td>
</tr>
<tr>
<td>Leucine oxidation</td>
<td>30 ± 2</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Nonoxidative leucine flux</td>
<td>106 ± 5</td>
<td>114 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFM, fat-free mass.

both groups under identical study conditions. There were also significant correlations between many changes in muscle protein synthesis and mitochondrial enzymes and GFRs.

Muscle mass is determined by the balance between muscle protein breakdown and synthesis; the two processes are essential for the remodeling of muscle tissue. The lack of increase in leucine flux (a measure of whole body protein breakdown) argues against an increase of protein breakdown. It is, however, possible that muscle protein breakdown may alter without any change in whole body protein breakdown estimated from leucine flux. The observed lower synthesis rates of mixed muscle proteins (an average of all muscle proteins) suggest that decreased muscle protein synthesis is likely the biochemical basis of muscle wasting in our patients with chronic renal failure. Although we studied only 12 patients, all showed a decrease in muscle protein synthesis without any change in whole body protein breakdown. In animal models of renal failure, the enhanced muscle protein breakdown that occurs with metabolic acidosis is believed to play a pivotal role in mass loss (3, 17, 28). The subjects who participated in our study were not acidic on the basis of serum bicarbonate concentrations. The chronic renal failure patients had slightly lower serum bicarbonate concentrations than the control group, but these bicarbonate levels were within normal range. Severe metabolic acidosis may cause increased muscle protein breakdown in humans, thus further accelerating muscle loss in decompensated renal failure. Because our subjects were relatively well compensated, we were unable to test this possibility. We cannot be certain that modest changes in muscle protein breakdown were not present, which can only be ascertained by direct measurement of muscle protein breakdown. A decrease in muscle protein synthesis alone is sufficient to explain the observed net catabolic state of muscle in humans with chronic renal failure. The decrease in muscle protein synthesis at the rate we observed during the postabsorptive state without any change in muscle protein breakdown could account for substantial muscle loss.

Recent technological advances in mass spectrometry (4) enabled us to measure the in vivo synthesis rates of individual muscle protein components by use of small needle biopsy samples. The decline in synthesis rates of mitochondrial proteins is of interest, as mitochondria are responsible for oxidative phosphorylation and ATP production. These mitochondrial functions depend on the integrity of electron transport, for which five protein complexes in the inner mitochondrial membrane are essential. The decline in mitochondrial protein synthesis and decreased levels of two crucial mitochondrial enzymes (cytochrome c-oxidase and citrate synthase) suggest a compromised ability to produce ATP. Durozard et al. (15), on the basis of their ³¹P NMR spectroscopic studies, reported a reduced ATP production, supporting impaired mitochondrial function in chronic renal failure patients. This reduced ATP production may not have much functional consequence in the
resting muscle because of the abundance of muscle mitochondria but may be a limiting factor for continued muscle contraction, thus limiting the endurance, as reported in chronic renal failure patients (36). In addition, decreased synthetic rate of myosin heavy chain, the contractile protein responsible for hydrolyzing ATP to ADP, may also affect muscle strength (7). An excellent correlation between muscle strength and synthesis rate of muscle myosin heavy chain has been previously observed (7). Reduced oxidative capacity of mitochondria and reduced synthesis rate of myosin heavy chain may contribute to the reported muscle weakness in patients with renal failure (36). Insulin levels were increased in patients with chronic renal failure. This finding, noted by several other groups in the past, could be due to tissue resistance to insulin and may contribute to some of the impaired muscle protein synthesis. Reduced bioavailability of IGF-I due to increased binding proteins, as observed in the current study and by others (44), may also play a role in decreasing muscle protein synthesis. In addition, there is reported resistance of muscle protein synthesis to IGF-I in chronic renal failure (13, 29).

In conclusion, the current study clearly demonstrated that, under identical study conditions, the patients with chronic renal failure have reduced synthesis rates of myosin heavy chain and mitochondrial protein, indicating that chronic renal failure causes a defect in the remodeling process of muscle proteins. Accumulation of toxins and altered hormonal levels, chemical milieu, and tissue redox potential, lack of exercise, or a decreased oxygen supply to muscle due to accelerated atherosclerosis are among many possibilities that need to be considered as the cause of reduced chronic muscle protein synthesis in chronic renal failure patients. We measured several hormones that could potentially affect muscle protein synthesis. The

Fig. 2. FSR of mitochondrial protein (A) and muscle activity of citrate synthase (B) and cytochrome c-oxidase (C) in people with renal failure and normal control subjects. Those with renal failure have a lower FSR of mitochondrial protein (* P < 0.05), citrate synthase (* P < 0.01), and cytochrome c-oxidase (* P < 0.01).

Fig. 3. FSR of myosin heavy chain (MHC, A), citrate synthase (B), and cytochrome c-oxidase (C) are significantly correlated to glomerular filtration rate (GFR).
current data do not show any correlation between the changes in muscle protein synthesis and any of the anabolic hormones or bicarbonate levels. The renal failure patients have elevated circulating insulin levels, which may reflect relative insulin resistance observed in these people (25). In conditions with insulin resistance to glucose metabolism, such as lipodystrophy (12), obesity, or type II diabetes (33, 37, 43, 45), there is no evidence of decreased protein synthesis. Increased insulin levels may inhibit protein breakdown in humans (31, 32), but the observed decline in muscle protein synthesis cannot be explained on the basis of insulin’s effect on muscle protein synthesis. Other hormones, such as cortisol, catecholamines, IGF-I, and IGF-II, which can potentially affect protein synthesis (41), have not changed significantly. The PTH levels are elevated, but to our knowledge PTH has never been shown to have any effect on muscle protein synthesis. The current study demonstrated decreased muscle protein synthesis as an underlying mechanism of muscle wasting in chronic renal failure. This decrease in protein synthesis could be related to multiple factors, including the effects of many metabolites and toxins not disposed by the kidney.

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