Proteasome Production in Human Muscle During Nutritional Inhibition of Myofibrillar Protein Degradation

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Protein undernutrition inhibits adenosine triphosphate (ATP)-dependent muscle protein degradation—a hallmark of the proteasome system. Here we report decreased myofibrillar protein degradation during dietary protein restriction without a concomitant decrease in proteasome gene expression, proteasome protein abundance, or proteasome in vivo fractional synthesis rate. Healthy human subjects consuming the average minimum adult protein requirement (0.71 g · kg^{-1} fat-free mass · d^{-1}) exhibited substantially lower (68%) excretion of 3-methylhistidine, an indicator of myofibrillar protein breakdown, when compared with subjects consuming an ample, American-style protein intake (1.67 g · kg^{-1} fat-free mass · d^{-1}). However, they displayed no difference in the expression of mRNA for proteasome subunits C2 or C3, in the content of C2 protein, or in the rate of incorporation of stable isotopically labeled L-[1-^{13}C]-leucine into proteasome proteins. The results demonstrate that nutritional inhibition of myofibrillar protein degradation does not involve suppression in vivo of proteasome production in man. This suggests that other elements of the ubiquitin-proteasome system, such as ubiquitination pathways, are more important than proteasome abundance in the nutritional regulation of skeletal muscle mass.

THE PROTEASOME, a multimeric multicatalytic protease that degrades ubiquitin-modified proteins, is responsible for the bulk of myofibrillar protein degradation.[1,2] Reports of muscle wasting in a wide variety of pathophysiologic conditions have demonstrated that increased expression of proteasome subunit genes is a forerunner to augmented muscle protein degradation in animals[3-6] and humans.[3,7-9]

Conservation of muscle mass during isoenergetic dietary protein deprivation involves inhibited degradation of myofibrillar proteins.[10-12] Nutritionally inhibited muscle protein degradation during dietary protein deprivation is the result of decreased activity of the ubiquitin-proteasome system.[13] By way of symmetry with disorders of muscle catabolism, it is expected that expression of proteasome subunit genes would be suppressed during dietary protein deprivation.[6] However, much remains unknown about the behavior of the ubiquitin-proteasome system in integrative physiology and the pathophysiology of disease.[14,15] The particular changes of the ubiquitin-proteasome system that spare myofibrillar proteins during the anticatabolic condition of isoenergetic protein deprivation have not been established. It is unclear whether, in direct contrast to the circumstance of muscle wasting, the decreased activity of the ubiquitin-proteasome system involves decreased transcription of proteasome genes and decreased myocyte content of proteasome proteins.

We examined the response of the proteasome in the skeletal muscle of healthy humans consuming the average minimum adult protein requirement, a dietary protein content particularly relevant for its use therapeutically to forestall declining renal function in certain conditions, such as diabetic nephropathy.[16-20] To establish the response of both the transcription of proteasome genes and the translation of proteasome proteins, we combined measurements of proteasome mRNA with measurement of proteasome protein content and the first in vivo isotopically-determined measurement of proteasome protein fractional synthesis rate.

MATERIALS AND METHODS

Subjects

We studied 14 healthy human subjects (9 men, 5 women) between the ages of 18 to 35 years who were randomly assigned to consume a diet containing an ample, American-style protein intake or one approximating the average minimum adult requirement for 4 weeks. Subjects in the 2 groups were matched for body composition, determined by hydrodensitometry. Subjects provided written informed consent as approved by the Institutional Review Board of the University of Illinois at Chicago.

Diet

Diets were isoenergetic as determined by 3-day diet diaries and adjusted Harris-Benedict estimates of energy requirements. Energy content of the diet over the 4-week dietary adherence was adjusted to maintain body weight. The diets contained a mixture of protein from animal and vegetable sources. The ample protein intake (high protein) contained 1.67 g · kg^{-1}fat-free mass (FFM) · d^{-1}. The restricted protein intake (low protein) contained 0.71 g · kg^{-1}FFM · d^{-1}. For a subject with the body composition of a typical college-aged male (ie, 15% fat), the former diet provided 1.5 g · kg^{-1} · d^{-1} protein and the latter diet provided 0.6 g · kg^{-1} · d^{-1}. This method of assigning dietary protein content assumes that the FFM is the relevant mass for dietary protein requirements and that indexing the dietary protein assignment to FFM minimizes the variation in protein intake resulting from variation in body composition. Subjects used a rotating menu program in which they followed menus that specified the food type and amount to be consumed. Food scales were provided to assure precision in portion control. One of the menus provided was meat free. It was used during the final 3 days of the study in which urinary creatinine and 3-methylhistidine was collected.

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Study Protocol

Subjects visited the General Clinical Research Center (GCRC) of the University of Illinois at Chicago at an initial visit for screening/randomization (baseline) and on 3 additional occasions during the 4-week period of dietary adherence. On each of these occasions to further establish dietary adherence, subjects submitted a 24-hour urine collection for measurement of nitrogen excretion. If a subject was thought to have deviated from strict adherence to the menu plan based on interview or urinary nitrogen excretion, he/she was excluded. Data are reported for 14 subjects.

Subjects used a meat-free menu for 3 days prior to the last occasion and extended the urine collection to 48 hours for measurement of creatinine and 3-methylhistidine excretion (an indicator of myofibrillar protein degradation). After 4 weeks of consuming the experimental diet, subjects were admitted to the GCRC overnight and received a primed infusion of L-[1-^{13}C]-leucine (6.86 μmol/kg bolus, 7.62 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ infusion) beginning at 4 AM in the postabsorptive state. We performed percutaneous muscle biopsies of the vastus lateralis muscle in the nondominant leg at 8 AM and 12 PM using a UCH skeletal muscle biopsy needle (Popper and Sons, New Hyde Park, NY). Female subjects were studied during the follicular phase of the menstrual cycle.

Analytical Methods

Urine nitrogen. Urine total nitrogen was measured by modified Kjeldahl analysis as previously described.[21]

Urine 3-methylhistidine. The urine excretion of 3-methylhistidine was measured by reversed-phase high-performance liquid chromatography (HPLC) (HP 1100; Hewlett Packard, Wilmington, DE) using a Pico-Tag C-18 column (Waters, Milford, MA). Urine aliquots were deproteinized with 10% sulfosalicylic acid. Amino acids were converted to phenylthiocarbamyl (PTC) derivatives by reaction with phenylisothiocyanate under alkaline conditions. The amino acids, including 3-methylhistidine, were separated using a gradient mobile phase in which a 50-mmol/L sodium acetate buffer (Buffer A; pH 6) was mixed with 7% to 60% of a 60% acetonitrile in water solution (Buffer B) over 40 minutes at 38°C. The amino acid PTC chromophore was detected at 254 nm.

Reverse transcriptase-polymerase chain reaction analysis of proteasome subunit mRNA content. We used 100 ng total RNA from a 10- to 20-mg skeletal muscle sample as a template for a 1-step reverse transcriptase-polymerase chain reaction (RT-PCR) using the LightCycler RNA Amplification kit with SYBR green1 (Roche Diagnostics, Basel, Switzerland). The RNA was extracted using TRI-reagent (Molecular Research Center, Cincinnati, OH) as described.[22] The PCR reaction conditions included a reverse transcriptase step of 15 minutes at 55°C and a denaturation step of 30 seconds at 95°C. We used a 4-step PCR amplification. We determined the temperature of the final step at which the fluorescence signal was acquired by performing melting curve analysis for each set of primers. The amplification steps were performed at 95°C for 1 second, 55°C for 10 seconds, 72°C for 13seconds, and signal acquisition at 80°C to 88°C for 2 seconds. The fluorescence signal was acquired after each cycle for 45 cycles. Samples were quantified against a range of in vitro transcribed mRNA standards generated separately for each proteasome subunit (hC2 and hC3) gene, to account for variability in the RT reaction and different primer efficiency. The primers used were: hC2 cDNA (Genebank accession # D00759): Forward 5′-AGATACCAACACAACGATATG-3′, Reverse 5′-CTCTCCAAGTAAGTACGAGC-3′. Human hC3 cDNA (Genebank accession #D00760): Forward 5′-TCAGGTGGTGTTCGTCCATT-3′, Reverse 5′-TTCAAAGCTTTCCTTTAGGGTTAAG-3′. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (Genebank accession # X01677):Forward 5′-GTGGACCTGACCTGCCGTCTA-3′, Reverse 5′-GCTTGACAAAGTGGTCGTTGA-3′.

Immunoblot analysis of proteasome subunit content. The total protein content of crude skeletal muscle homogenate protein was measured using the Bradford assay as previously described.[23] We loaded 10 μg protein into each lane of a sodium dodecyl sulfate (SDS) polyacrylamide gel containing a 12.5% separating component and a 4% stacking component. The protein was combined 1:1 with a Tris/SDS loading buffer and heated for 5 minutes at 100°C. We performed electrophoresis to separate the proteasome subunits using a constant current of 10/15 mA (stacking/resolving) for 4 hours. The proteins were then transferred to a polyvinyldifluoride membrane using the method of Towbin et al[24] as previously described. After incubating the membrane with reconstituted dried milk proteins to block nonspecific binding, the membrane was incubated with a mouse primary antibody against human C2 proteasome α-subunit protein (MCP20; Affiniti Research, Exeter, UK). After washing the membrane, it was incubated with an antimouse immunoglobulin G (IgG) secondary antibody conjugated to horseradish peroxidase (Sigma-Aldrich, St Louis, MO). The protein band was detected by subsequently incubating the membrane with ECL Plus reagent (Amersham Biosciences, Piscataway, NJ) and exposing x-ray film to the fluorescent signal for 1 minute. Equivalency of protein loading and transfer was assured by measuring the density of Ponceau S staining.

The C2 and C3 proteasome subunits were chosen for study because these noncatalytic subunits of the proteasome alpha ring maintain constant positions in the proteasome structure[25] and are not specifically induced or removed during physiologic changes. Thus, changes in expression or content of these subunits would indicate changes in expression or content of the core proteasome as a unit. Additionally, these subunits have been commonly used as indicators of proteasome expression in other studies of muscle catabolism/anabolism.[26]

Proteasome peptidase activity assay. Measurement of peptidase activity was performed as an initial step in isolating proteasomes for isotopic analysis and fractional synthesis rate calculation. Myofibrillar proteins were separated by centrifuging crude homogenate at 1,000 $\times$ *g* in a 20-mmol/L Tris buffer pH 8.7 with 1% Triton X-100 as previously described.[27] The concentration of myofibrillar proteins was measured using the Bradford method as above. The centrifugation of the supernatant was repeated at 15,000 $\times$ *g*. Proteasome fractions were prepared from this postmitochondrial fraction using ultracentrifugation in a 10% to 40% glycerol density gradient as previously described after measuring protein content with the Bradford assay.[28,29] To identify fractions with proteolytic activity characteristic of the proteasome, we removed 900-μL fractions from the top of the gradient and incubated 35-μL aliquots for 30 minutes at 37°C with the fluorogenic peptide succinyl-Leu-Leu-Val-Tyr-7-amidomethylcoumarin (Suc-LLVY-AMC) dissolved in 10 mmol/L dimethyl sulfoxide (DMSO) with 20 mmol/L Tris-base at a final pH of 8.5. In a second 35-μL aliquot of the same fractions, the incubation was performed with SDS added to the fluorogenic peptide solution at a final concentration of 0.05%. Methylcoumarin fluorescence was assessed at 350 nm excitation and 440 nm emission wavelengths.

Proteasome in vivo fractional synthesis rate. We combined fractions with peptidolytic activity and concentrated them using a Centricon-10 centrifugal concentrating unit. The concentrated proteasomes were further separated by electrophoresis on a 6% nondenaturing polyacrylamide gel using 150 V at 4°C for 1.5 hours.

The proteasomes were identified in the gel by overlaying glass paper saturated with the custom-synthesized fluorogenic peptide Succinyl-Ile-Ile-Val-Tyr-7-amidomethylcoumarin (Suc-IIVY-AMC; ChemImpex, Wood Dale, IL) at pH 8.5 and incubating for 30 minutes at 37°C in a tissue incubator. We established that the glass paper, in contrast to other vehicles for the peptide, produced no contamination of the gel with bacterial proteins. The use of Suc-IIVY-AMC in place of the commonly used Suc-LLVY-AMC avoided alteration of the proteasome leucine isotopic enrichment by leucine from the fluorogenic peptide. A

bright band produced by the 26S proteasome was identified along with a faint band corresponding to the core 20S proteasome. We confirmed the absence of other proteins in the proteasome region by identifying single bands in the location of fluorescence using Coomassie blue staining.

The portions of the acrylamide gel containing the proteasomes were excised and the protein in the gel was hydrolyzed using a modified proton attack method with 0.05 mol/L HCl and AG-50 Dowex cation exchange resin (BioRad, Hercules, CA) incubated for 24 hours at 100°C as previously described.[30] The resulting free amino acids were eluted with 1 mol/L NH_4OH from the cation exchange resin onto an AG-1 (BioRad) anion exchange resin to remove excess acrylamide. After washing with deionized water, we eluted the amino acids from the anion exchange resin with 1 mol/L HCl and subjected the sample to a second cation exchange step, washing with 10 mL 0.1 mol/L HCl before eluting again with NH_4OH.

The amino acids were dried in a centrifugal evaporator (SpeedVac; Savant, Holbrook,NY) and derivatized to their N-trimethylacetyl, n-methyl ester derivatives. Isotopic enrichment of the proteasome-derived leucine was measured by gas chromatography-combustion-isotope ratio mass spectrometry (Delta Plus; Finnigan MAT, Bremen, Germany).

We measured the isotopic enrichment of the precursor pool of leucine by analyzing the enrichments of skeletal muscle tissue fluid leucine and plasma α-ketoisocaproic acid (KIC), 2 surrogates for the true precursor for protein synthesis tRNA-bound leucine. Both surrogates have been shown to produce good approximations of true fractional synthesis rates when small tissue samples preclude the measurement of tRNA-bound amino acid isotopic enrichment.[31-33] Tissue fluid ^{13}C-leucine enrichment was measured by electron impact gas chromatography mass (EI-GCMS) spectrometry of its t-butyl, dimethylsilyl derivative (Shimadzu QP-5000; Shimadzu, Colombia, MD) and enrichment of plasma ^{13}C-α-ketoisocaproic acid by EI-GCMS of its t-butyl, dimethylsilylquinoxalinol derivative, both as previously described.[34,35]

Calculations. Fractional synthesis rate of proteasome protein was calculated using the precursor-product model as follows:

$$FSR = \frac{E_{13C-leu-proteasome-t2} - E_{13C-leu-proteasome-t1}}{E_{13Cleu-tissuefluid-steadystateor13C-KIC-plasma-steadystate} \times \Delta t}$$

where t2 and t1 refer to second and first skeletal muscle biopsies, respectively. The term Δt indicates the time interval between biopsies. E denotes isotopic enrichment.

Muscle mass was calculated as follows:

$$\text{Muscle mass (kg)} = 20 \times U_{creatinine}$$

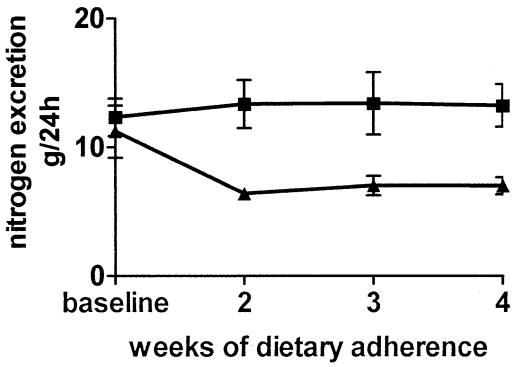
where $U_{creatinine}$ is the urinary creatinine excretion in grams/24 h as previously described.[36]

Statistical Analysis

The effects of the assigned diet were assessed by a 2-tailed *t* test for unpaired data. Where appropriate, means were adjusted for appropriate controls (eg, Ponceau S staining of immunoblot, protein loading in peptidase assay) using analysis of covariance (ANCOVA). Data with repeated measures (N excretion and balance) were analyzed using a 2-way analysis of variance (ANOVA). Data are expressed as mean ± SE.

RESULTS

Randomization procedures produced equivalent groups for dietary treatment. Muscle mass, calculated as 20 × creatinine excretion (24 hours) during meat-free intake was not different between the groups (31.23 ± 4.31 *v* 33.69 ± 3.71 kg; high-protein *v* low-protein groups). Hydrodensitometry revealed equivalent body compositions between the groups (19.8 ± 2.9 *v* 17.5% ± 2.0% fat; high protein *v* low protein).



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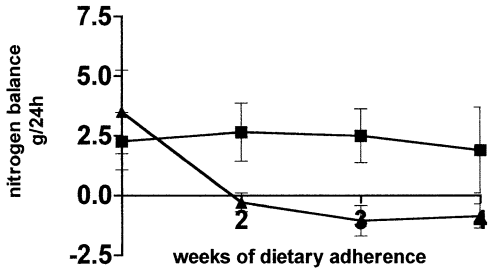

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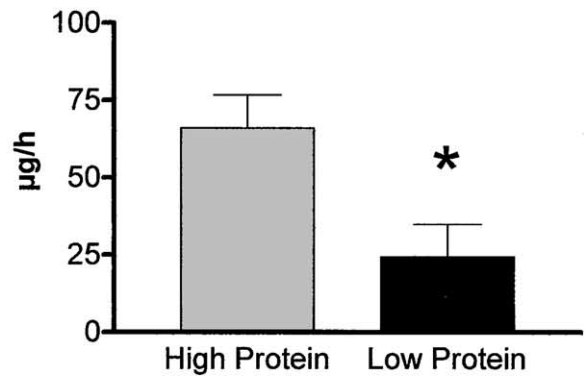
Fig 1. (A) Urinary excretion of total nitrogen expressed as g/24.h. Subjects randomly assigned to high-protein intake (■). Subjects assigned to low-protein intake (▲). Subjects assigned to low-protein exhibit lower excretion (ANOVA, $F = 24.81$; $P < .001$). (B) Nitrogen balance is displayed as g/24/h (NS).

Urinary nitrogen excretion was 47% lower in the group consuming the protein-restricted diet than the ample protein intake (Fig 1). The intraindividual coefficient of variation for nitrogen excretion over the 4-week course of dietary treatment was 13.5% (range, 3% to 25%) for the low-protein group, suggesting consistent adherence to the dietary program. Nitrogen balance did not change in the high-protein diet group, but trended lower in the low-protein diet group. The apparent difference between groups was not statistically validated. By study completion, the N-balance remained positive in the high-protein group, but not in the low-protein group (1.90 ± 1.80 g/24/h at study completion with high protein, −0.87 ± 0.52 in the low-protein group).

The concentration of skeletal muscle and myofibrillar proteins was not different between the treatment groups. Skeletal muscle protein concentration was 0.152 ± 0.007 versus 0.149 ± 0.009 mg protein/mg muscle weight in the high- and low-dietary protein groups, respectively. Myofibrillar protein was 69 ± 8 versus 66 ± 4 μg myofibrillar protein/mg muscle weight in the high- and low-dietary protein groups, respectively.

Urinary 3-methylhistidine excretion was 68% lower in the



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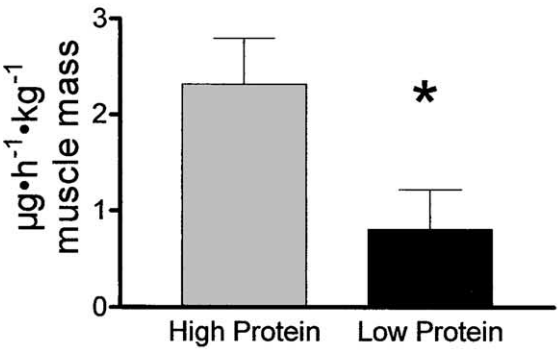

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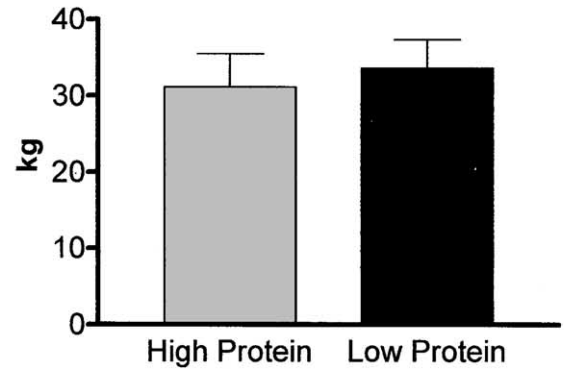


C

Fig 2. Urinary 3-methylhistidine excretion rate expressed as (A) μg/h and (B) $\mu g \cdot h^{-1} \cdot kg^{-1}$ muscle mass collected over 48 hours during meat-free intake. (C) Muscle mass, determined by creatinine excretion. Subjects assigned to low-protein intake exhibited a decreased excretion rate. *$P < .03$.

subjects consuming the minimum protein requirement than those consuming ample protein (2.32 ± 0.38 *v* 0.81 ± 0.42 $\mu g \cdot h^{-1} \cdot kg^{-1}$ muscle mass; Fig 2). As greater than 75% of urinary 3-methylhistidine is of muscle origin,[37] this suggests that the rate of myofibrillar protein degradation was significantly diminished by the low protein intake.

The steady-state content of proteasome subunits hC2 and hC3 mRNA, reported as a ratio with the control gene GAPDH, were not different in subjects consuming the different diets (0.028 ± 0.012 *v* 0.023 ± .004 hC2 subunit, 0.016 ± 0.004 *v* 0.013 ± 0.003 hC3 subunit; Fig 3). Expressed as raw data without indexing to GAPDH, results were similar (0.413 ± 0.207 *v* 0.37 ± 0.188 hC2 subunit, 0.22 ±0.15 *v* 0.233 ± 0.093 hC3 subunit; high *v* low protein, respectively). Although this measurement of transcript levels is not a measure of transcription rate, it suggests that the abundance of transcript available for proteasome subunit synthesis is not affected by dietary protein restriction.

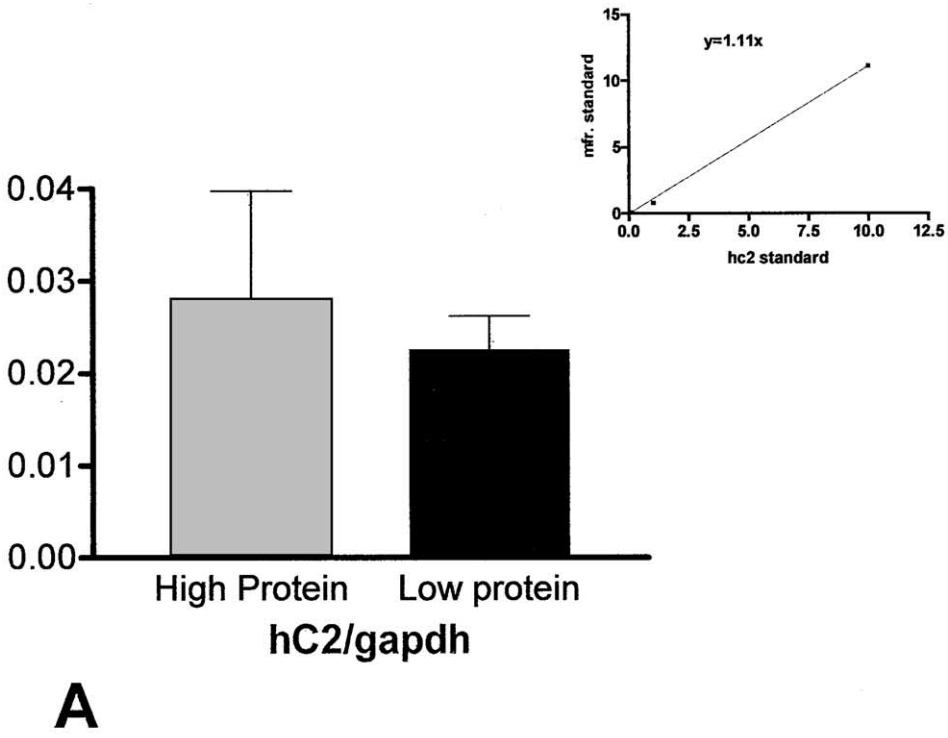
The content of proteasome subunit protein, inferred from the immunoblot band density of the hC2 subunit, was nearly identical in the 2 dietary treatment groups (6.80± 0.65 *v* 6.93 ± 0.93 arbitrary units in high- *v* low-protein groups; Fig 4). This indicates the absence of an effect of dietary protein deprivation to decrease the abundance of intracellular proteasomes. We have calculated that this measurement is capable of detecting a 27% difference between groups with a 1-β power of 0.8.

To provide a measure of the dynamics of proteasome production and its contribution to proteasome intracellular protein content, we herein report the in vivo fractional synthesis rate of proteasome proteins. Results of 6 subjects, meeting criteria for GC-IRMS leucine peak abundance, are reported here. The fractional synthesis rate was similar in the 2 dietary treatment groups (0.17 ± 0.049 *v* 0.211% ± 0.022 %/h, high- *v* low-protein diet using ^{13}C-leucine enrichment in the tissue fluid to represent precursor pool enrichment, not significant [NS]; Fig 5). The similarity in synthesis rates with the 2 treatments suggests that synthesis and degradation of proteasome proteins is not different between those consuming an ample or minimum protein intake. Rates were lower, but similar between groups when calculated using plasma ^{13}C-ketoisocaproic acid enrichment to represent precursor pool enrichment (0.063 ± 0.01 *v* 0.088% ± 0.021%/h, high- *v* low-protein diet, NS).

DISCUSSION

This investigation establishes that dietary protein deprivation at the average adult minimum requirement in human subjects is not associated with an altered abundance or production of proteasomes in muscle tissue. The maintenance of proteasome abundance in muscle in the face of markedly decreased excretion of 3-methylhistidine, a nonrecycled component of actin and myosin,[38] suggests that muscle proteasome content and production can remain unaltered during inhibited degradation of myofibrillar proteins. Taken together with reports that the ubiquitin-proteasome system is responsible for the majority of myofibrillar protein degradation[1,2,39] and is inhibited by dietary protein deprivation,[13] our findings suggest that activity of the ubiquitin-proteasome system can be markedly restrained in muscle tissue while maintaining the production and concentration of proteasomes.

The use of urinary 3-methylhistidine excretion as an indicator of myofibrillar protein degradation requires some caution.[40] Although more than 90% of 3-methylhistidine is found in



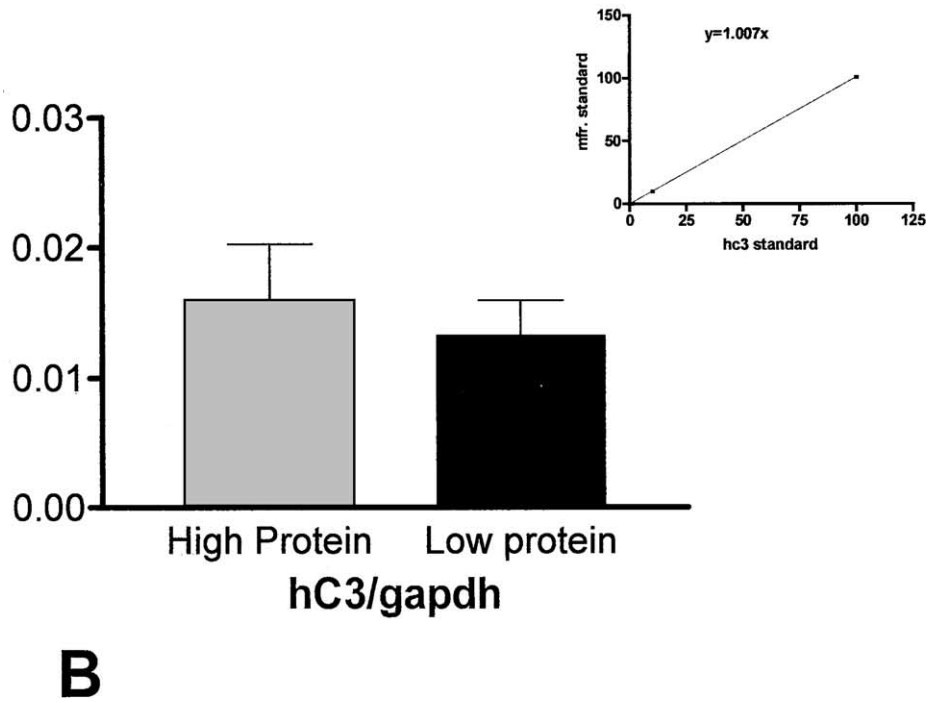


Fig 3. Expression of mRNA for proteasome subunits (A) hC2 and (B) hC3 expressed as the ratio of picograms proteasome subunit RNA: picograms of GAPDH control gene RNA. Gene-specific standards were used to quantitate RNA. Relationship between gene-specific standards and manufacturer standards for light cycler are illustrated in inset panels. There is no difference between the high- and low-protein groups.

muscle, it is present in nonmuscle tissue proteins of skin and gut with higher turnover rate than that of skeletal muscle protein. Thus, previous studies suggest that nonmuscle tissues may contribute from 8% to 24% of urinary 3-methylhistidine excretion.[37,41] The 68% difference in 3-methylhistidine excretion between the groups consuming ample and restricted protein intakes may not be precisely quantitative of the difference in skeletal muscle myofibrillar protein degradation.

In addition to our measurements of proteasome subunit content and gene transcript levels, we have supported the finding

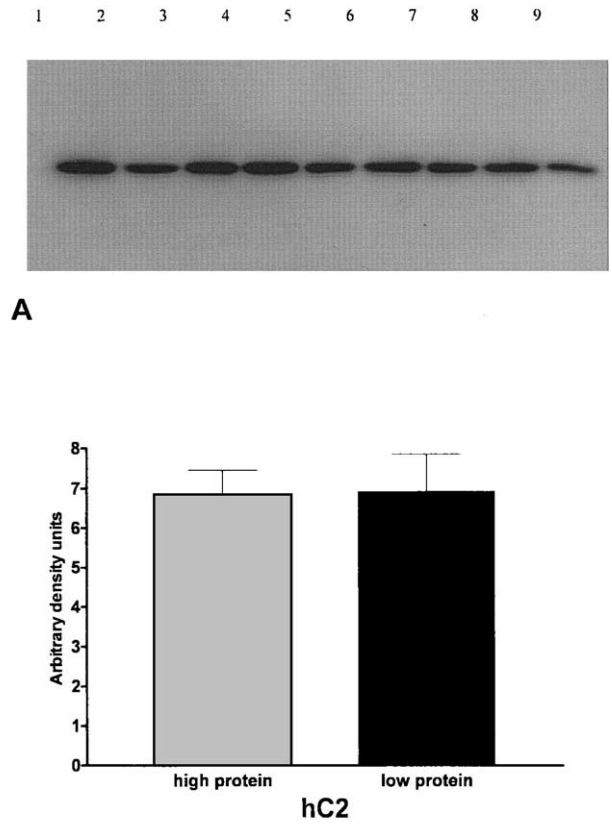


Fig 4. Quantity of proteasome C2 protein determined by immunoblotting. (A) Displays bands detected blotted after SDS-polyacrylamide gel electrophoresis (PAGE). Lanes 3, 5, 7, and 9 from subjects assigned to low-protein intake are interleaved with lanes 1, 2, 4, 6, and 8 from subjects assigned to high-protein intake. (B) Displays the result of densitometry measurements adjusted for protein transfer (indicated by Ponceau S staining). There is no difference between the dietary treatment groups.

that proteasome production remains unaltered during dietary protein deprivation by performing the first measurement in vivo of proteasome fractional synthesis rate. Quantities of proteasome protein were limiting in some measurements of synthesis rate taken from the percutaneous biopsies in this study. However, those with sufficient quantity of labeled protein established that the approximately 0.2%/h synthesis rate of proteasome proteins in humans is greater than that of other skeletal muscle proteins, such as myosin heavy chain (0.03% to 0.044%/h),[42] muscle mitochondrial protein (0.09%/h) representing synthesis of respiratory chain enzymes,[43] or matrix proteins, such as collagen (0.076%/h).[44]

There are potential limitations of our proteasome complex fractional synthesis rate measurement that should be considered. First, it must be recognized that the proteasome is not a single protein, but rather a protein complex. Present technology does not allow the measurement of isotope incorporation into the small quantity of individual proteasome components that can be purified from percutaneous human muscle biopsy material. The fractional synthesis rate of the proteasome represents an aggregate rate of synthesis of 14 distinct core proteasome components (~700 kd) and multiple additional proteins within the 19 S regulatory caps (~800 to 1,300 kd). The aggregate synthesis rate estimate will be biased toward those components with shorter half-lives. Second, it is likely that some nonprotease proteins were isolated along with proteasome complexes, as proteasomes are known to bind polyubiquitin and ubiquitin-tagged proteins. While the large size of the 26S proteasome complex (~2,000 kd) is likely to make bound proteins quantitatively small contributors to the synthesis rate measurement, it should be noted that the probable high representation of fast-turnover proteins among them may additionally inflate the fractional synthesis rate estimate. Despite these limitations, it is evident that the fractional synthesis rate of proteasome complexes is qualitatively high relative to that of other muscle proteins previously analyzed, and, as suggested by both synthesis rate estimates and subunit transcript measurements, it is not decreased by dietary protein deprivation. This suggests that proteasome production is not likely to be a rate-limiting step in the breakdown of muscle proteins. Furthermore, the maintenance of high proteasome production rate in the face of nutri-

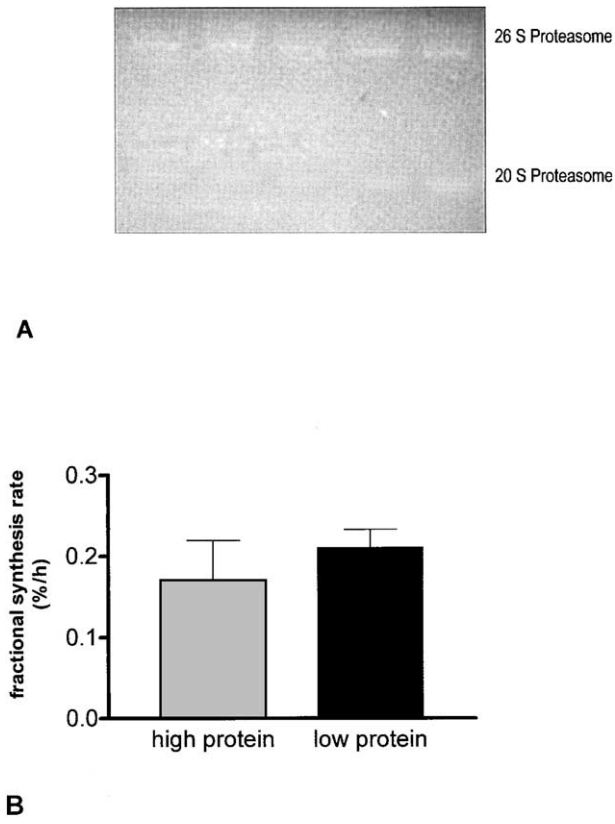


Fig 5. Fractional synthesis rate of proteasome proteins expressed as %/h for 6 of the 14 subjects for which there was adequate leucine quantity for isotopic analysis. (A) Displays the fluorescent bands of the nondenaturing polyacrylamide gel overlaid with the fluorogenic peptide Suc-llVY-AMC used to detect the presence of proteasomes. The top bands are produced by the active 26S proteasome, and the fainter lower bands are produced by activity of the core 20S proteasome. (B) Depicts the fractional synthesis rate of proteasomes expressed as %/h. There is no difference between treatment groups.

tional amino acid deprivation, a fundamental challenge to preservation of intracellular protein resources, may be indicative of its importance as a fundamental mediator of intracellular metabolism.

The estimate of proteasome synthesis rate among those consuming ample protein intake was similar to that among subjects consuming minimum protein intake. Given the identical intracellular proteasome protein content in the 2 groups, this suggests that rates of synthesis and degradation of proteasome proteins remains unaffected by the restriction of dietary protein to the minimum requirement. Our study measured the content of constitutive subunits of the proteasome. Inducible catalytic subunits may exchange. Until recently, this was reported to be a unique response to immune cytokines, particularly γ-interferon. However, others have recently reported a change in proteasome subunit composition, with unchanged content of proteasomes, in muscle of newly diabetic rats.[45] The latter findings suggest de novo proteasome biogenesis during unchanged proteasome abundance, an interpretation consistent with the high fractional synthesis rate reported in the present study. The possibility exists that subunit composition of proteasomes in our subjects could have been different between the 2 dietary treatments while proteasome abundance remained the same.

Of interest, there is a contrast between the mechanisms responsible for nutritionally induced myofibrillar protein conservation and those responsible for muscle wasting in catabolic conditions, such as sepsis, uremic acidosis, insulin deficiency, cancer cachexia, head trauma, and starvation. In these catabolic situations, expression of proteasome subunit genes is substantially increased.[3-5,8,9,46-48] Our data indicate that isoenergetic dietary protein deprivation, in what might be termed an anticatabolic circumstance of muscle protein preservation, does not induce a reciprocal decrease of proteasome gene mRNA levels.

In conclusion, our experiments show that the intracellular content of proteasomes is maintained in skeletal muscle during dietary protein deprivation. Estimates of proteasome synthesis suggest that proteasomes are produced at higher rates than other skeletal muscle proteins even in the face of nutritional deprivation. This can be interpreted to suggest an important role of the proteasome in cellular metabolism—that production of the proteasome is indispensable in the face of scarce amino acid resources. By corollary it suggests, given its unchanged tissue content during protein deprivation, that turnover of the proteasome, both synthesis and degradation, is important enough to cellular function to remain unaltered when degradation of myofibrillar protein is restricted. That myofibrillar protein degradation, a phenomenon attributed to the ubiquitin-proteasome system, is inhibited by dietary protein restriction, suggests that other elements of the ubiquitination pathway, the myofibrillar disassembly pathway, or proteasome subunit composition are likely to be altered by scarce availability of amino acid resources. The response of these elements of the ubiquitin-proteasome pathway to nutritional change has not been explored in the present study and is a reasonable area for further research.

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