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Increase in Oxidative Damage to Lipids and Proteins in Skeletal Muscle of Uremic Patients

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Muscle weakness and reduced exercise capacity are frequent complaints of patients with chronic uremia. Several lines of evidence have suggested that chronic uremia result in a state of increased oxidative stress. Reactive oxygen species (ROS) and free radicals are capable of damaging lipids and proteins but it remains unclear whether oxidative damage plays a role in the skeletal myopathy commonly seen in chronic uremia. In this cross-sectional study, we compared the levels of oxidative damage to proteins and lipids of skeletal muscle from 40 chronic uremic patients and 20 age- and sex-matched healthy subjects. Protein carbonyls were determined by a spectrophotometric method to assess the oxidative damage to proteins. Our results showed that the mean content of protein carbonyls in skeletal muscles was significantly elevated in the hemodialysis patients (3.78 ± 0.14 nmol of 2,4-dinitrophenyl-hydrazone per mg of protein) as compared to healthy controls (2.97 ± 0.28 nmol per mg of protein, $p = 0.017$ vs normal controls). In addition, we found that the mean malondialdehyde (MDA) level was also significantly increased in the uremic patients compared to healthy controls. Further analysis revealed that there was an age-dependent increase in both oxidative damages in these patients. Regression analysis between plasma protein carbonyl and MDA levels showed a significant correlation between these two parameters ($r = 0.43$, $p = 0.002$). The finding of increased oxidative damage to protein and lipids provide support that oxidative damage may play a role in the pathogenesis of skeletal myopathy in chronic uremic patients on hemodialysis.

Keywords: ROS; Free radicals; Protein carbonyls; Malondialdehyde

INTRODUCTION

Free radicals and reactive oxygen species (ROS) may impair intracellular organelles and damage lipids and proteins in the cells. The severity of oxidative damage may be gauged by the concentrations of products generated from oxidative modification of these macromolecules. Several markers of oxidative damage to proteins and lipids are elevated in various tissues of neurodegenerative diseases, cancer and ageing.[1–3] The process of lipid peroxidation is comprised of a set of chain reactions, which are initiated by the abstraction of a hydrogen atom (from carbon) in an unsaturated fatty acyl chain.[4] In an aerobic environment, oxygen will add to the fatty acid at the carbon-centered lipid radical to give rise to a lipid peroxyl radicals. These radicals propagate the peroxidative chain reactions by reacting with fatty acyl moieties in membrane phospholipids and result in altered structural integrity and ion channel function of the membrane. On the other hand, several *in vivo* studies have shown that the reaction of ROS with proteins results in the formation of carbonyl groups[5,6] which can be used as a marker of protein oxidation. Two tissues that may be particularly prone to oxidative damage are muscle and the central nervous system. Both tissues contain post-mitotic cells, which are liable to accumulate oxidative damage over time. Being the largest organ in the body, muscle tissues account for a

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large portion of the body's total oxygen consumption and are exposed to tremendous flux of oxygen free radicals.

Oxidative stress is a disturbance of balance between pro-oxidants and antioxidants. Several lines of evidence have accumulated over the last few years suggesting that patients with chronic renal failure have increased oxidative stress. It has also been shown that uremic milieu per se as well as the hemodialysis procedure may account for the increased oxidative stress observed in chronic uremia. Uremic patients on hemodialysis frequently suffered from easy fatigability and muscle weakness. Uremic myopathy as a clinical entity does exist but is probably under-investigated. There is substantial evidence that the uremic milieu adversely affects skeletal muscle metabolism. Current evidence suggests that malnutrition,[7] physical inactivity, inadequate contractile machinery, intrinsic mitochondrial defect[8,9] and impaired energy production and transport[10] are among the most important causes of myopathic changes in uremic patients. Enhanced oxidative stress in hemodialysis patients may impose a considerable threat to the integrity of the macromolecules of skeletal muscle tissues. Our hypothesis is that alterations of skeletal muscle dysfunction in uremic patients are a consequence of the accumulation of random oxidative damage to lipids, and proteins that are exposed to adverse uremic milieu. Evidence in support of this contention has accumulated in concert with advances in methodologies for measuring oxidative damage. To date, however, most of the evidence of oxidative damage to skeletal tissues comes from studies in non-uremic populations.[11,12] In the present study, we have investigated the increase in oxidative damage to proteins and lipids in skeletal muscles of uremic patients receiving hemodialysis.

MATERIALS AND METHODS

Forty skeletal muscle biopsies were obtained with informed consent from forty non-diabetic uremia patients undergoing forearm arteriovenous fistula creation. All the biopsies were cryopreserved immediately after excision. The studied patients were screened to exclude the possibility of being associated with any of the known mitochondrial diseases by reviewing the clinical history and biochemical criteria (SMA-12 analysis). On the basis of age, patients were divided into four age groups: group A, <40 years (four males, four females); group B, 41–60 years (five males, six females); group C, 61–80 years (six males, seven females) and group D, >81 years (four males, four females). We also obtained skeletal muscle biopsies from twenty healthy subjects as controls. Muscle biopsies were obtained during surgical intervention for forearm traumatic injury.

Preparation of Tissue Extract from Skeletal Muscle

The skeletal muscle was crushed under liquid nitrogen, resuspended in 0.5 ml RSB buffer per 100 mg tissue [RSB buffer: 10 mM Tris–HCl (pH 7.4), 10 mM NaCl, 25 μM EDTA (pH 7.4)] and 15 μl of 10% butylated hydroxytoluene (BHT) as antioxidant. The muscle tissues were digested with 1 μg proteinase K/μl and 1% SDS at 50°C for 2 h. An aliquot of 50 μl of 5 M NaCl was added and the DNA was extracted and precipitated. For measurements of MDA and protein carbonyls total extract of muscle tissues was used.

Quantification of Protein Carbonyls

Protein carbonyls were quantified by reaction with 2,4-dinitrophenyl-hydrazine (DNPH) as described by Reznick *et al.*[13] Briefly, 1 ml supernatant together with 10 μl of proteinase inhibitor (25 ×) and 10% BHT were pipetted into a 1.5 ml centrifuge tube (in the case of freshly collected plasma, the sample was diluted to 5 mg/ml protein). The solution was mixed by vortex at room temperature, and centrifuged at 5000 × g (gravitational force) for 15 min at 4°C. The supernatant was transferred into a 15 ml glass tube. For each run, one "blank" tube was always included in parallel with the "test". The "test" tube was prepared by adding 1 ml of DNPH (10 mM in 2 N HCl), while the blank tube was prepared by adding 1 ml of 2 N HCl without DNPH. The reaction mixtures were left at room temperature for 1 h in the dark and were mixed by vortex every 15 min. The proteins were precipitated by the addition of equal volume of 20% trichloroacetic acid (TCA). After mixing thoroughly, the tubes were centrifuged at 12000 × g at 4°C for 10 min. The supernatant was discarded and the pellet was washed three times with 4 ml of ethyl acetate: ethanol (1:1, v/v) to remove free DNPH and lipid contaminants. The final pellet was re-dissolved in 1 ml of 6 M guanidine hydrochloride and kept at 37°C for 1 h in a shaking water bath. Insoluble materials were removed by additional centrifugation. The absorbances at 280 nm (protein) and 364 nm (carbonyl hydrazone) were taken against the reagent blank on a spectrophotometer. Protein carbonyls content was calculated using a molar extinction coefficient for the hydrazone of $22{,}000\,M^{-1}\,cm^{-1}$ at the wavelength of 364 nm. The results are expressed as nmol protein carbonyl per mg protein.

Quantification of Lipid Peroxides

The content of lipid peroxides in muscle tissues was measured as MDA by an HPLC system described previously.[14] Fifty μl each of a plasma sample, reagent blank (distilled water), and 1,1,3,3-tetraethoxy propane (TEP) working standard solutions were, respectively, pipetted into a glass tube. After mixing, 750 μl of 0.44 M phosphoric acid and 250 μl of 0.6% thiobarbituric acid (TBA) were added. The mixture was heated for 1 h in boiling water (100°C), and then cooled at 4°C. This was followed by neutralizing 500 μl of the boiled sample with 500 μl of methanol-1N NaOH mixture (45.5:4.5, v/v) to precipitate proteins before injection into a C_{18} column. After centrifugation, 10–50 μl of the protein-free supernatant was injected to the HPLC system to separate the MDA–TBA adduct from interfering chromogens. The MDA–TBA adduct was quantified with a fluorescence detector using an excitation wavelength of 525 nm and emission wavelength of 550 nm. Assays were performed in triplicate and the concentration of lipid peroxides in the sample is expressed as nmol MDA/mg of protein. A calibration curve was prepared for each run by using TEP as standard.

Collection and Preparation of Blood Samples

After 12 h of fasting, venous blood samples were taken from each of the patients and control subjects. Following centrifugation at 300 × g for 10 min, the plasma was collected and stored in aliquots at −80°C or used immediately for enzymatic assays. Several aliquots of the same samples were transferred into other tubes to be used for the assays of other plasma or serum parameters, which were performed by routine laboratory techniques. In addition, an aliquot of 1 ml fresh blood was collected in a heparin vacutainer for biochemical analysis of the plasma level of protein carbonyls.

Determination of Total Glutathinone (tGSH)

An aliquot of 0.05 ml of 10% perchloric acid (PCA) was added to 0.1 ml of whole blood to remove proteins by precipitation and centrifugation. Total free GSH in whole blood was measured with the recycling enzymatic assay, which employs glutathione reductase to induce a kinetic colorimetric reaction of DTNB [5, 5′-dithiobis-(2-nitrobenzoic acid)].[15] The rate of change of absorbance at 412 nm was monitored at 30°C within 5 min. The concentration of tGSH in blood was calculated from a standard curve and is expressed as μg/ml.

Determination of Non-GSH Free Sulfhydryl Content in Whole Blood

The concentration of blood thiols was determined by a colorimetric method according to Ellman.[16] An aliquot of 0.05 ml 10% PCA was added into 0.1 ml whole blood to remove proteins by precipitation and centrifugation. The supernatant was added with 0.05 ml of 0.1 M DTNB. After thorough mixing, the mixture was left standing at room temperature. The final absorbance at 412 nm was recorded after 30 min reaction of DTNB with all the SH group-containing compounds. The blood concentration of total free thiols is expressed in the unit of μg/ml.

Measurement of Glutathione Peroxidase Activities in Plasma

Plasma, GSH reductase and GSH were incubated in 1.0 ml of 50 mM phosphate buffer (pH 7.0) at 37°C for 10 min. NADPH solution (1.0 mM) and 5 mM sodium azide were then added and allowed to equilibrate for 3 min at 20°C. The enzymatic reaction was initiated by addition of 5 mM *t*-butyl-hydroperoxide as the substrate. The conversion of NADPH to $NADP^+$ was then followed by continuous recording of the decrease of the absorbance at 340 nm for 5 min.[17] Glutathione peroxidase activity is expressed as U/l or U/g-Hb.

Statistical Analysis

All calculations were performed using the software package SPSS® running on an IBM-compatible personal computer. All data are presented as mean ± SE. Comparisons were made by one way ANOVA and Scheffe's *post-hoc* method. Correlation was examined using linear regression. In all comparative analyses, the difference is considered of significance when $p < 0.05$.

RESULTS

The demographic and biochemical data of our patients and controls are listed in Table I. There were significant differences in whole blood total glutathione, plasma protein carbonyls and glutathione peroxidase between the uremic patients and controls. The protein carbonyl content was used as a "general" index of oxidative protein damage and expressed as nmol of 2,4-dinitrophenylhydrazone per mg of protein. The mean concentration of protein carbonyls in skeletal muscle was significantly elevated in the uremic patients (3.78 ± 0.14 nmol per mg of protein) as compared to healthy controls (2.97 ± 0.28 nmol per mg of

TABLE I Clinical characteristics of patient groups and controls

Parameters	Controls	Patient
Number	20	40
Age (years)	57.3 ± 15.1	58.4 ± 17.1
Body mass index (kg/m^2)	25.4 ± 2.5	22.6 ± 2.9
Creatinine, mg/dl	0.9 ± 0.3	12.6 ± 3.6*
Blood urea nitrogen, mg/dl	15.2 ± 3.6	73.5 ± 19.6*
Hematocrit%	na	29.8 ± 5.6
Serum calcium	na	
Serum phosphate	na	
Urate, mg/dl	6.6 ± 1.1	8.1 ± 4.0
Total bilirubin, mg/dl	0.6 ± 0.3	0.7 ± 0.4
Total cholesterol, mg/dl	182.4 ± 30.0	175.6 ± 42.9
Triglycerides, mg/dl	127.6 ± 30.9	144.9 ± 90.8
Fasting blood glucose, mg/dl	107.7 ± 36.8	106.8 ± 37.7
Serum albumin, g/dl	4.53 ± 0.27	4.28 ± 0.39
Plasma protein-carbonyl(nmol/g)	0.92 ± 0.18	1.44 ± 0.20*
Total fSH, μg/ml	339.8 ± 12.1	291.1 ± 50.8*
Total GSH, μg/ml	221.0 ± 10.0	184.3 ± 46.3†
Plasma GSHPx, U/ml	635.6 ± 65.4	284.6 ± 61.5*

Values are expressed as mean ± SD. Abbreviations: tGSH, total blood glutathione; total fSH, total bloodnon-GSH free sulfhydryl compounds; pGSHPx, plasma glutathione peroxidase; na; not available. Comparison by Mann–Whitney test. *$p < 0.001$. †$p < 0.05$.

protein, $p = 0.017$ vs normal controls). A plot of skeletal muscle protein carbonyls levels of both patients and controls is shown in Fig. 1. The mean (±SEM) protein carbonyl levels (nmol per mg of protein) of controls and four groups of patients were 3.01 ± 0.24 in group A; 3.57 ± 0.02 in group B; 4.08 ± 0.26 in group C and 4.38 ± 0.16 in group D. Paired comparison of the plasma protein carbonyl levels of all four groups showed that groups C and D displayed a significantly higher levels of protein carbonyls than group A. There was also a positive correlation between age and skeletal muscle carbonyl content of the uremia patients ($r = 0.431$, $p = 0.002$) (Fig. 2).

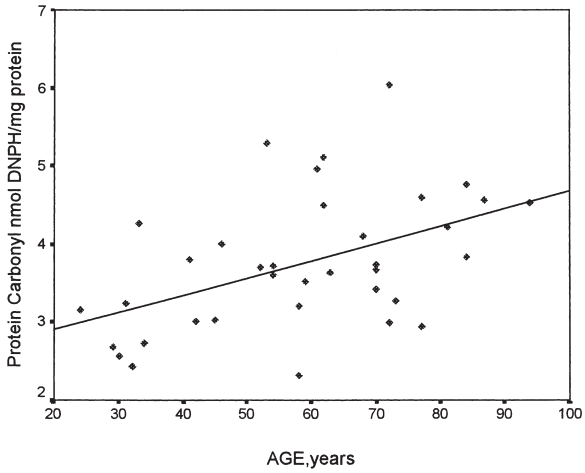

FIGURE 2 Correlation between protein carbonyl levels in skeletal muscles and age in 40 hemodialysis patients. ($r = 0.48$; $p = 0.003$)

We then measured MDA as an index of lipid peroxidation using HPLC with a fluorescent detector. BHT was added to prevent any peroxidation occurring in the homogenate or during the assay. The mean level of MDA in the skeletal muscle of uremia patients (0.065 ± 0.009 nmol/mg protein) was significantly higher than that of the healthy controls (0.043 ± 0.005 nmol/mg protein, $p = 0.032$ vs normal controls). A plot of skeletal muscle MDA levels of both patients and controls is shown in Fig. 3. The

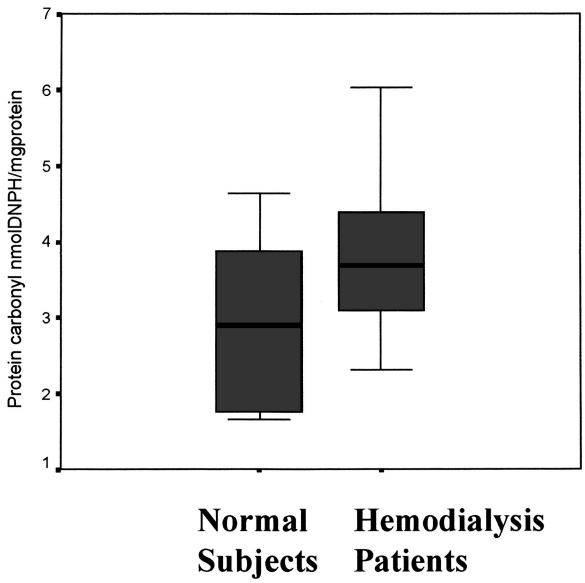

FIGURE 1 Whisker plot of protein carbonyl levels in skeletal muscles of normal subjects and hemodialysis patients. The difference is significant $p = 0.017$.

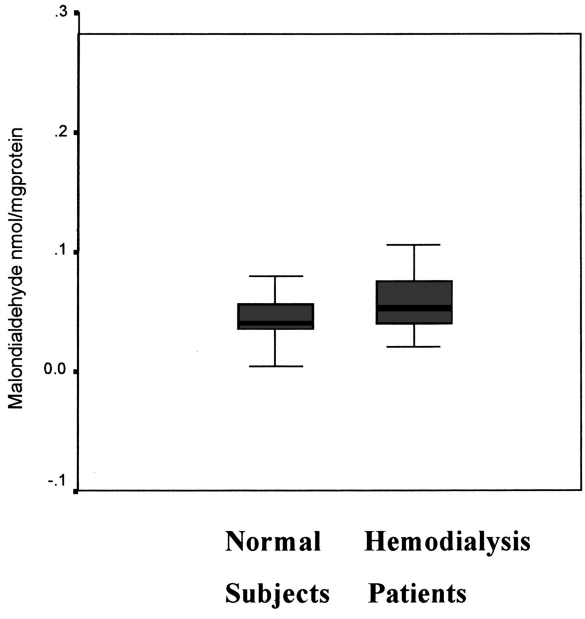

FIGURE 3 Whisker plot of malondialdehyde levels in skeletal muscles of normal subjects and hemodialysis patients. The difference is significant $p = 0.032$.

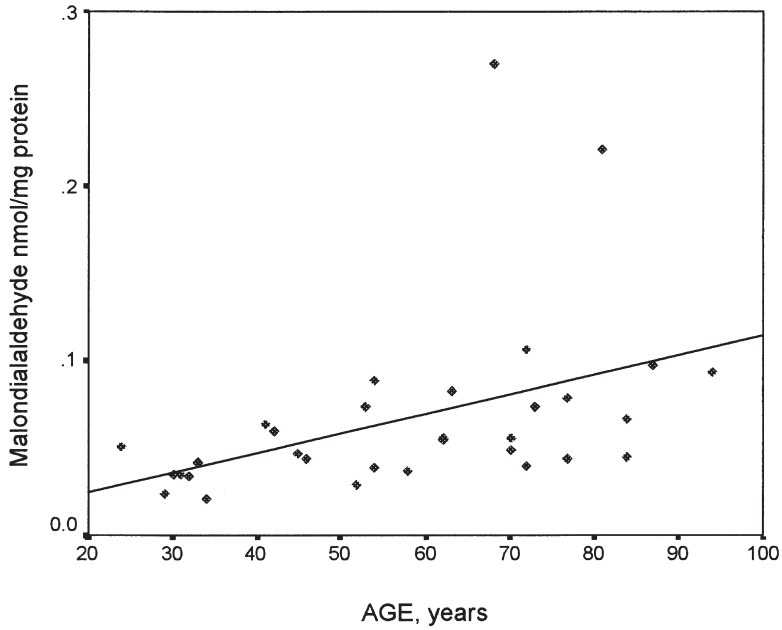

FIGURE 4 Correlation between malondialdehyde levels in skeletal muscles and age in 40 hemodialysis patients. ($r = 0.43$; $p = 0.015$)

mean (±SEM) MDA levels of controls and four groups of patients were 0.035 ± 0.004 in group A; 0.054 ± 0.006 in group B; 0.083 ± 0.002 in group C and 0.105 ± 0.003 in group D. Paired comparison showed that plasma MDA levels of groups C and D were significantly higher than that of group A. There was also a positive correlation between age and skeletal muscle MDA levels in uremia patients ($r = 0.43$, $p = 0.015$) (Fig. 4). Regression analysis between the protein carbonyl levels and MDA levels showed a significant correlation between these two parameters ($r = 0.43$, $p = 0.002$) (Fig. 5). No significant correlation was found between both oxidative damage indexes and sex or body mass index.

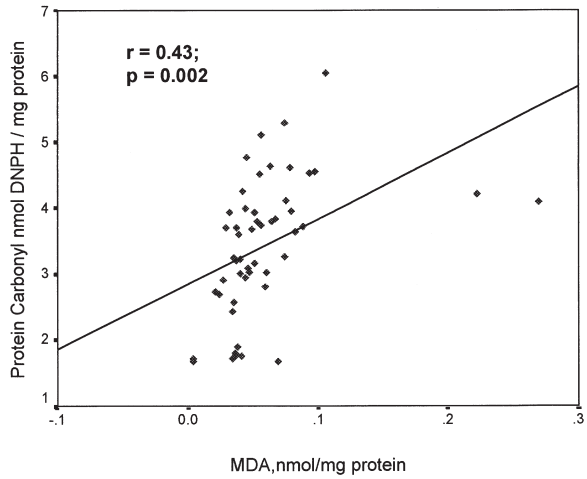

FIGURE 5 Regression analysis between MDA and protein carbonyls levels in skeletal muscles of 40 hemodialysis patients. ($r = 0.43$; $p = 0.015$)

DISCUSSION

In uremic patients receiving hemodialysis, muscle weakness is a common clinical symptom. Most of these patients suffered from lethargy, exercise intolerance[18,19] and easy fatigability. It has been suggested that altered skeletal muscle function is caused by capillary-myofiber dissociation, limitation of oxygen supply, inadequate contractile machinery, reduced mitochondrial content or an intrinsic defect of mitochondrial function. Numerous studies[20–22] have suggested that uremia is characterized by profound alterations of cell energy metabolism that results in an unavoidable decrease in physiological capacity for sustained muscle performance. An accrual of molecular oxidative damage with either hemodialysis or uremia per se has been ascribed as a major cause for loss of functional capacity of skeletal muscles. Evidence in support of this hypothesis comes from the observations that: oxidative stress is increased in uremic patients and oxidative damage to macromolecules of plasma accumulates in these patients.[23–26] Elevated levels of inflammatory cytokines such as tumor necrosis factor α (TNFα) observed in hemodialysis patients may also elicit an increase of oxidative response in myocytes.[27] In addition, experimentally induced oxidative stress in animals had been shown to cause myofibrillar muscle protein modification and degradation.[28]

Membrane phospholipids are continually subjected to oxidant challenges and membranes with peroxidative damage lose selective permeability and functional integrity, a process detrimental to cell viability. Lipid peroxidation of the mitochondrial

inner membrane may lead to leakage of ions and cause uncoupling of respiration from oxidative phosphorylation. In animal myocytes exposed to oxidative stress, a marked increase in intracellular calcium concentrations was observed, which is probably caused by a damaged sarcoplasmic reticulum membrane and impaired activity of calcium ATPase.[29,30] Conceivably, in the uremic patients, post-mitotic tissues such as skeletal muscles, which are likely to accumulate oxidative damage over time, may undergo structural and functional perturbation following repeated bouts of oxidative insults.

Proteins, which constitute major components of living cells, are also susceptible to oxidant damage. Oxidative modification of proteins may lead to alteration of its amino acid sequences and conformation as well as their rapid degradation. Among the various modifications of amino acids in proteins, carbonyl formation is considered as an early marker for protein oxidation. The most likely amino acid residues to form carbonyl derivatives are lysine, arginine, proline and threonine.[31] In the presence of massive oxidative stress and/or defective proteolytic system, aggregation and accumulation of damaged proteins may occur. There is good evidence that free radicals are involved in the initiation of muscle damage in some *in vitro* and animal studies.[12,32,33] The presence of nutritional myopathy in selenium-and vitamin-deficient animals is associated with increased free radical production in muscle tissue as determined by electron spin resonance.[32] It has also been shown that an intracellular calcium overload is involved in muscle atrophy[34] and that vitamin E deficiency facilitates muscle wasting and necrosis[35] which may be a free radical-mediated damage to proteins. Presumably, in certain pathological conditions associated with increased oxidative stress such as chronic uremia, the rate of protein turnover may be significantly altered and results in accumulation of oxidatively modified proteins. Recent studies have shown that plasma oxidative protein carbonyl levels were increased in hemodialysis patients.[25,26] Skeletal muscle contains high levels of fibrillar proteins and is susceptible to free radical oxidation. In the present study, we measured protein carbonyl groups as a marker of protein oxidation and found that there was substantial evidence for oxidative damage to protein in the skeletal muscle of uremic patients. In addition, we found an age-dependent increase in protein carbonyl groups in uremic skeletal muscle tissues similar to the trends seen in plasma protein carbonyls of hemodialysis patients and normal controls (unpublished data). A correlation between lipid peroxidation and oxidative modification to proteins in the skeletal muscles of uremic patients was also found.

In summary, we have demonstrated significant increases in oxidative damage to proteins and lipids in the skeletal muscle of uremia patients. These findings suggest that oxidative damage may play an important role in the pathogenesis of uremic myopathy, which often occurs in the skeletal muscle of uremic patients on long-term hemodialysis.

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References

[1] Chevion, M., Berenshtein, E. and Stadtman, E.R. (2000) "Human studies related to protein oxidation: protein carbonyl content as a marker of damage", *Free Radic. Res.* **33**, S99–S108.
[2] Lyras, L., Cairns, N., Jenner, A., Jenner, P. and Halliwell, B. (1997) "An assessment of oxidative damage to proteins, lipids and DNA in brain from patients with Alzheimer's disease", *J. Neurochem.* **68**, 2061–2069.
[3] Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1993) "Oxidants, antioxidants and the degenerative diseases of aging", *Proc. Natl Acad. Sci. USA* **90**, 7915–7922.
[4] Sevanian, A. and Hochstein, P. (1985) "Mechanisms and consequences of lipid peroxidation in biological systems", *Ann. Rev. Nutr.* **5**, 365–390.
[5] Garibaldi, S., Aragno, I., Odetti, P. and Marinari, U.M. (1994) "Relationships between protein carbonyls, retinol and tocopherols level in human plasma", *Biochem. Mol. Biol. Int.* **31**, 729–736.
[6] Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B., Shaltiel, S. and Stadtman, E.R. (1990) "Determination of carbonyl content in oxidatively modified proteins", *Methods Enzymol.* **186**, 464–478.
[7] Fahal, I.H., Bell, G.M., Bone, J.M. and Edwards, H.T. (1997) "Physiological abnormalities of skeletal muscle in dialysis patients", *Nephrol. Dial. Transplant.* **12**, 119–127.
[8] Thompson, C.H., Kemp, G.J., Taylor, D.J., Ledingham, G.G., Radda, G.K. and Rajagopalan, B. (1999) "Effect of chronic uremia or skeletal muscle metabolism in man", *Nephrol. Dial. Transplant.* **8**, 218–222.
[9] Lim, P.S., Cheng, Y.M. and Wei, Y.H. (2000) "Large-scale mitochondrial DNA deletions in skeletal muscle of patients with end-stage renal disease", *Free Radic. Biol. Med.* **29**, 454–463.
[10] Moore, G.E., Bertocci, L.A. and Painter, P.L. (1993) "31P-Magnetic resonance spectroscopy assessment of subnormal oxidative metabolism in skeletal muscle of renal failure patients", *J. Clin. Investig.* **91**, 420–424.
[11] Mecocci, P., Fano, G., Fulle, S., MacGarvey, U., Shinobu, L., Polidoro, M.C., Cherubini, A., Vecchiet, J., Senin, U. and Beal, M.F. (1999) "Age-dependent increases in oxidative damage to DNA, lipids and proteins in human skeletal muscle", *Free Radic. Biol. Med.* **26**, 303–308.
[12] Haycock, J.W., Jones, P., Harris, J.B. and Mantle, D. (1996) "Differential susceptibility of human skeletal muscle proteins to free radical induced oxidative damage: a histochemical, immunocytochemical and electron microscopical study", *Acta Neuropathol.* **92**, 331–340.
[13] Reznick, A.Z. and Packer, L. (1994) "Oxidative damage to proteins: Spectrophotometric method for carbonyl assay", *Methods Enzymol.* **233**, 357–363.
[14] Wong, S.H.Y., Knight, J.A., Hopfer, S.M., Zaharia, O., Leach, C.N. and Sunderman, F.W. (1987) "Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde–thiobarbituric acid adduct", *Clin. Chem.* **33**, 214–220.

[15] Anderson, M.E. and Meister, A. (1980) "Dynamic state of glutathione in blood plasma", *J. Biol. Chem.* **255**, 9530–9533.
[16] Ellman, G.L. (1969) "Tissue sulfhydryl groups", *Arch. Biochem. Biophys.* **82**, 70–77.
[17] Flohe, I. and Gunzer, W.A. (1984) "Assays of glutathione peroxidase", *Methods Enzymol.* **105**, 114–121.
[18] Diesel, W., Noakes, T.D., Swanepoel, C. and Lambert, M. (1990) "Isokinetic muscle strength predicts maximum exercise tolerance in renal patients on chronic hemodialysis", *Am. J. Kidney Dis.* **17**, 109–114.
[19] Painter, P., Messer-Rehak, D., Hanson, P., Zimmerman, S.W. and Glass, N.R. (1986) "Exercise capacity in hemodialysis, CAPD and renal transplant patients", *Nephron* **42**, 47–51.
[20] Brautbar, N. (1983) "Skeletal myopathy in uremia: abnormal energy metabolism", *Kidney Int.* **24**(Suppl. 16), 81–86.
[21] Metcoff, J., Dutta, A., Burns, G., Pederson, J., Matter, B. and Rennert, O. (1983) "Effects of amino acid infusions on cell metabolism in hemodialyzed patients with uremia", *Kidney Int.* **24**(Suppl. 16), 87–92.
[22] Del Canale, S., Fiaccadori, E., Ronda, N., Soderlund, K., Antonucci, C. and Guariglia, A. (1986) "Muscle energy metabolism in uremia", *Metabolism* **35**, 981–983.
[23] Loughrey, C.M., Young, I.S., Lightbody, D., McMaster, D., McNamee, P.T. and Trimble, E.R. (1994) "Oxidative stress in hemodialysis", *Q. J. Med.* **87**, 679–683.
[24] Peuchant, E., Carbonneau, M.A., Dubourg, L., Thomas, M.J., Perromat, A. and Clerc, M. (1994) "Lipoperoxidation in plasma and red blood cells of patients undergoing hemodialysis: vitamins A, E and iron status", *Free Radic. Biol. Med.* **16**, 339–346.
[25] Odetti, P., Garibaldi, S., Gurreri, G., Aragno, I., Dapino, D., Pronzato, M.A. and Marinari, U.M. (1996) "Protein oxidation in hemodialysis and kidney transplantation", *Metabolism* **45**, 1319–1322.
[26] Himmelfarb, J., McMonagle, E. and McMenamin, E. (2000) "Plasma protein thiol oxidation and carbonyl formation in chronic renal failure", *Kidney Int.* **58**, 2571–2578.
[27] Buck, M. and Chojkier, M. (1996) "Muscle wasting and dedifferentiation induced by oxidative stress in a murine model of cachexia is prevented by inhibitors of nitric oxide synthesis and antioxidants", *EMBO J.* **15**, 1753–1765.
[28] Nagasawa, T., Hatayama, T., Watanabe, Y., Tanaka, M., Niisato, Y. and Kitts, D.D. (1997) "Free radical mediated effects on skeletal muscle protein in rats treated with Fe-nitrilotriacetate", *Biochem. Biophys. Res. Commun.* **231**, 37–41.
[29] Astier, C., Rock, E., Lab, C., Gueux, E., Mazur, A. and Rayssiguier, Y. (1996) "Functional alterations in sarcoplasmic reticulum membranes of magnesium-deficient rat skeletal muscle a consequences of free radical-mediated process", *Free Radic. Biol. Med.* **20**, 667–674.
[30] Xu, K.Y., Zweier, J.L. and Becker, L.C. (1997) "Hydroxyl radical inhibits sarcoplasmic reticulum CA^{2+} -ATPase function by direct attack on the ATP binding site", *Circ. Res.* **80**, 76–81.
[31] Reznick, A. and Packer, L. (1994) "Oxidative damage to proteins: Spectrophotometric method for carbonyl assay", *Methods Enzymol.* **233**, 346–357.
[32] Arthur, J.R. (1998) "Free radicals and diseases of animal muscle", In: Reznick, A.Z., Packer, L., Sen, C.K., Holloszy, J.O. and Jackson, M.J., eds, Oxidative stress in skeletal muscle (Birkhause, Basel), pp 317–321.
[33] Hochman, A. (1998) "Oxidative damage after ischemia-reperfusion in skeletal muscle", In: Reznick, A.Z., Packer, L., Sen, C.K., Holloszy, J.O. and Jackson, M.J., eds, Oxidative stress in skeletal muscle (Birkhauser, Basel), pp 239–256.
[34] Soares, J.M., Duarte, J.A., Carvalho, J. and Appell, H.J. (1993) "The possible role of intracellular Ca^{2+} accumulation for the development of immobilization atrophy", *Int. J. Sports Med.* **14**, 437–439.
[35] Thomas, P.K., Cooper, J.M., King, R.H., Workman, J.M., Schapira, A.H., Goss-Sampson, M.A. and Muller, D.P. (1993) "Myopathy in vitamin E deficient rats: muscle fiber necrosis associated with disturbances of mitochondrial function", *J. Anat.* **183**, 451–461.