Resistance Training Increases Muscle Mitochondrial Biogenesis in Patients with Chronic Kidney Disease

Vaidyanatha S. Balakrishnan,* Madhumathi Rao,* Vandana Menon,* Patricia L. Gordon,† Monika Pilichowska,‡ Francisco Castaneda,§ and Carmen Castaneda-Sceppa¶

*Division of Nephrology, Tufts Medical Center, Boston, Massachusetts; †Department of Medicine, University of California San Francisco VA Medical Center Nephrology Section, San Francisco, California; ‡Department of Pathology, Tufts Medical Center, Boston, Massachusetts; §Max Planck Institute of Molecular Physiology, Dortmund, Germany; ¶Friedman School of Nutrition Science and Policy, Tufts University, Boston, Massachusetts; and ¶Bouve College of Health Sciences, Northeastern University, Boston, Massachusetts

Background and objectives: Muscle wasting, a common complication in chronic kidney disease (CKD), contributes to poor outcomes. Mitochondrial biogenesis is critical for the maintenance of skeletal muscle function and structural integrity. The present study—a secondary analysis from a published randomized controlled trial—examined the effect of resistance exercise training on skeletal muscle mitochondrial (mt)DNA copy number and determined its association with skeletal muscle phenotype (muscle mass and strength).

Design, setting, participants, & measurements: Twenty-three patients with moderate-to-severe CKD were randomized to resistance training (n = 13) or an attention-control (n = 10) group for 12 weeks. After a run-in period of a low-protein diet that continued during the intervention, mtDNA copy number in the vastus lateralis muscle was estimated by quantitative real-time PCR at baseline and 12 weeks.

Results: Participants mean age was 64 ± 10 (SD) years and median (interquartile range, IQR) GFR 27.5 (37.0) ml/min. There were no differences between groups at baseline. Median (IQR) mtDNA copy number was 13,713 (10,618). There was a significant increase in muscle mtDNA with exercise compared with controls (1306 [13306] versus 3747 [15467], P = 0.01). The change in muscle mtDNA copy number was positively correlated with previously reported changes in types I and II muscle fiber cross-sectional area.

Conclusions: In this pilot study, resistance training was highly effective in enhancing mitochondrial content in patients with moderate-to-severe CKD. This finding suggests that the mitochondrial dysfunction observed with chronic disease could potentially be restored with this exercise modality and should be investigated further.


Protein-energy wasting in chronic kidney disease (CKD) is defined as the loss of body protein mass and fuel reserves. Loss of body protein mass includes a reduction in muscle mass caused by wasting and/or sarcopenia (1). The wasting syndrome of CKD represents a significant public health concern given that approximately 19 million adults in the U.S. have CKD not requiring kidney replacement therapy and that the prevalence of kidney failure has increased by 51% during the last decade (2). Data from the Third National Health and Nutrition Examination Survey (NHANES III) suggest that sarcopenia is common in community-dwelling adults with CKD (3).

Mitochondrial biogenesis is critical in maintaining the functional and structural integrity of postmitotic tissues like skeletal muscle (4). Mitochondria are the main source of cellular energy. However, mitochondrial dysfunction caused by a reduction in the number of those functionally intact or DNA deletions is believed to play a role in the loss of skeletal muscle mass and alterations in contractile function (sarcopenia) seen with aging (5). Similar to the sarcopenia of aging, the muscle wasting of CKD may be associated with mitochondrial dysfunction (5).

Kidney failure is associated with mitochondrial abnormalities. Lim et al. (6,7) showed a high prevalence of somatic mitochondrial (mt)DNA mutations in skeletal muscle, specifically the 4977-bp deletion. The study by Rao et al. (8) in a cohort of prevalent maintenance hemodialysis patients from the Hemodialysis (HEMO) study found that mtDNA copy number, a measure of mitochondrial content, was significantly lower among older dialysis patients compared with older healthy subjects and was predictive of poor outcome and survival. In this study mtDNA-4977 deletions were present in 31% of the dialysis patients and seemed to predict survival (8). These observations raise important questions about the role of mitochondrial content and function in the development of uremic...
complications and clinical outcomes, which need to be investigated further.

Older adults may benefit through the exercise-induced adaptations in mitochondrial biogenesis and cellular antioxidant defense (9). Resistance exercise training is an exercise modality shown to reverse sarcopenia (10) and enhance mitochondrial function in aging muscle (11). We and others have examined the beneficial effects of resistance exercise on muscle mass and contractile function in CKD (12,13) and dialysis (14–18) patients. However, the effect of resistance exercise on mitochondrial biogenesis in CKD patients is not known. We undertook the present study to examine the effect of resistance exercise training on mtDNA copy number and to determine its association with skeletal muscle phenotype (i.e., measures of muscle mass and strength). This study represents a secondary analysis from a published randomized controlled trial that reported the anabolic effects of a 12-week high-intensity resistance exercise training program in patients with CKD stages 3 and 4 who were prescribed a low-protein diet (approximately 0.6 g/kg body wt per day) (12).

Materials and Methods

Study Population

Twenty-three participants (mean ± SD: 64 ± 10 years) with CKD stages 3 and 4 were studied (19). Median GFR was 27.5 ml/min/1.73 m². Study details were previously described (12). Briefly, eligibility criteria were serum creatinine concentrations between 1.5 and 5.0 mg/dL, and the ratio of mt:nuclear DNA reflects the tissue concentration of mitochondria per cell. A 120-bp-long mtDNA fragment within the ND1 gene and a 120-bp region of the lipoprotein lipase (LPL) gene were amplified. The ND1 forward primer used was (5'-3') CCTAAAACCCGCC ACATCT and the reverse primer was GAGCGATGGT-TGCTTCGA. Quantification was performed in a total reaction volume of 25 l containing: 2X SYBR Green (12.5 l), each primer (1.25 l), sample DNA (1 l), and water (9 l). Amplification and detection were performed in a Stratagene Mx4000 Multiplex Quantitative PCR System. PCR was initiated with 15 minutes at 95°C, 40 cycles of 15 seconds at 95°C, 30 seconds at 49°C, and 30 seconds at 72°C; 1 minute at 95°C; and 41 cycles starting at 49°C for 30 seconds, escalating by 1°C per cycle. Samples were assayed in triplicate. Data analysis was based on measurement of the cycle threshold (Ct), and the difference in Ct values was used as the measure of relative abundance: CTD1 – CTLPL or ΔCt, a quantitative measure of the mitochondrial genome. Results were expressed as the copy number of mtDNA per cell, provided by 2 × 10^-ΔCt, as a unitless ratio.

Outcome Measurements

Measurements were taken before randomization and after the 12-wk intervention by investigators blinded to participants’ group assignment, with the exception of muscle strength.

Muscle Biopsies

Percutaneous needle biopsies of the vastus lateralis muscle of the nondominant leg were obtained using a 5-mm Bergstrom needle with suction (24). Muscle tissue was frozen in embedding medium (Tissue-Tek OCT, Miles Laboratories, Elkhart, IN) in isopentane cooled to the temperature of liquid nitrogen. Tissue samples were obtained from all participants at baseline (n = 23) but only from 19 participants at week 12 after the intervention.

Skeletal Muscle Mitochondrial DNA Copy Number

Genomic DNA was extracted using a spin column method. In brief, muscle tissue was treated with proteinase K (20 µl) (Qiagen Inc., Valencia, CA), followed by SDS (200 µl) to lyse the cells. The homogenized solution was incubated at 56°C for 10 min and 100% ethanol (200 µl) was added to precipitate DNA. The mixture was applied to the QIAamp spin column, and after two washes with buffer (500 µl), genomic DNA was eluted with elution buffer (200 µl). Final DNA concentrations determined by mini-gel electrophoresis were 50 to 200 ng/ml. Mitochondrial (mt) copy number was estimated as relative amounts of nuclear and mtDNA by quantitative real-time PCR (Stratagene Mx4000Multiplex QPCR System; Stratagene, La Jolla, CA) (25).

Types I and II Muscle Fiber Cross-Sectional Area

Transverse tissue sections (10 µm) were prepared using a CM1850 Cryostat (Leica Microsystems, Nussloch, Germany) and stained for myosin adenosine triphosphatase at pH 4.3 (26). Stained muscle images were captured by a Hamamatsu CCD monochrome video camera (Model C2400, type 77) mounted on a Nikon light microscope (Labophot2), coupled to a computer through a LG-3 Scientific PCI Frame Grabber (Scion Corporation) data translation video capture board. Images were analyzed with the Image Software version 1.39 (Dr. W. Rasband, National Institute of Aging, Bethesda, Maryland), modified by Dr. Chun-Shan Yam (SyLoc Consulting LLP, Lexington, Massachusetts). At each time point, an average of 75 to 100 fibers per muscle fiber type per participant were measured for cross-sectional area (CSA) in µm². The coefficient of variation (CV) was 2% (27).

Participants met and performed the same stretching and flexibility exercises as those used during cool-down in the resistance exercise training group. Participants followed a low-protein diet (approximately 0.6 g/kg per day) (12). Study details were previously described (12). Briefly, eligibility criteria were serum creatinine concentrations between 1.5 and 5.0 mg/dl, and stages 3 and 4 were studied (19). Median GFR was 27.5 ml/min 1.73 m². The coefficient of variation (CV) was 2% (27).

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Muscle Strength

Baseline and 12-week whole-body muscle strength was calculated as the sum of 1RM measures for all machines used for testing at baseline and 12 weeks.

Metabolic Factors

Baseline GFR was measured in 18 participants using $^{125}$I-iothalamate (Glofil; Cypros Pharmaceutical Corporation, Carlsbad, CA) with a CV of 6.3% (28). For the remaining five participants, GFR was estimated using the Modification of Diet in Renal Disease (MDRD) study equation (29). High-sensitivity C-reactive protein (CRP) was measured in a Dade Behring BN II nephelometer (Dade Behring, Deerfield, IL). IL-6 concentrations were determined by quantitative sandwich enzyme immunoassay (Quantikine High Sensitivity Enzyme-Linked Immunosorbent Assay–ELISA kit; R&D Systems, Minneapolis, MN). Insulin concentrations were measured using Linco-specific human antibodies (Linco Research, St. Louis, MO) and glucose was measured using the hexokinase enzymatic method. Insulin resistance was estimated by the Homeostasis Model Assessment (HOMA-IR) method (30). IGF-1 concentrations were determined by RIA extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA). All measures had CVs <8%.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 for Windows (SPSS, Inc., Evanston, IL). Results were considered statistically significant with a $P < 0.05$ (two-tailed). Data are reported as mean and SD for normally distributed continuous data, median and interquartile range (IQR) for non-normally distributed continuous variables, and percentages for categorical data. Skewed variables (mtDNA copy number, GFR, CRP, IL-6, HOMA-IR, and IGF-1) were log-transformed for analyses. Baseline comparisons were assessed by independent sample $t$ test for normally distributed and log-transformed continuous variables or $\chi^2$ for categorical variables. Change (week 12 − baseline) in mtDNA copy number within group was assessed by paired $t$ test. Time × group effect on mtDNA copy number was determined by repeated measures ANOVA. Pearson’s correlation analyses of both groups combined were used to determine univariate associations at baseline and after the intervention (change: week 12 − baseline) between mtDNA copy number and variables of interest.

Results

Baseline Characteristics

Detailed subject recruitment was reported previously (12). In this study, 31 participants were enrolled in the run-in period (Figure 1). There were no significant differences between groups at baseline in any variable measured (Table 1).

Baseline Muscle mtDNA Copy Number and Its Correlates

Baseline mtDNA copy number was numerically higher in the attention-control group although the difference did not reach significance, $P = 0.08$ (Table 1). Table 2 shows baseline univariate correlations between mtDNA copy number and clinical, metabolic, dietary, and muscle phenotypic (muscle fiber CSA and muscle strength) variables. There was a significant negative association between mtDNA copy number and IL-6 plasma concentrations. A trend for significant positive associations were seen between mtDNA copy number and dietary protein and energy intakes, HOMA-IR, and type I muscle fiber CSA at baseline (Table 2).

Effect of Resistance Training on Muscle mtDNA Copy Number

The distribution of mtDNA copy number changed significantly after the intervention (Figure 2). Median (IQR) mtDNA copy number at baseline and 12 weeks were 13,125 (9904) and 14,099 (10,725) in the resistance training group, respectively, compared with median values of 14,762 (8577) and 12,094 (6884), respectively, in the attention-control group ($P = 0.01$, time × group interaction). The changes in mtDNA copy number within the study group were +1306 (13,306), $P = 0.22$, in the exercise group and −3747 (15,467), $P = 0.04$, in the control group.

Predictors of the Change in Muscle mtDNA Copy Number

Table 3 shows the univariate associations between the changes in mtDNA and each variable of interest. These measures have been published elsewhere (12,13). Noteworthy are the statistically significant positive associations between the change in mtDNA and the changes observed in type I ($P = 0.01$) and type II ($P = 0.05$) muscle fiber CSA (Figure 3). Additionally, significant positive associations were observed between the change in mtDNA copy number and the changes in energy intake ($P = 0.03$) and IGF-1 concentrations ($P = 0.01$). A trend for a positive association with the change in protein intake ($P = 0.06$) was also found (Table 3).

Discussion

To our knowledge this is the first study to investigate mitochondrial biogenesis in response to resistance exercise training among individuals with CKD stages 3 and 4 (moderate-to-severe disease). We found that median mtDNA copy number
increased significantly after 12 weeks of resistance training compared with that of the attention-control participants in whom a significant reduction in the median mtDNA copy number was observed.

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (n = 23)</th>
<th>Resistance Training Group (n = 13)</th>
<th>Attention-Control Group (n = 10)</th>
<th>p a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 10</td>
<td>65 ± 9</td>
<td>64 ± 12</td>
<td>0.83</td>
</tr>
<tr>
<td>Men (n)</td>
<td>17</td>
<td>8</td>
<td>9</td>
<td>0.34</td>
</tr>
<tr>
<td>White (n)</td>
<td>20</td>
<td>11</td>
<td>9</td>
<td>0.88</td>
</tr>
<tr>
<td>Etiology renal disease (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renovascular hypertension</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.92</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>27.5 (37.0)</td>
<td>24.8 (40.1)</td>
<td>32.0 (28.4)</td>
<td>0.95</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>3.7 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>0.87</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>80.7 ± 15.1</td>
<td>84.4 ± 16.8</td>
<td>77.3 ± 13.6</td>
<td>0.14</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.1 ± 4.9</td>
<td>29.6 ± 5.9</td>
<td>26.4 ± 2.7</td>
<td>0.11</td>
</tr>
<tr>
<td>Protein intake (g/kg per day)</td>
<td>0.64 ± 0.08</td>
<td>0.64 ± 0.08</td>
<td>0.65 ± 0.08</td>
<td>0.84</td>
</tr>
<tr>
<td>Energy intake (joules/kg per day)</td>
<td>76.6 ± 28.8</td>
<td>67.6 ± 26.9</td>
<td>87.1 ± 28.4</td>
<td>0.09</td>
</tr>
<tr>
<td>mtDNA copy number</td>
<td>13713 (10618)</td>
<td>13125 (9904)</td>
<td>14762 (8577)</td>
<td>0.08</td>
</tr>
<tr>
<td>Type I muscle fiber CSA (μm²)</td>
<td>4150 ± 1550</td>
<td>3887 ± 1566</td>
<td>4578 ± 1524</td>
<td>0.33</td>
</tr>
<tr>
<td>Type II muscle fiber CSA (μm²)</td>
<td>3752 ± 1120</td>
<td>3626 ± 1216</td>
<td>3957 ± 988</td>
<td>0.52</td>
</tr>
<tr>
<td>Muscle strength (kg)</td>
<td>282 ± 109</td>
<td>298 ± 136</td>
<td>265 ± 66</td>
<td>0.45</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>6.0 (3.0)</td>
<td>6.0 (3.5)</td>
<td>6.0 (3.0)</td>
<td>0.85</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>9.3 (4.9)</td>
<td>10.5 (5.7)</td>
<td>6.9 (6.1)</td>
<td>0.07</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.1 (1.9)</td>
<td>3.7 (2.3)</td>
<td>2.9 (1.8)</td>
<td>0.84</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>112 (90)</td>
<td>110 (100)</td>
<td>122 (90)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Data are mean ± SD, except for non-normally distributed variables for which median and interquartile range are shown. Variables log-transformed for analysis: GFR, glomerular filtration rate (measured n = 18 or estimated n = 5); mtDNA, mitochondrial DNA; CSA, skeletal muscle cross-sectional area; CRP, C-reactive protein; IL-6, interleukin-6; HOMA-IR, homeostasis model assessment of insulin resistance; IGF-1, insulin-like growth factor-1.

aBaseline comparisons between groups were assessed by two independent sample tests using t test for normally distributed variables and log-transformed continuous variables or χ² for categorical variables.

Table 2. Correlates of muscle mtDNA copy number at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>P a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.28</td>
<td>0.19</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>0.05</td>
<td>0.84</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>−0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>Protein intake (g/kg per day)</td>
<td>0.37</td>
<td>0.08</td>
</tr>
<tr>
<td>Energy intake (joules/kg per day)</td>
<td>0.36</td>
<td>0.09</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>−0.51</td>
<td>0.04</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.41</td>
<td>0.06</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>0.09</td>
<td>0.68</td>
</tr>
<tr>
<td>Type I muscle fiber area (μm²)</td>
<td>0.41</td>
<td>0.07</td>
</tr>
<tr>
<td>Type II muscle fiber area (μm²)</td>
<td>0.29</td>
<td>0.21</td>
</tr>
<tr>
<td>Muscle strength (kg)</td>
<td>0.01</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Univariate Pearson’s correlation coefficients of both groups combined were run between mtDNA copy number (dependent variable) and each independent variable of interest. Variables log-transformed for analysis: mtDNA; GFR, glomerular filtration rate; CRP, C-reactive protein; IL-6, interleukin-6; HOMA-IR, homeostasis model assessment of insulin resistance; IGF-1, insulin-like growth factor-1. Twenty-three participants were included in the analysis (13 from the resistance training group and 10 from the attention-control group).

Figure 2. Box plot showing the relative difference in the distribution of log-transformed skeletal muscle mtDNA copy number between groups at baseline (open bars) and after the 12-week intervention (gray bars). Time × group comparisons of the distribution of log-transformed mtDNA copy number after the intervention was determined by repeated measures ANOVA.
number was observed. Despite the prescription of a low-protein diet throughout the intervention, the anabolic response to resistance exercise training was robust, as evidenced by improved protein utilization, muscle hypertrophy, and increased muscle strength. The mitochondrial genome changes observed were positively correlated with phenotypic changes of skeletal muscle mass measured by cross-sectional area of type I and type II fibers in the vastus lateralis muscle.

We have previously shown that the anabolic effects of resistance exercise training in this patient population resulted in increased muscle accretion (i.e., enhanced muscle protein utilization) and reverted muscle weakness (12). These observations accompanied by the main finding of the present study, namely, enhanced mitochondrial content after 12 weeks of resistance training, suggest that uremic myopathy (31) may be improved with this exercise modality (32). This is of clinical relevance given that muscle wasting in CKD has been shown to be associated with mitochondrial dysfunction (5). Muscle mitochondrial biogenesis did not uniformly increase in all participants undergoing resistance training. There were a few participants in the exercise group that showed a decline in mtDNA copy number over the study period similar to that seen for most of the participants in the attention-control group who also received the low-protein diet but did not exercise. The level of mitochondria in cells is dependent on a tight regulation between synthesis and degradation in coordination with transcription factors and signaling pathways (33). There is evidence to suggest that amino acid mixtures enhance mitochondrial biogenesis in fat and skeletal and cardiac muscle of aged mice (34) and modify gene expression (transcription and translation) in mammalian cells (35). It is possible that the reduced dietary protein intake imposed by the study may have limited mitochondrial biogenesis in some participants despite exercise intervention. More studies are needed to better understand the causes and consequences of impaired mitochondrial biogenesis.

The increase in mtDNA copy number we found in the group undergoing resistance exercise training is in accordance with previous experimental studies. For instance, when electrical stimulation was used to induce chronic contractile activity in male Sprague-Dawley rats (36), it was observed that skeletal muscle responded to chronic contractile activity by modifying the rate of mitochondrial precursor protein import into the matrix of the organelle. The increase in protein import is likely

### Table 3. Predictors of the change in muscle mtDNA copy number

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<th>$P^{a}$</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>-0.08</td>
<td>0.74</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>0.28</td>
<td>0.23</td>
</tr>
<tr>
<td>Protein intake (g/kg per day)</td>
<td>0.42</td>
<td>0.06</td>
</tr>
<tr>
<td>Energy intake (joules/kg per day)</td>
<td>0.48</td>
<td>0.03</td>
</tr>
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<td>CRP (mg/L)</td>
<td>0.07</td>
<td>0.76</td>
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<td>-0.28</td>
<td>0.18</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.19</td>
<td>0.43</td>
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<tr>
<td>IGF-1 (ng/ml)</td>
<td>0.59</td>
<td>0.01</td>
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<tr>
<td>Type I muscle fiber area ($\mu m^2)$</td>
<td>0.56</td>
<td>0.01</td>
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<tr>
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<td>0.05</td>
</tr>
<tr>
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</tbody>
</table>

$^{a}$Univariate Pearson’s correlation coefficients of both groups combined were run between the change (week 12 − baseline) in mtDNA copy number (dependent variable) after the intervention and the change in each independent variable of interest. Variables log-transformed for analysis: mtDNA; CRP, C-reactive protein; IL-6, interleukin-6; HOMA-IR, homeostasis model assessment of insulin resistance; IGF-1, insulin-like growth factor-1. Nineteen participants were included in the analysis (11 from the resistance-training group and 8 from the attention-control group).
an important adaptation in the overall process of mitochondrial biogenesis. Ultimately, mitochondrial biogenesis involves the orchestrated expression of the mitochondrial genome and the nuclear genes that encode mitochondrial proteins. The increase in mtDNA copy number with resistance exercise training in patients with stages 3 and 4 CKD that we observed in the present study was similar to that observed in young healthy muscle (37). Resistance exercise has also been shown to be an effective countermeasure for aging-associated muscle atrophy, resulting in less oxidative stress and increased mitochondrial capacity that contribute to enhanced mitochondrial function (11). Taken together, these data suggest that muscle plasticity with resistance exercise training can result in increased skeletal muscle oxidative metabolic capacity in uremic muscle.

The effect of different exercise modalities on mitochondrial biogenesis needs to be investigated further. A study of endur-

ance exercise training in patients with mitochondrial myopathy showed exercise-induced adaptive responses similar to those training on mitochondrial content, energy metabolism, and work capacity in those patients when compared with a group of healthy controls (38). However, endurance training significantly reduced the expression of the DNA repair machinery with resistance exercise training has important clinical implications for CKD patients. Our findings suggest that the myopathic changes of CKD may be mitigated or reversed with a nonpharmacologic exercise modality like resistance exercise. Further study is needed in larger cohorts of patients to validate and refine these observations.

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Disclosures
None.

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