Kidney Medicine

Resistance-Based Muscle Therapy, Frailty, and Muscle Biopsy Findings in Kidney Transplant Candidates: A Clinical Trial

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Rationale & Objective: Frailty is associated with increased morbidity and mortality in kidney transplant recipients. We hypothesized that frailty may be attributable to diminished muscle function associated with muscle morphologic changes. This trial in kidney transplant candidates tested the reversibility of frailty by specifically targeting the affected major muscle groups.

Study Design: Randomized clinical trial.

Setting & Participants: Kidney transplant candidates.

Exposure: Supervised, resistance-based muscle therapy program delivered for 1 hour, 2 times per week for 1 year.

Outcomes: Baseline, 6-month, and 12-month Short Physical Performance Battery, gait speed, grip strength, sit-to-stand in 30 s, 36-item Short Form Survey, Patient-Reported Outcomes Measurement Information System-29, and muscle biopsy light and electron microscopy and immunohistochemistry.

Analytic Approach: Paired 2-tailed *t* test, 1-way repeated measures analysis of variance.

Results: Twenty-nine participants (mean age, 55 years; female, 55%; African American, 65%) were analyzed: 23 intervention and 6 control. Exercise intervention participants had significant improvements in Short Physical Performance Battery, baseline 5.2 (95% CI, 3.6-6.7) versus 6 months, 6.9 (95% CI, 5.2-8.5; $P < 0.001$) and 12 months, 7.2 (95% CI, 5.6-8.8; $P < 0.001$); baseline hand grip, 14.3 kg (95% CI, 10.3-18.4) versus 6 months, 16.9 kg (95% CI, 13.1-20.8; $P < 0.05$) and 12 months, 17.4 kg (95% CI, 13.9-21.0; $P < 0.05$); and baseline sit-to-stand in 30 s, 8.0 (95% CI, 3.8-12.2) versus 6 months, 12.7 (95% CI, 8.2-17.1; $P < 0.001$) and 12 months, 16.2 (95% CI, 10.7-21.7; $P < 0.001$). The exercise group 12-month muscle fiber diameter increased by 18.6 μm (95% CI, 8.4-28.5; $P = 0.003$). Expression of immunohistology markers of muscle atrophy decreased significantly. The mean difference in immunohistology score of mitochondrial oxidative function improved for cytochrome c oxidase complex IV, 1.00 (95% CI, 0.71-1.29; P < 0.001) and ATP5I increased by 0.74 (95% CI, 0.49-0.99; $P < 0.001$). Increased mitochondrial count did not achieve statistical significance ($P = 0.096$). Controls showed no improvement in either physical performance or histology.

Limitations: Significant under-enrollment in the control group required a paired *t* test analysis of experimental participants.

Conclusions: One year of muscle rehabilitation therapy resulted in significant improvements in physical performance metrics accompanied by significant improvements in muscle morphology.

Complete author and article information provided before references.

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Frailty, defined as a state of loss of physiological reserve related to senescent changes in organ systems, is a major determinant of adverse outcomes after major surgery. The system proposed by Fried et al[1] and modified by Bandeen-Roche et al[2] defines frailty using 5 determinants: weight loss, exhaustion, low physical activity, slowness, and weakness. In surgical patients, this is characterized by a reduced capacity to respond to surgical stress from both disease-related and age-related factors.[3] Frailty is associated with adverse outcomes following major surgery, including increased mortality,[4] morbidity,[5,6] wound infection,[7,8] non-home discharge,[9] hospital readmission,[10] and increased resource utilization.[11-13]

There is a strong association between kidney failure with replacement therapy (KFRT) and low activity levels and loss of strength and mobility with progressive frailty, and KFRT patients with low activity levels have higher rates of disability and mortality.[14] Several randomized trials have sought to reverse frailty with exercise interventions during or between dialysis sessions.[14-21] Importantly, most have not demonstrated significant benefit. An exception is a randomized study using a home-based walking intervention that was supervised by dialysis unit staff. The exercise group had statistically significant improvement in the 6-minute walking test and the 5-time sit-to-stand test, and this was accompanied by improvements in cognitive function and quality of social interaction.[16]

With the recognition that frailty is associated with poorer transplant outcome, there is increasing interest in exercise training as a form of prehabilitation in KFRT patients and kidney transplant recipients.[22] A pilot study of 24 patients at Johns Hopkins who underwent weekly

PLAIN LANGUAGE SUMMARY

Dialysis patients often develop muscle weakness and frailty that adversely impacts candidacy for and the results of kidney transplantation. Muscle rehabilitative therapy was tested in a randomized, controlled clinical trial in potential kidney transplant recipients. After 1 year of therapy, patients who exercised had marked improvements in muscle strength, walking speed, and self-assessed improvements in several domains of health and wellness; those outcomes did not improve in control patients. Muscle biopsies performed in exercise patients showed enlarged muscle fibers and evidence of improved muscle metabolism along with markedly decreased biological markers of muscle atrophy. Muscle rehabilitative therapy dramatically improved perceptions of health, objective measurements of strength, and the microscopic appearance of muscle tissue in kidney transplant candidates.

physical therapy sessions at an outpatient center had promising results. After 2 months of physical therapy, participants had a 64% improvement in physical activity, and they reported a high level of patient satisfaction.[23]

Improvement in frailty indices with exercise is likely rooted in reversal of pathologic changes in muscle tissue. Indeed, skeletal muscle loss can occur in several settings, including disuse, medication-related, cancer cachexia, aging, diabetes, and cardiovascular disease.[24] The University of Illinois at Chicago has designed a muscle rehabilitative therapy program based on enhancing the subject's cognitive connection with individual muscle groups undergoing training. Herein, we report the results of a 1-year pilot study of muscle rehabilitative therapy on physical function and patient-reported outcomes in 29 kidney transplant candidates. Our results demonstrate objective improvement in primary physical functioning endpoints along with marked improvement in self-reported levels of physical function with dramatic changes in muscle structure and function.

METHODS

Study Design

This pilot study evaluated the effects of a 12-month muscle rehabilitation therapy program, in addition to standard care, on pretransplant kidney failure patients undergoing evaluation for kidney transplantation. The study was approved by the Institutional Review Board (Protocol #2017-0784), ClinicalTrials.gov ID: NCT06374537.

Inclusion, Exclusion Criteria, and Randomization

Candidates were kidney transplant candidates who were on hemo- or peritoneal dialysis or had a glomerular filtration rate <20 mL/min. Participants were excluded if they were aged <18 years, had cardiac or pulmonary disease contraindicating exercise training, or were unable to cognitively cooperate. Consenting candidates had a planned randomization in a 1:2 ratio of control to intervention. The study site was the Integrative Physiology Laboratory at the University of Illinois at Chicago for 3 testing visits at baseline, 6 months, and 12 months.

Intervention

Muscle Rehabilitation

All participants underwent a personalized, 1-hour, 1-on-1 rehabilitative muscle therapy session, 2 days a week, for 12 months. The sessions consisted of a low-impact, resistance-based rehabilitation regime. The main aim of the intervention was to increase muscle strength and functionality by focusing on isolating each targeted muscle without increasing the patient's fatigue or pain level. Targeted muscle groups were shins (dorsiflexion), calves (plantarflexion), hamstring, quadriceps, deltoids, biceps, triceps, latissimus dorsi, gluteus, and rectus abdominis. All sessions were supervised by trained research personnel. Control group participants continued to receive standard of medical care management for end-stage kidney disease, as directed by their health care team.

Variables Measured

During each visit, participants were assessed for the following variables. The primary outcome was the Short Physical Performance Battery (SPPB).[24,25] The SPPB test requires assessment of the walking speed, a balance test, and a sit-to-stand test. Gait speed was determined by asking participants to walk 2.44 m as quickly as they could. Patients unable to walk (ie, wheelchair bound) were assigned a gait speed of 0.01 m/s. The balance test included standing in 3 different positions for 10 s each, tandem, semi-tandem, and side-by-side. The Timed Seated Chair Stand was performed by asking participants to stand up and sit down without using their arms for a total of 5 times. The time was recorded in seconds. Patients unable to perform this test were assigned a chair stand score of 32 s, the 99th percentile value among patients who were able to perform this test. Scoring was based on a scale of 0-12, with lower numbers representing worse performance and greater frailty.

Grip strength was measured in kilograms for each subject's dominant hand using a hand dynamometer (Jamar Hydraulic Hand Dynamometer, model 5030J1).[26] Contractions were performed in the seated position with the dominant arm at a 90° angle. The grip strength was recorded as an average of 3 attempts.

The 36-item Short Form Survey[27] was administered to assess 8 domains: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue, and general health perceptions. Scoring is on a scale of 1-100. A high score defines a more favorable health state.

The Patient-Reported Outcomes Measurement Information System (PROMIS)-29 global health survey was administered to all patients. The PROMIS-29 short form contains items from PROMIS domains (depression, anxiety, physical function, pain interference, fatigue, sleep disturbance, and ability to participate in social roles and activities) along with a single item on pain intensity.[28]

Muscle Biopsy

Subjects were separately consented for an optional biopsy of the forearm flexor muscle at baseline and 12 months. This biopsy was performed to measure muscle diameter; mitochondrial number; protein markers of muscle degradation, Atrogin-1, and MuRF-1; inflammation markers, NF-κB, Phospho-NF-κB, and mitochondrial markers, cytochrome c oxidase complex IV (COX IV) and ATP5I.The muscle biopsy was performed under local anesthesia by the study surgeon, JA, in the ambulatory operating room at the University of Illinois, Chicago. An approximately 600-mg tissue sample was fixed on a cord board to retain the resting muscle length. Images of paraffin-fixed, hematoxylin and eosin-stained muscle were captured at the same magnification (scale bar, 50 μm), and the muscle diameter was calculated from 3 separate measurements, keeping the scale bar as a reference.

Antibodies and Reagents

Antibodies against human Atrogin-1 (dilution 1:100), MuRF-1 (dilution 1:200), and COX IV (dilution: 1:1,000) were purchased from Abcam. Phospho-NF-κB antibody (1:100) was purchased from Santa Cruz Biotechnology. Anti-human RelA/NF-κB p65 antibody (dilution: 1:2,000) was procured from Novus Biologicals. Anti-human ATP5I antibody (dilution 1:500) was purchased from Invitrogen. Secondary antibodies, horseradish peroxidase-conjugated goat anti-rabbit IgG polymer, horseradish peroxidase-conjugated horse anti-mouse IgG polymer, and 3, 3′-diaminobenzidine were purchased from Vector Laboratories.

Immunohistochemistry Staining

Human muscle tissues were fixed in 4% formaldehyde and incubated in 70% ethanol for 48 hours. As described previously, immunohistochemistry (IHC) staining of paraffin-embedded muscle sections was conducted. Briefly, muscle tissue sections were rehydrated, followed by heat-induced antigen retrieval with sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) in a decloaking chamber (Biocare Medical). After blocking, tissue sections were incubated with primary antibodies overnight at 4°C and in secondary antibodies for 3 hours at room temperature. Images were captured using a fluorescence microscope (Nikon). Muscle fiber diameter was measured using hematoxylin and eosin images following the previously described method. IHC images were captured using bright field microscopy (Nikon). IHC scoring criteria were as follows: 0: no staining, 1+: weakly positive, 2+: moderate positive, and 3+: strongly positive.

Transmission Electron Microscopy

Muscles were analyzed by transmission electron microscopy (TEM) (University of Illinois at Chicago electron microscopy core) to determine the impact of exercise on the mitochondrial numbers. Briefly, the muscle tissues were fixed overnight in 2.5% buffered glutaraldehyde. The muscle tissues were then postfixed in osmium tetroxide (1% buffered) for 1 hour, followed by gradual dehydration. Ultrathin muscle sections were prepared for TEM imaging. Mitochondria were counted and compared in electron micrographs (magnification 25,000×) of patient muscle samples from visit-1 and visit-2.

Data Analysis

All data analyses were performed using IBM SPSS Statistics version 29.0.2.0. We provide descriptive statistics as mean ± standard deviation (SD) and/or mean difference with upper and lower 95% confidence interval (CI) in parentheses, unless otherwise noted (eg, median or percentage). The data analyses were performed separately per group (ie, intervention and control), as the greatly unbalanced sample sizes per group created problems for performing mixed-factor analysis of variance consisting of within (time) and between (group) participant factors; the imbalance and small sample sizes further created problems for applying a linear mixed model. The missing data for 6- or 12-month postintervention assessments were replaced using last value carried forward for a modified intention-to-treat analysis. We replaced missing 6-month data with baseline values and missing 12-month data with 6-month values. The performance and patient-reported outcome measure data were analyzed using a general linear model with repeated measurements over time (baseline, 6 months, 12 months), and follow-up analyses using pairwise comparison were performed separately per group (ie, intervention and control). $P \leq 0.05$ indicated statistical significance for all analyses. The histology data were analyzed using paired-samples t tests performed separately per group (ie, intervention and control), and changes are reported as the mean difference with 95% CI.

RESULTS

Sample Characteristics

The Consolidated Standards of Reporting Trials diagram in Figure 1 illustrates the flow of participants through the study. Enrollment began on May 22, 2019. With enrollment ongoing, the last patient analyzed was enrolled on March 20, 2024. We screened 204 patients, and of 117 who expressed interest, 48 participants meeting the eligibility criteria were consented and randomized. The intended randomization ratio of 1:2 control to intervention resulted in only 7 controls and 41 in the

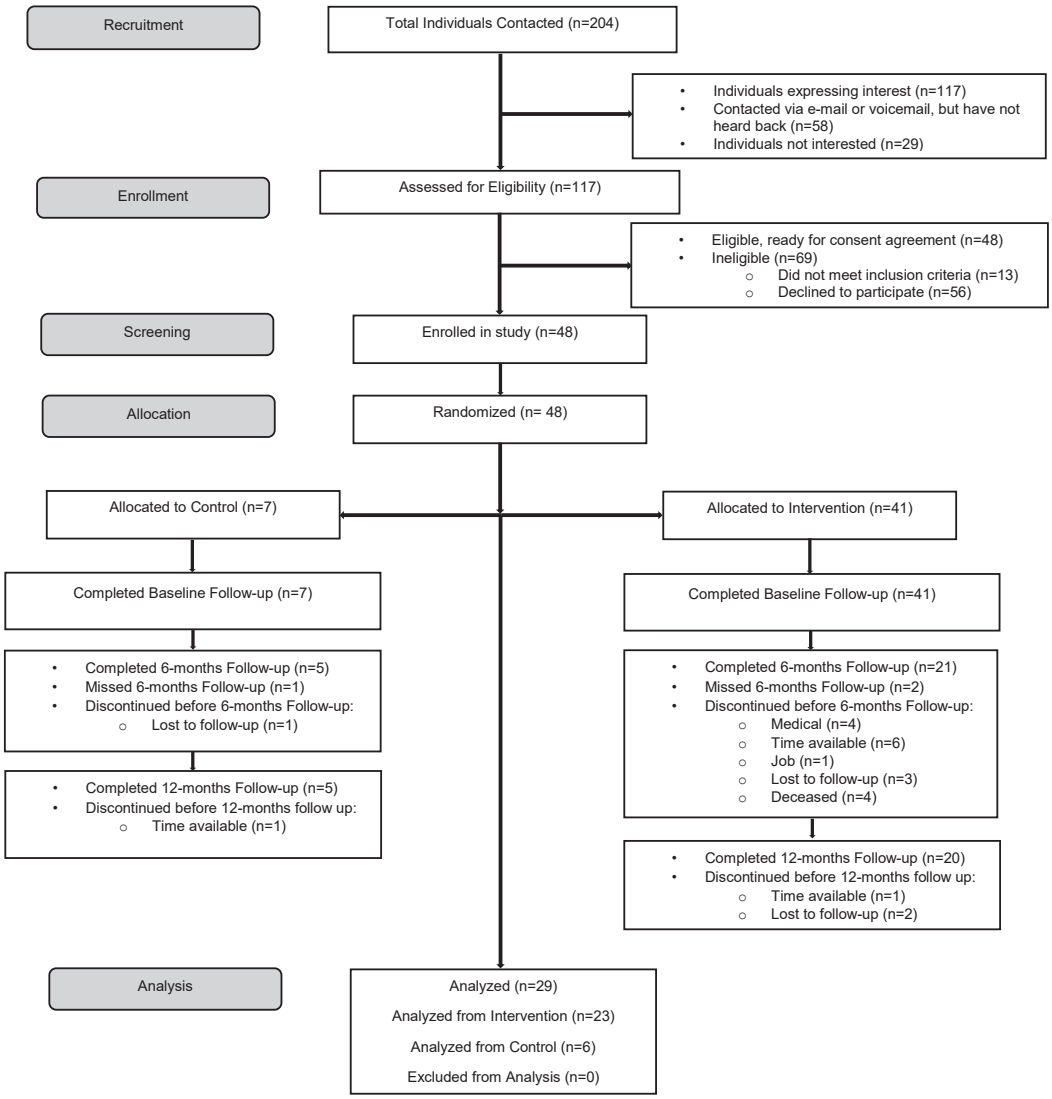

Figure 1. Consolidated Standards of Reporting Trials diagram: 204 patients were contacted, 117 were assessed for eligibility, 48 were enrolled and randomized; 23 intervention and 6 control patients completed the study and were the basis for analysis. One control patient did not have 6-month follow-up data due to COVID but did complete the 12-month follow-up. Six intervention patients had missing values at baseline, 6, or 12 months. Our intent-to-treat analysis used the last value carried forward method.

interventional arm, a 1:5.8 ratio. A detailed investigation by the 2 senior authors (STB, RWM) did not identify a procedural error leading to the imbalance in enrollment. The results of this investigation are detailed in Supplement Item S1.

Of the 41 participants in the intervention arm, 21 completed 6 months and 20 completed 12 months of the exercise program. Between 0 and 6 months, the intervention group participants completed a mean of 35 of 48 (73%) scheduled exercise sessions; from 6 to 12 months, adherence to scheduled exercise sessions declined to a mean of 26 of 48 (54%) scheduled sessions. Of the 23 who dropped out of the study before 12 months, 20 (87%) were non-White minorities, 14 African American, and 6 Hispanic. The reasons for discontinuing included insufficient time to complete the study (8), lost to follow-up (6), deceased (4), medical contraindication (4), and full-time employment (1). The characteristics of the 23 participants completing the study are provided in Table 1. Additional descriptive statistics for the subjects are provided in Table S1.

Patient Subjective Scores

Regarding the 36-item Short Form Survey subscales there were significant main effects of time for the intervention condition, but not control, on 7 of 8 subscales. The values generally improved from baseline at the 6- and 12-month assessments for the intervention condition. Regarding PROMIS composites, there were significant main effects of time for the intervention condition, but not control, on

Table 1. Descriptive and Clinical Characteristics of the Participants in the Interventional and Control Conditions

Variable	Intervention (N = 23)	Control (N = 6)
Age, y	55.3 (12.2)	52.7 (14.0)
Female, n (%)	13 (56.5%)	50 (3%)
African American, n (%)	16 (69.6%)	50 (3%)
Height, cm	170.5 (9.9)	165.8 (8.9)
Weight, kg	99.5 (23.4)	83.7 (35)
Using assistive device, n (%)	14 (60.9%)	2 (33.3%)
Median dialysis duration, mo (25th, 75th percentile)	26 (6, 72)	34 (21, 76.5)
Systolic blood pressure, mm Hg	132.6 (21.9)	158 (33)
Diastolic blood pressure, mm Hg	77.3 (13.5)	89 (7.9)

Note: Data shown as mean (SD) unless otherwise specified.

physical, but not mental, health. The value for physical health improved from baseline at the 6-and 12-month assessments in the intervention group. Regarding PROMIS-29 subscales, there were significant main effects of time for the intervention condition, but not control, on 4 of 7 subscales, namely physical function, depression, fatigue, and satisfaction (Table 2).

Physical Performance

The physical performance testing results are shown in Table 3. There was a statistically significant improvement in the SPPB (baseline, 5.2 [95% CI, 3.6-6.7] versus 6.9 [95% CI, 5.2-8.5] at 6 months [$P < 0.001$] and 7.2 [95% CI, 5.6-8.8] at 12 months [$P < 0.001$]); hand grip strength (baseline, 14.3 kg [95% CI, 10.3-18.4] versus 16.9 kg [95% CI, 13.1-20.8] at 6 months [$P < 0.05$] and 17.4 kg [95% CI, 13.9-21.0] at 12 months [$P < 0.05$]); and 30-s sit-to-stand (baseline, 8.0 [95% CI, 3.8-12.2] versus 6 months, 12.7 [95% CI, 8.2-17.1; $P < 0.001$] and 12 months, 16.2 (95% CI, 10.7-21.7; $P < 0.001$]). The 2.44-m walking speed improved but did not reach statistical significance ($P = 0.06$).

Muscle Biopsy Data

Exercise Increases Muscle Fiber Diameter

Muscle fiber diameter increased significantly between the baseline biopsy (59.8 ± 18.3 μm) (mean ± SD) and the biopsy performed at 12 months (78.3 ± 24.1 μm). The mean difference increase, 18.6 μm (95% CI, 8.4-28.5), in muscle fiber size was highly significant ($P = 0.003$) (Fig 2A). None of the intervention participants' muscle fiber diameters decreased during the study period. The growth of muscle fiber size at 12 months indicated a positive impact of exercise on the muscle health of transplant candidates. The control biopsies ($n = 3$) are shown in Figure S1. There was no improvement in the control muscle fiber size, IHC, or mitochondria number; however, given the small sample size, $n = 3$, the significance of this data is diminished.

Exercise Decreases Atrophy Markers in End-Stage Renal Disease Patients' Muscle

The IHC analyses of Atrogin-1 and MuRF-1 protein expression in visit-1 and visit-2 muscle samples showed a consistent decrease from visit-1 at baseline to visit-2 at 12 months (Fig 2B). The Atrogin-1 baseline of 2.22 ± 0.37 (mean ± SD) decreased to 0.89 ± 0.44; the mean difference from baseline to 12 months, 1.33 (95% CI, 0.79-1.87; $P = 0.001$), was highly significant. The MuRF-1 score decreased from 1.96 ± 0.45 at baseline to 0.67 ± 0.41 at 12 months. The mean difference, 1.30 (95% CI, 0.81-1.79; $P < 0.001$), was highly statistically significant. The decreased protein expression of Atrogin-1 and MuRF-1 at 12 months indicated that exercise significantly decreases muscle atrophy through pathways leading to muscle degeneration.

Exercise Decreases NF-κB Pathway Protein Expression in End-Stage Renal Disease Patients' Muscles

Both NF-κB and Phospho-NF-κB staining decreased significantly from baseline to 12 months (Fig 2C). NF-κB expression decreased from 2.22 ± 0.53 at baseline to 1.07 ± 0.49 at 12 months; the mean difference, 1.14 (95% CI, 0.61-1.67; $P = 0.003$), was highly statistically significant. Phospho-NF-κB expression decreased from 2.07 ± 0.57 at baseline to 1.04 ± 0.61 at 12 months. The mean difference, 1.04 (95% CI, 0.40-1.68; $P = 0.013$), was statistically significant. These results indicate that exercise might be downregulating the NF-κB pathway due to decreased inflammatory signaling.

Exercise Increases Mitochondrial Function

rBetween visit-1 and visit-2, COX IV expression increased significantly from 1.44 ± 0.37 to 2.44 ± 0.33. The mean difference between baseline and 12 months, 1.00 (95% CI, 0.71-1.29; $P < 0.001$), was highly statistically significant (Fig 3A). Mitochondrial count (baseline, 9.0 ± 3.2; 12 months, 11.8 ± 5.4) increased; however, the mean difference, 2.85 (95% CI, −0.10 to 5.80; $P = 0.096$), did not achieve statistical significance (Fig 3B). ATP5I protein expression increased from baseline to 12 months from 1.74 ± 0.46 to 2.44 ± 0.33. The mean difference, 0.74 (95% CI, 0.49-0.99; $P < 0.001$), was highly statistically significant. These results suggest that exercise plays an important role in regulating mitochondrial function (Fig 3C).

DISCUSSION

Our results demonstrate that rehabilitative muscle therapy results in significant improvement in physical functioning, strength, and several self-assessed wellness domains including physical health, emotional well-being, and social functioning. Given the connection between emotional well-being, social functioning, cognition, and executive functioning, additional studies that directly assess the effect of muscle therapy on

Table 2. Changes in Short-Form 36 and PROMIS-10 and -29 Subscale Scores Over the 12-Month Period for Intervention and Control Conditions

	Intervention (N = 23)				Control (N = 6)			
Measure/Subscale	**0 mo**	**6 mo**	**12 mo**	***P***	**0 mo**	**6 mo**	**12 mo**	***P***
Short Form-36 Subscales (T-scores 0-100)								
Physical function	35.0 (22.5, 47.5)	49.8 (38.5, 61.0)[a]	52.2 (40.6, 63.8)[a]	<0.001	47.5 (10.3, 84.8)	63.3 (24.9, 101.9)	57.5 (27.1, 87.9)	0.35
Role limitation physical	18.5 (6.2, 30.8)	37.0 (20.7, 53.2)[a]	46.7 (32.8, 60.7)[a]	<0.01	33.3 (−2.5, 69.2)	54.2 (0.6, 107.7)	41.7 (2.2, 81.2)	0.55
Role limitation emotional	62.3 (44.8, 79.9)	73.9 (58.3, 89.5)[a]	68.1 (50.5, 85.7)[a]	0.49	66.7 (22.4, 111.0)	72.2 (25.7, 118.7)	44.4 (8.3, 80.5)	0.36
Energy/fatigue	45.7 (35.4, 56)	56.7 (48.3, 65.2)[a]	52.2 (41.3, 63.0)	<0.05	47.5 (24.0, 71.0)	50.0 (28.2, 71.8)	45.0 (23.8, 66.2)	0.80
Emotional wellbeing	69.9 (60.9, 79.0)	71.5 (64.1, 79.0)	76.0 (69.0, 83.0)[a]	<0.05	78.7 (62.4, 95.0)	78.7 (67.5, 89.8)	72.7 (52.4, 93.0)	0.34
Social function	58.2 (44.3, 72.0)	74.5 (61.4, 87.5)[a]	70.1 (57.1, 83.1)	<0.05	70.8 (29.6, 112.0)	81.3 (54.0, 108.5)	58.3 (16.3, 100.4)	0.42
Pain	50.1 (39.9, 60.3)	71.1 (60.5, 81.6)[a]	64.8 (52.7, 76.9)[a]	<0.001	60.8 (42.4, 79.3)	78.8 (66.3, 91.3)	67.1 (37.2, 97.0)	0.10
General health	39.1 (32.2, 46.0)	47.8 (40.3, 55.4)[a]	44.6 (35.3, 53.9)	<0.05	54.2 (30.4, 78.0)	59.2 (45.3, 73.0)	53.3 (30.7, 76.0)	0.80
PROMIS-10 Composites (T-scores)								
Physical health	37.7 (34.7, 40.8)	42.7 (39.9, 45.6)[a]	42.6 (39.2, 46.0)[a]	<0.001	42.1 (34.1, 50.2)	45.2 (35.2, 55.2)	42.6 (30.3, 55.0)	0.71
Mental health	45.3 (41.1, 49.5)	46.6 (42.4, 50.8)	47.6 (43.4, 51.8)	0.14	47.3 (36.9, 57.7)	46.9 (40.3, 53.4)	44.1 (34.2, 53.9)	0.47
PROMIS-29 Subscales (T-scores 0-100)								
Physical function	35.3 (31.6, 39.1)	38.7 (34.6, 42.7)[a]	38.3 (34.6, 42.1)[a]	<0.05	42.6 (27.7, 57.5)	42.2 (27.2, 57.1)	43.7 (31.9, 55.5)	0.86
Anxiety	53.5 (48.6, 58.4)	51.7 (47.7, 55.6)[a]	50.5 (46.3, 54.7)	0.21	54.0 (41.0, 67.0)	50.1 (40.6, 59.7)	53.9 (45.1, 62.7)	0.66
Depression	53.1 (48.2, 58.0)	50.9 (46.0, 55.7)	49.3 (44.3, 54.3)[a]	<0.05	54.0 (45.1, 62.9)	50.6 (41.9, 59.3)	50.1 (38.6, 61.7)	0.62
Fatigue	55.9 (51.8, 60.0)	51.4 (46.9, 56.0)[a]	53.1 (49.0, 57.2)	<0.05	54.2 (43.8, 64.5)	48.2 (43.2, 53.2)	47.6 (41.1, 54.0)	0.12
Sleep disturbance	52.0 (48.2, 55.8)	51.4 (47.1, 55.6)	53.5 (48.7, 58.3)	0.48	54.9 (44.8, 65.0)	49.2 (35.4, 63.1)	48.6 (35.9, 63.2)	0.48
Satisfaction	42.7 (38.6, 46.8)	46.6 (42.1, 51.0)[a]	48.5 (44.7, 52.2)[a]	<0.01	47.3 (32.6, 62.1)	48.0 (37.9, 58.2)	46.5 (35.1, 58.0)	0.96
Pain interference	56.1 (51.5, 60.8)	51.9 (47.8, 56.0)	55.8 (51.2, 60.4)	0.08	52.9 (45.9, 59.9)	50.7 (42.5, 58.8)	52.2 (42.7, 61.7)	0.74

Note: The *P* value column reflects the statistical significance of the overall time main effect from the general linear model using a 1-way, repeated measures analysis of variance performed separately per group.
Abbreviations: PROMIS, Patient-Reported Outcomes Measurement Information System; SF-36, 36-item Short Form Survey.
[a]Denotes a significant difference in the mean value for either 6 or 12 months compared with the baseline mean value based on follow-up analyses using pairwise comparisons performed separately per group. The descriptive data are reported as mean (95% confidence interval).

Table 3. Changes in Physical Performance Outcomes Over 6- and 12-Month Periods for the Intervention and Control Conditions

	Intervention				Control			
Outcome	Baseline	6 mo	12 mo	*P*	Baseline	6 mo	12 mo	*P*
SPPB (0-12)	5.2 (3.6, 6.7)	6.9 (5.2, 8.5)[a]	7.2 (5.6, 8.8)[a]	<0.001	7.2 (2.9, 11.4)	7.2 (3.4, 10.9)	6.5 (2.5, 10.5)	0.56
2.44-meter walking speed, s	5.6 (4.2, 7.1)	4.4 (3.3, 5.5)[a]	4.4 (3.0, 5.8)	0.06	3.6 (2.4, 4.8)	4.1 (2.6, 5.6)	4.1 (1.8, 6.5)	0.64
Handgrip strength, kg	14.3 (10.3, 18.4)	16.9 (13.1, 20.8)[a]	17.4 (13.9, 21.0)[a]	<0.05	15.9 (6.5, 25.4)	15.3 (6.7, 23.8)	14.2 (7.2, 21.2)	0.75
30-s sit-to-stand, No.	8.0 (3.8, 12.2)	12.7 (8.2, 17.1)[a]	16.2 (10.7, 21.7)[a,b]	<0.001	21.8 (7.0, 36.5)	20.0 (8.9, 31.1)	21.8 (8.6, 34.9)	0.91

Note: The *P* value column reflects the statistical significance of the overall time main effect from the general linear model using a 1-way, repeated measures analysis of variance performed separately per group. Descriptive data are reported as mean (95% confidence interval).

Abbreviation: SPPB, Short Physical Performance Battery.

[a]Denotes a significant difference in the mean value for either 6 or 12 months compared with the baseline mean value based on follow-up analyses using pairwise comparisons performed separately per group.

[b]Denotes a significant difference in the mean value for 12 months compared with the value from 6-month based on follow-up analyses using pairwise comparisons performed separately per group.

objective measures of cognitive performance in the pretransplant population are warranted. Our study was not structured to determine whether muscle therapy can change the patient's candidacy for transplantation from exclusion due to frailty to inclusion after intervention. Thus, although significant improvement in objective physical performance was observed, enrollment was not limited to patients who had been deemed unacceptable candidates based on frailty.

The high study drop-out rate is noteworthy, particularly among minority patients. Of the 21 study patients who enrolled and did not complete the study, 20 of 23 (87%) were either African American or Hispanic. As noted, the incidence of KFRT among African Americans is 2.4-2.9 times that in non-Hispanic Whites, and the incidence of KFRT in Hispanics is 1.5 times. The high drop-out rate among minority enrollees further compounds the preexisting disparity in KFRT incidence. Future intervention studies for frail potential transplant recipients should adopt strategies to retain minority enrollees through the conclusion of the clinical trial. Importantly, the group from Calabria, Italy used a patient navigator role to assure adherence to the exercise program. We believe that culturally competent, same-language navigators will be critically important to retain minority patients in exercise therapy programs.

This study is unique in that it is the first study of a frailty intervention in pre-transplant patients that included muscle biopsy data. In a study of moderate to severe chronic kidney disease Castaneda et al found that 12 weeks of resistance training led to improvement in strength and an increase in Type I and Type II muscle fiber cross sectional area.[29] Castaneda et al identified the "malnutrition-inflammatory complex" in these patients as their study showed that the high levels of the proinflammatory cytokine IL-6 observed in the attention controls were ameliorated in the intervention arm. Our study extends these findings because we found that not only are strength and muscle fiber size increased as noted by Castaneda et al, but we also observed significant decreases in expression of multiple proteins associated with muscle atrophy and evidence of increased protein synthesis associated with mitochondrial ATP production.

The mechanisms of muscle catabolism and anabolism are well-established in the muscle physiology literature. The muscle-specific E3 ubiquitin ligases, Atrogin-1 and muscle RING finger 1 (MuRF1), are upregulated during muscle degeneration/loss.[30-33] MuRF1 and muscle atrophy F-box (MAFbx)/Atrogin-1 are 2 muscle-specific E3 ubiquitin ligases that are increased in skeletal muscle under atrophy-inducing conditions. Therefore, MuRF1 and Atrogin-1 are known as markers of muscle atrophy. The NF-κB pathway has been reported to induce muscle protein loss via the E2 ubiquitin carrier protein, UbcH2/E220.[34] Many transcription factors are activated/overexpressed during muscle atrophy.[34] NF-κB, STAT3, and

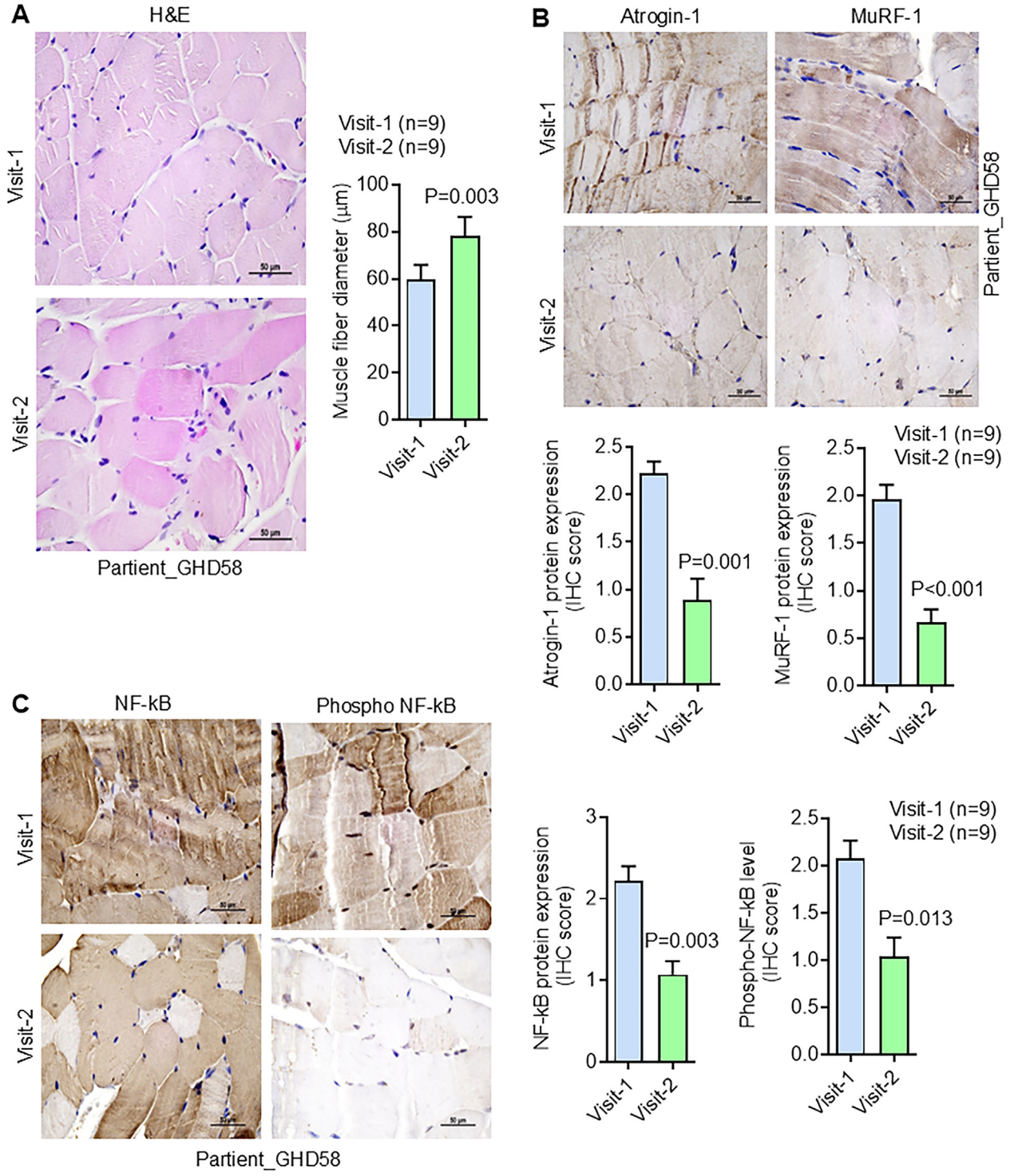

Figure 2. (A) Representative bright field images of hematoxylin and eosin (H&E) staining of patient muscle samples for visit-1 and visit-2. Patient GHD58 is shown. The scale bar is 50 µm. Measurement of muscle fiber diameter for visit-1 vs visit-2 (n = 9), paired samples *t* test, $P = 0.003$. Immunohistochemistry (IHC) staining and IHC score for (B) Atrogin-1, $P = 0.001$ and MuRF-1, $P < 0.001$; (C) NF-κB, $P = 0.003$ and Phospho-NF-κB, $P = 0.013$.

FOXO3A are either overexpressed or activated in atrophic muscles.[35,36] Data available from patients with muscle atrophy/cachexia showed upregulation in circulating TNFα, IL-1β, IL-6, and IFNγ levels and acute phase proteins.[37] These proinflammatory cytokines and acute phase proteins activate NF-κB and other signaling pathways. Activation of NF-κB signaling regulates downstream pathways that control muscle catabolism.

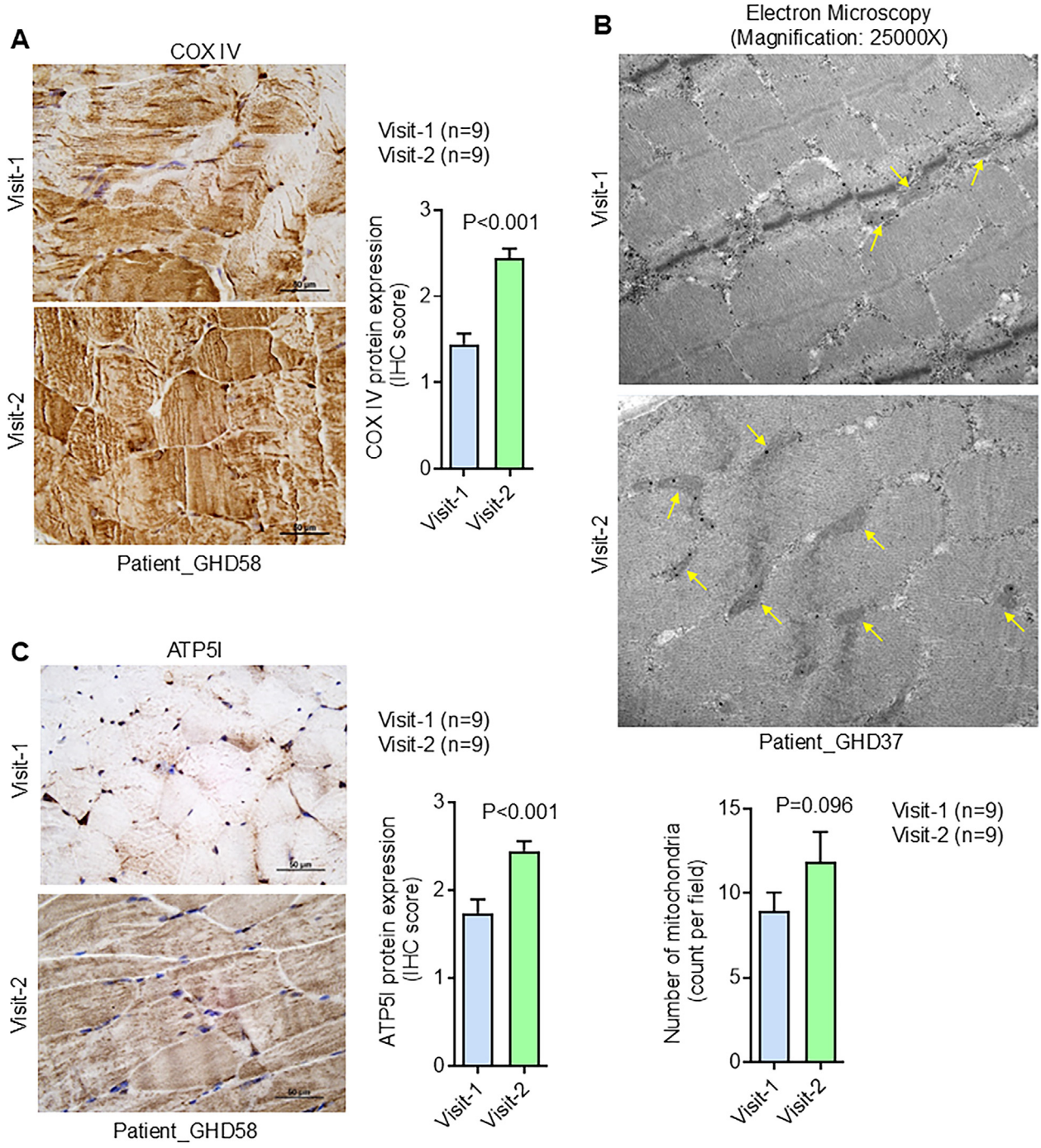

Figure 3. (A) Representative immunohistochemistry (IHC) images and quantification of COX IV expression in visit-1 and visit-2 muscle samples from patient GHD58. Scale bar is 50 μm. IHC scoring criteria: 0: no staining, 1+: weakly positive, 2+: moderate positive, and 3+: strongly positive. Data are presented as mean ± SD. Visit-1 vs visit-2, $P < 0.001$, paired samples *t* test. (B) Representative electron micrographs showing mitochondria (yellow arrows) in visit-1 and visit-2 muscle samples from patient GHD37. Magnification: 25,000×. Data are presented as mean ± SD. Visit-1 vs visit-2, $P = 0.096$, *t* test. (C) Representative IHC images and quantification of ATP5I in visit-1 and visit-2 muscle samples from patient GHD58. Scale bar is 50 μm. IHC scoring criteria: 0: no staining, 1+: weakly positive, 2+: moderate positive, and 3+: strongly positive. Data are presented as mean ± SD. Visit-1 vs Visit-2, $P < 0.001$, paired samples *t* test.

Mitochondria are the primary source of energy supply for skeletal muscle,[38] and their dysfunction is associated with skeletal muscle atrophy. Mitochondria have also been reported to play an important role in skeletal muscle remodeling.[39] COX IV is a marker of mitochondria, and its expression is indicative of mitochondrial oxidative capacity.[40] The protein, ATP5I, regulates ATP synthesis in the mitochondria.[41,42] For example, ATP5I is implicated in

mitigating high glucose-induced myocardial injury.[43] In agreement with this, our results suggest that ATP5I might be involved in improving muscle health in KFRT patients post exercise. Our data demonstrated a strong trend away from muscle atrophy markers to markers of muscle growth after 1 year of therapy.

The highly favorable results in this pilot study are encouraging and justify a clinical trial examining 2 pivotal questions. First, can interventions that include exercise therapy and prehabilitation favorably alter patients' clinical status from inactive to active transplant candidacy? Similarly, do patients who were previously frail and whose physical functioning consequently improves after intervention subsequently have a positive outcome after kidney transplantation? Although it is logical that resolution of frailty predicts a favorable clinical outcome, it is possible other factors play a role at a more fundamental level. Based on the immunochemistry evidence presented here, we hypothesize that biochemical markers of frailty may be discernable and validated in this target population. Biochemical markers of frailty will assist with transplant patient selection and identify those that needed additional pretransplant intervention. A longitudinal clinical trial should assess whether resolution of frailty carries forward into a positive clinical result with kidney transplantation. For this reason, because the resources required to apply exercise therapy to pretransplant patients are substantial, a controlled clinical trial designed to test resistance exercise therapy on transplant outcome is needed. A second critical question is whether this muscle rehabilitation therapy program can be easily implemented elsewhere. The equipment needed is widely available in most health systems that perform transplantation. The training needed for physical therapists is minimal because the exercises are part of physical therapy training. The details of resistance-based muscle training can be taught to others with demonstration videos. An example of the muscle therapy in shown in Supplement Item S2.The methodology for scoring patient performance can be shared as well. In short, wider implementation of this method is feasible. Our future studies will include facilitators and researchers experienced with community engagement and development.

In conclusion, our pilot study demonstrated a significant increase in multiple performance and self-report metrics of physical function in pretransplant patients with muscle rehabilitation therapy. Successful application of prehabilitation to expand candidacy for kidney transplantation to those with documented frailty will have a major impact in several ways. First, the opportunity to experience an improved quality of life after transplantation can be expanded to a greater fraction of potential recipients. Second, the national cost burden of KFRT care will be reduced by increasing the number of successfully transplanted patients. There is a significant disparity in the waiting time for transplantation for minority transplant recipients.[44] Long wait times are associated with progressive frailty and potential wait-list inactivation related to progressive frailty. An effective prehabilitation program would have important implications for addressing the racial disparity in access to kidney transplantation.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Figure S1: Control figure.

Item S1: Ad Hoc Analysis of the Randomization and Control Arm Data.

Item S2: Video of GH Therapy.

Table S1: Intervention and Control Patients' Exercise Compliance, Co-morbid Conditions, Transplant Status, Creatinine and Creatinine Clearance (GFR) at Six and Twelve Months, and Availability of Histologic Data.

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