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Effects of treadmill running in a rat model of chronic kidney disease

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ABSTRACT

Chronic kidney disease (CKD) progression results in musculoskeletal dysfunction that is associated with a higher likelihood of hospitalization and is predictive of hospitalizations and mortality. Despite this, there is a lack of effective interventions to treat the musculoskeletal dysfunction. We studied treadmill running as an intervention to improve musculoskeletal health in a translational rat model that has slowly progressive CKD. CKD rats were subjected to treadmill exercise or no treadmill exercise for 10 weeks (n = 8 each group). Animals ran for 60 min, 5 times per week starting at a speed of 8 m/min and ending at 18 m/min (1 m/min increase/week). Treadmill training had no effect on muscle strength (assessed as maximally stimulated torque), half-relaxation time (time from peak torque to 50%) or muscle cross-sectional area. Overall, there were no biochemical improvements related to CKD progression. Skeletal muscle catabolism was higher than non-exercised animals without a concomitant change in muscle synthesis markers or regeneration transcription factors. These results suggest that aerobic exercise, achieved via treadmill running was not protective in CKD animals and actually produced potentially harmful effects (increased catabolism). Given the high prevalence and dramatic musculoskeletal mobility impairment in patients with CKD, there is a clear need to understand how to effectively prescribe exercise in order to benefit the musculoskeletal system.

1. Introduction

Chronic kidney disease (CKD) is common in the United States, affecting > 30 million adults [1]. Chronic kidney disease progression is associated with muscle atrophy, reduced strength and impaired mobility; known as sarcopenia or frailty [2–4]. The progressive loss of muscle size and function leads to impaired physical function that is associated with a higher likelihood of being hospitalized [5] and is predictive of mortality in patients with CKD and those on dialysis [6,7]. Despite this, there is a lack of effective interventions to treat the musculoskeletal dysfunction.

Exercise is often viewed as a primary intervention to aide in musculoskeletal health. In persons with chronic disease conditions, exercise participation leads to a number of beneficial effects [8]. In CKD, a systematic review of clinical exercise studies for patients with stage 2–5 CKD, demonstrated beneficial effects on aerobic capacity and blood pressure, but no difference in muscle size, strength, or walking capacity [9]. In general, the extent to which exercise is beneficial for patients with CKD is inconclusive. Similarly, animal models of forced exercise have shown inconclusive evidence on the beneficial effects of exercise [10–14]. These animal studies mimic what is seen clinically with no effect on disease progression [10,13] or muscle protein synthesis [10,14], and mixed effects on antioxidants [13,15]. This lack of effect may be due to a number of factors surrounding the exercise prescription, mode, and disease model. We therefore intended to study the effects of aerobic exercise in a translational rat model with slowly progressive CKD with demonstrated muscle dysfunction [16,17]; to identify the effects of 10 weeks of treadmill aerobic exercise on skeletal muscle properties.

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2. Methods

2.1. Experimental Design

We used a rat model of CKD (Cy/+ rats) that develops progressive azotemia with terminal uremia by 40 weeks [18]. Animals were fed a casein diet (Purina AIN-76A, Purina Animal Nutrition, Shreveport, LA, USA; 0.53% Ca and 0.56% P) beginning at 24 weeks (approximately 50% normal kidney function) in order to promote consistent disease development. At 25 weeks, CKD rats were subjected to treadmill exercise or no treadmill exercise for 10 weeks (n = 8 each group). Animals ran for 60 min, 5 × /week starting at a speed of 8 m/min and ending at 18 m/min (1 m/min increase/week). Animals were acclimated for one week prior to initiation of training bout (from weeks 24–25). Animals received a mild electrical stimulus when failing to maintain prescribed speed and an individual treadmill session was terminated if electrical stimulation was given more than 3 times in one minute, or if the animal sat on the coils for greater than 10 s. The Indiana University School of Medicine Institutional Animal Care and Use Committee reviewed and approved all procedures prior to initiating the study.

2.2. Electrically stimulated torque

Prior to euthanasia at 35 weeks, muscle strength/torque was assessed (1305 A Whole Rat Test System, Aurora Scientific Inc., Aurora, ON, Canada). Animals were placed in a supine position and their right foot secured to a foot pedal attached to a servomotor [17]. Electrodes were placed near the common peroneal nerve to stimulate a dorsiflexion twitch response and input current was adjusted to achieve maximum twitch response followed by a 20% increase to ensure supramaximal stimulation of muscle fibers (~15 mA for dorsiflexion). Maximum isometric torque (N·m) was recorded for various stimulation frequencies ranging between 10 Hz and 200 Hz, with a pulse width of 0.2 ms and pulse duration of 200 ms. Following testing, time to maximum torque and half relaxation time were calculated from the peak isometric torque data.

2.3. Tissue procurement

Blood was collected prior to euthanasia, and the extensor digitorum longus (EDL) from the non-stimulated limb was collected. The middle third of EDL was isolated, placed in OCT media and frozen in liquid nitrogen chilled isopentane; then remaining tissue was snap frozen in liquid nitrogen. The remaining EDL was stored for protein and RNA analysis at −80 °C.

2.4. Assays

Blood plasma was analyzed for blood urea nitrogen (BUN), calcium and phosphorus using colorimetric assays (BioAssay Systems, Hayward, CA, USA) and intact PTH by ELISA (Alpco, Salem, NH, USA).

2.5. Muscle cross-sectional area

Immunostaining was performed on frozen EDL cryosections. Sections were incubated overnight with a polyclonal anti-laminin antibody (Sigma, St. Louis, MO) in order to label muscle fiber perimeters for measurement of fiber cross-sectional area. Skeletal muscle cross-sections were imaged at 10x magnification using a Spot RT Color Camera System mounted on an inverted Nikon Diaphot 200 microscope (Nikon Instruments Inc, Melville, NY), 3 images per Section, 3 sections per animals were analyzed. Prior to analysis, each image was inspected for sectioning artifacts, blood vessels, or poor image quality, and any sections that were not acceptable were omitted from the analysis. Muscle fiber cross-sectional area was measured with an automated, custom-written macro in ImageJ (NIH, Bethesda, MD), provided by Drs. Rick Lieber and Samuel Ward [19].

2.6. RNA isolation and real time PCR

Total RNA was isolated using miRNeasy Mini Kit (Qiagen) as previously described [16]. Gene expression was determined by real time PCR using TaqMan mRNA assays (Applied Biosystems, Foster City, CA). Target-specific PCR primers (Pax-7, MyoD, Myostatin, Myogenin, Atrogin 1, IGF-1) were obtained from Applied Biosystems. The cycle number at which the amplification plot crosses the threshold was calculated (CT), and the ΔΔCT method was used to analyze the relative changes in gene expression and normalized by β-actin.

2.7. Western blot

Western blotting was performed as previously described [16,20]. Briefly, the EDL muscle was homogenized and the total protein lysates analyzed for expression of markers for protein degradation using antibody against Ubiquitin and p70 (total and phosphorylated), respectively (1:500, Santa Cruz Biotechnology, Santa Cruz, CA). The blots were incubated overnight at 4 °C followed by incubating with peroxidase conjugated secondary antibody (1:5000 dilution) and immunodetection assessed with the Enhanced Chemiluminescence Prime Western Blot Detection Reagent (Amersham, Piscataway, NJ). Band intensity was analyzed by ChemiDoc MP Imaging System (Imaging Lab 4.0, Bio-Rad, Richmond, CA) and normalized to total protein expression using Ponceau S (Santa Cruz Biotechnology, Santa Cruz, CA) or phosphorylated to total p70 were utilized as the normalization for p70 expression.

2.8. Statistics

Two-tailed independent sample *t*-tests were used to compare means between CKD and CKD + exercise. Data are presented as mean ± SD.

3. Results

At 35 weeks of age (approximately 15% of normal kidney function), there was no difference between groups for muscle strength (via maximally stimulated torque; Fig. 1A) or half-relaxation time (time from peak torque to 50% torque reduction; Fig. 1B). There was also no difference in muscle fiber cross-sectional area between CKD rats (2821 μm ± 405) and CKD + exercise (2413 μm ± 449). Biochemical measures, indicative of disease severity were not significantly different between groups (Table 1) with the exception of elevations in Ca^{++} with exercise. Similarly, there was no difference in body mass.

In an attempt to better understand the lack of efficacy of treadmill running, we examined muscle protein turnover. Treadmill exercise did not alter the gene expression of IGF-1, an upstream, global marker for protein synthesis (Fig. 2A) or its downstream target p70 (Fig. 2B). However, treadmill exercise increased muscle catabolism, with levels of ubiquitin protein expression 50% higher compared to non-exercised animals (Fig. 3A). The propensity towards catabolism was also corroborated by higher gene expression levels of the ubiquitin pathway gene atrogin-1 (Fig. 3B, $p < 0.05$).

To determine if skeletal muscle regeneration was altered, we measured RNA transcription factors associated with muscle stem cell regeneration. Treadmill exercise resulted in 44% lower expression of the muscle stem cell quiescence marker PAX7 (Fig. 4, $p < 0.05$), but there was no difference in expression of the regenerative factors myoD or myogenin (Fig. 4).

4. Discussion

Exercise has demonstrated beneficial effects in a number of chronic