Oxidative stress contributes to muscle atrophy in chronic kidney disease patients

Kassia S Beetham[1], Erin J Howden[1], David M Small[2], David R Briskey[1,2], Megan Rossi[2,3], Nicole Isbel[2,3], Jeff S Coombes[1]

[1]School of Human Movement Studies, University of Queensland, Brisbane, Queensland, Australia, [2]School of Medicine, University of Queensland, Brisbane, Queensland, Australia, [3]Department of Nephrology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

Objectives: Patients with chronic kidney disease have impaired muscle metabolism, resulting in muscle atrophy. Oxidative stress has previously been identified as a significant contributor to muscle atrophy in other populations, but the contribution in chronic kidney disease is unknown. The aim of this study was to investigate the association between oxidative stress, grip strength, and lean mass in patients with chronic kidney disease.

Methods: This is a cross-sectional study of 152 participants with stage 3 or 4 chronic kidney disease. Outcome measures include grip strength, lean mass, plasma total F2-isoprostanes, inflammation, peak oxygen uptake, and standard clinical measures.

Results: Thirty four (22.4%) chronic kidney disease patients had elevated oxidative stress levels (plasma F2-isoprostanes >250 pg/ml), with 82% of patients below age-predicted grip strength normative values. There was a significant negative association between plasma F2-isoprostanes and grip strength ($r = -0.251$) and lean mass ($r = -0.243$). There were no associations with inflammation markers. Multiple linear regression identified plasma F2-isoprostanes as a significant predictor of grip strength independent of other predictors: sex, diabetes status, body mass index, body fat percent, and phosphate (adjusted $r^2 = 69.5$, $P < 0.001$).

Discussion: Plasma F2-isoprostanes were independently associated with reduced strength in chronic kidney disease patients.

Keywords: Oxidative stress, Muscular atrophy, Muscle strength, Kidney diseases

Introduction

Patients with chronic kidney disease (CKD) develop muscle wasting, which significantly influences muscular strength.[1] Skeletal muscle atrophy is associated with a three-fold increase in mortality over a 4–6-year period in dialysis patients.[2] The aetiology of muscle atrophy in CKD is multi-factorial and associated with metabolic acidosis, excess angiotensin-II, and inflammation, however, to our knowledge the contribution of oxidative stress in pre-dialysis patients has not been studied.[1]

Oxidative stress occurs when there is a disruption of redox signalling and control pathways.[3] A well-documented target of oxidative injury is lipid peroxidation of arichidonic acid, which produces F2-isoprostanes.[4] As such, plasma F2-isoprostanes are the gold standard for quantifying oxidative stress.[5] Other common assays or biomarkers of oxidative stress in CKD patients include advanced oxidation of protein products, protein carbonyls, γ-glutamyl transpeptidase, and malondialdehyde.[4] Protein carbonyls quantify reactive species damaged proteins, however, this assay is not a specific measure of oxidative stress as it also measures glycated proteins and bound aldehyde.[4] Reduced total anti-oxidant capacity (TAC) and glutathione peroxidase (GPX) may also indicate a disturbance of the redox signalling pathways. Elevated oxidative stress levels, measured by plasma-free F2-isoprostanes, protein carbonyls, and protein-reduced thiol content, have been reported in moderate-severe CKD.[6] Oxidative stress is suggested to be associated with inflammation, endothelial dysfunction, and malnutrition in the uraemic population, thereby synergistically contributing to atherogenecity and risk of a cardiovascular event occurring.[7] Despite the multi-factorial nature of oxidative stress in renal patients, it has been suggested that the retention of oxidized solutes is likely a major contributor to the disease process.[8] Only one small study has

Correspondence to: Jeff S Coombes, School of Human Movement Studies, The University of Queensland, Brisbane, Queensland 4072, Australia. Email: jcoombes@uq.edu.au

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investigated the effects of oxidative stress and muscle atrophy in renal patients. Crowe *et al.*[9] identified that haemodialysis patients ($n = 10$) had significantly reduced diameter size of type-I and -II muscle fibres when compared to sex-matched controls. However, the authors found no clear association between an oxidative stress marker, serum malonaldehyde, and muscle fibre diameter. Whether an increase in oxidative stress with CKD influences muscle atrophy and strength is yet to be examined.

Grip strength is well recognized as an indicator of overall upper body strength[10] and provides risk estimates similar to those of quadriceps strength.[11] It has been suggested that muscle strength is a greater predictor of mortality than muscle mass.[11] Moreover, it has been reported that hand grip strength is a reliable measure of lean body mass in both men and women with chronic renal failure.[12] Due to the strong validity and accessibility of grip strength measures in the community, this study focused on grip strength as a primary measure of strength.

The aim of this study was to investigate the association between oxidative stress, grip strength, and lean mass in patients with CKD. It was hypothesized that CKD patients would have impaired muscle function, evidenced by grip strength lower than age-predicted normative values. Furthermore, it was hypothesized that oxidative stress would be negatively associated with lean mass and grip strength.

Methods

The data from this study is a cross-sectional baseline analysis of the 'LANDMARK 3' study (Longitudinal Assessment of Multiple Discrete Atherosclerotic Risk Factors in Kidney Disease), looking at the effects of a 3-year multidisciplinary lifestyle intervention in CKD. This study included 152 subjects with stage 3 or 4 CKD (modification of diet in renal disease-175; estimated glomerular filtration rate (eGFR) 25–60 ml/min/1.73 m^2). Inclusion criteria were: aged 18–75 years and at least one of the following risk factors – blood pressure or lipids not at target, overweight (body mass index (BMI) >25 kg/m^2), and poor diabetic control (haemoglobin A1c>7%). Exclusion criteria were: intervention for, or, symptomatic coronary artery disease (within 3 months), current heart failure (New York Heart Association class III and IV) or significant valvular heart disease, pregnant or planning to become pregnant, and life expectancy or anticipated time to dialysis or organ transplant <6 months.

The study protocol was approved by the Princess Alexandra Human Research Ethics Committee (HREC 2007/190), and was registered at http://www.anzctr.org.au (Registration Number ANZCTR12608000337370). All patients gave written, informed consent to participate in this study.

Ethylenediaminetetraacetic acid vacutainers (BD vacutainers, Franklin Lakes, NJ, USA) were used to collect 10 ml venous blood samples following an overnight fast. Samples were stored on ice before being centrifuged at 750*g* for 10 minutes. Plasma was stored at −80°C with butylated hydroxytoluene (10 µl of 100 mM to each 1.5 ml eppendorf tube) to prevent artefactual oxidation. The complete methodologies for plasma total F2-isoprostanes, protein carbonyls, TAC, and GPX, including laboratory coefficients of variation (%CV), hve been previously reported by our group.[13] Patients were grouped by normal (≤250 pg/ml) or elevated (>250 pg/ml) oxidative stress based on plasma F2-isoprostanes. This was based on using a value 1.5 standard deviations (SDs) from mean values obtained from a previous study on apparently healthy 18–30-year old males and females from our laboratory.[13]

Inflammation markers interleukin-6 (IL-6), interleukin-1β, tumour necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) were measured by an electrochemiluminescence technique using Human Pro-inflammatory 4-plex Ultra-sensitive Kit (Meso Sector S 6000, Rockville, MD). The assays were performed according to the manufacturer's instructions with %CV less than 20% being considered acceptable, as previously described.[14]

Additional blood measures of lipids, haemoglobin, phosphate, creatinine, C-reactive protein (CRP), albumin, glucose, and insulin were conducted using standard laboratory techniques. The eGFR was estimated using the modification of diet in renal disease-175 formula.[15] Insulin resistance was computed using the homeostatic model assessment of insulin resistance method.[16] Stage 3 CKD was defined as eGFR $30 < 60$ and stage 4 was defined as ≤29 ml/min/1.73 m^2. Due to the small inclusion range of ≥25≤29 for stage 4 in this study, the majority of patients were classified as stage-3 CKD. Fasting blood samples were performed prior to any other testing. Beta blocker medication was withheld at least 12 hours prior to testing.

Grip strength was measured using a hand grip dynamometer (Jamar 5030 J1, Bolingbrook, IL, USA). Each participant received the same instructions after a demonstration by the tester, and the hand grip was adjusted accordingly for the patients comfort. Participants undertook the test six times, alternating between each hand. The maximum grip strength was attained from the highest reading of either hand. Age-predicted grip strength values were calculated by a predictive equation for males and females as reported by Desrosiers *et al.* (1995).[17] Cardiorespiratory fitness was measured as peak oxygen uptake (VO_2peak) using expired air analysis (Vmax29c, SensorMedics,

Yorba Linda, CA, USA) using the peak 20-second average of the final minute during a maximal treadmill test. The test protocol was determined by the Duke Activity Status Index, which was completed by the participants.[18] Based on participant's responses to this questionnaire, they performed either the Bruce, Balke, or Naughton protocols. Self-reported physical activity during the previous 6 months was determined using items from the Active Australia questionnaire.[19]

Dual energy X-ray absorptiometry (DEXA), using whole body composition analysis, was used to assess lean mass (Hologic QDR 4500A version 12.6, Bedord, MA, USA). Lean mass percentage was calculated from the percentage of lean mass from the total mass. Appendicular lean mass percentage was calculated from the average of the four limbs. DEXA was performed on a representative sub-set of patients due to limited machine availability ($n = 75$).

Statistics

Mean ± SD was used to describe baseline characteristics, with percentages used to describe frequencies for categorical variables. A one-way analysis of variance with a Bonferroni *post-hoc* analysis was used to determine between group differences of grip strength tertiles. Univariate associations between variables and grip strength were evaluated using Pearsons correlations. Not normally distributed variables were transformed using the natural logarithm. Spearmans Rho was used for not normally distributed variables that were not able to be transformed. Significant univariate associations were included in a multivariate model to identify independent correlates, using the enter method. Regression diagnostics were assessed for identification of collinearity and variance inflation factor issues. The power analysis (pnorm using R statistical program) was performed using the sample size and testing for a single correlation coefficient between F2-isoprostanes and grip strength. Statistical analysis was performed using the IBM SPSS statistics 21 (New York, 2012). Statistical significance was assumed at $P < 0.05$.

Results

One hundred and fifty two patients were recruited and eligible for study participation between March 2008 and February 2013. Table 1 shows the demographic and clinical data for this cohort. The mean age of patients was 60 years, the majority were obese and had low fitness. Nineteen patients were classified as stage-4 CKD and 141 patients were classified as stage-3 CKD. Sixty-eight (43.9%) patients had diabetes. There was no significant difference in F2-isoprostanes between patients with stage-3 and -4 CKD ($P = 0.91$) and patients with and without diabetes ($P = 0.75$). Thirty four patients (22.4%) had elevated F2-isoprostanes (≥250 pg/ml). There was no consequent increase in anti-oxidant status in patients with elevated F2-isoprostanes (GPX= normal F2-isoprostanes group 24.8 ± 2.9 (U/l) vs. elevated F2-isoprostanes group 19.6 ± 0.4, $P = 0.6$; TAC=normal F2-isoprostanes group 1.6 ± 0.1 (mmol/l) vs. elevated F2-isoprostanes group 1.8 ± 0.1, $P = 0.2$). There was also no significant correlation between F2-isoprostanes, protein carbonyls, GPX, and TAC (Table 2). There was, however, a moderate correlation between GPX and TAC ($r = -0.245$, $P = 0.003$).

Table 1 Patient characteristics

Variable	
Age (years)	59.7 ± 10.0
Female sex, *n* (%)	65 (42.8)
Diabetes, *n* (%)	68 (44.7)
eGFR (ml/min/1.73 m^2)	40.2 ± 9.0
Body mass index (kg/m^2)	33.7 [8.1]
Fat (%)	36.7 ± 7.7
Appendicular lean mass (%)	60 ± 9.2
Medications	
Beta-blockers, *n* (%)	47 (30.3)
Angiotensin converting enzymes inhibitor, *n* (%)	69 (44.5)
Statins, *n* (%)	83 (53.5)
Blood biochemistry	
F2-isoprostanes (pg/ml)	193 [108.5]
Protein carbonyls (nM/mg)	0.52 [0.14]
Glutathione peroxidase (U/l)	24.2 ± 4.3
Total anti-oxidant capacity (mmol/l)	1.7 ± 0.4
Albumin (g/l)	3.8 [5]
Homeostatic model assessment – insulin resistance* (%)	6.5 [15.8]
HbA1c*	7.2 [1.7]
Haemoglobin (g/dl)	13.2 ± 1.5
Phosphate (mmol/l)	1.1 ± 0.2
Bicarbonate (mmol/l)	26.0 ± 6.5
Inflammation	
Interferon-γ (pg/ml)	1.0 [0.7]
Interleukin-6 (pg/ml)	2.2 ± 2.1
Tumour necrosis factor-α (pg/ml)	6.8 ± 4.0
C-reactive protein (mg/l)	5.1 [4.8]
Exercise parameters	
VO_2peak (ml/kg/min)	23.3 [7.2]
Physical activity levels (hours/week)	2.3 [3.3]
Grip strength – best of either hand (kg)	30.7 ± 11.3
Haemodynamics	
Rest systolic blood pressure (mm/Hg)	137.4 ± 20.9
Rest diastolic blood pressure (mm/Hg)	81.8 ± 12.4

Mean ± standard deviation given for normally distributed variables, median [interquartile range] given for not normally distributed variables, number (%) given for categorical variables. eGFR, estimated glomerular filtration rate.
*In patients with diabetes only.

The association between F2-isoprostanes and other muscle atrophy markers are represented in Table 2. There was a negative association between F2-isoprostanes and grip strength (Fig. 1), and F2-isoprostanes and appendicular lean mass percentage (Fig. 2). There was no significant relationship between F2-isoprostanes and grip strength in patients with normal F2-isoprostanes ($r = -0.161$, $P = 0.102$). There were, however, significant associations between F2-isoprostanes and sex, albumin, Homeostatic model assessment-insulin resistance (HOMA-IR), haemoglobin,

Table 2 Muscle atrophy variables and univariate relationship with F2-isoprostanes

Variable	*r* value	*P* value
Sex	0.206	0.01
Diabetes status	−0.036	0.7
Age	−0.217	0.01
Glutathione peroxidase	0.019	0.8
Total anti-oxidant capacity	−0.046	0.6
Protein carbonyls	0.057	0.5
Albumin	−0.195	0.02
Homeostatic model assessment – insulin resistance	0.186	0.03
Interferon-γ	0.128	0.2
Interleukin-6	0.087	0.3
Tumour necrosis factor-α	0.045	0.6
C-reactive protein	0.069	0.4
Haemoglobin	−0.200	0.01
Grip strength	−0.251	<0.01
Appendicular lean mass %	−0.243	0.04
Body mass index	0.274	<0.01

and BMI. There were no associations ($P > 0.05$) with inflammation markers IFN-γ, TNF-α, IL-6, and CRP. The power of detecting a significant association between grip strength and F2-isoprostanes in 152 participants is 99.3%. The correlation between grip strength and F2-isoprostanes in patients with BMI $<30\ kg/m^2$ ($n = 41$) was statistically significant ($r = -0.353$, $P = 0.032$). Despite the significant difference in F2-isoprostanes between males and females (males = 177 ± 89.1 pg/ml vs. females = 214.4 ± 99.1, $P < 0.01$), the correlation between grip strength and F2-isoprostanes in males was only approaching significance ($r = -0.211$, $P = 0.067$). Likewise, the correlation between grip strength and F2-isoprostanes in females was only not significantly correlated ($r = -0.039$, $P = 0.772$).

Grip strength was separated into relative tertiles of low (≤25 kg, $n = 50$), moderate (26–35 kg, $n = 43$), and high strength (≥36 kg, $n = 44$) (Fig. 3). There was a significant group difference between grip strength tertiles ($P = 0.005$), with *post-hoc* analyses

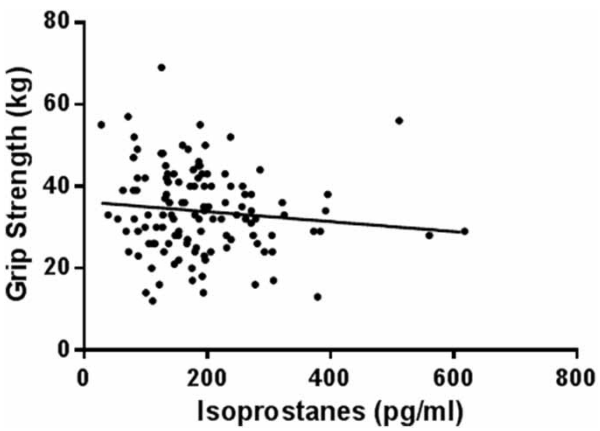

Figure 1 Association between F2-isoprostanes and grip strength. $r = -0.251$, $P < 0.01$.

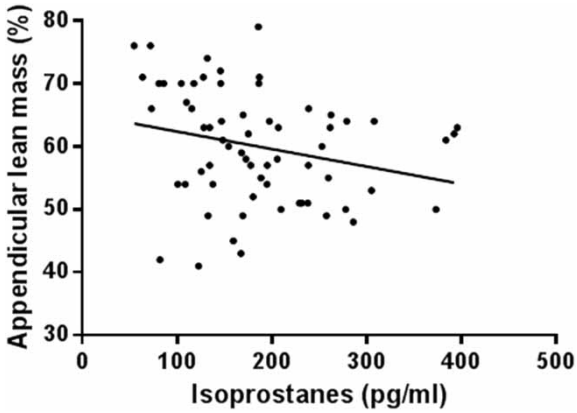

Figure 2 Association between F2-isoprostanes and appendicular lean mass percentage. $r = -0.243$, $P = 0.04$.

identifying the low-strength group to have significantly higher levels of plasma F2-isoprostanes than the high-strength group ($P = 0.006$). The difference between low strength and moderate strength was approaching significance ($P = 0.059$). It was identified that 82% of all patients had grip strength values below their age-predicted grip strength ($P < 0.001$). Factors associated with grip strength are shown in Table 3. There was a strong correlation between grip strength and appendicular lean mass. Multiple linear regression found F2-isoprostanes to be a predictor of grip strength ($\beta = -0.219$, $P = 0.032$) independent of other predictors: sex, diabetes status, BMI, body fat percentage, and phosphate in a model also including age, IFN-γ, appendicular lean mass, haemoglobin, and VO_2peak (adjusted $r^2 = 69.5$, $P < 0.001$).

Discussion

This is the first study to investigate the role of oxidative stress in reduced lean mass and grip strength in CKD

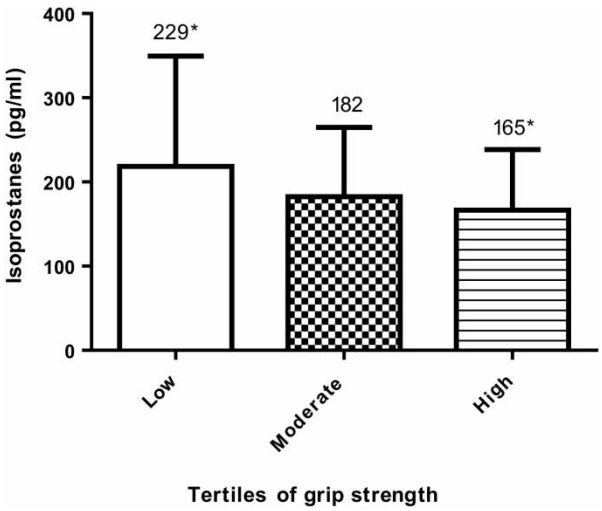

Figure 3 Plasma F2-isoprostane levels at low, moderate, and high tertiles of grip strength. Between group differences ($P < 0.01$). High grip strength has significantly lower plasma F2-isoprostanes than low grip strength ($P < 0.01$).

Table 3 Factors associated with grip strength

Variable	Univariate (r or r_s)	*P* value	Multivariate (β)	*P* value
Age	−0.215	0.01	−0.192	0.123
Sex_s	−0.729	<0.001	−0.592	<0.001
Diabetes $status_s$	−0.308	<0.001	−0.221	<0.01
eGFR	0.061	0.5		
Body mass index (Ln)	−0.226	0.01	0.373	<0.01
Total anti-oxidant capacity	0.012	0.9		
F2-isoprostanes (Ln)	−0.251	<0.01	−0.219	0.032
Protein carbonyls	0.041	0.6		
Glutathione peroxidase	0.013	0.9		
Interferon-γ_s	−0.222	0.02	0.048	0.606
Tumor necrosis factor-α	−0.103	0.3		
Interleukin-6	−0.091	0.3		
Phosphate	−0.408	<0.001	−0.181	0.05
Haemoglobin	0.481	<0.001	−0.040	0.707
Bicarbonate	0.006	0.9		
C-reactive $protein_s$	−0.063	0.5		
$Albumin_s$	0.128	0.1		
Homeostatic assessment model-insulin $resistance_s$	−0.162	0.07		
VO_2peak (Ln)	0.454	<0.001	0.053	0.673
Physical activity $levels_s$ (weekly)	0.097	0.3		
Appendicular lean mass (%)	0.855	<0.001	−0.056	0.776
Fat (%)	−0.576	<0.001	−0.592	0.013

Adjusted $r^2 = 0.695$, $P < 0.001$.

patients. Our main findings were: (1) 82% of CKD patients had grip strength lower than age-predicted normative values, (2) 22.4% of CKD patients had elevated F2-isoprostanes, (3) oxidative stress was negatively associated with variables associated with muscle atrophy, in particular, appendicular lean mass, grip strength, sex, age, albumin, HOMA-IR, haemoglobin, and BMI, and (4) plasma F2-isoprostanes were an independent predictor of reduced grip strength.

CKD results in significantly reduced muscle mass and strength.[20] Indeed, it was found that 82% of the CKD patients had grip strength lower than age-predicted normative values. It is likely that reduced strength is occurring at least partly as a consequence of muscle atrophy, evident by the strong positive correlation between grip strength and lean mass. Our findings are in support of the literature which identifies reduced strength in CKD patients, as a study on haemodialysis patients also found reduced strength to occur as a consequence of reduced muscle mass.[21] This is clinically relevant as reduced functional strength is closely associated with survival and low muscle mass is a potentially modifiable factor.[22] The almost linear relationship that occurs between grip strength and lean mass supports the use of grip strength as an inexpensive and easily used test in the field.

F2-isoprostanes were elevated in a significant proportion of patients; however, there was no subsequent increase in anti-oxidant status. In addition, there was no association between GPX, TAC, and F2-isoprostanes. These findings are supported by Karamouzis *et al.*[23] who found that as the stage of kidney disease increased, so too did the level of plasma F2-isoprostanes. This study also found that TAC did not change with advancing CKD stages. The disconnection between F2-isoprostanes and GPX and TAC in our findings suggests that an increase in oxidants is occurring without a compensatory increase in anti-oxidants.[23] This imbalance may be an important finding in elucidating the pathogenesis of oxidative injury in CKD patients. This is consistent with the findings from Dalla Libera *et al.*[24] who found an increase in muscle protein carbonylation and a blunted expression of stress proteins involved in the anti-oxidant defence in patients with disuse muscle dystrophy. As suggested by the authors of this study, future investigations are needed to identify the mechanisms responsible for the switching-off of these anti-oxidant defences, and whether challenging this mechanism can ameliorate muscle atrophy.[24]

Plasma F2-isoprostane levels were identified to be negatively associated with both lean mass and grip strength. This finding suggests a relationship between oxidative stress and resultant loss of strength through reduced muscle mass, which has not previously been identified in CKD patients. It has previously been reported in haemodialysis patients that inflammatory markers are associated with muscle mass.[25] However in the current study, we were not able to demonstrate an independent association between inflammatory cytokines and grip strength or lean mass. It is possible that in less severe CKD patients (i.e. stage 3–4) oxidative stress may have a more significant contribution to muscle wasting than inflammation.

The significant association of F2-isoprostanes and grip strength in patients with BMI $< 30\ kg/m^2$ ($n = 41$) suggests that F2-isoprostanes is not just a marker of muscle function in obese patients. However, future studies should identify whether this relationship still exists in CKD patients with a BMI of $<25\ kg/m^2$. Sex and F2-isoprostanes were strongly correlated in all patients, and yet when separated into males and females the correlation between grip strength and F2-isoprostanes was no longer significant. This may indicate that a larger sample size is needed to detect significant correlations due to the high variability in F2-isoprostanes. Prior studies have reported inconsistent findings on sex differences and oxidative stress levels.[26–29] We have shown that female CKD patients have higher levels of F2-isoprostanes than males. Therefore, future large-scale studies should identify whether the correlations between oxidative stress,

lean mass, and strength is dependent on sex and whether changes in muscle parameters after an intervention differs between males and females.

Oxidative stress was associated with other variables related to muscle atrophy: hypoalbuminemia, increased insulin resistance, anaemia, and obesity. These factors have previously been proposed as variables associated with muscle atrophy.[30] It has been reported that increased reactive oxidative species (ROS) bind specific muscle proteins and increases the degradation by the Ubiquitin–Proteasome System.[31] This increase in degradation results in muscle atrophy.[32] In genetic muscle diseases, oxidative stress is implicated as the initiator and driver of muscle damage.[33]

An obvious approach to the problem of reduced strength in CKD is exercise training. This potentially has a dual effect of not only strengthening muscle, but also correcting the imbalance between oxidative stress and protective anti-oxidant capacity. As exercise increases oxygen uptake, there is an acute increase in ROS production through the electron transport system.[34] With repeated exercise, anti-oxidant enzymes that combat this exercise-induced oxidative stress are upregulated to provide more protection against ROS.[35] Indeed, an investigation in exercise-induced malondialdehyde in rats also found a compensatory increase in GPX and superoxide dismutase.[36] Therefore, although exercise can cause acute increases in ROS, long-term exercise can prove to be a beneficial treatment in improving oxidative stress.[37] The randomized control trial following on from this baseline analysis involves an exercise intervention for 36 months and the impact of exercise training on oxidative stress will be examined. The current evidence on the protective effects of anti-oxidant therapies in improving kidney function remains equivocal.[38] However, using anti-oxidant therapy to attenuate ROS and increase muscle mass in the renal population has not previously been studied.[4]

Limitations

The analysis from this study employs a cross-sectional design and therefore causality between oxidative stress, reduced grip strength, and lean mass cannot be determined. Despite the well-recognized validity of grip strength as an indication of overall upper body strength,[10] additional measures of strength, such as 1-repetition maximum testing, may have reinforced the findings. It should be noted that while DEXA provides an estimate of lean body mass, skeletal muscle histology, or magnetic resonance imaging to assess fibre and muscle size, would have provided a better measure of muscle atrophy. Despite this limitation, the associations found with lean mass are encouraging, as DEXA has been reported to *underestimate* the loss of thigh muscle mass in comparison to MRI.[39] Further work looking at the expression of other factors involved in muscle loss, such as atrogin-1 and myostatin in muscle biopsies of patients with CKD, is required to better understand the role of oxidative stress in the pathogenesis of muscle atrophy.

Conclusions

CKD patients have below average grip strength when compared to age-predicted normative values. It was identified that plasma F2-isoprostanes were independently associated with reduced strength in CKD patients. The findings from this study may assist in ascertaining appropriately targeted treatments for muscle loss, such as resistance exercises to restore muscle strength and long-term exercise training to correct the oxidative stress imbalance.

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Disclaimer statements

Contributors KB conceived and designed the study, collected the data, analysed the data, interpreted the data and wrote the article in whole. EH collected the data, analysed the data, interpreting the data, revising the article. DS analysed the data and revised the article. DB analysed the data and revised the article. MR analysed the data and revised the article. NI obtained funding and ethics approval, interpreted the data and revised the article. JC conceived and designed the study, obtained funding and ethics approval, interpreted the data, wrote the article in part and revised the article.

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