ORIGINAL ARTICLE

Soy protein diet and exercise training increase relative bone volume and enhance bone microarchitecture in a mouse model of uremia

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Abstract Soy protein consumption and exercise training have been widely studied for their effects on the vasculature and bone in healthy populations, but little is known about the effectiveness of these interventions in chronic kidney disease (CKD). Cardiovascular disease and bone fracture risk are significantly elevated in CKD, and current pharmacological interventions have been unsuccessful in treating these conditions simultaneously. The purpose of this study was to compare the effects of a soy protein diet and endurance exercise training, alone or in combination, on cardiovascular and bone health in a mouse model of renal insufficiency. At 8 weeks of age, 60 female apolipoprotein $E^{-/-}$ mice underwent a two-step surgical procedure to induce uremia. These mice were then randomized at 12 weeks of age to one of four treatment groups for the 16-week intervention period: sedentary, control diet (n = 16); sedentary, soy protein diet (n = 18); exercise, control diet (n = 14); and exercise, soy protein diet (n = 12). There were no significant treatment effects on atherosclerotic lesion areas or aortic calcium deposits. We demonstrated a significant main effect of both diet and exercise on relative bone volume, trabecular number, trabecular separation, and trabecular connective density in the proximal femur as measured by microcomputed tomography. There were no treatment effects on trabecular thickness. We also showed a main effect of diet on plasma urea

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H. R. Chung · K. R. Wilund (⊠) Department of Kinesiology and Community Health, University of Illinois, 906 S. Goodwin Avenue, Urbana, IL 61801, USA e-mail: kwilund@illinois.edu levels. These data suggest that soy protein intake and exercise training exert beneficial effects on properties of bone and plasma urea levels in mice with surgically induced renal impairment.

Keywords Uremia · Atherosclerosis · Bone · Exercise · Soy

Introduction

Chronic kidney disease (CKD) patients have significantly elevated rates of osteoporosis [1], combined with a specific metabolic bone remodeling disorder known as chronic kidney disease–mineral bone disease (CKD–MBD) [2], leading to increased fracture risk, mortality, and morbidity [3]. Elevated parathyroid hormone, common to CKD patients, stimulates resorption of calcium from bone, leading to low bone density and strength, as well as to ectopic mineral deposition [4].

The abnormal mineral metabolism is not confined to bone but also affects the vasculature. Cardiovascular disease (CVD) is the leading cause of death in CKD patients, and the risk may be partially explained by excessive vascular calcification (VC) in CKD patients [5–7]. Historically, loss of mineral from the bone and VC have been considered independent disorders, but there is emerging evidence that they are mechanistically linked. VC is now recognized as an active, regulated process with many properties similar to bone formation [8–12].

Most strategies aimed at inhibiting the progression of VC and bone disorders in CKD have focused on pharmaceutical interventions known to modify traditional CVD risk factors or to improve bone density. However, many of these treatments have substantial side effects and are very expensive, indicating the need for additional therapies. A nonpharmacological approach, consumption of soy protein rich in isoflavones, has shown promise in improving risk factors for both CVD and bone disorders. Studies in animal models clearly demonstrate benefits of soy protein or isoflavones in reducing atherosclerosis [13] and increasing bone mineral density (BMD) [14, 15]. For CVD, soy isoflavones may reduce long-term risk in humans by improving blood lipids [16, 17] and blood pressure. However, many studies have shown no effect of soy supplementation [18–20] on CVD risk or bone health in humans, and a recently published review indicates that the benefits of soy in humans may be more modest than suggested by the animal studies [21]. In addition, it is not known if these effects persist in the context of CKD.

The benefits of exercise on cardiovascular risk and bone health have been well established in healthy populations, but few studies have considered the effect of weightbearing endurance exercise training for individuals with CKD, particularly in CKD stages 3-4, representing moderate to severe renal impairment. Exercise training in populations with normal kidney function improves traditional risk factors for atherosclerosis such as hypertension, plasma lipids, glucose, and inflammatory variables [22]. Many of these same factors may increase the development of VC, so it is reasonable to assume that exercise training may also reduce VC by improving the CVD risk factor profile. Few studies have considered the effects of exercise training on bone health in individuals with CKD, and this is the first study to consider the relationship between exercise, bone, and vascular health in a model of CKD.

Because of the complex pathogenesis of CKD, it has been suggested that multiple therapeutic interventions will be necessary and should be used simultaneously to reduce comorbidities in this population [23]. Previous animal studies have demonstrated prevention of bone loss and reduction of cardiovascular risk with a combination of soy isoflavones and exercise [24, 25], but this approach has not been examined in a model of kidney disease. Therefore, the purpose of this study was to evaluate the effectiveness of a soy protein diet and exercise training, alone and in combination, on vascular and bone measures in a mouse model of moderate to severe renal insufficiency. Apolipoprotein (apo) $E^{-/-}$ mice were chosen because when placed on a high-fat diet they rapidly develop atherosclerosis as the result of disordered lipid metabolism, allowing studying the relationship between CVD and CKD in this model of uremia-induced accelerated atherosclerosis [26]. Furthermore, Nikolov et al. [27] have demonstrated disordered bone metabolism in this model, which allows including this comorbid condition in our intervention study. We hypothesized both exercise and consumption of a soy protein diet would attenuate development of atherosclerotic lesions and bone disorders, and that the effects of the combined treatments would be additive.

Materials and methods

Animals

Female apolipoprotein $E^{-/-}$ (B6.129-*Apoe*^{tm1Unc}/J, #002052) (n = 60) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The mice were individually housed in plastic cages in a temperature-controlled facility and maintained on a 12:12 h light/dark cycle. Animals were provided ad libitum access to diet and water. All experiments and protocols were approved by the Illinois Animal Care and Use Committee at the University of Illinois, Urbana-Champaign.

Surgical creation of uremia

At 8 weeks of age all mice underwent a two-step surgical procedure to induce uremia. Animals were anesthetized with a combination of oxygen and 1-3% isoflurane. For the first procedure, the right kidney was approached through a 2-cm-long lumbar incision and exposed by fine dissection of the surrounding tissues. The anterior and posterior poles of the kidney were resected, leaving the middle segment of the kidney intact. At 10 weeks of age, following a 2-week recovery period, a total nephrectomy of the left kidney was performed by ligation of the renal artery with a 5-0 silk suture, followed by excision of the kidney. Establishment of the surgical model was determined by plasma urea concentrations between 12 and 25 mM (normal mouse plasma urea, <8 mmol/l). At 12 weeks of age, after an additional 2-week recovery period, animals with urea levels >12 mmol/l were randomized into one of the following four groups for the 16-week intervention: casein diet, sedentary (Cas/Sed, n = 16); soy protein diet, sedentary (Soy/Sed, n = 18); casein diet, exercise-trained (Cas/Ex, n = 14; or soy protein diet, exercise-trained (Soy/Ex, n = 12). The different number of animals in each group reflects the number that survived the high-intensity surgery until the completion of the 16-week intervention.

Diet and exercise protocol

The compositions of the experimental diets are outlined in Table 1. Both diets were purified diets that derived 15% of kilocalories from fat and differed in the protein source. Protein content in the diet was chosen on the basis of the protein level provided in standard rodent chow. The soy protein diet (TD.06653; Harlan Teklad, Madison, WI, USA) contained 17.8% w/w soy protein isolate, resulting in 19.0%

Table 1 Composition of study diets Image: Composition of study	Selected components	Casein diet	Soy diet
	Isolated soy protein ^a (g/kg)	0.0	200.0
	Casein (g/kg)	200.0	0.0
	Sucrose (g/kg)	150.0	150
	Cornstarch (g/kg)	371.8	368.8
	Maltodextrin (g/kg)	120.0	120
	Cellulose (g/kg)	50.0	50.0
 ^a Contains 250 mg/kg isoflavones ^b Soybean oil does not provide any isoflavones 	Soybean oil ^b (g/kg)	60.0	60.0
	Mineral mix AIN-93G (g/kg)	35.0	35.0
	Vitamin mix AIN-93G (g/kg)	10.0	10.0

of kilocalories from protein, and provided 250 mg of isoflavones/kg diet; the control diet (TD.06650; Harlan Teklad) contained 17.7% w/w casein, resulting in 19.2% of kilocalories from protein, and contained no isoflavones. The diets were approximately matched for sulfur amino acids, available phosphorus, calcium, sodium, potassium, magnesium, iron, and choline. The animals received standard chow until 12 weeks of age (2 weeks after second surgery), at which time they were randomized to a purified experimental diet.

The exercise protocol consisted of running on a motorized treadmill (Jog-a-Dog, Toledo, OH, USA) 5 days per week for 45 min/day during the intervention period. The mice were acclimated to the treadmill exercise such that by the second week of training they ran at 15 m/min. This level corresponds to 60-75% VO₂max in C57BL/6J mice [28], which represents "moderate-intensity" exercise in these animals [29–31]. Negative reinforcement was not used, but rather gentle prodding with a blunt instrument was employed to encourage the mice to exercise. Sedentary mice were not provided access to the treadmill.

Serum assays

Fasting blood samples were drawn from the retroorbital vein on two occasions: before the start of the dietary intervention and before euthanasia. Plasma and serum was collected by centrifugation, aliquoted into microfuge tubes, and stored at -80° C until analyzed. Plasma urea (BioAssay Systems, Hayward, CA, USA) and total cholesterol (Infinity Incorporated, Melbourne, Australia) were measured enzymatically at both time points.

Quantification of aortic calcium and atherosclerotic lesions

Following the intervention period, each mouse was killed by CO_2 asphyxiation. The heart, including the proximal aorta, was removed, washed in phosphate-buffered saline to remove the blood, placed in freezing medium (OCT; Fischer Scientific, Pittsburgh, PA, USA), and stored at -80° C until sectioning. Serial sections of heart tissue measuring a thickness of 8 μ m from the start of the aortic sinus to the ascending aorta were sliced, mounted on glass slides (Fischer Scientific), and frozen at -20° C, as described by Daugherty and Whitman [32].

Calcium in the cryosections was identified by Alizarin red at three specific anatomic regions of the proximal aorta, each separated by approximately 200 μ m, which coincide with the start of the aortic sinus, the orifices of the coronary arteries, and the start of the ascending aorta. For Alizarin red staining, slides containing the cryosections were rinsed in 70% ethanol, placed in Alizarin red stain for up to 5 min, and rinsed in distilled water twice. Quantification of calcium staining was graded on a scale of 0–4 by blinded investigators: 0 = no staining, 1 = weak, non-discrete staining, 2 = light, discrete staining, and 4 = multiple, intense areas staining [33, 34]. Scores were determined by averaging the scores of four blinded investigators.

Additional slides containing cryosections of the same regions of the proximal aorta were stained for neutral lipids using Oil Red O to detect and quantify intimal atherosclerotic lesions at the same sites. For Oil Red O staining, slides containing the cryosections were rinsed with 60% isopropyl alcohol for 5 min, blotted, and then stained with filtered Oil Red O for 10 min and blotted once more. Slides were rinsed again with 60% isopropyl alcohol for 2 min, blotted, rinsed with distilled water, blotted, and then stained with hema-toxylin for 10 s. Quantification utilized image analysis software (Microsoft Photoshop) and was expressed as total area of the aorta covered with lipid-filled lesions.

Measurement of bone microarchitecture by micro-computed tomography (μ CT)

The right femur was removed from each animal after death, cleaned of surrounding tissue, and stored in ethanol at -20° C. High-resolution images of the femur were acquired using a desktop microtomographic imaging system (μ CT40; Scanco Medical, Basserdorf, Switzerland). Each tissue

sample was scanned at 45 keV with an isotropic voxel size of 6 μ m, and the resulting two-dimensional cross-sectional images were shown in gray scale. Scanning began in the midepiphysis and extended proximally for 3.6 mm (600 CT slices/specimen). The scans resulted in reconstructed threedimensional (3-D) data sets with the μ CT Evaluation Program. Trabecular bone was determined by specifying regions of interest with the provided software program. Using these regions of interest, bone volume, trabecular volume, and composition were calculated by the program using nondestructive three-dimensional reconstruction as described [35].

Statistical analysis

All statistical tests were conducted using SPSS software with two-tailed significance set at $\alpha = 0.05$. Plasma variables, atherosclerotic lesions, and bone outcomes were assessed using a general linear model univariate two-way analysis of variance (ANOVA) with diet (casein or soy) and activity (sedentary or exercise) as between-subjects factors. In any analysis, if significant interactions were observed, variables were analyzed with the post hoc Tukey's test. Main effects were only considered when interactions were not significant, as a significant interaction indicates that the effect of one independent variable depends on the value of the other. The ranked calcium scores were analyzed using the Kruskal–Wallis test for nonparametric data. Data are presented as mean \pm SEM unless otherwise noted.

Results

Body weight and plasma variables

Body weight and plasma variable values are presented in Table 2. There were no interactive or main effects on

change in body weight from baseline to final measurement. There was no treatment effect on change in plasma cholesterol, although all groups had a decrease in measured plasma cholesterol from baseline to the end of the intervention period. There was no interactive effect on plasma urea, but there was a significant main diet effect for the change in plasma urea values ($F_{1,49} = 0.614$; P = 0.013), with greater reduction of plasma urea in the Soy/Sed and Soy/Ex groups compared to Cas/Sed and Cas/Ex.

Atherosclerotic lesion area and aortic calcium

There was no significant effect of diet or exercise on atherosclerotic lesion area at the position in the proximal aorta corresponding to the cusps of the aortic valves or at the branch point of the coronary arteries (Fig. 1a). At the position of the proximal aorta corresponding to the start of the ascending aorta, there was a significant interaction effect ($F_{1,54} = 4.945$; P < 0.05), with the Cas/Ex animals tending to have the greatest lesion area at this site. Non-parametric analysis of ranked calcium scores revealed no significant differences between groups, although the mean rank for the control group was the highest at each of the three aortic sections just described (Fig. 1b).

Bone microarchitecture

There was a significant interaction effect of diet and activity ($F_{1,51} = 4.006$; P = 0.05) on total volume (TV) but not for log-transformed bone volume. However, there were significant main effects of diet ($F_{1,50} = 4.086$; P < 0.05) and exercise ($F_{1,50} = 9.007$; P = 0.004) for log-transformed bone volume (BV), resulting in overall main effects of diet ($F_{1,50} = 8.596$; P < 0.01) and exercise ($F_{1,50} = 10.070$; P < 0.01) on the total volume to bone volume ratio (BV/TV), or relative bone volume (Fig. 2a).

Table 2 Body weight, plasma cholesterol, and plasma urea at baseline and final measurement for all treatment groups

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	Cas/Sed	Soy/Sed	Cas/Ex	Soy/Ex
Body weight: baseline (g)	17.5 ± 0.8	17.2 ± 0.7	16.9 ± 1.1	17.8 ± 0.8
Body weight: final (g)	21.3 ± 0.7	20.2 ± 0.9	19.2 ± 1.6	21.0 ± 1.1
Body weight: delta (g)	3.8 ± 0.8	3.0 ± 0.8	2.3 ± 1.1	3.2 ± 1.0
Cholesterol: baseline (mg/dl)	409.9 ± 25	484.4 ± 25	466.5 ± 22	429.1 ± 15
Cholesterol: final (mg/dl)	328.2 ± 14	407.5 ± 18	378.4 ± 19	385.3 ± 12
Cholesterol: delta (mg/dl)	-79 ± 25	-78 ± 33	-105 ± 17	-44 ± 15
Urea: baseline (mmol/l)	16.3 ± 0.9	15.9 ± 1.0	15.9 ± 0.7	17.0 ± 0.6
Urea: final (mmol/l)	17.0 ± 0.7	14.1 ± 0.6	15.6 ± 0.5	15.3 ± 0.7
Urea: delta (mmol/l)	0.71 ± 0.9	$-1.78 \pm 0.8*$	-0.26 ± 0.5	$-0.73 \pm 0.5*$

Values are presented as mean \pm SEM

Cas casein, Sed sedentary, Soy soy protein, Ex exercise trained

* P < 0.05 for diet main effect



Fig. 1 Atherosclerotic lesions and aortic calcium in sections corresponding to the cusp of the aortic valves (*Area A*), the branch point of the coronary arteries (*Area B*), and the ascending aorta (*Area C*). There was a significant interaction between experimental diet treatment and exercise training on atherosclerotic lesions at the site

The Soy/Ex group showed a 61% increase in relative bone volume compared to the Cas/Sed animals. There were no interactive effects on measures related to bone architecture, but there were several main effects of our treatments. which included a significant main effect of diet $(F_{1.50} = 13.112; P = 0.001)$ and activity $(F_{1.50} = 11.325;$ P = 0.002) on trabecular number, with the Soy/Ex group tending to have the greatest number of trabeculae compared to Cas/Sed (Fig. 2b). There were also significant main effects of diet $(F_{1.50} = 9.990; P = 0.003)$ and activity ($F_{1,50} = 5.873$; P = 0.02) on trabecular separation (Fig. 2c). Again, the Soy/Ex group tended to have less separation compared to Cas/Sed. There were also significant main effects of diet ($F_{1,51} = 9.950$; P = 0.003) and exercise ($F_{1.51} = 4.149$; P < 0.05) on the log transformation of trabecular connective density (Fig. 2d), with a trend for an interaction effect (P = 0.089). In a manner similar to trabecular number and separation, the highest connective density was in the Soy/Ex animals compared to Cas/Sed. There were no effects of treatment on trabecular thickness $(0.0375 \pm 0.001, 0.0482 \pm 0.001, 0.0515 \pm 0.001, and$ 0.0492 ± 0.001 for Cas/Sed, Soy/Sed, Cas/Ex, and Soy/ Ex, respectively).

Discussion

The primary findings in this study were that both a soy protein diet and exercise training significantly increased relative bone volume and improved bone microarchitecture in a mouse model of disordered lipid metabolism and surgically induced renal insufficiency. The effect tended to be greatest when these interventions were administered in combination. Although the interaction effect was not significant for any of the bone measures (with the exception of total volume as a component of relative bone volume, or



corresponding to the ascending aorta (*Area C*) with significantly higher lesion area in Cas/Ex (**a**). There was no significant interactive or main effects of diet or activity on aortic calcium score (**b**). *P < 0.05 for an interaction effect at this site. *Cas*, casein; *Sed*, sedentary; *Soy*, soy protein; *Ex*, exercise trained

BV/TV), the Soy/Ex combination group tended to have more favorable bone variables when compared to the other treatment groups. Furthermore, we found a main effect of diet on the change in plasma urea levels during the intervention period, with animals on the soy diet having lower plasma urea levels. Finally, neither soy nor exercise had much effect on the vasculature. There were no group differences in aortic calcium in any of the three proximal aorta sections, and no interactive or main treatment effects in atherosclerosis in two of the three proximal aorta sections. Surprisingly, we observed an interaction effect of diet and exercise on atherosclerotic lesion area in the section of the proximal aorta corresponding to the start of the ascending aorta, with the exercise-only animals having the greatest lesion area. However, this effect was ameliorated with the combination treatment. These data suggest that a combination of a soy-rich diet and endurance exercise training may be beneficial for protection of bone health and preservation of renal function in individuals with CKD, although the effects in the vasculature remain unclear.

Observational studies have linked high soy protein consumption with lower osteoporotic fracture risk, but the treatment effect of soy protein on bone density and microstructure remains controversial in healthy populations, and even less is known about the effects of soy protein on bone health in the context of CKD. Intake of soy protein has been shown to improve BMD whereas other studies have shown no effect on a variety of bone parameters, and much attention has been focused on the phytoestrogens contained in soy protein. Furthermore, recent research suggests that the efficacy of soy protein on bone depends on the ability of the body to convert the soy isoflavone daidzein to eqoul, a potent estrogenic metabolite. Only 20-35% of the population has been reported to have the ability to metabolize daidzein to eqoul via intestinal microflora [36], which may partially explain the differential responses to soy protein

Fig. 2 Relative bone volume and bone microstructure in response to dietary and activity intervention. There were no interactive effects for diet and activity on any variable measured. However, there were significant main effects of soy protein diet (*P < 0.05) and exercise training (*P < 0.05) on relative bone volume (**a**), trabecular number (**b**), trabecular separation (**c**), and connective density (**d**)



interventions in the context of bone health. The effectiveness of equol production in the context of kidney disease is not known. In addition, mice produce equol prolifically when fed diets containing soy protein [37], which may explain why the efficacy of soy protein is more modest in human studies. In this study, we found a significant main effect of diet not only on relative bone volume, but also on bone microstructure including trabecular number, thickness, and density.

Furthermore, we also found a main effect of activity on the same parameters, with a trend toward a greater effect in the combined soy plus exercise group. Several studies have found a synergistic effect of phytoestrogens and exercise on improving bone parameters in ovariectomized mice, rats, and premenopausal women (reviewed in [38]). Exercise is known to stimulate the estrogen receptor on bone, and this action may contribute to the enhanced bone microarchitecture seen in the soy and exercise combination group. Oh et al. [25] have suggested soy isoflavone supplementation protects against exercise-induced oxidative stress, which may explain why the benefits demonstrated in this study were more pronounced in the combination group compared to exercise training alone. Although many of these animal studies used subcutaneous injections of soy isoflavones in doses ranging from 0.4 to 6.4 mg/day, we have demonstrated beneficial effects of intact soy protein on bone health in our uremic mouse model using 250 mg isoflavones/kg diet with an effective dose of about 0.625 mg isoflavones per day. We believe the distinction between soy protein use and isoflavone supplementation to be important, as studies have suggested that the benefits of soy may be the result of other components of the intact protein in addition to the phytoestrogenic effects of soy isoflavones.

Most studies have investigated the effects of soy protein on bone loss in women, targeting the stages before and after menopause. However, metabolic and hormonal conditions and the subsequent consequences on bone may be different for an individual with CKD compared to healthy pre- or postmenopausal women. Thus, the benefits of soy in pre- and postmenopausal women may not translate to this population. Specifically, CKD patients can experience both high- and low-turnover bone disease in response to the metabolic changes induced by declining kidney function. To date, no study has looked at the efficacy of soy protein for bone parameters in the context of CKD, either with animal models or in human populations. Furthermore, soy protein may represent a valid therapy in this population for treating CKD associated bone conditions, as studies have shown that soy proteins do not effect glomerular filtration rates or postprandial renal blood flow in the same way that animal proteins do after a high-protein meal [39]. Therefore, with soy protein consumption, CKD patients are able to obtain the benefits of protein without the negative effects on the kidneys that have been reported for animal proteins.

In addition to the mechanisms already listed, soy protein supplementation may have effects on CVD that are specific to CKD. For example, soy supplementation has been associated with reduced oxidized low density lipoprotein (LDL) in hemodialysis patients [40], reduced urinary albumin excretion and LDL/high density lipoprotein (HDL) cholesterol ratio [41] in patients with diabetic nephropathy, and preservation of renal function in moderate kidney disease

[41, 42]. In this study we chose plasma urea as a measurement of the kidney's ability to metabolize nitrogenous waste; this ability is impaired with renal insufficiency, and the consequent buildup of urea and other toxins may contribute to the common comorbidities of CKD. We found a main effect of diet on change in plasma urea levels measured at baseline and before death, suggesting that soy protein may improve uremic conditions. Protein restriction as a means to preserve renal function has been widely practiced for many years, but the efficacy of this treatment has recently been called into question [43]. As individuals with CKD progress to renal failure, the incidence of protein wasting sharply increases [44] and is associated with low quality of life, low physical functioning, and even mortality [45]. The high prevalence of protein malnutrition in CKD patients may be caused, in part, by clinical recommendations to restrict protein during moderate to severe renal impairment. As this study suggests, the addition of soy protein in moderate CKD may not have any detrimental effects on kidney function and has the potential to slow or prevent the occurrence of protein wasting in later stages of CKD.

We did not see any improvements in the vasculature, either in atherosclerotic lesion area or aortic calcium score, for any of the treatment groups compared to the control animals. In fact, we found an interaction effect of diet treatment group and exercise training on atherosclerotic lesions at the site of the proximal aorta corresponding to the ascending aorta, with the exercise-trained animals having a significantly higher lesion area compared to the other groups. Combining the soy protein diet with exercise seemed to ameliorate this effect, but the lesion areas in the combination group were not significantly different from those of the control sedentary animals. Exercise training has been shown to reduce CVD risk in individuals with normal kidney function and to improve physical function in CKD, but virtually no studies to date have investigated exercise training on CVD outcomes in CKD patients, and these studies have focused on patients with renal failure (reviewed in [46]). Phan et al. [47] showed that pharmacological treatment in an identical animal model slowed the progression of uremia-associated atherosclerosis, whereas the same group found a reduction in atherosclerosis with the antioxidant *n*-acetylcysteine [48], highlighting the high burden of oxidative stress in CKD. However, we did not see such an effect with dietary or exercise intervention on aortic atherosclerosis or calcification, which may have been caused by the severity of the surgical procedure used to create this model of renal insufficiency. It is possible that the deficiency of apoE combined with the high stress of the 5/6 nephrectomy was too severe to warrant endurance exercise training as a therapeutic means to reduce uremia-associated CVD with this particular model. Studies on the beneficial effects of exercise on cardiovascular risk in humans with varying degrees of kidney function are clearly needed.

There were several limitations to this study. First, we believe that the severity of the animal model may have prevented the dietary and exercise intervention from having any effect in the vasculature. As these are primarily preventative measures, this may provide a rationale to begin such treatments before the development of severe renal impairment. Additionally, it is not known whether the same effects on the vasculature or bone would be seen in male mice, accounting for the estrogenic properties of soy isoflavones. As mentioned earlier, the efficacy of soy protein may depend on the ability to metabolize the isoflavone daidzein to eqoul; all mice are eqoul producers whereas only some humans possess this capability. Therefore, it is unclear if these results would be seen in humans with CKD. Food intake was not measured with these animals, so we are not able to determine the effect of food intake on the results of this study. Finally, apo $E^{-/-}$ animals have been shown to have an increase in bone mass compared to wild-type mice; renal insufficiency in this model decreases bone volume. It is not known, on the basis of this current study, if the increase in bone volume would translate to improved fracture risk or if it represents a pathological disorder of bone remodeling. Furthermore, without indices of bone formation and resorption, we are unable to make these determinations.

In summary, we found beneficial effects of soy protein and exercise on properties of bone and plasma urea in mice with surgically induced renal impairment, showing a trend toward a stronger effect with a combination approach. Further research involving individuals with CKD is needed to test the efficacy and practicality of these lifestyle interventions on preventing the decline in cardiovascular and bone health associated with CKD and perhaps the preservation of residual renal function in early stages of the disease.

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