Exercise to Prevent Muscle Mass and Functional Loss in Elderly Dialysis Patients

NCT #01990495

Date: 5/31/2020

02a. Research Plan

Background and Significance.

Prevalence and Consequences of Chronic Kidney Disease. In the United States, the proportion of the population over 65 years is predicted to grow from 12.4% (35 million) in 2000 to 19.2% (71 million) in 2030 (1). Thus, the proportion of elderly dialysis patients that currently comprise approximately half of the incident adult US dialysis population can be expected to increase (2). Aging alone leads to a loss of muscle mass and strength with decreased function, increased atrophy, and eventually frailty following bed rest (1; 3; 4). Many of the causes of age-related wasting are the same as those in maintenance hemodialysis (MHD) patients, including nutritional and hormonal derangements, oxidative stress, inactivity and co-morbid conditions (5;6). In *elderly* dialysis patients the situation is compounded, since uremia *per-se* causes a loss of muscle mass and function and together with the aging process predisposes these patients to the development of frailty with an increase in fall-related injuries, a decrease in quality of life (QOL) and an increase in morbidity and mortality (7; 8). In chronic kidney disease (CKD) physical function declines as renal failure progresses (9) and in those new to dialysis the vast majority (up to 95%) report significantly impaired physical function and reduced activity which is worse in the elderly (10). Older dialysis patients have even more co-morbidities and functional limitations; the death rate of prevalent dialysis patients increases by 2-fold in those aged 66-74 and approximately 3-fold in those aged 75-79 vs. those <65 years (2;11). Moreover, Kurella-Tamura (12), studying elderly nursing home residents with multiple co-morbidities, noted that during the first dialysis year, 58% died, 29% had a decrease in functional status, and physical function was maintained in only 13%. Current recommendations to prevent and manage aging events in patients with MHD include provision of an adequate nutrient intake and regular exercise (3-6). Exercise-based rehabilitation is cost effective and has been demonstrated to improve muscle mass, muscle function, and QOL in MHD patients (13;14). This highlights the need for rehabilitation during treatment in patients with CKD, whether they undergo dialysis or conservative management. However, implementation of rehabilitation as a standard treatment modality in patients with CKD is lacking (13).

Mechanisms of Loss of Muscle Mass in CKD. Several processes operative in uremia contribute to loss of muscle mass and function by affecting protein turnover. These processes include reduced nutrient intake, inflammation, acidosis, anemia, oxidative stress, resistance to and/or deficiencies in anabolic hormones (15), and reduced physical activity and disuse due to concomitant illnesses. Together these alterations lead to an increase in muscle proteolysis, largely because of activation of the ubiquitin-proteasome system (16), while protein synthesis may also be diminished (17; 18). Other proteolytic systems including lysosomal and caspase systems may also be involved (16-18). Maneuvers to correct or prevent muscle wasting in uremia have included correction of acidosis, anabolic hormone therapy, and exercise (14; 15; 19; 20). Provision of adequate dietary protein and calories can help, but in some patients supplements may be required (21; 22). Abnormalities in amino acid (AA) levels are present in CKD (23; 24). Plasma levels of several essential AA (EAA) especially branched-chain AA (BCAA) including leucine, are reduced (25), while non-EAA levels may be abnormally elevated (26). Numerous metabolic abnormalities at the cellular level have also been described in CKD (25). Regarding the increased catabolism and loss of AA that occurs during MHD, intradialytic parenteral or enteral AA and calories acutely stimulate whole body and muscle protein synthesis and inhibit whole body (22; 26), but not muscle proteolysis (27). Of note, net muscle protein accretion can be augmented by concurrent exercise (20; 28). The nutrient effect appears to arise from correction of AA deficiencies and stimulation of insulin release. In addition, EAA, especially the BCAA leucine, directly activates the mTOR signaling pathway, which promotes translation initiation of protein synthesis. Whether the AA stimulated mTOR signaling response is intact in patients with end-stage renal disease (ESRD) is at present unknown. In rats with CKD, it has been reported that leucine-stimulated insulin secretion is impaired (29). Nevertheless, in

a recent study with rats with CKD we observed that leucine does stimulate the mTOR anabolic signaling pathway, albeit less effectively than in normal controls (30). In normal humans, EAA are highly effective in stimulating protein synthesis if ingested at the time of resistance exercise, a maneuver that also activates mTOR signaling, but earlier in the signaling pathway (31) (Fig 1).

Effects of Exercise Training in CKD. While not a uniform finding (32), several studies in advanced CKD and ESRD have reported that exercise increases the mass of the exercised muscle, though not necessarily lean body mass (33-35). Failure to do so may reflect the local response to exercising just one set of muscles (e.g. lower limbs). In contrast, regular exercise involving trunk, leg and arm muscles increases total body K content, a measure of body cell mass (33). In general, resistance exercise in particular is effective in increasing muscle mass, muscle fiber area, improving strength and function and even reducing inflammation in CKD (33-35). Interestingly, it was reported that prolonged endurance exercise can increase muscle fiber size in ESRD (32-36), which is not the usual response in normal subjects. Nevertheless, endurance exercise appears to be therapeutic, since it improves muscle strength as well as cardiopulmonary function (13; 14; 35-37). Regular endurance exercise also has a marked effect on cardiovascular risk factors in ESRD subjects (14). Both intensive and moderate aerobic training have been shown to improve cardiovascular performance in MHD subjects, and studies have consistently shown an increase in peak VO_2 following rehabilitation programs, with an average increase of 17% (14; 36; 37). Peak VO_2 is the gold standard for cardiopulmonary fitness since it incorporates elements of cardiac output, metabolic changes in skeletal muscle, vascular function, and muscle strength (38; 39). Moreover, peak VO_2 is an independent predictor of survival in MHD patients (40). In studies employing *combined endurance and resistance exercise* in MHD, significant increases in muscle mass and function have been demonstrated (41; 42). Indeed, clinical trials and guidelines on physical activity and health in non-renal subjects suggest that peak VO_2, muscular strength, and endurance are optimally improved by programs using combined aerobic and resistance exercise vs. either one alone (38; 39). Because of its potential benefits, there have been numerous short-term studies of resistance and/or endurance exercise training in CKD patients in different venues including facility-based, MHD unit-based and even home-based exercise training (14). Overall, studies in patients with CKD have suggested that in addition to improving muscle mass, strength and functionality, regular exercise has a salutary effect on cardiac and respiratory function, endothelial function, insulin sensitivity, lipid levels, inflammation, psychosocial status and QOL, and has been shown to be safe (14; 43). The potential cardiovascular benefits of exercise may be particularly important for ESRD patients, who have a high prevalence of cardiovascular disease that accounts for nearly half their deaths (2; 44).

However, most of the exercise studies in patients with MHD have involved small numbers, lack randomized controls, were of short duration, and enrolled relatively young subjects in relatively good condition. *Notably, there is a dearth of studies specifically targeting the elderly (5)*. In addition, there is limited information on the cellular mechanisms underlying the salutary effect of exercise in humans with ESRD (45-47). Moreover, the precise relationship between muscle wasting with loss of function and poor long-term outcomes in elderly ESRD patients remains to be established. The extent to which muscle atrophy is a consequence of underlying disease processes and the extent to which it actually contributes to outcomes is also unclear (47). Thus, there is a need for large-scale prolonged studies to establish the true long-term benefits of exercise on morbidity and mortality in this population. It may be that given the natural limit of lifespan and the co-morbid conditions common in elderly MHD patients (8), the main benefits of exercise are improvements in physical function, activities of daily living, QOL, and a decrease in adverse events (e.g. falls and hospitalizations, which are more frequent in the elderly dialysis patient than the general population) (7). Sorely needed as a prerequisite to such large, long-term studies is development of a low-cost exercise program that will be relatively easy to administer and adhere to, and that will form part of routine ESRD care (13; 47). In this regard a recent short-term study reported the feasibility and potential effectiveness of home-based exercise in dialysis patients, half of whom were over

age 60 (48). Lastly, a small number of studies of exercise training among elderly CKD subjects, *not yet on dialysis*, have also shown promising results on multiple domains of physical function (5).

Protein Signaling in CKD. The mechanism whereby resistance exercise induces muscle enlargement in uremia is incompletely understood, though from our work and others (45; 49), a change in the balance between local IGF-1 (up), and myostatin (down) and also activation of anabolic signaling pathways, especially the PI3K/ mTOR pathway, which is depressed in CKD, appear to be important (50). Exercise also increases muscle androgen receptor expression in normals (51). Among the factors regulating muscle mass, IGF-1 plays a key anabolic role (52; 53). IGF-1 is synthesized under the influence of growth hormone (GH) and nutrition (54). In skeletal muscle, expression of IGF-1, including the IGF-1Ea mRNA transcript and the splice variant mechano growth factor (MGF), is also regulated by mechanical stimuli induced by exercise independent of GH, and plays a key role in exercise-induced hypertrophy (52; 53). Indeed, in uremic rats we showed that muscle work overload effectively stimulates local IGF-1 expression, despite resistance to GH-induced IGF-1 expression (49). In addition, expression of myostatin, an inhibitor of muscle growth which is elevated in CKD, decreased. These responses provide explanations for the beneficial effects of exercise in MHD patients. Indeed, the same response to exercise was subsequently observed in MHD subjects (45). In ESRD skeletal muscle IGF-1 and IGF-1 receptor mRNA levels are reported to be reduced (55) and in patients on MHD with muscle wasting, IGF-1 peptide levels are low, even when serum levels are unchanged (56). Generally, serum and muscle IGF-I levels are normal or even elevated in adults with ESRD (55; 56), but are reduced in the severely malnourished wasted subject (54). IGF-1 bioavailability may be reduced because of increased serum IGF binding proteins (57). Also contributing to uremic muscle wasting is impaired IGF-1, and insulin signaling, mediated through the IRS-PI3K/Akt signal transduction pathway (50; 58). Normally, activation of Akt/ PKB, leads to mTOR phosphorylation which then activates the 70-kDa ribosomal S6 kinase (p70S6K), eventually leading to increased protein synthesis (59; 60). Akt activation also suppresses proteolysis (26). *Notably, the human studies described above were performed in relatively young ESRD populations and data specifically for the elderly are not available.*

The signaling pathways through which IGF-1 stimulates muscle growth are similar to those activated by mechanical stimuli alone [Fig 1], and it is difficult to distinguish between these stimuli, especially as mechanical stimulation leads to a local increase in IGF-1 (53; 61). Both mechanical and IGF-1 induced activation of the Akt/mTOR pathway are essential for promoting muscle growth and repair (53; 59). How mechanical stimuli activate the PI3-kinase/Akt/mTOR pathway and cause muscle hypertrophy are poorly understood (61). One way is through growth hormone-independent stimulation of local IGF-1 expression (52; 53). mTOR signaling is also directly activated by EAA, particularly leucine, and then mTOR via its downstream signaling proteins, up-regulates mRNA translation initiation and thereby stimulates protein synthesis (Figure 1) (30; 62). Thus mTOR appears to serve as a nutrient sensor for EAA, but how this occurs is unknown (104). AA also elicit an anabolic response by increasing insulin secretion, but the signaling responses to AA and insulin are independent though complimentary (62). From these studies it is clear that AA function more than simply serving as a key protein synthesis substrate.

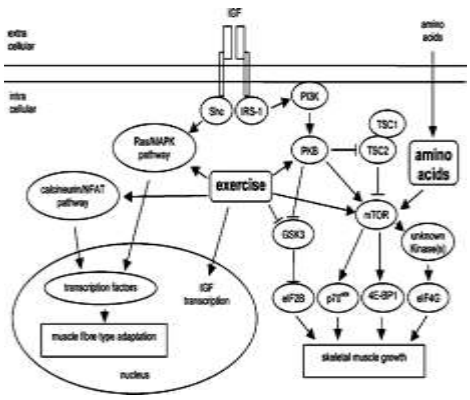


Figure 1. IGF-1/insulin, exercise and amino acid activated signaling pathways in skeletal muscle. *IGF-1 and insulin* receptor binding activates the IRS-1 PI3K-mTOR and the calcineurin/NFAT and RAS/MAPK pathways leading to increased protein synthesis and decreased proteolysis. IGF-1 also induces muscle repair and growth. *Exercise* acts upstream of mTOR via Akt/PKB to promote muscle fiber adaptation and growth. Mechanical stress also acts by stimulating local IGF-1 production. *Branched chain amino acids*, especially leucine, directly activate mTOR and in turn its downstream mediators, eukaryocytic initiation factors (eIF)4E binding

protein 1 and possibly eiIF4G to enhance assembly of eIF4G with eIF4E and 70-kDa ribosomal S6 kiinase 1, thereby stimulating mRNA translation initiation and ultimately protein synthesis (62). Figure from (30).

The activated PI3K/Akt pathway also inhibits protein degradation. It does so by inactivating the FOXO transcription factors that increase expression of the atrophy-related ubiquitin ligases, atrogin-1/MAFbx and probably MuRF-1, and thus suppresses proteolysis mediated through the ubiquitin-proteasome pathway (16). Caspases, an important component of the degradation process, cleave complex proteins into smaller products rapidly degraded via the ubiquitin-proteasome system (16). In CKD and other catabolic states, PI3-kinase/Akt signaling is depressed (50; 58) and consequently protein synthesis and FOXO inactivation are depressed. This permits FoxO to stimulate the E3 enzymes atrogin-1 and muscle ring finger-1 (MuRF-1), favoring proteolysis through the ubiquitin pathway. Inflammatory cytokines such as TNF-α, may also increase degradation by activating NFK-κB, which leads to increased expression of the E3 enzyme MuRF-1 (16). Protein synthesis is attenuated and degradation through the ubiquitin-proteasome pathway is accelerated, especially if acidosis is present. Caspase-3 activity is increased, accelerating actinomysin cleavage with increased formation of a 14-kD actin fragment, a muscle proteolysis marker (16; 46). Interestingly we and others noted in uremic rodents that by simply increasing muscle workload, the IRS-1/PI3-kinase/Akt signaling pathway can be fully activated and this leads to a decrease in proteolysis, with a reduction in actin fragment level, increased protein synthesis and muscle hypertrophy (50; 63). In MHD patients, endurance exercise decreases the elevated fragment levels (45).

There has been intense interest in establishing whether the anabolic response to exercise can be enhanced by providing supplemental EAA (64-66). In normals, leucine-rich supplements are especially effective in promoting muscle protein synthesis and when combined with resistance exercise cause an even greater increase in muscle protein synthesis than exercise alone (64; 67). A similar response is seen with endurance exercise (68). Among commonly used protein supplements, whey is of special interest. It is readily digestible, is rich in EAA (~50g/100g/protein), particularly leucine (14g/100g), has a low Na, K, and phosphorus content, is inexpensive, and is effective in enhancing muscle protein synthesis (66; 69; 70). Hulmi (71) reported that whey acutely increased anabolic signaling in resistance exercise-trained normal subjects over 10.5 weeks. Regular exercise with whey supplementation caused a greater increase in muscle mass than exercise alone. Commonly a carbohydrate is added to the whey or AA supplement to stimulate insulin release and inhibit proteolysis induced by exercise (72). This combination also stimulates anabolic signaling induced by endurance exercise (68). Since in ESRD alterations in serum and intracellular AA profile with reduced serum BCAA levels are common (17; 23), provision of EAA at the time of exercise is likely to be especially effective. However, it is conceivable that there may be resistance to the signaling response to AA as there is for insulin/IGF-1 in CKD (50; 58). In healthy elderly subjects there is some impairment to AA-induced protein synthesis (73), and this can be overcome by increasing the leucine load (66), particularly in the form of whey protein (74). In a study with CKD rats, described below, we found that leucine alone largely corrects and stimulates the anabolic signaling defect present in these rats (30). Since leucine also stimulates protein synthesis in non-exercising humans including the elderly, it is recommended that the diet of the elderly include leucine-rich foods to prevent and treat sarcopenia (6; 75).

Specific Aims:

Specific Aim 1. To devise a safe, low-cost aerobic and resistance exercise regimen to counteract the loss of muscle mass and function common in elderly MHD patients that is easily implemented, suitable for the home and to which most subjects will adhere.

Specific Aim 2. To elucidate the mechanisms whereby a program of exercise-based rehabilitation counteracts muscle wasting in elderly ESRD patients.

Specific Aim 2A. To determine whether the mTOR anabolic signaling response to exercise or a high-calorie leucine-rich protein load is intact in elderly MHD patients and whether the response to exercise is enhanced by providing this supplement at the time of exercise.

Hypotheses:

Hypothesis # 1. Regular exercise, by counteracting muscle wasting, enhancing cardiopulmonary and muscle function, and reducing cardiac risk factors, will improve muscle mass, cardiopulmonary function, and psychosocial health in elderly MHD patients. We anticipate that our approach to implementing a home-based rehabilitation program, modeled on a program effective in cardiac patients, in a rigorous randomized clinical trial will provide an innovative and relatively low-cost approach to the management of ESRD patients. It is our hope that this proposal will lead to future studies in which long-term outcomes of elderly MHD subjects will be improved.

Hypothesis # 2. The mechanisms whereby regular exercise counteracts muscle wasting in elderly ESRD patients are explained by changes in local skeletal muscle expression of IGF-1, androgen receptors and myostatin, and by stimulating Akt signaling pathways that promote protein synthesis and inhibiting proteolysis.

Hypothesis # 2A. Provision of leucine-rich protein at the time of acute exercise will stimulate skeletal muscle anabolic signaling in uremia. By augmenting exercise-induced signal transduction, this will increase the anabolic response to exercise.

Significance:

Significance of Specific Aim 1. As described above, muscle wasting with frailty and loss of muscle function and reduced exercise capacity are common in advanced CKD. This is particularly true in elderly CKD patients, and these factors have been shown to be associated with increases in morbidity and mortality (5; 11). These issues also compromise the elderly patient who might otherwise be suitable for transplantation (75). One simple approach to these problems is an exercise program, an approach that has been effective in the long-term management of patients with cardiovascular disease (76), including the elderly (77; 78). Such programs in patients with cardiovascular disease have been consistently associated with improved survival rates (79). Exercise programs, at least in the short term, also appear to be effective in the management of MHD patients (14; 80), but there have been logistic, economic and compliance barriers to implementing exercise regimens in these patients, and the long-term impact on morbidity and mortality has not been established. Thus, despite evidence of short-term benefits, at least in younger patients, exercise is not part of routine MHD patient care. The primary thrust of this application is therefore to devise an effective, safe and low-cost home-based exercise regimen that is easily implemented and in which a substantial number of elderly MHD patients could potentially participate and adhere to, and that improves surrogate markers of long-term benefit. We do realize that given natural lifespan limits and the co-morbid conditions common in the elderly, the main benefits of exercise in elderly MHD patients are likely to be improvements in physical function, activities of daily living and QOL, and a reduction in adverse events such as falls and need for medical attention. If the present study yields a positive outcome, then it will form the basis of a broader study to examine the long-term outcomes of this regimen in both elderly and younger patients on MHD. The results could also have important implications for non-renal patients with other catabolic diseases. Thus, this study holds the potential for serving to increase QOL and to reduce morbidity and possibly mortality rates in many dialysis patients and at considerably lower cost than other approaches under consideration, such as daily dialysis. Moreover, if effective, the study may serve as a model for implementing home-based exercise for advanced CKD patients not needing MHD, and for establishing clinic-based exercise programs for MHD patients unable to undergo home exercise.

Significance of Specific Aim 2. We propose that regular exercise acts largely by: (a) inducing changes in local skeletal muscle expression of IGF-1, androgen receptors and myostatin; and (b) by stimulating Akt signaling pathways that promote protein synthesis and inhibiting proteolysis. This aim will provide novel information regarding the impact of uremia on molecular events that regulate muscle mass in response to exercise training in CKD. In addition, this may lead to novel strategies for preventing muscle wasting in patients with CKD, and provide an impetus for the use of rehabilitation as a treatment strategy for patients suffering from this condition.

Significance of Specific Aim 2A. We postulate that leucine supplementation will stimulate muscle anabolic signaling in uremia by augmenting exercise-induced signal transduction, and will increase the anabolic response to exercise. This information will help elucidate the mechanisms whereby exercise counteracts muscle wasting in elderly ESRD patients. This analysis will also provide new information regarding nutrient supplementation and exercise-induced molecular events in uremia and may well serve as the basis for a more extensive study that combines regular exercise and dietary intervention as treatment options for managing protein energy wasting in elderly patients.

Relevance to the VA Patient Care Mission

CKD is a common condition in the VA; there are approximately 18,000 such individuals with end-stage CKD requiring MHD currently being treated in the VA system, and this condition is responsible for approximately 18% of all deaths in the VA (81,82). The number of persons affected by CKD is projected to increase by 35% between 2005 and 2015 (83; 84). Moreover, CKD is typically associated with other co-morbid conditions, including cardiovascular disease, resulting in frequent hospitalizations and a mortality rate ranging between 15 and 30 times that of age-matched healthy individuals (85). It has been estimated that a patient with end-stage chronic renal failure requires healthcare resources costing the VA Health Care System more than $50,000 annually, making CKD the second most costly condition in the VAHCS, second only to spinal cord injury (82). Notably, the majority of these costs are not attributable to dialysis, but rather to renal and other medical/surgical hospitalizations. This underscores the frequent and costly co-morbidities that are associated with advanced CKD. Moreover, total Medicare expenditures in 2005 for an elderly (≥67 years) dialysis patient (similar in age to our population) have been estimated to be >$100,000 per year. *Costs for MHD treatment in the VA system are expected to double over the next 10 years (81).* Exercise-based rehabilitation programs have been demonstrated not only to improve functional capabilities of patients with CKD (including those requiring MHD) through improvements in muscle mass, strength and cardiovascular function, but rehabilitation has also been shown to reduce disability and hospitalizations (14; 43; 86). However, these results have come largely from relatively small research-based studies and a major gap exists between these research findings and the clinical implementation of rehabilitation as a treatment modality in MHD patients. A home-based rehabilitation program that can be easily applied at minimal cost has the potential to substantially reduce healthcare costs associated with this condition.

We have recently implemented a National Institutes of Health-sponsored home-based rehabilitation program in patients with abdominal aortic aneurysm (AAA) disease, in which we observed marked improvements in exercise capacity, and in which no adverse events related to exercise testing or training occurred (87). Exercise training as a treatment modality in this population was novel, and the home-based, case management exercise model provided a training stimulus that would not have been possible with a conventional center-based program. We similarly anticipate that that a home-based exercise program in patients with MHD will be “user-friendly” and will effectively counteract muscle wasting, improve cardiopulmonary and muscle function, quality of life, and surrogate markers of long-term outcomes. New insight into mechanisms whereby exercise and nutrient supplementation induce an anabolic response in CKD will be provided that may assist in developing novel strategies to improve outcomes. Our broader goal is to assess the long-term effects of such a program at multiple centers. If the program described in the current proposal were to be implemented on a wider scale, the overall health of patients with advanced CKD requiring MHD could be markedly improved, and healthcare cost savings in the VAHCS could be

substantial. Moreover, if effective, the study may serve as a model for implementing home-based exercise for advanced CKD patients not needing MHD, and for establishing clinic-based exercise programs for MHD patients unable to undergo home exercise. Finally, this program could serve as a model for the management of Veterans with other chronic disease states such as advanced COPD.

Research Design and Methods

General study design: MHD subjects aged 65-80 years with impaired exercise capacity and function will be randomized into two groups (30/group). One group will undergo a home-based exercise program and the other usual care over a 12 week period. This subject number allows for a drop-out rate of 20%, while maintaining 80% power to detect a significant difference in our primary end-point, peak VO_2. After enrollment, all subjects will be encouraged to consume at least 1.2 g/kg/protein/day throughout the study. The 12-week program will include a 2-week clinic-based exercise instruction period with testing at baseline before intervention and after 6 and 12 weeks. Measures at baseline and 12 weeks will include body composition by anthropometrics and dual energy X-ray absorptiometry (DEXA), thigh muscle volume and composition by CT scan, muscle strength and function, cardiopulmonary exercise testing, QOL, cognitive function and nutritional, inflammatory, lipid and biochemical status. Muscle biopsies will be obtained at baseline and at 12 weeks and will be assayed for signaling proteins, and gene and protein expression of key muscle mass regulators. More limited interim testing will be performed at 6 weeks. In addition, two to three days after the 12-week study, a pilot sub-study will be performed to test whether a high-calorie leucine-rich protein supplement enhances exercise-stimulated anabolic signaling. At this time muscle biopsies will be taken. Overviews of the study measurements and timeline are presented in Appendices I and II.

For recruitment we will draw on a large patient population within 30-40 miles of the VA Palo Alto Health Care System (VAPAHCS) with travel reimbursement provided. *For example, the nearby Satellite Healthcare Centers have agreed to actively support the identification and recruitment of subjects, with their Chief Medical Officer, Dr. Schiller, serving as a significant contributor to this study. They operate 30 stand-alone dialysis centers within a 40 mile radius the VA Hospital in Palo Alto. These centers treat a total of 5,000 dialysis patients, with approximately 25% of these patients on home dialysis. At any given time, approximately 2% of their total patient population is enrolled in a clinical trial. There are 6 Satellite Healthcare clinics within 10 miles of the VA Hospital in Palo Alto, which provide MHD services to roughly 1,000 patients; these clinics will be a primary source of patients for the study.* At Santa Clara Valley Medical Center, a Stanford affiliate, ≈300 patients are treated with MHD and this facility has agreed to refer study subjects. Smaller dialysis clinics such as VAPAHCS (Dr. Rabkin), Stanford Medical Center (Dr. Chertow) and community nephrologists (letters attached) will also serve as resources. Combined, these resources provide a pool of ≈2,000 subjects in close proximity to our center. From prior experience, ≈ 5% of subjects will meet inclusion criteria and agree to participate. This will provide an adequate enrollment pool. While we expect the recruitment of subjects to reflect the distribution of women and minorities in our area, a concerted effort will made to recruit and include them in the study.

Study population and inclusion/exclusion criteria: Males and females aged 65-80 years undergoing MHD for at least three months and without other active/uncontrolled disease will be studied. Exercise and usual care groups will be *matched by age, body mass index (BMI), MHD duration, and protein intake* using a stratified randomization approach.

Inclusion criteria. MHD subjects with impaired exercise capacity and function will be considered for the study. Subjects will be required to be in the peak VO_2 range of 10 to 20 ml/kg/min, equivalent to moderate functional impairment in patients with heart failure (88; 89). Subjects will be required to have dialysis treatment for >3 months with an average Kt/V ≥ 1.2, and be able to perform exercise safely. Racial

and ethnic composition will reflect the diverse composition of the ESRD population in counties from which patients are referred.

Exclusion criteria. Current activity > 2 hrs/wk of moderate intensity exercise, temporary vascular access, uncontrolled diabetes mellitus, active vasculitis, active autoimmune disease, malignancy, severe obesity (BMI > 35), alcoholism or other recreational drug use, unstable cardiac disease (abnormal exercise test, angina, uncontrolled arrhythmias or myocardial infarction within three months), peripheral vascular disease (claudication with exercise), lung, liver or intestinal disease, those who are medically unstable and subjects who have received anabolic, catabolic or cytotoxic medications in the past 3 months. We will also exclude subjects with excessive previous exposure to radiation. Although “excessive" in regards to previous radiation exposure has not been defined by consensus panels and there are no national standards or regulatory guidelines that define this, we will not consider subjects for the study who have undergone a CT scan or any procedure with similar radiation exposure within the last year.

Screening. MHD subjects will be screened for eligibility and to ensure that they can safely complete the exercise program. Screening tests will include: Part 1: (a) cardiovascular risk factors (including smoking history, blood lipids, CRP, blood pressure), and VA cardiovascular disease score; (b) resting 12 lead ECG; (c) medical history and physical function questionnaires; (d) Kidney Disease QOL-36 instrument (90); (e) the VAPAHCS Physical Activity Questionnaire (91); (f) chair raise test; and (g) six-minute walk test. Part 1 screening results will be collected and presented for evaluation by the investigators in the Cardiology Service. If the subject is approved, they will proceed with Part 2 testing, which will include a treadmill exercise test with 12 lead ECG and measurement of peak oxygen consumption. If the subject is considered sufficiently low-moderate risk following the exercise test and shows no evidence of severe functional impairment on the 6-min walk test (<200 meters) and cardiopulmonary exercise test (<10 ml/kg/min), he/she will be scheduled for additional baseline testing.

Group Assignment. After screening and baseline testing, an independent third party investigator (Dr. Heidenreich, letter attached) will *match MHD subjects on age, body mass index (BMI), MHD duration, and protein intake,* and assign them randomly to the exercise or usual care study groups. At the end of 12 weeks of study this investigator will assign half of the subjects in each group to receive a blinded nutritional supplement (high calorie whey or placebo). The blind will be broken on completion of the study.

Dietary Control. Prior to entry into the study the subjects will be encouraged to consume 1.2 g protein/kg/day, per Kidney Disease Outcomes Quality Initiative (KDOQI) recommendations (92) and advised to maintain a steady intake. However, we realize that the recommended intake may not be achieved in some subjects. In order to adjust for variation in protein intake we will measure the subject’s protein intake on 3 occasions during the month prior to entry into the study. Then when assigning subjects to the study groups, measured protein intake will be taken into account by the third part study assigner, so that the protein intake in the 2 study groups will be similar. Protein intake will be monitored by three-day dietary records and the normalized protein equivalent of nitrogen appearance (nPNA,), a reflection of protein intake in stable conditions. nPNA is calculated from the urea generation rate by means of kinetic modeling using measurements of pre- and post-dialysis urea levels plus any urea lost in the urine in patients with residual renal function. After entry into the study nPNA is calculated weekly for the first two study weeks, then monthly. During the study period, there will be regular bi-weekly follow up counseling by a dietician in an effort to ensure that both groups meet target dietary protein intakes

Safety Measures. Before screening, subjects will be informed of the nature of the study and the procedures to be performed, and will sign an approved consent form. Measures to monitor and ensure safety include a screening ECG, maximal exercise test, and medical approval (see screening). The cardiovascular exercise lab has extensive experience in conducting clinical studies and has a staff of experienced personnel. We do not expect any long-term side effects from the proposed interventions. The exercise program is well balanced and varied with aspects of strength and aerobic training. Subjects will

have an adaptation period of several weeks in which they will progress at their own rate toward the desired intensity and duration goals. During the lead-in period, exercise variety and relatively modest energy output of the program will minimize risk of musculoskeletal and cardiovascular injury. Subjects will not take part in any other research project without investigator approval. All procedures will be performed by trained personnel in a hospital setting with emergency medical staff available. All samples and data will be stored and analyzed using subject codes to assure participant confidentiality. Interventions employed, including exercise testing and training, are of low to moderate risk.

Retention of Subjects and Compliance to the Intervention. We will select subjects highly motivated to improve and maintain their health. Thus, we expect high compliance and low attrition rates (up to 20% drop out). During recruitment, emphasis will be placed on the importance of completing the 12-week program. According to our power calculations (detailed below), we will need to retain at least 23 subjects/group to maintain a power of 80%. On completion of the study, subjects in the exercise group will receive a small payment ($350) for the time and inconvenience of 14 exercise clinic and lab visits. Subjects in the usual care group will receive $75 for their participation (4 clinic and lab visits). Participants in the muscle biopsy substudy will receive an additional $250. To encourage compliance and continued participation in the exercise program, the investigators will provide regular feedback including weekly telephone calls and encouragement regarding exercise and assistance with diets, scheduling etc., and will encourage subjects to return to the exercise clinic for evaluation and reinforcement every two weeks during the study.

A. Specific Aim 1 is designed to develop an effective, safe, low-cost aerobic and resistance exercise regimen. Because of the focus on improving cardiopulmonary function as well as muscle function and mass, both resistance and aerobic exercise will be components of the rehabilitation program. The program is modeled on a successful combined in-home/in-center study program administered by Dr. Myers at the VAPAHCS rehabilitation center for patients with cardiovascular disease with a mean age of 72 years (87). In general, exercise rehabilitation is highly effective in patients with cardiovascular disease (76), including the elderly (77; 78), and is associated with improved survival rates (76; 77; 79). Subjects randomized to the exercise group will undergo 12 weeks of exercise, including a 2-week ramp-up period conducted at the VAPAHCS rehabilitation facility. Subjects will be required to attend the facility twice a week on non-dialysis days during the initial 2 weeks of the study. These sessions will consist of 45-60 min of supervised exercise that combines aerobic and resistance training, including warm-up and cool-down. Throughout the study, subjects will be asked to perform 45 minutes of unsupervised aerobic exercise of their choice (such as walking) on non-dialysis days. Following the initial 2-week supervised period, subjects will be instructed to exercise on their own, and surveillance methods will be employed as previously described (87; 93) and outlined below. Following the 12-week study period, all subjects will undergo muscle biopsies. The following end-points will be measured at baseline and after the 12 week study period:

Primary Endpoint. Peak VO_2 is the primary outcome measure and is the gold standard for cardiopulmonary fitness. It incorporates elements of cardiac, pulmonary and muscle function known to improve with exercise.

Secondary Endpoints. These include lower and upper body strength, body composition, thigh muscle mass, muscle biopsy for protein signaling, quantitative muscle morphology and gene expression, daily activities, QOL, laboratory measures of cardiovascular risk factors, nutritional and inflammatory parameters as detailed below.

Together, these measures should allow us to determine whether the home-based exercise regimen is effective in counteracting loss of muscle function and mass common in elderly MHD patients, along with reducing cardiovascular risk.

Exercise training, detailed description. Initially, all subjects will undergo four supervised exercise sessions over two weeks. The purpose of these sessions is to familiarize the patients to their individualized training program, assess stability during exercise, ascertain that they understand their exercise prescription and how to use activity and heart rate logs, and provide guidelines and education in terms of what is expected of them during the study. Subjects will then exercise at home, with an in-clinic exercise session repeated every two weeks for the duration of the study. The biweekly exercise sessions will be used to ensure stability and compliance, to review activity logs, and to modify the exercise prescription as appropriate. Guidelines for patient monitoring, safety, and prescription outlined by the AHA, American College Sports Medicine, and AACPR will be followed (76; 94; 95).

Supervised in-clinic exercise *sessions*. These will include 5 min warm-up and cool-down sessions before and after a combination of continuous aerobic (treadmill walking, cycle ergometry, arm ergometry, rowing, stair climbing) and resistance exercise. Exercise intensities will initially be targeted to achieve 60% of HR reserve for 30 min; exercise intensity will be documented for each patient by frequent (5 min) recordings of heart rate and perceived effort. Progression of exercise intensity will be individualized in accordance with established guidelines (76; 94-96), but in general the goal will be to increase intensity and duration to 70 to 80% heart rate reserve and 45 min, respectively. Perception of effort will be targeted to 12-14 on the Borg scale. All in-house sessions and activity surveillance will be supervised by certified exercise physiologists. A nephrologist (R. Rabkin), cardiologist (V. Froelicher) and a clinical fellow will address patients' clinical concerns as necessary. As many MHD patients have a blunted HR response to exercise or are taking β-blockers, measured maximal heart rate will be used to develop exercise prescriptions, and patients will remain on their β-blockers for testing.

During the initial supervised sessions, resistance exercise will involve an introduction to a low-resistance, high-repetition regimen including upper and lower body major muscle groups under individualized supervision in accordance with established guidelines (76; 96). Resistance exercises will include leg press, leg extension, leg flexion, chest press, shoulder press, row, and lat pull-down. During the initial sessions, subjects will perform 12 to 15 repetitions at 70% of the 5-repitition maximum with a minimum 2-minute rest period between sets. Subjects will gradually increase to two sets of these exercises, with increasing resistance as tolerated. Subjects will be given hand-held weights and Thera-Bands in accordance with their capabilities, and instructed on their use at home based on their individualized prescription (see attached VAPAHCS brochures, Appendices II and III). Thera-Bands are special latex bands used for resistance exercise and have been effectively employed in MHD subjects (48).

Home training sessions. Subjects will receive detailed instructions on individualized exercise prescriptions, including how to monitor exercise intensity using heart rate and perceived exertion, and how to use heart rate and activity tracking devices (see below). Cycle ergometers will be provided for home use, but the subjects will be encouraged to achieve an individualized, targeted exercise stimulus using walking, stair stepping, and other available modes of exercise. Activity logs will be given to each patient to record activities, their intensity, duration, HR, and pedometer steps.

Detailed description of measurements:

(1) Vitals: Body weight, height, resting blood pressure and resting heart rate will be measured at the start of each testing appointment.

(2) Fitness, functional and cardiopulmonary testing:

(a) ***Peak aerobic capacity (peak VO_2):*** Peak VO_2 is considered the gold standard for cardiovascular fitness (39). Aerobic capacity will be determined on a treadmill using an individualized ramp protocol (97) with collection of continuous ventilatory gas exchange responses (38; 39).

(b) ***Exercise testing:*** Symptom/sign limited ramp treadmill tests will be performed on all patients at baseline and after 12 weeks of participation (97). A thorough review of clinical history, medications and risk factors will performed at the time of baseline exercise testing. Exercise capacity will be measured directly

using ventilatory gas exchange techniques. The Duke Treadmill and VA Prognostic Scores (38; 98) will be used to help estimate risk and assess suitability for exercise. Patients with evidence of significant coronary artery disease as determined by profound ischemic markers, ominous arrhythmias, or resting ECGs that confound the recognition of ischemia (bundle branch block, more than 1.0 mm resting ST depression, paced rhythm) will be excluded. Patients with hemodynamic instability or inability to exercise, those with complicating illnesses or questionable motivation to sustain prolonged training will also be excluded. The exercise tests will be performed, analyzed, and reported according to a standardized protocol and utilizing a computerized database (99).

(c) ***Measurement of strength:*** Strength will be measured at baseline and after the 12-week study period using leg extension 1 repetition maximum for lower body strength and chest press 1 repetition maximum for upper body strength. Maximal isometric strength will be measured using a hand-grip dynamometer. All assessments will be performed using standardized guidelines, including appropriate warm-up and familiarization trials, instruction, joint positioning, and rest periods between repeat tests (100).

(d) ***Six-minute walk test:*** This test incorporates elements of balance as well as strength and aerobic capacity. A standardized 6-min walk test (39) will be performed at baseline and after the 12 week study period. A 100 foot distance will be marked in a corridor, and patients will be instructed to walk from end to end at their own pace, attempting to cover as great a distance as possible within 6 minutes. The test will be supervised by a research assistant, who will provide standardized verbal encouragement at 30-second intervals. Distance covered is expressed in meters.

(e) ***Chair raises:*** Balance and strength will be measured using chair raises, a surrogate for muscle power. Subjects will be asked to rise with arms folded on the chest from a standard seat (46 cm high) and then return to a sitting position. Time to complete five repetitions and number of repetitions that can be completed in 60 seconds will be recorded. Chair raise capacity, validated against more extensive assessments (101), correlates strongly with muscle cross-sectional area and is reduced in ESRD (102).

(f) ***Additional monitoring of daily activities:*** In addition to formal training sessions, subjects will be encouraged to increase their daily activities and to exercise at a moderate individualized intensity for a minimum of 45 min each day they do not exercise at the hospital facility. Energy expenditure will be documented with a pedometer, a heart rate monitor and by intermittent accelerometer usage. We have recently validated these methods of estimating energy expenditure during home-based exercise (93). Brochures that we have used for instructing patients on the use of TheraBands at home are presented in Appendices III and IV, respectively. Patients will complete daily activity logs (Appendix V), and phone calls will be made weekly to monitor compliance, to complete the Veterans Exercise Testing Study (VETS) 7-day Activity Recall Questionnaire (Appendix VI), and to address any clinical concerns. Energy expenditure will be expressed in terms of kcals/week and MET/hours/week. If patients in the exercise group fail to meet their exercise goals, they will be strongly encouraged to increase their activity and monitoring will be increased. This approach is used at our facility (87; 93) and the PI is experienced in monitoring and surveillance of physical activity with these devices (93).

(3) Body composition and muscle area:

(a) ***Anthropometrics:*** Abdominal girth and thigh circumference.

(b) **Total body lean mass and percent body fat** will be assessed using whole body DEXA. Scans will be performed in duplicate at baseline and 12 weeks. DEXA has been effectively used for measuring body composition in ESRD patients, is recommended by the National Kidney Disease Outcomes Quality Initiative (K-DOQI) as a reference method to assess body composition in ESRD (92), and has been shown to be responsive to exercise training in numerous studies, including conditions associated with muscle wasting such as HIV and osteoporosis (103). As water is measured by DEXA in the fat-free compartment and thus may confound compositional assessment, MHD subjects will be carefully examined for fluid retention before scanning. Scans will be performed the day after dialysis at estimated “dry weight”.

(c) **Thigh muscle mass and fat composition** by computerized tomography (CT). Mid-thigh CT of the non-dominant limb will be performed a day after MHD, with subjects in the supine position with the thigh muscle relaxed. Axial CTs will be obtained at the midpoint between the trochanter of the femur and the superior border of the patella, using slice thickness of 2.5 mm. The radiographic film will be digitally scanned for analysis with a personal computer. The analyses will be performed by Dr. McIntire (Co-Investigator), who has extensive experience analyzing CT images in patients with CKD. He will be blinded to study group when processing the images. Thigh muscle cross sectional area (a measure of muscle mass) and interstitial adipose tissue embedded in muscle (reflects muscle quality) will be measured and computed. Measurements of mid-thigh subcutaneous and total fat will also be obtained.

(4) Muscle biopsy measurements:

(a) ***Biopsy procedure****.* Vastus lateralis muscle will be biopsied under local anesthesia and sterile conditions with a Bergstrom needle. This procedure will be performed by Dr. Rabkin (Co-PI), who has extensive experience in this area. A ~150 mg sample will be obtained by applying suction to the needle at the time of biopsy as described previously (104), trimmed of fat and connective tissue and bisected. One part will be frozen at -70°C, and the remainder will be mounted in embedding medium and frozen. Biopsies will be taken at the beginning and end of the 12 week study period as detailed below.

(5) Biochemical tests:

(a) ***Nutritional parameters:*** Serum albumin, pre-albumin and glucose will be measured in the PAVAHCS clinical lab. Serum IGF-I and IGF binding proteins (IGFBP1, 2, 3 and 5) will be measured by ELISA and Western ligand blots as previously described in our laboratory at 0 and 12 weeks (105).

(b) ***Insulin sensitivity from fasting serum glucose and insulin levels at 0, 6 and 12 weeks*****:** Insulin resistance appears in early CKD and is associated with an increase in cardiovascular disease (106). Resistance is characterized by an increase in serum insulin levels relative to serum glucose levels and will be evaluated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Fasting glucose and insulin samples will be obtained and the HOMA-IR calculated using the formula of fasting insulin (μU/ml) times fasting glucose (mmol/l) divided by 22.5. HOMA-IR is used frequently in epidemiologic studies and has been validated against a euglycemic-hyperinsulemic clamp technique (r=0.82-0.88) as an estimate of insulin sensitivity (107). We anticipate that insulin sensitivity, measured by HOMA-IR, will improve with exercise, though a recent study in MHD subjects failed to show any benefit of aerobic exercise (107). However, it is possible that this may have reflected a suboptimal exercise program. Insulin samples will be batched and analyzed with commercial kits in the PIs lab.

(c) ***Lipid panel:*** *(Fasting total cholesterol, LDL, HDL, ApoB, very low density lipoprotein, lipoprotein(a) and triglycerides)*. Individuals with ESRD are reported to have a high prevalence of dyslipidemia, particularly hypertriglyceridemia, and are at greater risk for CVD (44). The benefits of aerobic exercise on lipid profiles in non-CKD patients are well established, but the results in MHD patients are mixed (14). Lipid panels will be performed by the VA clinical laboratory at baseline and 12 weeks.

(d) ***C-reactive protein (CRP), inflammatory cytokines and complete blood count (CBC):*** Serum CRP and inflammatory cytokines such as IL-6 and TNF-alpha are elevated in many individuals with advanced renal failure, but can be reduced with resistance exercise (34). Batched serum samples will be assayed in the Co-PIs lab (Dr. Rabkin) for high sensitivity CRP levels by nephelometry, and IL-6 and TNF-alpha with ELISA kits at 0, 6 and 12 weeks.

(e) ***Total Testosterone, steroid hormone binding globulin and bioavailable TT*** will be measured in the VAPAHCS clinical laboratory. Measurements will be made at baseline and after 12 weeks.

(f) ***Renal biochemistry:*** BUN, Creatinine, CA, phosphate electrolytes, and bicarbonate will be monitored monthly.

(6) Questionnaires:

(a) ***Activity questionnaires & logs*** (Appendices IV and V). All subjects will keep **daily activity and treatment logs**. Daily logs will record compliance to the exercise regimen and quantify any changes in

physical activity during the study. Daily activity logs are helpful in completing the VETS 7-Day Activity Recall Questionnaire, which will be completed during a weekly phone call with a staff member (93).

(b) ***Diet*:** Subjects will be instructed on completing accurate **three-day food records** at the start of the study. Dietary records will be collected at 0, 4, 8, and 12 weeks. Dietary analysis will be performed using Nutritionist 5 (First Data Bank, San Bruno, CA) and subjects meet with a clinical dietician after each analysis to discuss results and make proper adjustments. nPNA will be used to assess protein intake.

(c) ***Health Related QOL (HRQOL):*** **HRQOL is a sensitive and validated means to assess a patient's perceived well-being in physical, psychological, and social areas of health** (90). ESRD patients have depressed HRQOL scores, but the literature indicates that physical activity can reduce depression, improve physical function, psychological well-being and social and family interactions, thus also improving HRQOL in ESRD (109). The Kidney Disease QOL-comprehensive instrument to evaluate QOL will be used (90). Dr. Kurella-Tamura (Co-Investigator) has published extensively in the area of QOL in CKD, and she will oversee these evaluations.

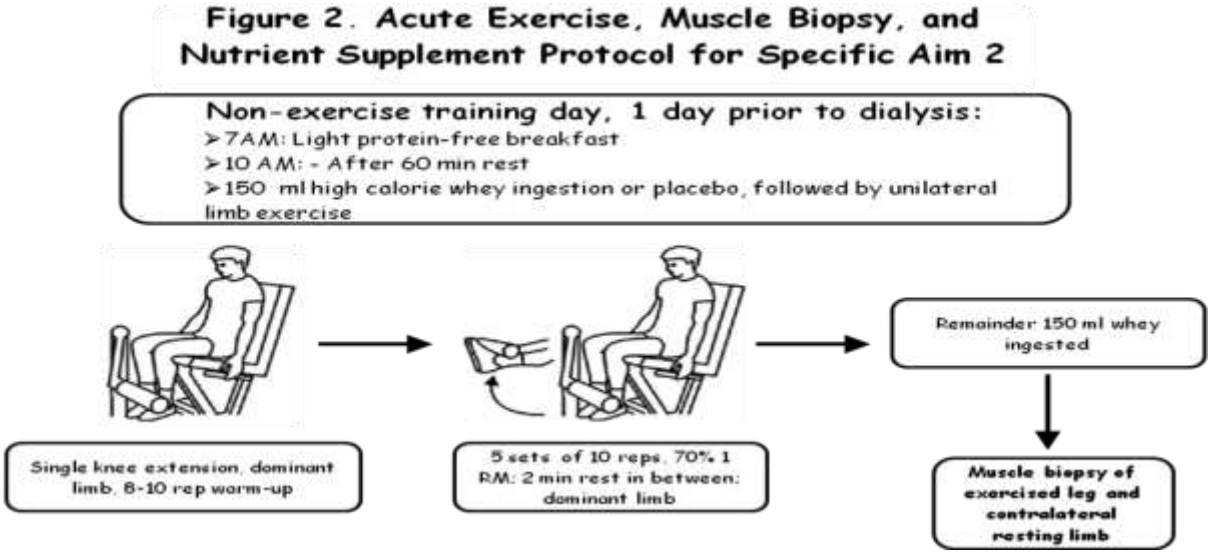
B. <u>Approach to Specific Aim 2. This pilot sub-study is targeted to elucidate the mechanisms whereby exercise training ameliorates or protects against muscle wasting in elderly ESRD patients and to test whether a nutrient supplement can enhance the anabolic response to exercise.</u>

Since this is a pilot sub-study carried out with a limited number of subjects after completion of the main 12 week study, we have adopted a unilateral exercise protocol in which the resting contralateral limb serves as a control. This protocol will allow us to gain considerable insight into the salutary molecular processes activated by exercise in ESRD subjects and to test whether these processes can be enhanced by provision of a leucine-rich supplement. In brief, two to three days after completing the primary 12-week study, subjects from both the exercise and usual care groups will be studied in which one limb is exercised for 15 minutes, after which the vastus lateralis muscles of both the exercised and the contralateral resting limb will be biopsied. *Half the subjects in each of these 2 main groups (12 subjects from each group) will be matched by BMI and age and randomized to either a placebo mixture or a high calorie nutrient supplement.* The nutrient supplement will consist of a high-calorie leucine-rich protein (whey protein) given at the start and end of the exercise session. The study consists of the following 4 groups, each undergoing acute, unilateral exercise:

Group 1. Regular exercise group plus placebo
Group 2. Regular exercise group plus nutrient supplement
Group 3. Usual care group plus placebo
Group 4. Usual care group plus nutrient supplement

An outline of the experimental protocol is illustrated in Figure 2, and a detailed protocol follows.

Figure 2. Acute Exercise, Muscle Biopsy, and Nutrient Supplement Protocol for Specific Aim 2



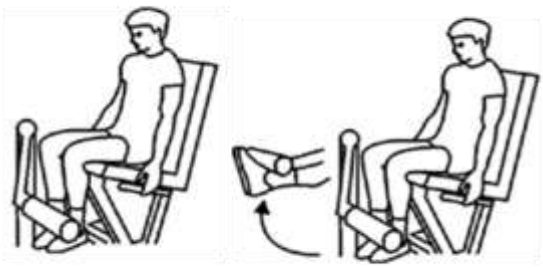
Biopsies will be obtained from the dominant limb after acute exercise and from the non-dominant limb following rest. A *single* biopsy of each limb will taken from each subject. Tissue taken from the biopsies will undergo molecular analysis to answer the following questions:

1. What sustained molecular processes, activated by regular exercise, might be contributing to the maintenance or improvement of muscle mass in MHD subjects who participate in a 12 week exercise program? To this end, molecular events in the ***resting*** dominant limb of participants given placebo in regularly exercised (Group 1) and usual care (Group 3) subjects will be compared. We will test the postulate that regular exercise acts largely by inducing local changes in the expression of key regulators of muscle mass and also by stimulating the Akt/mTOR signaling pathways that serve to promote protein synthesis and inhibit proteolysis. We anticipate that a 12-week program of rehabilitation will increase muscle mass as measured by CT and DEXA scans, and improve skeletal muscle function via several interrelated mechanisms. These include changes in the balance between IGF-1 and myostatin, increased androgen receptor expression, changes in expression of components of the ubiquitin-proteasome and calpain proteolytic systems, suppression of inflammatory cytokines, and increased anabolic signaling. This is illustrated in Figure 3.

Figure 3. Assessment of 12 weeks of exercise training vs. usual care in resting muscle among subgroups receiving placebo.

2. What impact does acute exercise have on anabolic signaling in regular exercise versus usual care subjects? To answer this question we will measure activity (phosphorylation) of the IRS-1/AKT and mTOR signaling transduction pathways in the ***acutely exercised*** (and the contralateral resting) limbs of regular exercised subjects ingesting placebo (Group 1). The same measurements will be made in the muscle of the corresponding usual care group (Group 3) (Figure 4). Activity of the anabolic IRS-1/AKT and mTOR signaling pathways in the exercised limb will be compared with these responses in the contralateral resting limb of the same subject. We anticipate that acute exercise will activate anabolic signaling in both groups and that the response will be more vigorous in the regular exercise group.

Figure 4. Assessment of the response to acute exercise in trained vs. usual care subjects



3. Does ingestion of a high-calorie leucine-rich protein (whey) supplement enhance acute exercise-stimulated anabolic signaling? (Specific Aim 2A). The effect of a nutritional supplement on acute exercise-induced signal transduction will be assessed by comparing IRS-1/AKT and mTOR signaling in a unilateral acutely exercised limb of regularly exercised subjects ingesting the supplement (Group 2) versus the acutely exercised limb of comparable subjects ingesting placebo (Group 1). A similar comparison will be made between the exercised limbs of usual care subjects ingesting the supplement (Group 4) versus placebo (Group 3) (Fig 5).

We anticipate that the leucine-rich supplement will enhance the acute exercise-stimulated mTOR signaling pathway and that the response will be more marked in the group undergoing the 12 week exercise program. This thesis is based on reports that in healthy young subjects, a leucine-rich supplement added to a resistance exercise program stimulates muscle protein synthesis, mass and strength (64; 72) and although delayed in the elderly, the anabolic response is effectively stimulated by this regimen (73). In this regard, Hulmi reported that whey protein enhanced exercise-induced signal transduction (phosphorylation of p70S6K) by nearly five-fold over exercise alone in young healthy subjects (71).

Figure 5. Assessment of the effect of nutrient supplementation on the response to acute exercise.

In summary, we anticipate that a 12-week program of exercise rehabilitation will increase muscle mass and improve skeletal muscle function and that this is mediated by several different processes that we aim to identify. In this regard we expect that basal anabolic signaling will be higher in the resting muscle of the regular exercise group versus the resting muscle of the usual care group, *a potential carry over effect of regular exercise*. In addition, we anticipate that while the high calorie whey supplement will cause an increase in anabolic signaling in the resting muscle of both groups, the response will be greater in the regular exercise group, *again possibly reflecting a carryover effect of regular exercise*. *If a carryover effect is indeed present, then this would support the hypothesis that regular exercise is beneficial even in ESRD.* We also anticipate that acute exercise alone will increase anabolic signal transduction, and that this response will be enhanced by the high calorie whey supplement. If true, **this holds the prospect of developing a simple therapeutic strategy to boost the anabolic response to regular exercise and thereby enhance the functional and cardiopulmonary benefits of exercise in patients with ESRD.**

Detailed Protocol for Nutrient Supplementation, Acute Exercise, and Biopsy for Specific Aim 2. An outline illustrating the acute exercise, biopsy, and nutrient supplementation protocol for Specific Aim 2 is presented in Figure 2. On a morning the day before regular MHD and after an overnight rest, both regular exercise and usual care subjects will consume a light protein-free breakfast between 7 and 8 am and will be studied three hours later after a 60-min rest. Subjects in both groups will then will begin a unilateral dominant lower limb exercise session lasting ≈15 min, with the contra-lateral limb at rest. At the start of the exercise session, subjects will ingest half the high calorie whey or placebo so as to compensate for AA deficiencies common in MHD subjects. The other half of the supplement will be ingested at the end of the exercise session so as to enhance anabolic signal transduction. This will be followed by 60 minutes of rest, then muscle biopsies will be performed on both limbs to assess anabolic signal transduction.

The unilateral exercise will be performed using seated single knee extension exercise against resistance. Subjects will be seated with the hips flexed at 90° with their back against a backrest at a ≈110° angle. The leg pad will be positioned ≈5 cm above the ankle and the rotation arm will be positioned so that the knee is bent to 90°. After a warm-up consisting of 8-10 repetitions with minimal resistance, the weight will be adjusted to 70% of the one repetition maximum. The unilateral 1-RM will be determined by a post-training period strength test, performed on a different day. Five sets of 10 repetitions will be performed, with 2 minutes of rest periods between sets. The load will be adjusted during the course of the session due to fatigue such that each subject is able to perform 10 repetitions during each set.

The supplement, high-calorie whey, (300 ml) comprises 24 g whey protein (contains ≈3 g leucine) with 30g carbohydrates (sucrose) in liquid, and the placebo contains a liquid, dextrose 24 g plus sucrose 30g. The amount of whey (Nestle Healthcare) prescribed is guided by studies in normal subjects (66; 69; 70; 110) including the elderly (70; 74). We do not anticipate any adverse effects from nutrient or placebo if taken as prescribed. Whey has a low NA, K and phosphorus content (60,140 and 60 mg/serving respectively). The vehicle for dissolving the powdered supplement will be of the subject's choice, but is limited to fluids permissible for ESRD such as apple juice and will be pre-tested for taste by the subject. Whey is also available in a variety of flavors and in long term studies has been included in baked goods or sprinkled over foods.

Detailed molecular analysis of skeletal muscle. The following items will be measured using methods established in Dr. Rabkin's laboratory. Note that receptor and signaling proteins will be measured by western immunoblots; mRNA levels by real-time quantitative PCR, and selected degradative proteins by immunobloting and serum and local IGF-1 by ELISA.

- mRNA levels of local regulators of muscle mass: Total IGF-1 (all transcripts), the Ea transcript (a generalized tissue form and main form in liver, and the IGF-1Eb transcript, MGF, a loading sensitive and more potent form than the Ea form). Myostatin, and receptors for IGF-1, insulin, growth hormone and androgens.
- mRNA levels of inflammatory cytokines IL-6 and TNFα.
- Signaling protein levels and their phosphorylated forms activated by IGF-1, insulin, EX or AA, including IRS-1, Akt, mTOR, p70S6K, rpS6, and 4EBP-1.
- Protein degradative pathway; mRNA levels of FOXO1 and 3, the atrogenes Murf-1 and MAFbx/atrogin-1, E3α, and the 20S proteasome α7 subunit and their proteins when antibodies are available. Muscle specific calpain 3 (p84) mRNA and if sufficient tissue, the mRNA for the ubiquitous calpains calpain 1 and 2 and calpastatin) will be determined. The 14 kDa actin protein fragment, a caspase-3 activity product that serves as an indirect measure of its activity (170), and LC3-II protein, a marker of autophagy, will be determined (111).
- DNA content as an indirect measure of myonuclear number (112).
- RNA content, an indirect measure of total tissue ribosomal content and protein translational capacity (49).

Subject Burden. We anticipate that baseline evaluations for each subject will require two-2 hour visits, with the exercise test and questionnaires performed on a separate day from the CT and biopsy evaluations. The initial visit will include questionnaires, health history, DEXA, exercise test and blood panel. The second visit will include the CT scan. For the exercise group, the total number of visits to the hospital will be approximately 14 (including exercise training sessions), and for the usual care group the total number of visits will be approximately 4.

Limitations of the study and anticipated problems.

(1) Though subject number is relatively small, the study is rigorous and sufficiently powered to achieve our aims and takes into account subject compliance. To our knowledge this is the first randomized controlled study to test whether home-based exercise is feasible and effective in elderly MHD subjects. Ideally we would have liked to include healthy sedentary subjects to serve as controls for comparison and also more extensive testing of nutrient supplementation, but this would have increased the required group sample sizes dramatically and encumbered the study. However, there is a large body of rigorous studies documenting the effects of exercise in healthy individuals, including signal transduction.
(2) The study is relatively short in duration (3 months/subject), and surrogate markers of long-term outcomes such as peak VO_2, serum albumin, and cardiac risk factors are used. Nevertheless, if the study outcome shows that our exercise training program is efficacious, then this will form the basis for a multicenter, long-term outcomes study. Thus, this study could be of great value in determining whether a long-term study of home-based exercise with or without nutrient supplementation is merited, and will be invaluable in the design of such a study.
(3) Recruitment of MHD subjects that meet our eligibility criteria and are prepared to undergo a large battery of tests and an exercise program involving considerable time requires highly motivated subjects and dedicated personnel. In terms of recruitment, the PIs and Co-investigators are accomplished in this area, with Dr. Schiller and staff at Satellite Healthcare being particularly experienced in recruiting and conducting ESRD clinical trials. Furthermore, as discussed earlier under the general study design, we will be able to draw on a large patient population, ~2,000, within 30-40 miles of the VAPAHCS.
(4) The study sample will include subjects who, in addition to MHD, will have other co-morbidities. We recognize that a great deal of motivation will be required for compliance with the exercise program. Some subjects may not be able to sustain 30 continuous minutes of aerobic exercise initially, and will need to be gradually progressed as tolerated. The cardiac rehabilitation group at the VAPAHCS under Dr. Myers' direction has significant experience with monitoring, surveillance, and motivating patients to sustain physical activity, and will apply similar techniques in the current proposal. We have also built a 20% dropout rate into the sample size estimates, as we anticipate that some patients will not complete the program.
(5) Study subjects, though functionally impaired, are likely to be highly motivated and in better condition than many other elderly MHD patients. However, given the consequences of losing muscle function, a low-cost regimen beneficial to even a modest subset of patients, would seem worth implementing. Also, this program could serve as a model to test in younger patients, among whom many more may be able to participate.
(6) Since the exercise training program consists of endurance and resistance exercise, and we anticipate that the daily activities of the subjects will increase in response to the exercise, we will be unable to define the role played by each type of training in any of the responses that occur. To define these respective roles, separate studies of resistance and endurance exercise would be required, but this is beyond the scope of this study.
(7) The study of nutrient supplementation is limited to the acute response at the time of exercise on one occasion only. It would be preferable to also study the long-term effects of this combination, but to ensure recruitment to achieve our primary goal of testing home-based exercise training, we have limited this study to a pilot. A wider study would require increasing the subject number several fold. If positive, this pilot will form the basis of a more extensive study.
(8) As the nutrient supplement to be administered contains several soluble proteins and AA, we will not be able to attribute any responses to a specific AA. This is well beyond the scope of the present proposal.
(9) While each of the questionnaires has been widely used and shown to be valid, each is subjective. The accuracy of the responses depends upon subject's recollection and the judiciousness with which researcher and subject conduct the interview. For example, though the KDQOL has been demonstrated to be reliable and valid (90), some domains have a higher internal reliability than others. In addition, there is some evidence to suggest that subjects participating in a study tend to overestimate their compliance to

exercise and diet (113). Our research group has extensive experience in using these tools; particular emphasis will be placed on taking the necessary time to obtain responses as accurately as possible.

Statistics. ***Sample Size Computation and Power Analysis.***

Primary endpoint, Specific Aim 1. For this analysis we have used data from a recent 12-week study by Storer et al (36) on the effects of endurance exercise versus no exercise (usual care) on cardiorespiratory and skeletal muscle function in MHD patients. Change from baseline for peak VO_2 was 22% (an increase) in the exercise group and 6% (a decrease) in the usual care group. These findings are consistent with an average increase from baseline of 17% in MHD patients participating in exercise-based rehabilitation programs reported in the literature (14). Similarly, our primary outcome will be comparison of the change in peak VO_2 for patients randomized to exercise vs. usual care. To calculate the desired sample size we used a two group t-test of equal means and calculated the per group sample size (East 5, Cytel Inc, copyright 2007). Given the desired values for the standardized effect size (the absolute value of the difference in group mean changes divided by the common standard deviation) and overall level of significance and power of the test, the required sample was calculated. To detect a difference in group mean changes of 17% in peak VO_2 for exercise vs. usual care, given a common standard deviation of 20% (based on Storer et al (36), using a standardized effect size of 0.85), 23 subjects/group would be required to complete the study to achieve statistical power of 80% with a two-sided test of significance at the 0.05 level. Appendix VII shows how the required sample size varies by the standardized effect size for power of 0.80 (solid curve) and 0.85 (dashed curve). Appendix VIII provides curves showing how the power varies by the standardized effect size for a per group sample size of 23 (solid curve) and 26 (dashed curve). For example, a per group sample of 23 provides power of about 0.17 and 0.38 to detect a small (0.3) and medium (0.5) standardized effect size, respectively. To allow for a 20% dropout rate we plan to enroll 30 subjects per group, or a total of 60. The 20% dropout rate is based on the PI's experience (87), and other similar research studies.

Secondary analyses, Specific Aim 2. In this specific aim we will analyze molecular changes in muscle biopsied at baseline and at the end of the study. IGF-1 is a key muscle growth factor; Kopple et al (45) reported that IGF-1Ea mRNA levels rose ~100% in 12 MHD subjects undergoing 21.5 weeks of combined resistance and endurance exercise. We plan to compare changes in IGF-1 mRNA levels from baseline to those levels at the end of 12 weeks of rehabilitation or usual care. Muscle will be obtained from limbs at rest in the exercise and usual care subjects as described above (n=12/group) and we will consider a 50% change in IGF-1Ea mRNA to be a clinically meaningful difference. While there are no data available in MHD subjects to estimate the standard deviation of the change in these molecular indices from baseline, Appendix VIII may be used to estimate the power associated with an assumed standardized effect size that is small (0.3), medium (0.5) or large (0.8).

Secondary analyses, Specific Aim 2A. In this specific aim we will assess the nutrient-enhanced exercise-induced protein signal transduction pathway in subjects undergoing exercise versus usual care. Specifically, we will compare the change in activity (phosphorylation) of the mTOR signal transduction pathway induced by unilateral exercise alone versus unilateral exercise plus nutrient supplementation. This will be a pilot study of 12 subjects in each group. Since this is a novel measurement and there are no data in MHD subjects describing the variance of this response, a power analysis has not been carried out. Change in activity will be taken to equal the difference in activity between the unilateral exercise limb and the contra-lateral resting limb of the same subject. We will also examine the effect of the supplement alone vs. placebo on signal transduction in the resting limb of unilateral exercise and usual care subjects. Of note, Hulmi (71) reported that whey protein enhanced exercise-induced signaling (phosphorylated p70S6K)

by nearly 5-fold over exercise alone in young healthy subjects, while Drummond (73) noted a similar increase in the elderly following EAA ingestion.

Statistical analysis plan. Comparisons of clinical and demographic data between groups at baseline will be performed by the appropriate statistical test (for continuous variables, t-test for normally distributed data and Wilcoxon test for non-normal data; for categorical variables, chi-square test or Fisher's exact test). These comparisons will be performed at baseline only to assess adequacy of randomization. Comparisons between groups for cardiopulmonary exercise test, muscle biopsy, energy expenditure, cognitive function, and other functional measures before and after the study period will be performed by multivariate ANOVA; post-hoc comparisons will be made using the Bonferroni method. To analyze continuous variables measured at three or more time points we will use linear mixed effects regression (114). To explore the relationships between a given outcome and other variables, we will use multiple regression techniques. Since the reliability of a fitted regression model may be reduced if too many predictors are used (114), we will limit the number of predictors to four variables in each model (≤1/10 of the total). For the primary outcome, change in peak VO_2, the specific independent variables in the model will include exercise compliance (average energy expended in kcals/week), change in muscle strength, and change in muscle mass. Missing data will be managed using the multiple imputation method which is preferable to older methods and described in detail by Schafer & Olsen (1999).

For Specific Aim 2 (effect of acute and chronic exercise on muscle wasting), we will employ a linear model, using period (baseline vs. 4 months) and treatment (exercise training vs. usual care) as independent variables along with their interaction (Bonferroni method). The intention to treat principal will be applied for all analyses.

Timeline. We anticipate that we will recruit ≈15 MHD subjects/year during the 4 years of the study. We expect that it will take four years to complete the study, with much of the final year dedicated to completing sample processing and data analysis. We do not expect much delay in getting started as there is an established exercise research program at our facility. In addition, the techniques for biopsying muscle as well as measuring muscle structure and cellular biology are established in the PI's (Rabkin) research laboratory. Finally, both the Satellite Healthcare Dialysis research group (Dr. Schiller) and Dr. Chertow's group (Stanford University) have vast experience in recruiting and performing clinical trials in MHD patients. *A study Timeline of Project Tasks is presented in Appendix I.*

Plan for Dissemination. Dissemination will be a major focus of year 4. We believe the results of this study will lend themselves well to presentations at the VA RR&D meeting, American Society of Nephrology, and a number of rehabilitation meetings (AACVPR, ACSM). The Co-PIs will devote efforts during years 3 and 4 to abstract presentations and manuscript preparation. We anticipate that there will be results on functional and psychosocial outcomes that will be appropriate for publication in the Journal of Rehabilitation Research and Development, Journal of Cardiopulmonary Rehabilitation and Prevention, and Archives of Physical Medicine and Rehabilitation. Physiological results will be appropriate for the Journal of Applied Physiology and Nephrology journals, including Journal of the American Society of Nephrology, American Journal of Kidney Diseases, and others.

PRELIMINARY STUDIES

Exercise training studies. The Co-PI (Dr. Myers) and one of the Co-Investigators on the current proposal (Dr. Froelicher) have previously conducted a 3-year NIH-funded Specialized Center of Research (SCOR) project of exercise training in patients with cardiovascular disease (115; 116), and are currently conducting an NIH Specialized Center of Clinically-Oriented Research (SCCOR) project on the effects of exercise therapy in patients with abdominal aortic aneurism disease (87; 93). The proposed project will use existing exercise testing and training facilities at the VA Palo Alto Medical Center, and methods similar to the current NIH SCCOR project will be used in the current proposal.

Dr. Myers' research focus has been in the areas of exercise testing, training, and epidemiology in patients with coronary artery disease, chronic heart failure, and spinal cord injury. Since January 1992, he has directed the exercise research laboratory at the Palo Alto VAHCS. Dr. Myers has a more than 25 year body of work related exercise testing and training in patients with cardiovascular disease. He has served on writing groups for guidelines developed by the American Heart Association (AHA), American College of Sports Medicine (ACSM), American Thoracic Society (ATS), American Association of Cardiovascular and Pulmonary Rehabilitation (AACVPR), and the European Society of Cardiology Working Group on Prevention and Rehabilitation. He is a recent recipient of the AACVPR Established Investigator Award, and is a Senior Research Career Scientist Award recipient from the VA. The fifth edition of his textbook co-authored with Dr. Froelicher, "Exercise and the Heart: Clinical Concepts" was published in April 2006.

Dr. Myers has also published extensively in the area of exercise training among patients with chronic heart failure (CHF). The focus of these studies has been on the application of MRI to quantify the effects of training on the myocardial remodeling process. He and his colleagues were the first to employ this technology to address a controversy that arose in the 1990s, in which it was suggested that exercise training could lead to myocardial damage. Using a high intensity training protocol and a series of randomized trials, it was demonstrated that training did not cause further myocardial damage among patients with CHF (117-120). These results have since been confirmed by studies performed in Italy, Canada, the US and elsewhere, and have been influential in the development of rehabilitation guidelines in these patients. Dr. Myers' followed these initial studies by applying a novel MRI tagging technique, in which the myocardium could be visualized more closely in 3 dimensions, including rotational displacement during systole and diastole (120). These studies demonstrated no adverse effects of training on myocardial size, function, or rotation velocity during systole up to one year after undergoing training, and an improvement in relaxation velocity in a subgroup of patients with non-ischemic cardiomyopathy.

An ongoing NIH SCCOR project entitled, "Effects of exercise therapy in abdominal aortic aneurysm (AAA) disease" (Myers and Dalman, Co-PIs, Froelicher, Co-Investigator) is in its 6th year, and employs exercise testing and training methods that are similar to those in the current proposal (87; 93). The purpose of the SCCOR project is to test the effectiveness of exercise training to reduce abdominal aortic aneurysm risk, limit small aneurysm progression, and modify biologic markers of AAA disease. The study includes 3 arms; one to identify signature protein profiles of AAA disease, another the application of MRI to develop and validate hemodynamic computational models in AAA progression, and the 3rd to investigate the effects of 3 years of exercise training on AAA size, aneurysm risk, and physiologic responses to exercise. Some early results of this trial are presented below.

Safety, efficacy, and adherence to a home-based exercise training program in patients with cardiovascular disease. Myers J, et al. Effects of exercise training in patients with abdominal aortic aneurysm (AAA): Preliminary results from a randomized trial. J Cardiopulm Rehabil Prev 30: 374-383, 2010, and Myers J et al. Cardiopulmonary exercise testing in abdominal aortic aneurysm: Profile, safety, and mortality estimates. Eur J Cardiovasc Prev Rehabil 18:459-466, 2011. Figure 6 below shows changes in exercise capacity in 57 subjects with AAA disease after 1-year of home-based exercise training at the VA Palo Alto rehabilitation facility. At the 3-year evaluation point, significant differences between exercise and usual care groups were also observed for peak VO_2, VO_2 at the ventilatory threshold, and other indices of the training response, such as a reduction in submaximal heart rate. Using a case-management approach and the application of weekly phone calls, activity logs, heart rate monitors and pedometers, subjects in the exercise group expended an average of >2,000 kcals/week of recreational energy expenditure. In a substudy, these methods of activity surveillance have been validated (Myers J, Dupain M, Vu A, Powell A, Smith K, Dalman R, on behalf of the Stanford AAA SCCOR Investigators. Agreement between activity monitoring devices during home rehabilitation: A substudy of the AAA STOP Trial. J Phys Act Aging, in press, 2012).

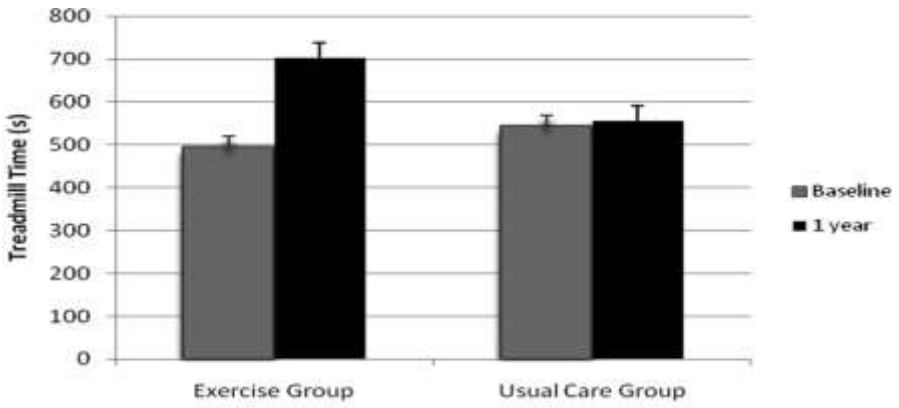


Figure 6. Bar graphs showing 42% increase in exercise time in the exercise group and no change in usual care subjects following 1-year of exercise training in patients with abdominal aortic aneurysm disease (86). Changes in exercise capacity were similar at the completion of the full trial, after up to 3 years of home-based exercise training.

Data on the approach to activity monitoring and surveillance. In the current proposal, methods for monitoring and surveillance of physical activity will be employed that are similar to the above-mentioned ongoing NIH trial. In our NIH trial, we observed that subjects achieved 98±7% of their target heart rates during monitoring (87). Using 7-day activity recall instruments to quantify weekly activity, the mean overall energy expenditure for subjects in the exercise group after 1 year of participation was 2269±1207 kcals/week (shown in the figure below). This is roughly double the CDC/AHA recommendations for physical activity, and is significantly higher than the recently-published HF-ACTION trial (≈500 kcals/week) and other rehabilitation studies. This degree of exercise stimulus resulted in a 42% increase exercise capacity on the 1-year treadmill test, while no changes were observed in controls. Thus, the approach proposed is likely to be successful in achieving an adequate training stimulus.

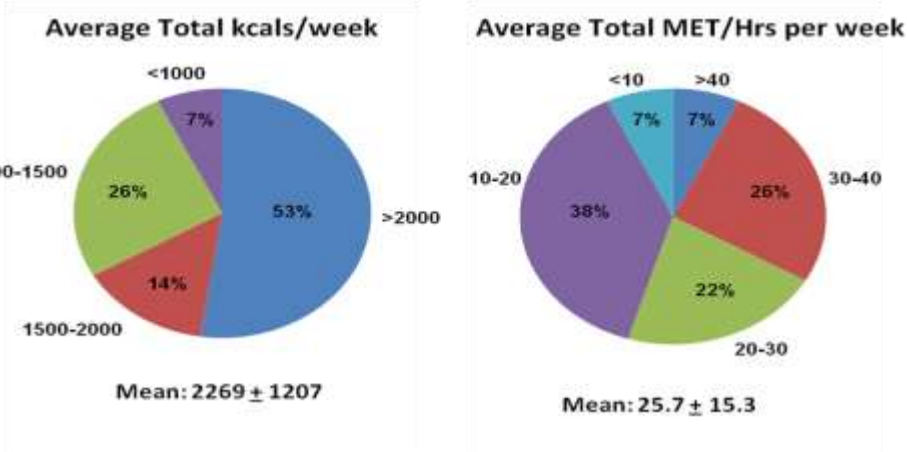


Figure 7. Average energy expenditure over 1 year in the exercise group (from reference #86)

Studies on muscle wasting in kidney failure. Dr. Rabkin (Co-PI) is a Professor of Medicine, Emeritus Active at Stanford University, and a Staff Nephrologist at the Palo Alto VAHCS. The focus of his research is on the mechanisms of muscle wasting in CKD with the goal of identifying potential strategies for the prevention and treatment of this disorder. The current proposal reflects an effort to translate his many animal findings to a clinical population with CKD. The following briefly highlights some of this work relevant to the current proposal.

Chronic uremia attenuates growth hormone induced signal transduction in skeletal muscle. *Sun D, Zheng Z, Tummala P, Oh J, Schaefer F,* ***Rabkin R:*** *J Am Soc Nephrol 15:2630-6, 2004*. Resistance to growth hormone contributes to muscle wasting in uremia. To test whether impaired growth hormone signaling is a cause of the resistance to growth hormone and to elucidate its mechanisms, we studied

muscle growth hormone signaling and action in rats with surgically induced chronic renal failure. As we observed previously, growth hormone stimulated IGF-1 expression was depressed in CRF and evidence is provided that the resistance to growth hormone is caused at least in part by impaired JAK2-GHR-STAT5 phosphorylation and nuclear STAT5 translocation. Furthermore, this study demonstrated that the attenuated JAK2 –STAT5 signaling may be caused by at least two different processes. One involves depressed phosphorylation of the signaling proteins because of increased suppression of cytokine signaling-2 expression that may be linked to low-grade inflammation. The other may involve increased signaling protein dephosphorylation because of heightened protein-tyrosine phosphatase activity.

Work-induced changes in skeletal muscle IGF-1 and myostatin gene expression in uremia. Sun DF, Chen Y, **Rabkin R**. Kidney Int 70:453-9, 2006. Resistance to GH induced IGF-1 gene expression contributes to uremic muscle wasting. Since exercise stimulates muscle IGF-1 expression independent of growth hormone, we tested whether work overload could increase skeletal muscle IGF-1 expression in uremia and thus bypass the defective growth hormone action. In addition, to provide insight into the mechanism of uremic wasting and the response to exercise we examined myostatin expression. Unilateral plantaris muscle work overload was initiated in uremic and pairfed normal rats by gastrocnemius tendon ablation with the contralateral plantaris as a control. Some rats were growth hormone treated for 7 days. Work overload stimulated a gain in weight of the plantaris muscle in both groups and corrected the uremic muscle atrophy. As shown in Figure 8 below, growth hormone increased plantaris IGF-1 mRNA levels >2 fold in pairfed rats but the response in chronic renal failure was severely attenuated.

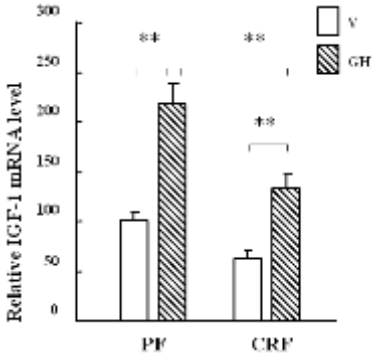


Fig 8. Effect of GH on muscle IGF-1 mRNA levels

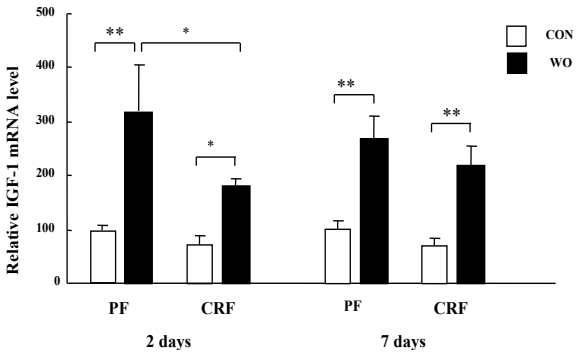


Fig 9. Effect of work overload on muscle IGF-1 mRNA levels

Work overlaod increased the IGF-1 mRNA levels significantly in both the chronic renal failure and pairfed groups, albeit less brisk in renal failure (Figure 9). However after 7 days IGF-1 mRNA levels were elevated similarly, >2 fold, in both groups. Of note, the atrophied uremic plantaris muscle basal myostatin mRNA levels were increased significantly and normalized after an increase in work-overloaded rats suggesting a myostatin role in the wasting process (Figure 10).

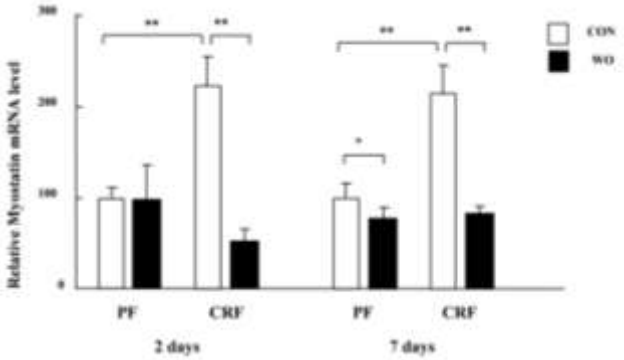


Fig 10. Myostatin levels in CRF and response to work overload

In summary, these findings provide insight into the mechanisms of skeletal muscle wasting in uremia and the hypertrophic response to exercise, and suggest that alterations in the balance between IGF-1 and myostatin play an important role in these processes.

Increased workload fully activates the blunted IRS-1/PI3-kinase/Akt signaling pathway in atrophied uremic muscle. Chen Y, Sood S, Biada J, Roth R, **Rabkin R.** Kidney Int. 2008. In this study of muscle wasting and recovery in chronically uremic rats, we set out to characterize the mechanisms whereby resistance exercise increases muscle mass, despite the presence of impaired signaling through the IRS-1/PI3-kinase/Akt pathway and insensitivity to IGF-1. Uremic and sham nephrectomized (SN) rats with surgically induced unilateral plantaris muscle work overload were studied, with the contralateral plantaris muscle serving as a nonloaded control. In these control muscles signaling protein levels were similar in the chronic renal failure and SN groups despite atrophy of the chronic renal failure muscle. However, basal state activation (phosphorylation) of the IRS-1/PI3-kinase/Akt signaling pathway was depressed in the uremic rat muscle. Of note, even though the basal activity of the IRS-1/PI3-kinase/Akt signaling pathway was depressed in the uremic atrophied plantaris muscle, simply increasing the muscle workload effectively activated this signaling pathway and phosphorylation of the signaling proteins in the work-overloaded plantaris muscle was similar in both groups of rats (Figure 11 below). Work overload induced a significant increase in local IGF-1 and mechano-growth factor expression and also a significant increase in signaling protein levels. Together these changes provide an explanation for the work-induced increase in signal transduction. Importantly, the work-induced signaling response in the uremic rats was similar to that observed in non-uremic rats and promoted changes that we observed consistent with a decrease in muscle protein degradation, an increase in protein synthetic capacity and an increase in myonuclear number.

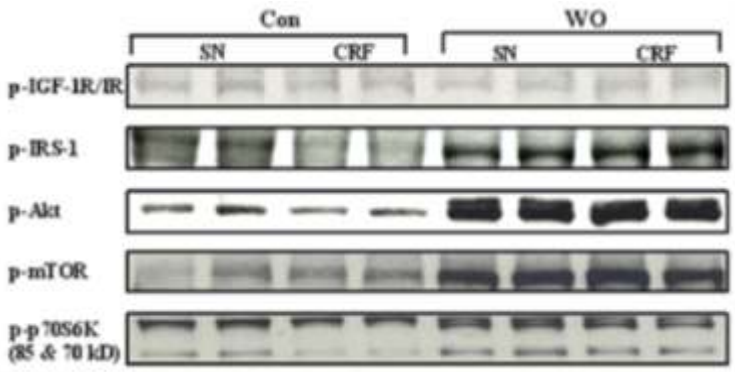


***Fig 11**. Levels of phosphorylated signaling protein levels in control and work overloaded plantaris muscle of SN (sham nephrectomy) and chronic renal failure rats. Phosphorylation of IRS-1 and Akt is depressed in the control (con) non-loaded muscle of the renal failure group. Work overload stimulated signaling protein phosphorylation similarly in both groups of rats.*

We concluded that increasing the work of atrophied muscle in uremia can fully activate the depressed **basal IRS-1/PI3-kinase/Akt signaling pathway and it appears to do so by increasing** IGF-1 and mechano growth factor expression and increasing the levels and phosphorylation the IRS-1/PI3 kinase/Akt/ mTOR signaling proteins. This in turn appears to account for the increase in the reduced uremic muscle mass, which reached a level comparable to that seen in normal rat.

Leucine stimulated mTOR signaling is partly attenuated in skeletal muscle of chronically uremic rats. Chen Y, Sood S, McIntire K, Roth R, **Rabkin R.** Am J Physiol Endocrinol Metab. 2011. The branched chain amino acid leucine stimulates muscle protein synthesis in part by directly activating the mTOR signaling pathway. Furthermore, leucine, if given in conjunction with resistance exercise, enhances the exercise-induced mTOR signaling and protein synthesis. In this study we tested whether leucine can activate the mTOR anabolic signaling pathway in uremia and whether it can enhance work overload-induced signaling through this pathway. CKD and control rats were studied after 7 days of surgically induced unilateral plantaris muscle work overload and a single leucine or saline load. In the basal state 4E-BP1 phosphorylation was modestly depressed in non-work overload muscle of CKD rats while rpS6

phosphorylation was nearly completely suppressed. After oral leucine mTOR, S6K1and rpS6 phosphorylation increased similarly in both groups while the phospho-4E-BP1 response was modestly attenuated in CKD. In work overload CKD muscle leucine augmented mTOR and 4EBP1 phosphorylation, but its effect on S6K1 phosphorylation was attenuated and it failed to stimulate rpS6 phosphorylation. ***In summary, this study has established that the chronic uremic state impairs basal signaling through the mTOR anabolic pathway***, an abnormality that may contribute to muscle wasting. ***However despite this abnormality, leucine can stimulate this signaling pathway in CKD***, though its effectiveness is partially attenuated, especially in skeletal muscle undergoing sustained work overload. Thus, even though there is some resistance to leucine in CKD, these results suggest a potential role for leucine rich supplements in the management of uremic muscle wasting.

Description of key relevant studies published by co-investigators.
Dr Chertow (Co-Investigator) is Professor Medicine /Nephrology and Chief of Nephrology at Stanford University and a leader in the field of outcomes research in CKD. Dr. Kurella-Tamura (Co-Investigator) is a Staff Nephrologist/Gerontologist VAPAHCS and Assistant Professor of Medicine/Nephrology at Stanford University. She is a widely recognized authority on cognitive disorders in the CKD population**.** Some representative studies relevant to the current proposal are presented below.

Cognitive impairment in chronic kidney disease. **Kurella-Tamura M, Chertow GM**, Luan J, and Yaffe K. J Am Ger Soc; 52:1863-1869, 2004. The prevalence of cognitive impairment and its association with the severity of kidney disease has not been defined. A cross-sectional study of cognitive function was performed in 160 ambulatory persons with ESRD and CKD not yet on dialysis. The performance on three cognitive function tests assessing global cognitive function (3MS), executive function (Trails B), and verbal memory (California Verbal Learning Trial, or CVLT) was compared to published norms. Overall, 17% of subjects met established criteria for global cognitive impairment. Global impairment was present in 27% with ESRD and 15% with advanced CKD, but in no subjects with mild to moderate CKD ($p=0.02$). Unadjusted and adjusted mean scores on the 3MS, Trails B, and CVLT immediate and delayed recall were significantly worse for subjects with ESRD than for subjects with CKD or published norms ($p<0.001$ for all comparisons). Scores on the Trails B ($p<0.001$) and CVLT immediate ($p=0.01$) and delayed recall ($p<0.001$) were significantly worse for subjects with CKD not requiring dialysis than for published norms. In addition, the fraction of subjects with impairment on the 3MS and Trails B increased with decreasing kidney function. These results demonstrate that significant impairment in cognitive function is present in both CKD and ESRD, and suggest kidney disease directly contributes to cognitive impairment.

Significance of frailty among dialysis patients. Johansen KL, **Chertow GM**, Jin C, Kutner NG. J Am Soc Nephrol. 2007 Nov;18(11):2960-7. The aim of this study was to determine the prevalence and predictors of frailty among a cohort of incident dialysis patients and to determine the degree to which frailty was associated with death and hospitalization. A cohort of 2,275 adults was studied who participated in the Dialysis Morbidity and Mortality Wave 2 study, of whom two-thirds met our definition of frailty: a composite construct that incorporated poor self-reported physical functioning, exhaustion/fatigue, low physical activity, and undernutrition. Multivariable logistic regression analysis suggested that older age, female sex, and hemodialysis (rather than peritoneal dialysis) were independently associated with frailty. Cox proportional hazards modeling indicated that frailty was independently associated with higher risk of death (adjusted hazard ratio [HR] 2.24, 95% confidence interval [CI] 1.60–3.15) and with the combined outcome of death or hospitalization (adjusted HR 1.63, 95% CI 1.41–1.87). Therefore, frailty is extremely common and is associated with adverse outcomes among incident dialysis patients. Given its prevalence and consequences, increased research efforts should focus on interventions aimed to prevent or attenuate frailty in the dialysis population.