COMIRB Protocol

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I. Hypotheses and Specific Aims:

Due to the success of combination antiretroviral therapy (ART), more than one-half of the estimated 1.2 million persons with human immunodeficiency virus-1 (HIV) in the United States are now age 45 or older[1]. Although they are living longer, current evidence suggests that persons with HIV infection have higher than expected rates of aging complications including physical function impairment, frailty, and falls[2-5]. The pathogenesis of aging complications in HIV, such as physical function impairment, is not well understood.

Insulin-like growth factor (IGF)-I is the primary mediator of growth hormone (GH) effects on muscle and bone. Low IGF-I is associated with frailty, sarcopenia (low muscle mass and strength), and mortality in some aging cohorts. In our cohort of middle-aged HIV+ adults on effective ART, lower IGF-I was associated with a significantly greater odds of functional impairment [6]. Based on our data, we believe impairment in the GH/IGF-I axis may be an important pathway leading to aging complications in HIV. Early evidence of an HIV-GH interaction was illustrated in a rodent model where injection of the HIV envelope protein (gp120) into the hypothalamus suppressed GH[7]. GH deficiency is seen with lipodystrophy and GH resistance with AIDS wasting, but most studies of the GH/IGF-I axis in HIV+ persons are limited to: 1) cross-sectional analyses; 2) young subjects with wasting or lipodystrophy; 3) studies conducted prior to the era of combination modern ART treatment; 4) low numbers of women; and 5) significant subject and treatment heterogeneity. ***Little is known about the GH/IGF-I axis in the current era of HIV and ART, the role of GH/IGF-I in physical function in HIV, or the role of the GH/IGF-I axis on the aging trajectory in HIV.***

My *long-term goal* is to promote successful aging among persons with chronic HIV-1 infection by strategies to optimize physical function and minimize disability and frailty. The *primary objective* of this proposal is to investigate the GH/IGF-I axis as a potential pathway of early functional impairment in persons aging with HIV. My research is anticipated to advance understanding of the mechanisms and interactions of the GH/IGF-I axis in physical function. My *central hypothesis* is that a phenotype of accelerated functional impairment observed among some persons with HIV infection will be associated with disruption in the GH/IGF-I axis (the somatopause). This hypothesis will be evaluated through the following *specific aim:*

Specific Aim 1: Determine the impact of high or moderate intensity combined resistance (REx) and cardiovascular exercise (CEx) on physical function in older HIV+ (n=36) and HIV- (n=36) adults.
Approach: Previously sedentary older adults (aged 50-75 years) will undergo a 24 week REx + CEx exercise training intervention.

Hypothesis 1a: Both HIV+ and HIV- older adults will have a significant improvement in physical function (chair rise time) with REx + CEx, but HIV+ will have a *lower* increase than HIV- older adults.

Hypothesis 1b: Both HIV+ and HIV- older adults randomized to a high intensity REx + CEx intervention will have greater improvement in physical function than those randomized to the moderate intensity exercise.

Hypothesis 1c: Utilizing established techniques for screening and training, moderate intensity exercise followed by randomization to either continue a moderate- or progress to high- intensity exercise will result in minimal or no adverse events and will result in similar completion rates, with 80% of participants completing the training program.

Specific Aim 2: Determine the impact of high or moderate intensity combined REx and CEx on local and systemic IGF-I, as an explanatory factor in the physical function changes of older HIV+ and HIV- adults.
Approach: Previously sedentary older adults (aged 50-75 years) will undergo a 24 week REx + CEx exercise training intervention.

Hypothesis 2a: HIV+ older adults will have lower *systemic* IGF-I at baseline and at week 20 compared to HIV- adults, with blunted response to exercise training.

Hypothesis 2b: HIV+ older adults will have lower baseline and lower week 20 *skeletal muscle* IGF-I mRNA and IGF-I protein, compared to HIV- adults

Specific Aim 3: Demonstrate a dose-response relationship between exercise intensity and chronic inflammation in HIV+ and HIV- adults aged 50 to 75 years.

Hypothesis 3a: Older HIV+ adults will have a lower acute response to exercise (lower systemic IL-6 and TNFα) at baseline and with 12 weeks training, in comparison to HIV- older adults.

*Hypothesis 3b. O*lder HIV+ adults training on a high-intensity exercise regimen for 12 weeks will experience a greater initial increase in systemic IL-6 without a detrimental ncrease in TNFα in response to an acute bout of exercise, compared to those randomized to moderate-intensity exerci*se Hypothesis 3b:* After 24 weeks of training, older HIV+ adults randomized to high-intensity exercise will experience a greater relative decline in chronic (resting) markers of systemic inflammation (IL-6, sTNFRI, TNFα) compared to those randomized to moderate-intensity exercise and compared to HIV- adults.

Specific Aim 4: Use qualitative methods to understand the barriers and facilitators to starting and maintaining an exercise program among older, HIV-infected population with multiple health disparities.

Hypothesis 1. Barriers to exercise will be identified among older HIV-infected men and women; these issues may focus around themes such as access to exercise facilities, body image, income/resources, stigma of HIV, and social support. Facilitators to routine physical activity and to continuation of exercise after completion of the exercise program will include greater exercise self-efficacy.

Methods: Focus group and individual interviews will be conducted among 1) participants who are currently exercising or who recently completed the exercise intervention study (K23 AG050260), or 2) participants not enrolled in the study. Participants will be men or women with HIV infection, aged 50 and older who are either exercising regularly or not exercising, similar to the recruitment criteria for the current participants. Semi-structured interviews for group interviews will be framed around the theoretical framework of behavior change, self-efficacy, and the Social Ecological Model.

II. Background and Significance:

Complications of aging with HIV. ART has dramatically extended the life expectancy of HIV-infected people. Longer life expectancy is changing the demographics of the HIV epidemic and by 2015 half of those living with HIV in the US will be 50 years or older[8]. In comparison to HIV-uninfected populations, HIV-infected persons, even when on effective ART, experience excess morbidity and mortality[9-11]. Persons on ART have early onset of aging complications including osteoporosis and fractures[12-14], impaired physical function[15-17], frailty[18-22], and falls[23]. Preservation of physical function is a cornerstone of geriatric medicine and will be essential in promoting successful aging among people with HIV.

HIV-infected persons are at risk for early development of frailty or a frailty-like syndrome, especially if not taking antiretroviral therapy or if CD4+ T-cell count is low[20-22, 24-26]. Measures of physical performance have identified greater than expected impairments in walking speed, balance, ability to rise from a chair and peak exercise capacity[16, 17] among middle-aged and older adults with HIV-infection. Impaired physical function may result in decreased quality of life, social isolation, nursing home placement, hospitalizations, and ultimately, higher mortality[27-30]. As preliminary data to support this proposal, we found that *38-53% of middle-aged HIV-infected adults who have undetectable plasma HIV-1 RNA on ART in the Infectious Diseases Group Practice (IDGP) at the University of Colorado Hospital (UCH) have moderate to high physical function impairment*[31]. We found that physical function impairment was associated with significantly lower insulin-like growth hormone, lower lean muscle mass, and lower bone mineral density (BMD). Furthermore, although we did not find the IGF-I was

Table 1. Odds of poor performance on functional testing per 100 ng/mL↓ in IGF-1

Variable	*OR (95% CI)*	*P value*
Frailty	**2.5 (0.8, 7.9)**	**0.13**
Grip	1.8 (0.5, 5.9)	0.35
Activity	3.1 (1.0, 9.3)	0.04
Fatigue	7.6 (2.0, 28.7)	0.003
Weight loss	1.6 (0.4, 7.0)	0.54
Slowness	2.3 (0.4, 14.2)	0.36
SPPB	3.2 (1.0, 10.2)	**0.05**
Chair rise	4.3 (1.3,14.1)	0.02
Tandem	2.2 (0.5, 9.3)	0.30
Walk	4.7 (0.7, 34.0)	0.12

associated with frailty overall, we did find significant associations between IGF-I and chair rise time, a measure of lower extremity strength, self-reported physical activity, and fatigue (table). *Based on our findings in elderly HIV- populations, we hypothesize that impairment in the growth hormone (GH)/insulin-like growth factor (IGF)-I axis is an underlying, and potentially modifiable, component in this aging process.*

GH/IGF-I axis in "normal" aging. Regulation of the GH/IGF-I axis is complex: pulsatile secretion of GH is primarily regulated through hypothalamic GH-releasing hormone (GHRH) secretion and feedback through somatostatin and ghrelin. In "normal" aging, decreased hypothalamic release of GHRH results in a ~1% per year decline of GH levels after age 30, referred to as the "somatopause"[32, 33]. The decline in mean GH, GH pulse amplitude, and GH pulse frequency result in a subsequent decline in hepatic IGF-I production[34]. As a result, up to 30% of older adults could be considered GH-deficient by IGF-I levels[32]. IGF-I is considered one of the most important mediators of muscle and bone growth[35], and low systemic IGF-I has been associated with frailty[36, 37], slowed walking speed, decreased strength, and increased mortality[38, 39]. IGF-I is directly involved in the muscle adaption to exercise, and may explain, in part, impaired exercise tolerance seen in older individuals, or adults with HIV[16]. One of the most potent stimuli of GH release and subsequent skeletal muscle IGF-I production is acute exercise. The endogenous release is attenuated with age, obesity, poor sleep or nutrition[40, 41]. The impact of HIV on the endogenous release of GH/IGF-I is not known.

Therapies that target the GH/IGF-I axis have an anabolic effect on lean body mass (LBM) and bone mineral density (BMD). Shorter-term GH therapies (≤ 6 months) typically result in increases in LBM without subsequent improvement in muscle function. However in studies of longer GH administration in GH-deficient adults (ie, >12 months), increases in muscle strength are consistently found[42-46]. GH replacement over 5-10 years nearly normalizes muscle strength among GH deficient adults with low baseline LBM and strength[42, 47]. Whether the effect of GH/IGF-I-axis therapy on muscle function is sustained after cessation of therapy is unknown. GH replacement is also associated with improved aerobic capacity, lipid profiles, insulin sensitivity, and quality of life, suggesting that the effects of GH on physical function in GH deficient adults may be multifactorial[48-52]. Limited efficacy, expense, and adverse effects of continuous GH-axis replacement suggest that mitigating the decline in GH through enhanced endogenous release (i.e. exercise) or improved local response to IGF-I may be better strategies to maintain physical function.

GH/IGF-I axis in HIV. Many factors can contribute to alterations in the GH/IGF-I axis, but whether long-standing HIV infection is an independent risk is not clear. HIV proteins are present in the hypothalamus of deceased subjects with advanced HIV[53]. Injection of the HIV envelope protein (gp120) into the third ventricle of rodents suppressed GH secretion compared to saline, suggesting HIV may have a *direct* effect at the hypothalamic level[7]. A GH-resistant state is seen in AIDS patients, with or without wasting, with higher baseline GH but markedly lower IGF-I and/or IGFBP-3 levels compared to HIV- controls[54-57]. Conversely, lower basal GH, reduced pulsatility, and blunted response to exogenous GHRH but similar IGF-I was seen in HIV-associated lipodystrophy compared to HIV- controls[54, 58, 59]. Most of these studies were conducted >10 years ago, and involved few women, few contemporary ART regimens, and few persons aged >50 years. ***None of these studies investigated a potential association between GH/IGF-I and functional impairment or aging outcomes***.

Multiple mechanisms could explain the association between physical function and GH/IGF-I axis changes with age and HIV. First, little is known about the effect of different ART on the GH/IGF-I axis. In initial GH studies, HIV+ subjects were either untreated or on ZDV monotherapy. The largest study of the GH/IGF-I axis in HIV+ subjects (N=164) did not detect a difference in GH between the NRTIs and protease inhibitors, but individual ART drugs were not compared[60]. We are currently investigating the impact of ART in a different study to help answer this question. Second, the role of sleep in the GH-HIV relationship is unknown. Although GH secretion is circadian, the link between slow wave sleep and the GH/IGF-I axis is weak in most cohort studies[61-64]. Insomnia and or other sleep disturbances, however, are very common and may be independently associated with HIV[65-70], and both low IGF-I and poor sleep are associated with physical function impairment[71-73]. Therapies targeting the GH/IGF-I axis among HIV+ persons (for lipodystrophy or wasting treatment), have resulted in increased LBM [74, 75] and gain in muscle function and exercise capacity[76, 77]. The newer GHRH analogue, tesamorelin, appears to be effective at decreasing central fat and increasing LBM with minimal side effects compared to prior GH therapies [78]. Among both HIV+ and HIV- persons, cessation of therapy almost inevitably reverses the LBM gain, supporting a role of the GH/IGF-I-axis in mediating physical function changes among aging, HIV+ adults[78, 79]. *Since low IGF-I, low muscle mass[80-82] and functional impairment are predictors of mortality and morbidity in both HIV-infected and -uninfected elderly[83-85], our findings of frequent low LBM and BMD in middle-aged HIV-infected persons raise concerns about potential risks for loss of independence as HIV-infected persons age.*

***Exercise as an intervention to promote successful aging*.** In addition to the benefits on the GH/IGF-1 axis, the health benefits of regular physical activity for older adults are recognized by national and international organizations including the National Institute on Aging (Go4Life), the Agency for Healthcare Research and Quality (STEP Physical Activity Program), and the World Health Organization[86]. The Department of Health and Human Services- Physical Activity (DHHS-PA)

guidelines recommend that adults perform 150 min of moderate- or 75 min of vigorous-intensity endurance exercise (CEx) per week, and moderate-intensity resistance exercise (REx: i.e., weight training) on at least 2 days/week, to regularly stimulate the cardiorespiratory, musculoskeletal, and metabolic systems[87].

The DHHS-PA guidelines can be met by sedentary older adults or adults with chronic disease via progressive (i.e., incremental) CEx and REx exercise training methods. Exercise training interventions that combine CEx and REx are associated with improved physical function[88, 89], muscle strength[90], or both[91]. Beyond these direct effects of exercise, training-related improvement in physical function was associated with better quality of life (QoL)[90] and lower IL-6[92] in older adults. A greater reduction in inflammatory markers with exercise may result in greater improvement in muscle mass, strength, or exercise capacity among persons with chronic inflammation[93, 94].

Existing studies of exercise interventions among HIV-infected adults have primarily focused on gain in LBM among persons with wasting syndrome, or metabolic and cardiovascular benefits among persons with lipodystrophy. REx with or without additional interventions (e.g., testosterone, whey protein, carnitine) improves LBM in HIV-infected adults[95-100] while CEx improves insulin resistance, exercise capacity, and body composition changes associated with lipodystrophy[101-109]. Among HIV-infected adults, exercise interventions improved sit-stand time and short-walk speed[110] and increased 6-minute walk[111]. As in HIV-uninfected older adults, exercise or lifestyle modification reduces inflammatory markers in middle-aged HIV-infected individuals[104, 112].

Exercise as an intervention to increase IGF-I. Exercise is a potent stimulus of IGF-I production, although the effect appears to be most strongly mediated through the local production at the level of the muscle. Changes in *systemic* IGF-I with exercise are inconsistent across study populations[113], partly limited by variability in the type and intensity of exercise intervention. For example, changes in serum IGF-I with 6 months of cardiovascular exercise among 52 older, healthy adults did not result in significant changes in serum IGF-I, however, the intervention did not include REx[114]. In contrast, other studies have found increases in serum IGF-I with REx. More consistently, REx results in an increase in skeletal muscle IGF-I or IGF-I mRNA[115-118]. When skeletal muscle is subject to mechanical loading, two splice variants (IGF-IEa and MGF or IGF-IEc) are expressed. IGF-IEa isoform is expressed in both resting and working muscle and may serve more of an endocrine action similar to IGF-I production in the liver. In contrast, the MGF is more muscle specific, with autocrine or paracrine actions, and may be more involved in the early muscle repair and satellite cell activation. Similarly, IGF-IEa is thought to be more responsive to GH whereas MGF is relatively insensitive to GH[119, 120].

Of 28 post-op older adults were randomized to REx, nerve stimulation, or function exercises, only the REx arm had significant gain in both IGF-IEa and MGF as well as muscle fiber hypertrophy. These findings suggest that routine physical activity or rehabilitation focused on functional improvement alone is not enough to overcome the sarcopenia found in many older adults[121]. Furthermore, in a recent publication, 15 men with abdominal obesity treated with rhGH for 12 weeks experienced an increase in both skeletal muscle mRNA and serum IGF-I, with serum changes associated with improvements in body composition and muscle mRNA associated with changes in skeletal muscle mitochondrial function, suggesting differing mechanisms between systemic and local IGF-I[122]. The increase in IGF-I mRNA and protein is blunted with aging, but the impact of HIV on the ability to respond to the exercise stimulus is not known. Further examples of the impact of REx, CEx or both on IGF-I are shown in the table below.

	Cohort	Intervention	Outcome	Notes
Vitiello, [114]	67 healthy older adults	CEx x 6 mo	Serum IGF-I (No difference)	
Hameed [118]	19 healthy 74 y/o men	REx, GH or REx + GH	All groups had increase in mRNA IGF-1, GH alone increased IGF-IEa, but MGF was enhanced with REx added	No change in serum IGF-I with REx alone -REx also increased IGF-IEb
Hameed [117]			Young subjects had increase in MGF but not IGF-IEa mRNA 2.5 h after single-bout exercise; older adults did not	
Singh[123]	Older men & women	10 wks REx	500% increase in IGF-I within muscle but did not distinguish isoforms	
Kim[124]	20 young and 18 older adults	1x REx	IGF-IEa increased 34%; detected a load and age interaction with an increase of only 16% among older adults	
Hambrecht[125]	18 with CHF	½ to EX, ½ to none	Increase in IGF1 at muscle, decrease in IGFR at muscle	Systemic IGF-1 fails to respond to GH stimulus with disease,

				suggesting peripheral GH resistance not overcome with ex
Bamman[116]	10 healthy subjects (mean age 24 years)	8 sets concentric or eccentric REx	Single bout of mechanical loading increased IGF-1 mRNA by 62% and were greater in eccentric group	
Gregory[126]	Healthy women	8 weeks REx, CEx, or both	8 weeks of R or R+E increased total IGF and decreased IGFBP-1	
Kraemer [127]	8 younger, 9 older men, all physical active but no REx	REx 3x/wk x 10 weeks	Exercise induced increases in T were more pronounced in younger, IGF didn't change with EX	
Lovell [128]	32 older men	12 REX, 12 AEX, 12 control to 16 weeks	No differences in serum GH, IGFI, testos, SHBG, FT, but did have transient T elevation immediately after REx	

Exercise as an intervention to reduce inflammation.

Regular physical activity is associated with reduced risk of dementia, cancer, cardiovascular disease, and insulin resistance, among other diseases associated with heightened inflammation in epidemiologic studies. In contrast, prolonged inactivity is associated with accumulation of visceral fat and elevation of multiple cytokines. Some of the benefit of exercise may be explained through alteration in systemic cytokines[129, 130]. Acutely, an exercise session stimulates the production and release of IL-6 into circulation, independent of the TNFα pathway (Figure 1, top panel). After repeated episodes of acute IL-6 elevation (i.e., chronic exercise training), a paradoxical reduction in the resting level of systemic inflammatory cytokines is seen. An exercise intensity that is too low may not generate a great enough IL-6 stimulus acutely, and result in minimal to no decline in inflammatory cytokines over time (middle panel). Alternatively, under certain conditions, (e.g., in individuals with compromised immune function or heightened systemic inflammation), a high-intensity exercise may cause muscle damage, resulting in a maladaptive acute increase in TNFα and an increase in chronic systemic inflammatory cytokines (bottom panel)[131, 132]. For example, among subjects with chronic obstructive pulmonary disease, the resting plasma TNFα increased after 8 weeks of moderate intensity cardiovascular exercise (CEx) training[133], suggesting that exercise training may have a detrimental effect on systemic inflammation among some individuals with heightened systemic inflammation[132]. Insufficient evidence exists to support whether this effect is true in other diseases of heightened inflammation.

Few studies have explored the impact of exercise on acute or chronic markers of inflammation or immune activation in younger or older HIV+ adults. In a study of younger HIV+ men, a bout of moderate-intensity CEx + resistance exercise (REx) in 14 subjects resulted in significant increases in 30 and 60 minute post-exercise IL-6 and significantly lower soluble TNF receptor (sTNFR)-II levels compared to 11 subjects performing low-intensity CEx[134]. These findings suggest that at least a moderate-intensity of CEx + REx may be required to stimulate an adequate acute exercise response expected to ultimately decrease systemic inflammatory markers among younger HIV+ men (top vs middle panel). Similar acute exercise responses have not been studied on older, HIV+ adults. With 16 weeks of high-intensity CEx training, significant decreases in IL-6, hsCRP, and TNFα were measured among 18 younger HIV+ adults[104]. Promising preliminary results presented at the Conference on Retroviruses and Opportunistic Infections (Boston, March 2014) found a significant decreases in IL-6, hsCRP, d-dimer, and IL-18 among 35 older adults completing a 12-week intervention of either CEx or CEx + REx (Longo, et al, Abstract #763); these results were limited by combined CEx and CEx + REx results and intensity defined only as "brisk".

Need for evidence-based recommendations for using exercise as an intervention for successful aging in older HIV-infected adults. Despite the evidence that exercise increases physical function in younger persons living with HIV infection, available care guidelines for treating HIV infection provide few, if any, specific recommendations about the type, duration, frequency and intensity of exercise for older HIV-infected persons. The *HIV and Aging Consensus Project* suggests that interventions to prevent or reverse functional compromise should "support exercise", but acknowledges that "further studies are needed to determine the role and optimal type of exercise training in HIV-infected patients, particularly older patients with concomitant comorbid diseases"[135]. A recent attempt to develop evidence-based exercise recommendations for older HIV-infected adults extrapolated data obtained from younger HIV-infected persons and older HIV-uninfected adults with frailty or metabolic syndrome to propose exercise recommendations for older, HIV-infected adults[136]. However, *available data are not sufficient to formulate evidence-based specific exercise recommendations for older HIV-infected adults, including the type, duration, frequency and intensity of exercise.* Since the biology of aging with HIV may be affected by multiple unique factors (e.g., residual viral replication, ART toxicities including mitochondrial toxicity), it is important to establish whether the health benefits of exercise are similar in HIV+ and HIV- adults. Furthermore, it is imperative that the level of exercise intensity does not exaggerate the chronic inflammatory response in

HIV+ persons. *Further investigations are needed to document the benefits of exercise and compare specific exercise recommendations, including the impact on hormonal pathways such as IGF-I, between HIV-infected and –uninfected adults. Studies need to evaluate current exercise recommendations in older HIV-infected persons*. Such studies will inform the development of exercise guidelines and promote implementation in clinical practice.

Will HIV-infected participants continue to engage in cardiovascular and strength training after completion of an exercise intervention? Despite extensive evidence that exercise has proven health benefits, only 20% of adults aged ≥18 years in the US meet DHHS guideline recommendations for both cardiovascular and resistance exercise[33]. Exercise rates among older adults are even lower: < 20% of older adults meet cardiovascular exercise goals and <15% meet recommendations for resistance exercise [34].To the best of our knowledge, no data exists on rates of cardiovascular or resistance exercise uptake among *older*, HIV-infected adults. In a cohort of intravenous drug-users with and without HIV, HIV-infected individuals on ART had the lowest reported time spent in vigorous activity compared to those with untreated HIV or without HIV[35]. A convenience sample of 70 HIV-infected adults (mean age 40.4 years) in an urban US clinic found that nearly half of participants met guidelines for resistance exercise, but 40% did not meet the Healthy People 2010 recommendations for moderate or vigorous physical activity; in this study, greater annual income, stronger social support, and greater self-reported physical health were significantly correlated with a greater amount of vigorous physical activity[36]. In contrast, among 191 younger (mean age 44), mostly male (93%) HIV-infected individuals in Melbourne, Australia, recommended physical activity levels were achieved in almost 75% of those surveyed, but the whether these same rates would be found in older, HIV-infected adults in the US is not known[37]. The study participants in our current exercise intervention provide a unique opportunity to expand our understanding of 1) the decision process that led to exercise initiation, 2) motivations and barriers for continued exercise during a controlled experimental exercise intervention, and after completion of the intervention and 3) the contributions of race/ethnicity and SES, as well as chronic disease burden and sexual preference to exercise uptake and maintenance. Additional individuals not currently enrolled in the study will also be included to provide additional insights into motivations and barriers to exercise, particularly among individuals who may have been excluded from the exercise study due to current physical activity or several medical conditions, HIV medical non-compliance, or social barriers.

Older, HIV-infected adults epitomize healthcare disparities associated with poor exercise uptake and greater chronic disease burden in the general population. Of 15 HIV-infected participants enrolled in our study thus far, 40% are ethnic or racial minorities. Epidemiologic data suggest that racial or ethnic minorities may be less likely to engage in regular physical activity than their white counterparts[38]; or may have different motivations for participating in physical activity and barriers for not participating[39-41]. Racial and ethnic minorities suffer from a disproportionate number of chronic illnesses that could be prevented or attenuated by routine physical activity[38,42], but availability to physical activity resources is significantly lower in predominantly minority neighborhoods[43]. In an exercise intervention, adherence to exercise was significantly lower among HIV-infected black African compared to HIV-infected white or black British or Caribbean participants (mean age 42 years) [14]. Less is known about the factors that influence exercise uptake and adherence in *older*, racially/ethnically diverse adults with or without HIV. In a recent meta-analysis of effectiveness of exercise interventions in older adults, racial and ethnic minorities comprised only 15% of the study population[44].

Low SES is consistently associated with lower physical activity, due to a combination of limited access to safe exercise facilities, time constraints, lack of social support, and less encouragement by healthcare providers to participate in physical activity[45-47]. In our prior study of middle-aged (45-65 years of age) HIV-infected adults on effective ART[7], more than half were unemployed or on disability and more than two-thirds reported an annual household income of <$25,000/year (Erlandson, unpublished data), thus many of these barriers are prevalent in our population. Comorbidity and perceived disability are common barriers in initiating exercise among older adults[48,49]; both comorbidities and disability are reportedly higher among HIV-infected older adults, even among those on effective ART [50-53].

Lastly, studies suggest that gay men are less likely to seek medical care (and thus, receive counseling to achieve adequate physical activity goals), and gay or lesbian persons may experience unique exercise barriers including lack of same-sex partner family memberships at fitness facilities or shared locker/shower facilities [54-56]. Whether similar barriers to exercise or physical activity are perceived among older gay or bisexual individuals is not known. A goal of the Healthy People 2020 campaign is to “improve the health, safety, and well-being of lesbian, gay, bisexual, and transgender individuals” [57] . Since 80% of our study population are gay or bisexual men, our cohort provides a unique opportunity to assess these barriers particularly by examining the context in which the gay participants make decisions, experience barriers and perceive potential facilitators. Qualitative methods are particularly effective at understanding meaning and situations of individuals and groups. Improved understanding of the motivations and barriers for initiation and continuation of cardiovascular and resistance exercise in older HIV-infected adults is needed to enhance scientific knowledge of exercise implementation in older adults with a multitude of health disparities.

Gaps in the current literature support a need for a <u>qualitative and quantitative</u> understanding of exercise motivators and barriers in older adults with healthcare disparities. In the initial interaction with our study participants, we have identified multiple barriers and motivators to exercise that are not captured on routine questionnaires, and not described in scientific literature. Dominant themes underlying barriers and motivators for exercise will be identified best by using qualitative interview techniques. We have noted a few of these themes during conversations with our participants. For example, many participants had never used a treadmill or lifted weights: the lack of familiarity with gym equipment is likely a significant barrier to initiating resistance exercise. These themes could be common in older adults in general, but we have become aware of themes specific to aging with HIV infection. Lack of familiarity with community recreation center or gym senior discount programs may be a barrier to continuation of exercise. The social support networks that provide encouragement for exercise initiation and maintenance are often fragmented among older, HIV-infected adults[58]. Many of our current participants are on disability from prior complications of HIV: the cycle of disability may lead to the perception of disablement, lower exercise self-efficacy, and self-imposed limitations to physical activity. Furthermore, many older, HIV-infected adults assumed they would die in the early AIDS (pre-antiretroviral) era and the idea of "healthy living" has been difficult to grasp and implement (personal communication from one of our participants). Body weight and body image motivators may differ: obesity may be associated with prosperity and health among some racial/ethnic groups, and thus may not serve as a motivating factor for exercise. In contrast, both older HIV-infected men and women tend to be thinner than HIV-uninfected populations (Erlandson, et al, unpublished data), and the lack of obesity may limit physician-prompted encouragement for routine physical activity.

Quantitative differences in response to exercise differ between racial/ethnic groups, gender, and by socioeconomic factors: Persistent elevation of inflammatory markers following a bout of exercise may limit the anti-inflammatory benefits of exercise among black compared to white runners[59]. Cigarette smoking and substance abuse, more common among persons of low socioeconomic status[60,61], are associated with impairment in the diffusion capacity of carbon dioxide, which may impede the cardiorespiratory responses with exercise and lead to an altered perception of exertion. Poor sleep, commonly associated with lower socioeconomic status[62]contributes to fatigue, a major barrier to initiation of exercise. Indeed, fatigue is a very common, often crippling symptom among HIV-infected adults: a third of middle-aged HIV-infected adults on effective ART in our prior work reported that everything they did was an effort, or they just couldn't get going at least 3-4 times/week[7]. *While some of these motivators and barriers may be specific for HIV or other chronic disease processes, we hypothesize that much of what can be gleaned about exercise barriers and facilitators through a combination of both qualitative and quantitative assessments will advance the understanding of exercise uptake and maintenance across health disparities, and among populations at the greatest risk for chronic diseases.*

Expected contribution of the proposed research. As outlined above, gaps in our knowledge of the effects of exercise in older HIV-infected persons have limited general implementation of exercise as an intervention to maintain and/or improve the health of people as they age with HIV infection. The investigations proposed in the current application will help to close these gaps and contribute to a more robust evidence base to support use of exercise as a method to improve successful aging with HIV infection. *This overarching goal will be accomplished through 1) measuring the effects of an exercise intervention on physical function in HIV+ and HIV- older adults, 2) determining the role of IGF-I expression and production in the response to exercise training in HIV+ and HIV- older adults, 3) determine the relationship of the IGF-I changes to other predicted responses to exercise: change in LBM, muscle quality (fat infiltration), visceral fat, BMD, and inflammation.*

We expect that our findings will ultimately lead to exercise recommendations to prevent or reverse impaired physical function, decrease morbidity and mortality, and thereby achieve "successful aging" in persons with and without HIV infection.

III. Preliminary Studies/Progress Report: As above.

In our case-control study of 80 HIV+ low and high-function subjects aged 45-65 years, matched on age, gender, and duration of HIV, lower levels of serum IGF-I and the primary binding protein, IGFBP-3, were associated with significantly greater odds of poor physical function (OR 5.0, 95% CI 1.4-20.0, p=0.02; OR 3.3, CI 1.6-6.3, p=0.001, respectively)[6]. Levels of IGF-I in our low-function group (mean 99 ± SD 8 ng/mL) were comparable to those reported in healthy men

aged 70-80 years (125 ± 48 ng/mL)[32] and frail women aged 70-79 years (104 ± 2 ng/mL)[137]. LBM and appendicular LBM were also significantly lower (all $p<0.05$) among low-function compared to high-function subjects.

The theories underlying exercise uptake and adherence often focus on behavior change and self-efficacy. Among persons that have opted to change behavior (such as those volunteering for participation in our study), a *sustained* change in behavior is driven by 1) **self-efficacy**, or a perception that an individual has the skills to complete a task in a variety of circumstances and 2) **outcome expectation**, an individual's belief that an action will achieve a given specific outcome[49,64,65]. For example, when an individual is given the choice to begin resistance exercise, she must believe that she has the knowledge and skills to safely and appropriately use a resistance weight machine *and* that use of the resistance machine will result in an improvement in strength with no injury. A higher level of exercise self-efficacy is associated with a greater likelihood of initiating exercise, greater perceived benefit from the exercise, and improved maintenance of exercise over the long-term[66,67]. Self-efficacy increases through (a) recognition of individual achievements (performance accomplishments), (b) recognition of success experienced by others, particularly of similar background (vicarious learning), (c) positive support from healthcare providers, family, or friends (verbal encouragement), and (d) recognition of the positive physical and emotional changes associated with exercise (physiological and emotional responses)[49,68]. The relative importance of these aspects of self-efficacy can vary significantly by individual or group. Among persons experiencing the similar successes of peers (vicarious learning), and continued support from family or friends, the impact on self-efficacy will be great (evaluated in Aim 1). Similarly, appreciable gains in personal performance (performance accomplishments or physiological/emotional responses through improved exercise capacity, strength, and speed) will contribute to greater improvement in self-efficacy and outcome realization (evaluated in Aim 2).

The Social-Ecological Model, commonly used in disease prevention/health promotion strategies, provides a framework for understanding barriers for exercise initiation and adherence, particularly among groups with health disparities[69,70]. According to the Social-Ecological Model, behavior change is influenced by multiple levels including individual (intra- and interpersonal), organizational (e.g., churches, work environment, etc.), community norms and expectations (e.g. neighborhoods), and public policy (e.g., equality of legal rights)[71]. Better understanding of the influence of these levels on exercise value among HIV-infected individuals with health disparities can provide valuable insight in the multiple levels of change necessary for implementing effective, large-scale interventions.

Combined mixed methods research, utilizing both quantitative and qualitative approaches have proven successful in understanding exercise preferences in a variety of different populations. For example, the People Reducing Risk and Improving Strength through Exercise, Diet, and Drug Adherence (PRAISEDD) program incorporated results of mixed methods research to tailor an intervention to meet the cultural and social needs of a community[72]. African American older adults often prefer to exercise in groups, particularly in faith-based group exercise programs, while some communities have expressed safety concerns about walking in the surrounding neighborhood. The PRAISEDD Program incorporated a group-based exercise program within a senior living facility, enhanced by educational and self-efficacy training components. The program is now led by community members and has been successfully maintained for several years after initial conception. Mixed methods research such as those underlying development of the PRAISEDD Program can also provide insight into study participation: In an primary care-based physical activity clinical trial of older adults, patients that were offered but declined participation tended to have less support from others to exercise (verbal encouragement), felt that underlying medical problems would worsen with exercise, and tended to be male, non-white, and less affluent than participants[73]. Others report that older adults are less likely to exercise because they may have misconceptions about the benefits of starting exercise later in life (outcome expectation), and associate exercise with intense physical exertion rather than as a method to maintain physical function[49]. Furthermore, poorer perceived health[74,75] and a fear of falling[76] (self-efficacy) are often barriers in exercise confidence among older adults.

Through a mixed methods approach, we will integrate the qualitative input that is not readily captured in a close-ended questionnaire to understand the self-reported reasons for initial engagement in exercise outside of the on-going study, motivating factors for study participation and engagement, and continuation of exercise after completion of the intervention. The addition of qualitative methods will provide insight into the aspects of health and wellness that are most valued by persons who are aging with HIV infection.

IV. Research Methods

A. Outcome Measure(s):

The objectives of this study are to

1) demonstrate an improvement in physical function among HIV+ and HIV- older adults,

2) determine whether differences exist in the responsiveness of the GH/IGF-I axis to an exercise stimulus in treated HIV infection, and, if so, whether these differences mirror those of aging (decreased IGF-I), or are unique to HIV, and
3) determine whether exercise results in a reduction in inflammatory and activation markers both acutely and chronically
4) determine whether these responses have a dose-response to exercise intensity such that a higher intensity intervention is associated with a greater improvement in function.
5) explore barriers and facilitators to exercise maintenance.

The proposed studies will test the working hypotheses that a) physical function response to exercise will be less in HIV+ versus HIV-, b) serum IGF-I and skeletal muscle IGF-I mRNA will be lower in HIV+ compared to age-matched HIV- controls prior to exercise and that c) serum and skeletal muscle IGF-I will increase in response to exercise, but will be lower in HIV+ compared to HIV- and d) higher intensity (vs moderate intensity) exercise will be associated with a greater decline in markers of inflammation. Successful completion of the proposed research will first contribute to the development of routine exercise recommendations in older HIV+ adults. On a mechanistic level, the research will contribute to the understanding of the local IGF-I response to exercise in skeletal muscle, and relationship to changes in physical function in persons aging with or without HIV. This knowledge is critical to designing and implementing interventions to effectively prevent or reverse functional decline for multiple reasons. If the IGF-I response to exercise stimulus is reduced in the HIV+ participants compared to HIV- participants, then the exercise stimulus may need to be more intensive to preserve/improve function with aging, exercise in younger HIV+ adults may need to employ specific exercises that enhance the IGF-I response (ie, heavier resistance training), or exercise may need to be accompanied by weight loss to create the same level of response. If higher intensity exercise is associated with lower compliance, higher drop-outs, and poorer responses on measures of depression, self-assessed physical function, and sleep, with increased sedentary time, then the higher intensity exercise may have detrimental effects to overall health in this older population. Reasons for the blunted exercise response in HIV such as heightened inflammatory cytokines, greater levels of visceral fat, deficiency in other hormones, impaired GH response, or impaired hepatic IGF-I should be further investigated. If the response to exercise is impaired but IGF-I responses are similar, then other mechanisms are driving the exercise impairment and should be further investigated.

B. Description of Population to be Enrolled:

Participants will be sedentary men and women between the ages of 50 and 75 years (*performing < 2 days/week of 30 minutes of CEx or REx during the prior 6 mo*). HIV+ subjects will initially be recruited from the University of Colorado Infectious Diseases Group Practice which provides care for approximately 1700 HIV+ adults, with 528 patients aged 50-59, 170 patients aged 60-69, 25 patients aged 70-79. Subjects will be on effective ART for a minimum of 2 years with HIV-1 viral load primarily below the limit of detection with no HIV RNA >200 copies/mL. Recruitment of HIV- subjects will first target high risk HIV- partners, friends, or family members of HIV+ subjects, current research participants of IMAGE (Investigations in Metabolism, Aging, Gender and Exercise) research group, the Internal Medicine Clinic, and the Family Medicine Clinic. Inclusion and exclusion criteria were selected to enroll the most appropriate study population while maximizing the generalizability of the findings.

- Inclusion:
  - Between the ages of 50-75 years at study entry
  - HIV+ subjects must be on an ART regimen (change in regimen permitted for preference/tolerability but not for virologic failure) for a minimum of 2 years, with a viral load < 200 during that 2 year period
    - ☐ Initial recruitment will target those on ART for at least 5 years; this will be removed as needed to achieve target sample size
  - Sedentary (< 2 days/week of CEx and/or REx over the prior 6 months)
  - CD4 T-cell count greater than 200 cells/mm3
  - All participants must be able to perform activities of daily living without assistance, and ambulate independently.
  - BMI ≥ 20 and <40 (initial goal, will expand if difficulties recruiting)
  - Among females, must be post-menopausal (no menses for >12 months, or if prior hysterectomy, FSH >30 IU/l)

- Exclusion criteria:
  - Due to interaction with the GH/IGF-I axis, persons ***with diabetes with a hemoglobin A1c >7%, on insulin, on GLP-1 analog, or DPP IV inhibitors. Well-controlled diabetics on other oral medications with hemoglobin A1c of 7.5 or less are eligible.*** .
  - Persons on **growth hormone or growth hormone axis therapy (i.e., tesamorelin)** will be excluded.
  - Post-menopausal use of stable doses of estrogen/progesterone (at least 3 months) will be permitted.

- Persons on intramuscular testosterone will be excluded; stable doses of patches or gel will be permitted
- Corticosteroid use, including intra-articular, will be excluded (within 3 months for oral; within 6 months for intra-articular); inhaled or intranasal will be permitted.
- Immunosuppressive medications within 6 months, including methotrexate, infliximab, azathioprine, etc.
- Women that are pregnant, breast-feeding, or intend to become pregnant will be excluded.
- Known hepatitis B or C ***with detectable viremia*** within in the past 6 months will be excluded
- Severe liver disease other than hepatitis B or C due to interference with systemic IGF-I production
- uncontrolled hypertension defined as resting systolic blood pressure (BP) >180 mmHg or diastolic BP >100 mmHg
- indicators of unstable ischemic heart disease
- New York Heart Association Class III or IV congestive heart failure, clinically significant aortic stenosis, uncontrolled angina, or uncontrolled arrhythmia
- pulmonary disease requiring the use of supplemental oxygen ≥ 4L with physical exertion
- current diagnosis of malignancy (excluding non-melanoma skin cancers) within 48 weeks prior to enrollment
- surgery/trauma/injury/fracture within 24 weeks prior to enrollment that may impact a subject's ability to exercise
- Acute infection (skin infection, sinus infection, uncomplicated upper respiratory infection, dental infection, etc) within 2 weeks of study entry will be excluded until the infection has resolved for at least 2 weeks.
- Chronic, deeper infections (complicated pneumonia, bone infection, liver abscess, deep wound infection, etc) must have completed treatment and show no evidence of ongoing infection for at least 12 weeks
- history of stroke with residual deficits that may impact ability to exercise; orthopedic problems (e.g., severe osteoarthritis, rheumatoid arthritis) that greatly limit the ability to perform moderate-intensity REx (e.g., unable to be properly positioned in exercise equipment or to have severely restricted range of motion even after modifications have been made)
- weight over 300 pounds (due to limitations of the DXA machine)
- Montreal Cognitive Assessment (MOCA) score ≤ 18 (will be evaluated at screening visit after consent obtained; MOCA score of 18 is equivalent to a MMSE score of ~24)
- AIDS-defining opportunistic infection[75] within the 24 weeks prior to enrollment
- Person who appear to have unstable health, are incapable of safely participating in the exercise intervention, or are felt to have a life expectancy of < 1 year.
- Participants on anticoagulants (clopidogrel, Coumadin, etc) will be excluded from the muscle biopsy. Patients on short-acting anticoagulant therapy requiring dose cessation for only 48-72 hours (rather than 1-2 weeks for clopidogrel or Coumadin) for can be considered for muscle biopsy with approval by their hematologist or treating physician.
- Aspirin and Non-steroidal anti-inflammatory agents are not exclusions but should be stopped 1 week prior to biopsy.
- For the substudy only, participants experiencing a diarrheal change in bowel habits consisting of greater than one loose stool a day within the last two weeks will be excluded. Patients with chronic loose stools will be included.

- Focus Group Substudy will be more inclusive, as participants will only be participating in a group meeting and not exercising as part of the study:
  - Inclusion:
    - Age 50 or older at study entry
    - Either 1) currently exercising 2 days/week or more, or sedentary < 2 days/week of CEx and/or REx over the prior 6 months
    - Willing to participate in a group discussion with other HIV-infected individuals
    - HIV-infected for at least 2 years.
  - Exclusion criteria:
    - Non-English speaker (group discussions will be in English only)
    - Unable to participate in any form of exercise. Participants with severe activity limitations will still be eligible, as they could still participate in balance training, band resistance, etc.
    - Unable to provide own consent.
    -

C. Study Design and Research Methods

Potentially eligible subjects will be screened during routine clinic visits or over the telephone. Potentially eligible and interested subjects will return for informed consent and a screening/baseline visit. No research procedures will occur prior to consent, including the MOCA.

After obtaining informed consent, participants will undergo a graded exercise test to screen for potential underlying cardiovascular disease. V02 peak will be measured at this time to develop the exercise prescription. All participants will begin with a low intensity lead-in for 1-2 weeks, then advance to a moderate intensity exercise during first 12 weeks. Randomization to continue on moderate versus advance to high intensity will occur at week 13.

1. **Consent & baseline visit**. After obtaining consent, the participant will be evaluated

-After obtaining consent, the participant will be evaluated by

- medical history, physical examination including vital signs
- Montreal Cognitive Assessment (MOCA)
- Resting BP, 12-lead resting ECG, and then a graded exercise test (GXT) administered by research personnel and a clinician experienced in exercise stress testing of older adults (K. Erlandson) to screen out volunteers with evidence of ischemic heart disease, serious arrhythmias, or abnormal HR/BP responses to exercise. Briefly, the test will begin at a comfortable walking speed and 0% elevation; speed will be maintained and the grade increased by 2% every 2 min, until the participant reaches 85% of age-predicted maximal heart rate or the test is otherwise terminated. The absolute and relative indicators for terminating an exercise test that are recommended by the American College of Sports Medicine (ACSM)[76] will be employed.
- Baseline physical function assessments will be administered.

2. **Procedure visit #1 (within 14 days of visit #1)**. All participants will arrive between 7:30-8am for:
    - DXA scan
    - Vital signs
    - Anthropomorphics (waist/hip circumference)
    - Blood draw (fasting)
    - 36 (up to 46, if needed to ensure 36 paired biopsy samples) volunteer participants (18 HIV+/18 HIV-) will have a muscle biopsy
        - If muscle biopsy at the end of the study cannot be done (patient refuses, change in mediations, etc), then the number of biopsies will increase to a maximum of 46 in order to ensure each group has at least 18 paired (entry/completion) biopsy samples.
    - Questionnaires
    - Between the procedure #1 visit and the first exercise visit, participants will be sent home with an ActiWatch Spectrum monitor for activity and sleep.
    - Between consent/baseline visit and procedure visit #1, patients will be sent home with a specimen cup with biosafety bag to collect stool and a 3 day dietary log. The log should be collected in real time during a 3 day time period preceding the procedure visit.
    - Participants will be asked to collect stool within 72h period (if possible) prior to procedure visit #1 and place sample in a biosafety bag in their home freezer. They will bring the sample to procedure visit and it will be placed immediately in a -80C freezer within the RC2 11th floor lab.

3. **Exercise training**. The exercise intervention will begin at least 24 hours following, and within 14 days of the procedure visit (+/- 14 days for illness, scheduled vacation, etc).
    - At the first visit, all participants will repeat physical function testing & 1-RM
    - The first 2 weeks will be a lead-in period with low intensity exercise to gain familiarity with the machines. He/she will then be advanced to a moderate intensity exercise for an additional 10 weeks.
    - During the exercise training, participants will come to the research center 3 to 4 days/week.
    - Chair rise will be measured at the first visit of the week, every 2 weeks and pedometer data will be downloaded.
    - To account for unforeseen absences from exercise (due to death of family/friends, illness, etc), the duration of exercise can be extended by up to 4 weeks. This will allow the participant to slowly acclimate back to the prior exercise intensity and minimize risk for injury. The exercise will only be extended if the participant is in agreement. If he/she wishes to complete the exercise intervention within the 24 weeks as planned, then the duration will not be extended.

4. **Acute inflammation visit 1**. At 3 time points during the study, participants will have an "acute exercise visit".
   - The first will occur during week two of exercise
   - Participants will be asked to exercise on Monday, Thursday, and Friday.
   - Thursday (**beginning between 7:30-10am**) will consist of a high intensity CV + REx exercise stimulus, and a blood draw prior to exercise, and 0, 60, and 90 minutes following an exercise stimulus.
   - Participants will be encouraged to avoid a large meal before exercise (snack is ok and encouraged). Blood pressure will be monitored before exercise, after the CVEx, and after the REx. As the expected response to exercise would be an increase in blood pressure, the PI will be contacted for any decreases in blood pressure after exercise.

5. **Week 12**.
   - Full physical function, anthropomorphics, questionnaires will be administered on Monday of week 12
   - The **Acute Inflammation visit 2** will occur on Thursday morning of week 12 (**beginning between 7:30-10am**). Arrangements for alternative dates can be made, but the participant must have at least 48 hrs from the prior exercise session). Participants will be encouraged to avoid a large meal before exercise (snack is ok and encouraged). Blood pressure will be monitored before exercise, after the CVEx, and after the REx. As the expected response to exercise would be an increase in blood pressure, the PI will be contacted for any decreases in blood pressure after exercise.

6. **Randomization/Week 13 visit**. At week 13, participants remaining on study will be randomized to either continue moderate intensity or advance to a higher-intensity exercise regimen with the randomization sequence generated by the study statistician (Sam MaWhinney).
   - V02, 1-RM will be measured on Monday of this week to develop a new prescription.

7. Week 23.
   - V02, 1-RM will be measured between Wed-Friday of this week.

8. **Week 24.**
   - **Acute inflammation visit 3**. During the week 24 of exercise, participants will be asked to exercise on Monday, Thursday, and Friday.
   - **Thursday (between 7:30-10 am)** will consist of a high intensity CV + REx exercise stimulus, and a blood draw prior to, and 0, 60, and 90 minutes following an exercise stimulus. Participants will be encouraged to avoid a large meal before exercise (snack is ok and encouraged). Blood pressure will be monitored before exercise, after the CVEx, and after the REx. As the expected response to exercise would be an increase in blood pressure, the PI will be contacted for any decreases in blood pressure after exercise.
   - Full physical function testing will occur on the final day of the exercise intervention

9. **Procedure visit #2**. Participants will return >48 hours after the last exercise session for a 2nd procedure visit for muscle biopsy (n=36), DXA scan, and blood draw.
   - DXA
   - Fasting for muscle biopsy (n=36)
   - Complete questionnaires. If the participant is not part of the muscle biopsy substudy, he/she may complete the procedure visit #2 procedures on the same visit of their final exercise session if preferred.
   - Between consent/baseline visit and procedure visit #1, patients will be sent home with a specimen cup with biosafety bag to collect stool and a 3 day dietary log. The log should be collected in real time during a 3 day time period preceding the procedure visit.
   - Patients will be asked to collect stool within the 72h period prior to procedure visit #2 and place sample in the freezer. They will bring sample to procedure visit and it will be placed immediately in a -80C freezer.

10. Focus group discussions
    - Participants agreeing & consenting to participation in this additional part of the study will meet at an agreed upon time in a University of Colorado Hospital conference room for up to 2 hours.
    - Discussion will be audiorecorded.
    - Consent will be obtained prior to beginning focus group discussions

D. Description, Risks and Justification of Procedures and Data Collection Tools (apply only to the main study participants unless otherwise specified):

1. **Muscle biopsy**. Subjects participating in the muscle biopsy portion will have a biopsy prior to beginning the exercise intervention and after completing the 24 week intervention. Subjects will be advised to refrain from any physical activity for at least 48 hours following the biopsy and return within 14 days of the screening/consent visit. Non-steroidal anti-inflammatories or aspirin are held for 7 days prior to the biopsy. If participants are taking direct oral anticoagulants, this will be held for 48 hours prior to the procedure (72 hours if creatinine clearance is 30-50 and taking dabigatran). Therapy can be resumed 24 hours after the procedure, or as instructed by their physician. At study completion, subjects will be asked to return within 7 days of the final exercise session for a muscle biopsy. The exercise intervention will begin >24 hours after the initial biopsy, but within 14 days. Participants will be asked to abstain from alcohol or caffeinated beverages after 1300h and to fast from any food or drink beginning at 9pm with the exception of water. Subjects will arrive at 8am in the CTRC for the biopsy. Percutaneous samples of the vastus lateralis muscle will be obtained. After cleansing the area of the thigh with antiseptic (e.g., chlorhexidine, Betadine solution), 1% lidocaine (without epinephrine) will be injected under the skin. A 3-5 mm incision will be made in the skin and fascia over the belly of the vastus lateralis, and 100-150 mg of muscle tissue will be removed using a Bergstrom side-cutting biopsy needle with suction applied. The incision will be closed and a compression wrap and ice will be applied to the incision area for 20 minutes. The patient will be given instructions for wound care before discharge.

Muscle specimen processing. Tissue specimens will be immediately placed in RNAse free vials, immersed in liquid nitrogen, and then stored at -80^{o} C for batch analysis. Frozen tissue will be homogenized using a MagNA Lyser system (Roche Molecular Systems, Branchburg, NJ). Total RNA will be extracted from the supernatant using the Trizol Plus RNA Purification Kit (Life Technologies, Carlsbad, CA). RNA quantity and quality will be determined by spectrophotometry (NanoDrop ND-1000, Thermo Scientific, Rockford, IL). Total DNA will be measured using the MagnaNA Pure LC DNA Isolation kit (Roche Applied Science). MtDNA and genomic DNA in muscle tissue will be assayed by RT-PCR using standard curves generated using plasmid controls for both mtDNA and nuclear genes as described previously[138]. The number of mtDNA genome copies per cell for muscle, which is multi-nucleated, will be calculated.

Skeletal muscle analyses. Total RNA will be reverse transcribed and amplified using the TaqMan RNA to CT 1-Step Kit and TaqMan Gene Expression Assays (primers and probes; Life Technologies). mRNA from the following genes will be measured: IGF-1, androgen receptor (AR), mechano growth factor (MGF), *SOCS-3, PGC-1α , IL-6R, TNFR1, TNFR2, MFN-1, MFN-2, and FIS-1 mRNA*. Gene-specific assays will be plated into triplicate wells of 96-well plates with serial dilution of GAPDH as reference for cross plate comparison and no-template controls for each gene. Reactions will be run using a real-time PCR detection system (Bio-Rad; Hercules, CA). Validation experiments will be performed to demonstrate that efficiencies of target and reference genes are approximately equal. The target genes will be normalized to GAPDH RNA concentrations to control for sample degradation and other factors that could reduce quantitative PCR efficiency. Linear regression will be used to determine the relationship of relative target RNA concentrations, including high-, medium- and low-abundance mRNAs, determined by real-time RT-PCR to the relative abundance of specific gene sequence reads in RNA-seq. Differences in selected gene expression will also be tested using two-way ANOVAs with the target gene as the dependent variable, intervention timing (before or after) and HIV serostatus (infected or uninfected) as independent variables.

Muscle protein expression. The protein expression of IGF-I, IGF receptor, IL-6R, SOCS-3, TNFα, TNFR-1, *NF-kB and MuRF1*; *PGC1-α and Mitochondrial Transcription Factor (mt biogenesis), citrate synthase (mt function); Mfn-1 and -2, Opa1, FIS-1 and Drp1 (mt fusion/fission)* will be measured by established Western Blot techniques[139] using commercially available antibodies (Cell Signaling Technology, Inc., Beverly, MA).

Risks: The risks associated with muscle biopsy include brief, mild burning pain from the local anesthetic (lidocaine), more than mild discomfort during the acquisition of tissue (about 10% of cases), and infection (less than 0.4% of cases). Allergy to the anesthetic (skin swelling or rash) occurs rarely. There may be persistent numbness in the biopsy area.

Plan to Minimize Risk: Risks of the muscle biopsy procedure will be *minimized* by having a trained clinician perform the procedure on participants who meet the biopsy-specific criteria listed above. As part of her career development award, Dr. Erlandson is obtaining the skills in muscle biopsy. She is currently being trained in the procedures and will not begin performing the biopsies until her skills have been approved by an experienced clinician (Rebecca Benson and/or Dan Bessesen) with expertise in muscle biopsies. Participants will be observed following the procedure for any adverse events and given strict instructions for wound care. A phone call will be made within 48 h of the biopsy procedure to assess wound healing with a study clinician. Only non-diabetics participants and participants with a CD4 count >200 cells/mm^3 will be eligible to participate which will further minimize risk for post-biopsy infection. Additionally, the study will have a safety officer (Jose Castillo-Mancilla, a physician in the Infectious Diseases clinic). Dr. Erlandson will review

biopsy and exercise safety with Dr. Castillo-Mancilla every 6 months.

Justification: Systemic IGF-I does not appear to be nearly as strongly linked to muscle adaption to exercise as local IGF-I at the level of the muscle. Additionally, skeletal muscle mitochondria are reflective of insults that may have occurred decades earlier, whereas mitochondrial studies obtained through peripheral blood would only reflect changes over a much shorter time period. Thus the muscle specimens provide a window into mechanisms well beyond what can be collected less invasively.

2. Exercise testing and training: All exercise intervention components will include 3 to 4 weekly, supervised sessions of combined CEx + REx at the University of Colorado-Denver (UCD) Exercise Research Laboratory (ERL). All participants will begin with a 2-week supervised, low-intensity exercise acclimation with an experienced research assistant. The initial goal will be to walk on a treadmill for 20-30 continuous minutes at low-intensity (30-40% of maximal aerobic power [VO_2max]) and complete 3 sets of 8 repetitions at low-intensity (40-50% of the one-repetition maximum; 1-RM) 3 or 4 days/week. This will facilitate comfort with study equipment and minimize injury.

At 2 weeks, all participants will complete an additional 10 weeks of moderate- intensity exercise, with a goal of 50 minutes of cardiovascular + 3 sets of 8 resistance exercises by the end of the 12 week initial intervention. This will ensure that every participant meets at least the DHHS-PA recommendations in a supervised setting. At week 13, participants will be randomized to continue moderate intensity exercise, or progress to a high-intensity intervention for an additional 12 weeks.

Maximal aerobic power (VO_2 max). The measurement of VO_2max (or peak) will be used to formulate the prescription for CEx at the screening visit. Increases in VO_2max in response to exercise training will also verify that the exercise was of sufficient intensity to induce cardiorespiratory adaptations. During an initial 5-min warm-up, walking speed will be adjusted to elicit a HR that is ~70% of maximal HR (from the screening GXT). Speed will then be held constant and the grade of the treadmill will be increased by 2% every 2 minutes until volitional exhaustion or until the test is stopped (see section 4.1.2b). The O_2 and CO_2 content of expired air will be measured continuously by open circuit spirometry and averaged every 30 sec using an automated online system (TrueMax 2400; ParvoMedics, Sandy, UT). Objective evidence that VO_2max has been attained will include at least 2 of the following: a plateau in VO_2 despite an increased energy demand, a respiratory exchange ratio in excess of 1.10, and a maximal HR within 10 beats of the age-predicted value. In the absence of these benchmarks, the maximum measured VO_2 will be deemed VO_2 peak.

Muscle strength (1-RM). Muscle strength will be evaluated as the 1-RM (the maximal weight that can be lifted only 1 time using correct lifting form through the full range of motion) for the upper and lower body REx performed during weeks 1-20. 1RMs will be measured at week 0 and every 4 weeks to week 20. The change in muscle strength from week 0 to 20 will be a secondary outcome measure.

Exercise intervention. CEx duration will be increased to 50 minutes of treadmill walking/jogging and exercise intensity will be gradually increased to either 40-50% VO_2max *(moderate intensity)* or 60-70% VO_2max (*high intensity*). By completing 50 minutes of CEx within the 3 weekly visits, we will ensure that all subjects comply with the DHHS guidelines, regardless of their physical activity outside of the routine visits. REx intensity will increase every 2-3 weeks to a target of 3 sets of 8 repetitions at 60-70% of 1-RM (*moderate intensity*) or ≥80% of the 1-RM (*high intensity*).

Supervised exercise sessions. Each session will begin with CEx and proceed to REx. Three sets of resistance exercises will include leg press, knee extension, knee flexion, lunges (forward and side), lateral pulldown, chest press, seated row, and dips on a weight-assisted machine. Weight-stack and free weight equipment will be used. Stretching for 10 minutes will conclude each session. Research assistants will supervise exercise, interact frequently with participants, and encourage feedback regarding any discomforts or undue fatigue experienced during or after the exercise sessions. The individualized exercise prescriptions will be geared toward increasing the exercise stimulus while avoiding unfavorable events.

Physical activity recommendations outside of supervised sessions. Each participant will be provided with a pedometer (Omron) and recommendations to achieve at least 5,000 steps/day. Walking paths near the participants' home will be mapped for each participant. Information from the NIH Healthy Aging site (exercises for the older adults, nutrition advice, etc) will be provided to each participant at study entry and tip sheets for ways to decrease sedentary time will be provided. Participants will be encouraged to utilize fitness apps (MyFitnessPal, ec) and each subject will receive instructions to partake in physical activity "most" days of the week, outside of their 3x/week supervised sessions.

Risks: The potential risks of exercise testing and training include development of ventricular arrhythmia, myocardial

infarction, cardiac arrest, and death, as well as the less serious problems of injury to tendons, ligaments, joints, and muscles.

Plans to minimize risk: The risks of exercise testing and training will be minimized by adhering to the following strategies:

Endpoints for exercise tests: In asymptomatic individuals who do not develop cardiovascular abnormalities, the endpoint for the maximal exercise tests will be achieving 85% of age-predicted maximal heart rate, fatigue that forces cessation of exercise, or increasingly unstable gait. The criteria that will be used to stop the exercise test before meeting the above criteria include the development of: (a) ST-segment depression of more than 0.2 mV that is either horizontal, downsloping, or slowly upsloping (less than 1 mV/sec) and lasts for 0.08 sec, or ST-segment elevation greater than 0.1 mV; (b) chest pain or discomfort; (c) serious arrhythmias, including multifocal PVCs, ventricular tachycardia, or sustained atrial tachyarrhythmias; (d) development of A-V block or other conduction defects; (e) a fall of systolic blood pressure of 10 mmHg or greater from the peak level with increasing exercise intensity; (f) diastolic blood pressure above 110 mmHg or systolic above 220 mmHg; (g) dizziness; (h) ataxic gait; and (i) pallor or cyanosis. If a subject is noted to have osteoporosis (T-score <-2.5, upon screening, he or she will be referred to their primary care physician to discuss the potential risks and benefits of exercise and the need for immediate or delayed treatment for osteoporosis prior to initiating the study. The risks associated with the muscular strength tests are musculoskeletal pain, fatigue, dizziness, and syncope. These risks will be minimized by providing one-on-one instruction in proper exercise technique on multiple days prior to testing. Workloads will be increased gradually to elicit moderate to maximal effort, and a brief rest period will follow each attempt. Appropriate warm-up and cool-down periods will be included in the strength test procedures. The potential for a serious adverse event to occur with resistance exercise testing is minimal in persons who have undergone prior medical screening. In 20,000 maximal dynamic strength tests in women and men aged 18 to 93 yr, no cardiovascular events were reported [140].

Training of personnel: All personnel assisting with the administration of screening treadmill tests will have Basic Life Support certification and at least 1 member of the testing team (Dr. Erlandson) will have Advanced Cardiac Life Support certification. In accordance with current recommendations of the American Heart Association and American College of Sports Medicine, a clinician with exercise stress testing experience in older adults or an exercise specialist technician with experience testing older adults will directly supervise screening treadmill tests; a physician will be immediately available in the facility if not directly administering these screening tests. A physician will interpret ECG and blood pressure responses for all screening treadmill tests. For subsequent exercise testing (e.g., strength testing), the physician will provide written orders, based on the results of the screening test, regarding whether a clinician must be present for, or directly supervise, the follow-up tests. Dr. Erlandson will be trained in exercise stress testing and certified by Dr. Judy Regensteiner, who trains residents, fellows, and faculty in administration of these tests on a regular basis. Regular exercise sessions will be with a trained, exercise research assistant who is routinely involved with exercise interventions in older adults. During acute exercise visits with multiple blood draws, blood pressure will be monitored immediately after the exercise session, If the SBP rises > 190 or drops <85 immediately following the session, the CTRC nurse or PI (if in the facility) will evaluate the patient and determine if additional blood draws is appropriate. If dizziness occurs during any other exercise session, blood pressure will be monitored and the nurse contact if a participant has SBP <85, or experiences any unexpected post-exercise symptoms.

Screening of volunteers: To minimize the risks of exercise testing in older subjects, testing will be conducted only after the volunteer is examined by a clinician and after a resting ECG is obtained and evaluated. During exercise testing, the ECG is monitored constantly and BP is measured frequently. Exercise tests are terminated if any of the American College of Sports Medicine absolute (and, in some cases, relative) stopping criteria are met [141]. The American Heart Association-recommended emergency equipment and supplies will be available, including: automatic external defibrillator; portable oxygen tank, nasal cannula, ventimask, non-rebreathing mask, and appropriate tubing to connect to the oxygen tank; oral airways; bag-valve-mask hand respirator; syringes and needles; IV tubing, solutions, and stand; and adhesive tape. The Exercise Research Laboratory (ERL) operates as a 911 emergency response facility, as do all other UCH outpatient clinics.

Justification: Exercise is routinely recommended in older adults for its known benefits on multiple systems. Furthermore, stress testing is a routine clinical screening test if recommending that older adults with risk of cardiovascular disease plan to begin an exercise intervention. Thus the procedures are consistent with routine clinical practice.

3. DXA: Three densitometry (DXA) scans will be performed: Femur and spine for bone density, and full body for composition. Urine pregnancy will not be performed prior to DXA scans, as all women will be post- childbearing state (ie, post-menopausal).. Body composition comparisons will include total body fat free mass, body fat mass, trunk fat, trunk to limb fat ration, and appendicular lean tissue mass. Determination of sarcopenic obesity will be calculated from

appendicular skeletal lean tissue mass divided by squared height (kg/m^2), with < 7.26 kg/m^2 in men or < 5.45 kg/m^2 in women suggesting presence of sarcopenic obesity based on limited prior studies.

Risks: The DXA (for measures of body composition) involves exposure to ionizing radiation. The DXA scans (total body, hip, and lumbar spine scans) at 2 times during the study involve a total effective radiation exposure of about 90 mrems. DXA involves <2% of the annual maximum non-therapeutic radiation exposure to the whole body recommended by the FDA (5000 mrem/year). This risk is minimized by having trained DXA technicians administer the procedure, thereby reducing the likelihood of needing repeat assessments.

Plans to minimize risk: The risk of radiation exposure is minimized by having trained technicians administer the DXA, thereby reducing the likelihood of needing repeat assessments.
Justification: DXA is a routine test obtained in clinical practice and will provide important data regarding the impact of exercise on lean and fat mass.

4. Physical function tests: The standard SPPB and a modified version of the SPPB (mSPPB)[142] will be used to measure physical function. These physical function tests have been modified from the performance battery originally used in a the Established Populations of Epidemiologic Studies of the Elderly (EPESE)[143], the Women's Health and Aging Study (WHAS)[144], the Health, Aging, and Body Composition Study (Health ABC)[142, 145], and the Baltimore Longitudinal Study of Aging (BLSA)[146]. The mSPPB improves discrimination of physical function at the higher end of the functional spectrum by increasing the number of repeat chair stands from 5 to 10, standing balance test for 30 seconds instead of 10, and adding a single leg stand. Split times will be obtained, allowing for direct comparison to the standard SPPB, and time it takes to stand 5 times and 10 times will be recorded. *The repeated chair stand test, a measure of lower extremity strength, will be the primary physical function outcome and will be administered every two weeks.* Secondary functional outcomes will be 4-m and 400-m walk times, grip strength, standing balance, and time to climb a flight of 10 stairs (a measure of lower extremity muscle power[147]). These measures will be performed at consent/entry, week 10, and visit 20. The use of assistive devices will be allowed except for the chair rise test.

Risk: The risks associated with the physical function tests are minimal and include a) slight muscular soreness or strain; b) a small possibility of loss of balance or fall, and c) a very small possibility of angina from overexertion.

Plans to minimize risk: Physical function assessments are meant to mimic a patient's daily activities, such as walking across the room, rising from a chair, and balancing to reach for an overhead object. These assessments pose little risk beyond what would be encountered in a day's activities. To minimize the risks of physical function testing, an experienced examiner will administer the tests and instruct participants to stop if they are uncomfortable or fearful at any time during the test. The tasks within the modified short physical performance battery (mSPPB) are ordered from the least to the most challenging and the test will be stopped at the earliest failure. The 400-m walk and stair climb tests will be conducted after the mSPPB. Participants will be allowed ample rest time between tests.

Justification: Physical function measures are predictive of subsequent disability, hospitalizations, falls, and mortality and are safely administered in multiple large cohorts of aging individuals.

5. Venipuncture:

Laboratory analyses will include: CBC, CMP at procedure visit #1 only.

HIV antibody for participants that are not known to be HIV+ if no HIV testing available within the past 1 year.

Fasting IGF-I, IGF-BP3, total/free testosterone, IL-6, sTNFRI and sTNFRII, insulin and glucose will be evaluated at procedure visits #1 and #2, and at week 14, at least 48 hours following the last exercise session.

Inflammatory markers (IL-6, sTNFR-1, and TNF-alpha) will be measured pre-exercise, and 0, 60, or 90 minutes, and ~ 24 hours after a high-intensity exercise stimulus at week 2, 12, and 24.

For the optional microbiome substudy, EDTA plasma samples will be assessed for LPS, heparin plasma samples for sCD14, iFABP) and CRP. LPS levels will be measured using the Limulus Amebocyte Lysate (LAL) assay (Lonza, Switzerland) with plasma samples diluted 1:10 in endotoxin free water and heat-inactivated at 80°C for 15min. Commercially available ELISAs will be used to evaluate levels of IL-6, sCD14, and iFABP (all R&D Systems, Minneapolis, MN). Serum LBP levels were assessed using a custom ELISA all as reported in Dillon 2014.

Peripheral blood mononuclear cells (PBMCs) will be collected at each of the pre-exercise acute visits (weeks 2, 12, and 24) and stored. Future analyses (pending funding) will include an assessment of markers of T-cell and B-cell activation

Samples will be stored for additional analyses. These assays will be performed from stored specimens (in batch to minimize inter-assay variability) in the Clinical Translational Research Center (CTRC) core laboratory at UCD or by Rick Rapaport in Dr. Thomas Campbell's laboratory**. All participant samples will be drawn between 7:30-10am. We will draw approximately 70 mL at the initial visit and 75 mL at the subsequent 3 visits.**

Risk: There is a small risk of local hematoma or infection associated with blood sampling.

Plans to minimize risk: The risks of hematoma and infection are minimized by having trained clinical personnel perform the procedures using sterile techniques.

Justification: We will be evaluating the response of HIV+ vs HIV- individuals to an exercise intervention, in hormone levels and markers of inflammation. Additional samples will be collected for storage. These safety and efficacy markers will be imperative in the design of future, larger intervention studies of exercise in HIV.

6. Intravenous line placement:

For the acute exercise visits at weeks 2, 12, and 24, participants will have the option of having an intravenous line (IV line) placed rather than having venipuncture at the 4 time points. The same studies outlined above will be drawn. The participant does not have to have the same procedure at each of the acute visits (ie, can have 4 venipunctures at one and then opt to have the IV line at the next visit).

Risk: There is a small risk of a local hematoma, infection, superficial blood clot formation, or inflammation of the vein associated with intravenous catheter placement.

Plans to minimize risk: The risks of hematoma and infection are minimized by having trained clinical personnel perform the procedures using sterile techniques.

Justification: Some participants express a desire to have an IV placed rather than undergoing 4 venipunctures, while others note that they would not want an IV line while exercising. We will allow either to meet participant preference.

7. HIV testing.

Risk: A positive HIV test may be identified during routine screening.

Plan to Minimize Risk: Dr. Erlandson is an HIV care provider in the Infectious Diseases Clinic and is trained in the counseling of new HIV+ patients, linkage to care of newly identified HIV+ patients, and care of HIV+ patients. If a person is identified as HIV+, she will directly provide counseling on the diagnosis and ensure that the participant is linked to care. As the study is restricted to HIV- or HIV+ on antiretroviral therapy for 2 years, that individual will no longer be eligible for study participation.

Justification: Every adult should have an HIV test at least once, thus HIV testing is often a part of routine clinical care. Because our outcome is focused on differences between HIV+ and HIV-, it is imperative that we correctly categorize subjects.

8. Questionnaires and other assessments:

Food and nutrition. The "All-Day" screen for fruit and vegetable consumption will be administered at procedure visits #1 and #2. Additionally, a 3 day diet record will be provided the patient at baseline and 24 weeks. Analysis of 3 day diet record and correlation and quantification of dietary fiber intake will be performed by the Nutrition section of the CCTSI in collaboration with Dr. Janine Higgins. Participants that provide stool samples will also complete the Diet History Questionnaire. This will be completed at Acute Visit #1 while participants are waiting for the post-exercise blood draw.

QoL and depression. QoL and depressive symptoms, using the Medical Outcomes Study 36-Item Short-Form Health Survey (SF36), and Centers for Epidemiologic Studies Depression (CES-D) Questionnaire, respectively, will be assessed at procedure visits #1 and #2 and week 12.

Body Image Ideals Questionnaire. This questionnaire derives from a "self-discrepancy theory" where body-image satisfaction will depend upon (1) the extent to which an individual believes that his/her physical characteristics match his/her physical ideals, and (2) the importance associated with having or attaining those ideals.

Physical activity, life space, sedentary time, and wake/sleep time. The International Physical Activity Questionnaire (IPAQ) will be used to assess self-reported physical activity and sedentary time at weeks 0, 4, 8, 12, 16, 20, 24. Changes in spatial mobility will be measured using the Life-Space Assessment (LSA) Questionnaire[148], which is a modification of the original life space questionnaire developed by Stalvey et al.[149]. The LSA assesses the range, independence, and frequency of movement, is highly reproducible, and is sensitive to changes over a 6 mo. period[148]. Participants are queried about the number of times of the previous 4 weeks that they have travelled outside of the bedroom (or room that they sleep), and life-space is evaluated in series of levels (bedroom, other rooms in the home, outside house, within neighborhood (~1/2 mile), within town/city (5 mile), and outside town/city) radiating from that room.

Sedentary time and sleep quality will be measured using two tools. Actiwatch Spectrum, accelerometers to collect physical activity *and* wake/sleep time through additional sensors for light and dark, will be worn for 1 week between the 1st procedure visit and the first exercise visit, and for 1 week between the week 24 and the procedure visit #2. Accelerometers have been calibrated in Dr. Helen Burgess' laboratory at Rush University, and data will be analyzed in conjunction with her laboratory. We will assess change in sedentary time and change in sleep using the actiwatch. The Pittsburgh sleep index and the Munich Chronotype will assess usual sleep habits and restfulness following sleep.

In addition, each participant will be provided with a pedometer *(Omron Tri-Axis USB Pedometer, HJ-324U)* to wear every day through the study to monitor overall activity patterns and changes with an exercise intervention. This monitor stores 22 days of data. The participant will be asked to bring the monitor every other week and data will be downloaded during the exercise session.

Evaluation of health status, medication use, and falls. A brief health status questionnaire will be completed by participants every 2 weeks to document potential study-related problems/concerns (e.g., exercise discomforts); changes in health status (e.g., hospitalizations), prescription and over-the-counter medications (including non-steroidal anti-inflammatory agents); and falls. The surveys will be reviewed by the study nurse and appropriate follow-up pursued.

Post-intervention follow-up. Participants will be contacted by phone 3 months following study completion. Continued frequency of exercise/physical activity (IPAQ), intent to continue exercising, overall well-being will be assessed.

Risk: A person with a very high depression score may be identified on the CES-D depression screening questionnaire.

Plans to minimize risk: CES-D questionnaires will be briefly reviewed prior to the study participant leaving the visit. If an individual indicates a risk for suicidal ideations or intent on the CES-D, the participant will be referred immediately to their primary care provider or the emergency room.

Sample collection: Stool samples will be collected from participants at the first procedure visit (baseline) and second procedure visit as described above. Participants will be given a sample container and a biosafety bag and asked to give a sample within 72 hours prior to the procedure visit and store it in their freezer in the biosafety bag. The samples will be collected by Dr. Jay Liu who will transfer to a -80 degree freezer in the 11th floor RC-2 lab for future analyses (pending future funding).

Risk: There a small risk of embarrassment due to collection and handing in stool samples.

Justification: The microbiome of stool can provide valuable data on dietary intake, and is hypothesized to play a role in the inflammatory profile of older adults with or without HIV infection. To understand the actual components of the microbiome, stool specimens or rectal swabs are required. Stool specimens are less invasive for participants, and thus will be utilized for collection for future studies.

Confidentiality and privacy: The use of questionnaires, interviews, and collection of personal medical information poses a risk to confidentiality and privacy and may cause embarrassment. These risks will be minimized by not including personal identifying information on the forms, when possible, and by conducting interviews and collection of personal information in a private setting. We will be using the REDCap, a Web-accessible database platform, to store data in a manner compliant with IRB and HIPAA standards for security. Data are backed up regularly and automatically and changes to data are logged, creating an audit trail indicating which data were changed, by whom, and when. REDCap is offered to investigators and supported through the CCTSI.

9. Focus group discussions (main study and sub-study participants). Participants will be interviewed in a quiet conference room with round-table seating, located on the Anschutz Medical Campus for convenience. Focus group interviews will be conducted in group sessions, with up to 8-12 HIV-infected participants in each group. We estimate 3 group interviews with ~ up to 24 participants total for current exercise study participants, and up to 5 group interviews for HIV-infected participants not currently exercising (anticipate 6 group sessions: 1 noon and 1 evening meeting for women, 1 noon and 1 evening meeting for non-exercising men, 1 noon and 1 evening meeting for exercising men). Because recruitment for K23 AG050260 is targeting initial recruitment of HIV-infected participants, Aim 1 will focus on HIV-infected participants only. Interviews will be audio-recorded and will last up to 120 minutes. Additional individual interviews lasting 30-60 minutes with select participants (up to 8) with unique perspectives may be conducted. We will continue interviews until further interviews are felt unlikely to provide novel themes.

Risk: The main risk with the discussion groups is confidentiality.

Plan to minimize risk: Participants will have completed the full study consent prior to enrollment (if in the main study), and will complete an additional consent if interested in the qualitative assessments. All the data generated from the study will be kept strictly confidential. Group interviews will be conducted among HIV-infected participants only. Participants will be notified clearly in the consent and prior to scheduling the focus group sessions that all involved participants will be HIV-infected, and therefore their HIV status will be known to others. Participation within the focus groups will be entirely voluntary. All data will be collected using unique patient identification codes.

Justification: We will integrate the qualitative input that is not readily captured in a close-ended questionnaire to understand the self-reported, individual reasons for initial engagement in exercise outside of the on-going study, motivating factors for study participation and engagement, and continuation of exercise after completion of the intervention. The addition of qualitative methods will provide insight into the aspects of health and wellness that are most valued by persons who are aging with HIV infection. The addition of the qualitative interviews allow each participant the opportunity to share his/her personal experiences, and gain insights from those that were unable or uninterested in joining the exercise study.

2. Potential Scientific Problems:

The primary problem that we anticipate is difficulty with enrollment. As of 10/30/2014, the HIV clinic at the University of Colorado cares for 724 HIV+ adults aged 50 or older. Thus, we do not anticipate that enrollment will be a significant barrier. However, we will plan to advertise the study at additional venues including HIV patient advocacy groups (On the TEN), the AIDS Education and Training Center twice year updates (for HIV providers, social workers, advocates), and encourage word-of-mouth advertising. If recruitment appears to be lagging significantly, we will addend our proposal and expand recruitment at the Denver Health HIV clinic. By targeting recruitment at HIV- controls, we will try to maintain similar demographic characteristics between the HIV+ and HIV- groups. Additionally, we limited our enrollment criteria with BMI restrictions—if we have difficulty recruiting due to a larger proportion of participants with a BMI <24, we may choose to enroll lower BMI, and then target recruitment of the HIV- to include participants with a lower BMI.

Other problems that we anticipate: lack of adequate sample on muscle biopsy due to low baseline muscle mass. The primary outcome will only require minimal muscle sample (IGF-1 protein and mRNA). Thus, we recognize that all muscle analyses may not be able to be completed, but will target the studies on the primary analyses of interest.

F. Data Analysis Plan:

Statistical Considerations

Sample Size Calculations: The primary outcome for sample size calculations is change from baseline in chair rise pace. We assume a change from baseline of 1.5 (SD=1.54) seconds or larger, based on differences observed by fall history [85]: Sample sizes of 36/group will yield an estimate of 29 patients per group at week 16 (~20% dropout). 29 subjects per group achieves over 80% power to detect a between group difference of 1.5 seconds with a significance level of 0.05 and the standard deviation conservatively estimated at 2.0 seconds[110] using a two-sided two-sample equal variance t-test.

Matching and Randomization: Enrollment in the uninfected arm will be constrained to lag behind HIV-enrollments. Upon the enrollment of every 5th HIV-infected patient, HIV-uninfected enrollment will be evaluated and recruiting goals updated to ensure a similar age (<60; >=60), gender, BMI, and smoking

distribution. Given the smaller proportion of women in the HIV-infected population, we plan to enroll fewer women. The randomization will be blocked by gender and exercise intensity to ensure balance according to the following scheme:

Exercise Intensity	Moderate (N=36)		High (N=36)	
HIV-Infected (N=36)	Men:	15	Men:	15
	Women:	3	Women:	3
Controls (N=36)	Men:	15	Men:	15
	Women:	3	Women:	3

Analysis Plan: Given repeated measurements over time, a linear mixed effects model will be used with change from week 0 as the primary outcome. The inclusion of additional time points will provide additional power. Groups defined by HIV-status (Exercise Intensity) will be evaluated. The baseline value and a group and time interaction term will be included as model predictors. The primary analysis will be intent to treat. Baseline (week 0) characteristics will be compared between groups prior to comparisons of endpoints, with log transformation for normality, as appropriate. The primary analysis will consider adjusting for age, smoking, exercise intensity (HIV-status) and body mass index (BMI); although if matching and/or balance by group is achieved, they may be precision variables, but they cannot be confounders. Finally, given the potential for non-ignorable dropout, we will accommodate dropout in the analysis of functional measures using a semi-parametric varying-coefficient model approach [150]. The primary analyses will assume that the slope beyond dropout is linear with sensitivity analyses utilizing a slope is attenuated by 50% and a zero slope beyond dropout, the latter being similar to a last value carried forward. Additionally a sensitivity analysis using only data from study completers will be conducted.

Change in IGF-I mRNA will be a primary outcome in the subset of participants undergoing muscle biopsy.

Outcome measures will be available bi-weekly for chair rise, and three times for other functional measures. Serum IGF-I, IGFBP3, muscle IGF-1 mRNA/protein, visceral fat, LBM, and inflammatory cytokines will be measured at procedure visits #1 and #2. Secondary outcomes will include change in other cytokines and hormones, sleep quality, quality of life, V02 peak, strength, and modified SPPB. The inflammatory markers will be tested as mediators on the effect of exercise on changes in function and LBM. Additional secondary outcomes will determine if sedentary time increases as the physical activity intensity decreases, the impact of the intervention on the affect and efficacy of exercise. These factors will be important in design of larger interventions and dissemination to identify points of low exercise efficacy and poor affect and attempt to prevent drop-outs from the intervention. An important outcome is to ensure that intensive 3x/week physical activity does not lead to a decrease in usual activity and an increase in sedentary time due to exercise-related fatigue.

For the inflammatory analyses:

Table 3. Detectable % change from geometric mean needed to achieve 80% power

Outcome	Geometric Mean	Mean (SD) log scale	Within Group Change*		Between Groups Change	
			Increase	*Decrease*	*Increase*	*Decrease*
IL-6 (pg/mL)	0.98	-0.025 (0.629)	52%	34%	110%	52%
TNFa (pg/mL)	1.48	0.392 (0.274)	20%	17%	37%	28%
hs-CRP (mg/L)	1.55	0.440 (1.21)	120%	55%	300%	75%
sTNFRI (pg/mL)	997.9	6.91 (0.25)	24%	19%	34%	25%

*standard deviation (SD) adjusted based on a 0.7 correlation between paired measurements.

Analysis. We propose that no subjects in either arm will experience a major injury (e.g., muscle strain or other injury requiring self-prescribed or provider prescribed treatment) and no more than 1 subject in each group will drop out because of exercise-induced discomfort (i.e., fatigue, muscle or joint pain). The step counts between arms will be compared to determine if the high-intensity group limits non-study activity. For Aim 3, we will compare the change between arms in IL-6, TNFα and sTNFRI (pre-exercise, and 0, 60, and 90 minutes, and 24 hours) with a single bout of exercise at weeks 2, 12, and 24. We also will compare relative changes in serum IL-6, TNFα and sTNFRI from week 2 to week 24 between groups using geometric means and 95% confidence intervals. Secondary outcomes will include the change in other inflammation (sTNFRII, hsCRP, IL-10) and immune activation (sCD14, sCD163) markers, change in physical function, QoL, depressive symptoms, and exercise self-efficacy between study arms. *Sample size and power*. Sample size calculations were conducted for the within and between group percent change in IL-6, TNFα, hs-CRP, and sTNFR-I levels. Data from 49 HIV+ subjects with higher function were used to estimate sample size parameters. We anticipate additional power using

the summary measure AUC. Table 3 contains the within and between group detectable percent change from the geometric mean was calculated assuming a sample size of 13 subjects per group, 80% power, a significance level of 0.05 and a two-sided two-sample t-test.

For the qualitative interviews:

Data Analysis: We will analyze transcribed interviews using a mixed inductive and deductive approach. To develop the codebook, a qualitative analyst and the PI will code a subset of interviews separately using ATLAS.ti software. Coding differences will be adjudicated by Dr. Jones (Co-I). The analyst will then code the remainder of the interviews. After open coding, key meanings and insights will be identified and then reduced to identify patterns. Themes will be explored and tested using iterative techniques across subsequent individual and group level interviews.

G. Summarize Knowledge to be Gained:

Successful completion of this study (additional aims evaluated outside of the CTRC and UCD) will clarify whether the changes in the GH/IGF-I-axis among HIV+ persons are expected age-related GH changes, or represent a unique pathway. For the exercise intervention specifically:

1. If HIV+ have less improvement in chair rise compared to HIV-, we have confirmed that something is interfering with the ability to response appropriately to a similar exercise intervention (IGF-I, testosterone, inflammation, nutrition, motivation, etc).
2. If HIV+ have similar response in chair rise time, then there may be no issues with response to exercise, however this would be in contrast to prior studies in younger adults. Peak V02 and 1-RM differences between groups will also be evaluated to ensure this is not a sensitivity limitation of chair rise vs more intensive measures.
3. If only the HIV+ high-intensity group have improvement in chair rise, this supports that future interventions should target higher-intensity exercise rather than simply lifestyle modifications if improvement in function is the intended outcome. If the HIV+ high-intensity group has improvement in chair rise, but this is associated with greater sedentary time and less improvement on other measures (sleep, mood, exercise efficacy, etc), this raises concerns regarding the long-term durability and compliance with an exercise intervention of this intensity.
4. If #1, then we will look at measures that may interfere with the exercise response including
    a. Systemic IGF-1. Based on our findings and published results from HIV- interventions, we expect that systemic IGF-1 will be lower in the HIV+ group and have less change in an intervention than the HIV- group. However, due to changes in visceral fat which tends to be more pronounced in HIV+ persons with similar BMI, we may see a greater change in IGF-1 in HIV+ vs HIV. This will then be compared to the changes that occur at the level of the muscle, thought to mediate the majority of GH/IGF-I effects on strength and function
    b. If IGF-I increases in HIV+ but not to the extent seen in HIV-, this confirms our hypothesis. Future studies can investigate whether certain exercise (higher intensity or more intensive REx can result in a greater improvement in function, mediated by a greater increase in IGF-I.
    c. If there are no differences in IGF-I mRNA, then it is likely that impairment to an exercise stimulus in HIV is through other pathways. We will also be investigating the inflammatory pathways at the level of the muscle, and stored samples for DNA can lead to future investigations into potential mechanistic pathways.

Skeletal muscle IGF-1 interfering with exercise capacity. If this is associated with an attenuated increase in systemic IGF-I or other hormones in the HIV+ compared to HIV- group, if compared to HIV- persons, a low GH peak amplitude and frequency and low IGF-I will support GH deficiency while high GH peak amplitude and frequency but low IGF-I supports GH resistance; future investigations are described in Aim 1.

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