INTRODUCTION

This is a two-site, placebo-controlled, randomized, clinical trial that will determine the effects of testosterone replacement on atherosclerosis progression in older men with low testosterone levels. The primary outcome measure is the rate of atherosclerosis progression, as measured by common carotid artery intima-media thickness and coronary artery calcium scores by electron beam tomography. The secondary outcome measures include plasma lipids, apolipoproteins, lipoprotein particles, inflammation sensitive markers and insulin sensitivity. We will also assess the effectiveness of testosterone replacement in improving health-related quality of life and its three major determinants, namely, physical, sexual and cognitive functions. While the focus of this proposal is on atherosclerosis progression, we feel that this project provides an outstanding opportunity to establish the efficacy of testosterone replacement in improving health-related quality of life.

A. SPECIFIC AIMS

Although short-term administration of testosterone in replacement doses is relatively safe, the risks of long-term testosterone administration in older men remain poorly understood. The two major areas of concern include the potential for increased risk of atherosclerotic heart disease and exacerbation of a pre-existing, subclinical prostate cancer (1-3). There is a widespread perception that testosterone supplementation adversely affects plasma lipoprotein profile and increases the risk of atherosclerotic heart disease; this premise is not supported by data (4). Thus, the long-term consequences of testosterone supplementation on the risk of atherosclerosis progression remain unknown. While supraphysiological doses of testosterone and non-aromatizable androgens frequently employed by body-builders undoubtedly decrease plasma HDL-cholesterol levels (5-9), physiologic testosterone replacement in older men has been associated with only a modest or no decrease in plasma HDL-cholesterol (10-13). Cross-sectional studies of middle-aged men (14-16) find a direct, rather than an inverse, relationship between serum testosterone levels and plasma HDL-cholesterol concentrations as well as an inverse correlation between serum testosterone levels and visceral fat volume. Testosterone supplementation of middle-aged men with truncal obesity is associated with a reduction in visceral fat volume, serum glucose concentration, blood pressure, and an improvement in insulin sensitivity (17-19). These data suggest that serum testosterone levels in the range that is mid-normal for healthy young men are consistent with an optimal cardiovascular risk profile at any age, and that testosterone concentrations either above or below the physiologic male range may increase the risk of atherosclerotic heart disease. Studies in a LDL-receptor deficient mice provide compelling evidence that testosterone retards early atherogenesis, and that testosterone effects on atherogenesis are mediated through its conversion to estradiol by the action of aromatase enzyme that is expressed in the vessel wall. The effects of testosterone replacement on cardiovascular risk in humans have never been directly examined. **Therefore, the primary objective of this study is to examine directly the effects of testosterone replacement on atherosclerosis progression in men by measuring common carotid artery intima-media thickness (CCA IMT) and coronary artery calcification (CAC) by multidetector computed tomography (MDCT), two independent measurements of generalized atherosclerosis.**

The second objective of this study is to determine whether physiologic testosterone replacement of older men with low testosterone levels improves health-related quality of life. Aging-associated decline in physical, sexual, and cognitive functions contributes to diminished quality of life in older men (20-31). Although the pathophysiology of impairment in each of these subdomains of health-related quality of life is complex and multifactorial, one correctable cause of the diminished quality of life in older men is the decrease in serum testosterone concentrations (32-54). Total and free testosterone (T) levels decline with advancing age in normal men (13-37), with a significant number of men meeting usual criteria for hypogonadism by the sixth to seventh decades (55). Spontaneous (56) and experimentally-induced (57) androgen-deficiency in young men is associated with decreased muscle mass and strength and impaired sexual function. Because loss of muscle mass and function contributes to diminished health-related quality of life (HRQOL) in older men, anabolic therapies such as testosterone that increase muscle mass and strength, would be expected to improve physical function. In older men with low testosterone levels, testosterone might also improve sexual function and marital interaction (11-13, 53, 58-62). A growing body of literature suggests that testosterone impacts neuronal functioning and may affect cognitive performance. Because physical, sexual, and cognitive functions are important determinants of health-related quality of life, testosterone replacement of older men with low testosterone levels would be expected to improve general health perceptions.

Hypotheses

1. Compared to placebo group, testosterone replacement will be associated with either similar or a slightly lower rate of progression of subclinical atherosclerosis, assessed by measurement of CCA IMT and MDCT CAC.
2. In comparison to the placebo group, physiologic testosterone replacement in older men with low baseline testosterone levels will have a neutral or slightly beneficial effect on known cardiovascular disease risk factors,

including insulin sensitivity and the circulating concentrations of plasma lipids, lipoproteins, apolipoproteins, lipoprotein particles and inflammation sensitive markers.

3. Testosterone replacement of older men will be associated with greater improvements in physical function, sexual function, marital interaction, and some aspects of cognitive function than those associated with placebo treatment; this will translate into improvements in health-related quality of life in testosterone-treated men.

This study will not determine the effects of testosterone replacement on the risk of prostate cancer. That issue will require longer treatment duration and a substantially greater sample size than is planned for this study, and is being addressed in a separate VA-Cooperative Initiative.

B. SIGNIFICANCE

The aging of humans is a recent evolutionary event. Of the thousand generations of men and women who have lived on this planet, only the humans of the last two generations could have hoped to live past the age of 50! The population is getting proportionally older. The number of people 85 years of age and older today is substantially greater than at the beginning of the 20th century. Advancing age is associated with decreased muscle mass and strength, and impairment of physical, sexual, and cognitive functions. Diminished muscle mass and strength increases the risk of falls, disability and poor quality of life. Age-related impairment of sexual and cognitive functions also contributes to overall reduction in quality of life. Testosterone replacement, by improving some aspects of physical, sexual and cognitive functions, would be expected to improve health-related quality of life.

Previous studies have established that testosterone replacement in older men with low testosterone levels increases muscle mass and strength. However, lack of information in two areas has prevented formulation of general recommendations about wider use of testosterone replacement in older men. First, the effectiveness of testosterone in improving physical function, quality of life, and other health-related outcomes has not been demonstrated. Second, while there is agreement that short-term administration of testosterone in replacement doses is safe, the long-term risks of testosterone supplementation in older men remain unknown. The areas of major concern are the risks of prostate cancer and heart disease. Because of the high prevalence, even small increases in the incidence rates of atherosclerotic heart disease associated with testosterone supplementation will have significant impact on overall morbidity and mortality, and health care costs. The study will evaluate one important aspect of the long-term safety of testosterone administration by directly examining its effects on the rate of progression of atherosclerosis. If the study demonstrates that testosterone retards atherosclerosis progression, then that would provide one additional reason for testosterone supplementation of older men with low testosterone levels. If the study demonstrates a neutral effect of testosterone on atherosclerosis progression, that information would also be reassuring and useful to regulatory agencies. This study will establish the efficacy of testosterone replacement in improving physical, sexual and cognitive functions that are major determinants of health-related quality of life in older men.

In spite of the paucity of efficacy and safety data, the sales of testosterone and other androgenic products have witnessed explosive growth because of increased media attention and public interest. During the summer of 2000, testosterone-related stories were on the cover of Time, Newsweek, New York Times, and Los Angeles Times! The **prescription sales of testosterone that had been growing at 25-30% annual rate since 1993, almost doubled in the year 2000, and have cumulatively increased 500% since 1993** (Source: IMS Sales Data, provided by Reed Selby, Marketing Director for ALZA Corporation). The growing testosterone use in older men, without a clear understanding of its benefits or long-term risks, has raised concern among regulatory agencies. The proposed study by providing definitive information on the effects of testosterone replacement on several measures of efficacy and safety in older men would facilitate an analysis of its risk: benefit ratio.

C. BACKGROUND

1. Serum Testosterone Levels Decrease as Men Grow Older. There is agreement that even after accounting for the potential confounding factors such as time of sampling, concomitant illness and medications, and technical issues related to hormone assays, serum testosterone levels decrease with advancing age (46). Cross-sectional (31, 33-52, 54) and longitudinal studies (53, 55) demonstrate a gradual but progressive decrease in serum testosterone from age 20 to age 80. Because SHBG concentrations increase with increasing age (31, 34, 35), free testosterone levels decline to a greater extent than total testosterone.

2. **Risks and Benefits of Long-term Testosterone Administration Remain Unknown.** In spite of the growing use of androgenic products, the efficacy and safety of long-term testosterone administration has not been demonstrated in older men. The efficacy of testosterone supplementation in improving health-related outcomes such as health-related quality of life, fracture risk, and physical, sexual and cognitive functions has not been studied. The long-term risks of testosterone administration in older men also remain unknown. In this study, we will determine the effects of testosterone replacement on one efficacy end point - health-related quality life and its determinants, physical, sexual and cognitive functions- and one safety end point –atherosclerosis progression.

2. Testosterone and the Risk of Atherosclerosis Progression

a. Supraphysiological Doses of Androgens Decrease Plasma HDL-cholesterol. Androgen effects on plasma lipids depend on the dose, route of administration, and metabolism (aromatizable or not) (5-9, 63-65). Supraphysiological doses of androgens decrease plasma HDL-cholesterol, apolipoproteins (apo) AI, apoB, and Lp(a) levels and increase LDL-cholesterol. Non-aromatizable androgens, especially when given orally, produce a greater reduction in HDL-cholesterol levels than aromatizable, parenterally administered androgens (63, 64). There are anecdotal reports of myocardial infarction, cerebrovascular accidents, and sudden death in athletes taking large doses of androgens. The uncontrolled nature of these reports, the variety of androgens used, and poor description of patient characteristics such as the possible pre-existence of cardiovascular disease and genetic factors, make it difficult to establish a cause-and-effect relationship between prior androgen use and subsequent clinical events.

b. Physiologic Testosterone Replacement has Little or no Effect on Plasma HDL-Cholesterol Levels. Testosterone replacement in healthy, young hypogonadal men is associated with a small decrease in plasma HDL-cholesterol, while LDL-cholesterol does not change (66-68). Three placebo-controlled studies (10-13) of older men demonstrated no significant change in plasma HDL-cholesterol levels during long-term testosterone administration.

c. Physiologic Testosterone Replacement and Apolipoprotein and Lipoprotein Particles. Plasma lipoprotein cholesterol or triglyceride constituents are not specific markers for discrete lipoprotein families, and the measurement of these lipids alone does not describe adequately the concentration profiles of lipoprotein families (69, 70). A meaningful insight into the profile of individual apoA- and apoB-containing lipoprotein families can only be obtained by direct measurements of these lipoproteins. Chemically distinct apoA- and apoB-containing lipoproteins have unique metabolic properties and atherogenic potentials. The concentration of Lp-AI, but not Lp-AI: AII particles, is significantly lower in normolipidemic men with angiographically documented coronary artery disease (CAD) than in subjects without CAD suggesting that Lp-AI containing particles may represent the antiatherogenic fraction of HDL (71, 72) Although cholesterol-rich apoB-containing lipoproteins of decreasing size and increasing densities (cholesterol-rich LDL equivalent to Lp-B particles) may have the greatest atherogenic potential, (73-76) there is evidence that partially delipidized triglyceride-rich lipoproteins (small VLDL and IDL) may be atherogenic to a similar degree. Cholesterol-rich apoB-containing lipoproteins contribute to the progression of severe atherosclerotic lesions, while triglyceride-rich lipoproteins enhance the progression of mild/moderate lesions (77). Among triglyceride-rich lipoprotein families, partially delipidized Lp-B: C and Lp-AII:B:C:D:E particles may be more atherogenic than Lp-B:C:E or Lp-B:E particles. The rates of neutral lipid and apoB accumulation in human THP-1 macrophages are higher for Lp-B:C than Lp-AII:B:C:D:E or Lp-B particles. The apoA- and apoB-containing lipoproteins differ metabolically and in their relative antiatherogenic potential.

Apolipoproteins control lipoprotein transport and correlate with the risk of myocardial infarction, severity of CAD (78), and progression of CCA-IMT (79, 80). Apolipoprotein B levels correlate with CAD as assessed by angiography (81) and changes in carotid IMT (79, 80). Apolipoprotein C-III levels were correlated with angiographic CAD (77, 82), change in CCA-IMT (79, 80), and recurrent coronary events (83). Women on estrogen replacement therapy (ERT) have lower ApoC-III and higher ApoA-1 levels compared with those not receiving estrogen (Preliminary Data). Whether testosterone, either directly or through its conversion to estradiol, has similar effects on these apolipoproteins is unknown.

There is a paucity of data on the levels of apoA- and apoB-containing lipoprotein families in older men. We do not know how levels of lipoprotein families and triglyceride-rich lipoprotein particles respond to testosterone replacement in older men. We will examine the effects of testosterone on regulation of lipoprotein metabolism by specific apolipoprotein and lipoprotein particle determinations in relation to progression of atherosclerosis.

d. Fat Mass Increases as Men Age, and Testosterone Replacement Decreases Fat Mass in Androgen Deficient Older Men. Testosterone is an important regulator of regional fat metabolism. As men age, their testosterone levels decline and fat mass increases (21, 24, 84). Serum testosterone levels are correlated inversely with fat mass, particularly visceral fat area (15, 85). The induction of androgen deficiency in young men is associated with a decrease in lipid oxidation rates and an increase in total fat mass (57). Testosterone replacement of young (58, 86) and older hypogonadal men (9-12) is associated with reduction in fat mass. Testosterone inhibits uptake of labeled triglycerides and enhances lipid mobilization from the visceral fat (87, 88).

e. Testosterone Level and Risk of Type 2 Diabetes. Testosterone levels are lower in patients with type 2 diabetes mellitus compared with controls (89). Low total and free testosterone levels correlate with a higher risk of type 2 diabetes (90). Free testosterone levels are negatively correlated with glucose, insulin, and C-peptide levels independent of body mass index (91). However, free testosterone levels in many studies were measured by a tracer analog method that is susceptible to changes in SHBG concentrations induced by high insulin levels.

f. Low Testosterone Levels and Heart Disease. Whether variation of testosterone within the normal range is associated with risk of CAD remains unclear. Of the 30 studies reviewed by Alexandersen (4), 18 reported lower testosterone levels in men with CAD (92, 93), 11 found similar testosterone levels in controls and men with CAD and 1 found higher levels of DHEAS. Prospective studies (4) have failed to reveal an association of total testosterone levels and onset of CAD. Lower testosterone levels in men are associated with higher levels of dense LDL particles (94) and prothrombotic factors (95). In one study (96), testosterone undecanoate given orally improved angina pectoris in men with CAD. Testosterone infusion acutely improves coronary blood flow. Thus, cohort and cross-sectional studies suggest a neutral or favorable effect of testosterone on coronary heart disease in men (4).

g. Testosterone Replacement Decreases Visceral Fat and Improves Insulin Sensitivity. Testosterone replacement of middle-aged men with mid-segment obesity decreases visceral fat, plasma glucose and insulin levels, and blood pressure (19, 63, 64). Surgical castration in rats impairs insulin sensitivity; testosterone replacement reverses this derangement (97). However, high doses of testosterone impair insulin sensitivity in castrated rats. Androgens increase insulin-independent glucose uptake (98) and modulate LPL activity (32).

h. Testosterone Replacement and Inflammatory Markers of Atherosclerosis. Higher concentrations of inflammation sensitive markers, particularly high-sensitivity C-reactive protein (CRP), have emerged as important markers for cardiovascular disease risk (99-102). These effects are independent of other risk factors and are of similar magnitude to those reported for plasma lipids. CRP adds to the predictive value of lipids in determining myocardial infarction risk (103). Drugs and hormones may influence cardiovascular risk through inflammation-related mechanisms. Both low dose aspirin and pravastatin lower CRP and cytokine levels (101, 104). The postmenopausal hormone regimens increase CRP (105), suggesting that proinflammatory effects might be related to early adverse effects of HRT on cardiovascular events rates, such as those observed in the HERS trial (106). A major determinant of CRP concentration is obesity (107). Adipose tissue is a major source of interleukin-6 (IL-6) (108), a proinflammatory cytokine, which controls CRP production in the liver. IL-6 concentration also predicts all-cause mortality in the elderly (109). Because testosterone decreases visceral fat in older men (19, 63, 64), it is possible that testosterone replacement may lower CRP and IL-6 levels.

i. Testosterone Retards Atherosclerosis Progression in an Animal Model of Atherosclerosis. In a LDL receptor-deficient mouse model of atherosclerosis (110), orchiectomy is associated with accelerated formation of early atherosclerotic lesions in the aorta. Testosterone supplementation retards the progression of atherosclerotic lesion, an effect that is blocked by concomitant administration of an aromatase inhibitor (110). Testosterone effects on atherosclerosis progression were independent of plasma lipids. These data provide compelling evidence that testosterone, through its conversion to estradiol, can retard the progression of atherosclerosis in this animal model. The proposed study will extend these observations to humans and assess the effects of testosterone on atherosclerosis progression in older men, and determine whether testosterone effects on atherosclerosis are mediated through its conversion to estradiol.

3. Ultrasound Imaging of CCA IMT as a Primary Atherosclerosis End Point. Serial coronary angiography is impractical in asymptomatic men because of an unacceptable risk from this procedure. In contrast, high resolution B-mode ultrasonography is a safe, sensitive, and accurate method for the measurement of changes in subclinical atherosclerosis. Several studies such as CLAS (79, 111), MARS (80), and the Estrogen in Prevention of Atherosclerosis Trial (EPAT) (Preliminary Data), have shown that repeated measurements of carotid IMT reduces the sample size necessary for study when computerized edge detection method of image analysis is applied to B-mode images (112). Pignoli and others (113, 114) have shown, through comparison with excised vessel segments, that accurate ultrasonographic estimates of carotid atherosclerosis are feasible. Non-invasive measurement of distal CCA IMT is informative about coronary artery status. Carotid artery atherosclerosis in men and women is significantly correlated with the degree of atherosclerosis in coronary arteries at autopsy (115). There is a strong relationship of carotid wall thickness with angiographic presence of coronary artery disease (116-118) and with confirmed history of CAD (119) in both men and women. Epidemiological studies have shown an association of carotid IMT with cardiovascular risk factors in both men (120) and women (121).

Carotid IMT progression is associated with clinical progression of atherosclerotic disease and reduction in carotid IMT progression mirrors reduction in clinical events. For instance, the reduction in progression of subclinical atherosclerosis measured by carotid IMT observed in primary prevention trials using lipid-lowering therapy (e.g., ACAPS (122), KAPS (123), and CAIUS (124)) mirrors the reduction in clinical events in primary prevention trials (e.g., WOS (125) and AFCAPS (126)). Similarly, the carotid IMT trials conducted in subjects with established cardiovascular disease (PLAC II (127), CLAS (79, 111), MARS (80), and LIPID substudy (104)) show that a reduction in atherosclerosis progression with lipid-lowering mirrors the reduction in clinical event rates seen in 4S, CARE, and LIPID (128)secondary prevention trials. There is a parallel reduction in carotid IMT progression and coronary atherosclerosis with lipid-lowering therapy (129). Similarly, reductions in carotid IMT progression have

been demonstrated with beta-blockers (BCAPS (130)) and ACE inhibitors (SECURE (131)). Over the last decade, carotid IMT has emerged as a robust, highly tested non-invasive imaging end point for assessing drug effects on the progression of subclinical atherosclerosis.

In a randomized trial, we demonstrated that ERT reduces progression of subclinical atherosclerosis as determined by CCA IMT in healthy postmenopausal women without preexisting cardiovascular disease (**Preliminary Data**). We have also shown a significant correlation of CCA IMT progression with CAD progression measured by serial coronary angiography (129). Further, CCA IMT progression is predictive of clinical events and correlates with direct assessment of coronary atherosclerosis by sequential coronary angiography (132). The relation between clinical events and progression of carotid IMT is as strong as the relation between events and progression of coronary atherosclerosis as determined by angiography (132). These data are consistent with 4 other studies in which carotid IMT has been found to be predictive of cardiovascular events in symptomatic and asymptomatic men and women <65 and >65 years old (129, 133-136). There is a consistent relationship between carotid IMT and relative risk of clinical events of 1.2 to 1.4 for each 0.1 mm increment of IMT. These data confirm the close association between carotid and coronary atherosclerosis and support the usefulness of B-mode ultrasound measurement of IMT for quantitation of subclinical atherosclerosis in studies of agents that affect the progression of atherosclerosis. When high-resolution B-mode ultrasound imaging is focused on CCA IMT, small changes in subclinical atherosclerosis can be measured. Several atherosclerosis intervention trials have demonstrated that a 2 to 3 year intervention period is sufficient for detecting treatment group differences (137).

4. MDCT as an Atherosclerosis Endpoint. The ability of MDCT to quantitate coronary calcium has been validated in many studies (138-144); there is a direct relation between coronary calcium as measured by MDCT, and histologic (145, 146) and *in vivo* intravascular measures of atheromatous plaque (141). Although there are differences in the design of studies that used MDCT to track longitudinal changes, including the duration of follow-up and the method used for quantitating calcium (Agatson vs. volumetric method), recent studies are in agreement that MDCT can be used successfully to track changes in coronary atherosclerosis over time (147, 148). The development of novel, calcium volume scoring system (149) and novel ECG-gating algorithms have allowed for a higher degree of reproducibility between scans making it possible to use MDCT to track changes in atherosclerotic plaque by sequential scans (148). We have demonstrated (147) that coronary calcium scores in asymptomatic men and women increase by a mean 33% per year, predicting that the coronary calcium scores would double every 2.5 to 3 years. Several studies have shown that individuals with hypercholesterolemia treated with statins have lower rates of atherosclerosis progression, as measured by MDCT, than those not receiving statins. Changes in calcium scores measured by MDCT also predict the progression of coronary artery disease (147); MDCT measured progression of coronary calcium is associated with 5-13 fold greater risk of cardiac events (150).

5. Health-Related Quality of Life Declines with Advancing Age and the Effects of Testosterone Replacement on Health-Related Quality of Life (HRQOL) are Unknown. Health-related quality of life refers to how health impacts an individual's ability to function, and his or her perception of well-being in physical, mental, emotional and social domains of life. HRQOL is a multi-dimensional construct that includes perceptions of physical function, role limitations caused by physical health problems, emotional well-being, general health perceptions, role limitations caused by emotional problems, energy/fatigue, and social functioning. The impairment of physical function is an important determinant of the deterioration in quality of life in older men and in men with chronic illness. In HIV-infected individuals, lean body mass and function correlate with measures of health-related quality of life. Importantly, advancing age is associated with decreased scores for health-related quality of life; the greatest changes are noted in the subdomains of physical function. Interventions such as exercise that improve physical function also improve HRQOL. Therefore, we posit that testosterone replacement, by improving muscle mass and physical function, would be expected to improve an individual's perception of their general health.

Impairment of sexual function in men is associated with a significant decrease in emotional domains of quality of life. Sexual function correlates significantly with general health perceptions and role limitation due to emotional problems. Sexual function also significantly affects marital interactions. Cognitive impairment affects an individual's ability to live independently, and correlates inversely with HRQOL. Because testosterone might have beneficial effects on some aspects of physical, sexual and cognitive functions in older men, testosterone replacement would be expected to improve several subdomains of HRQOL and marital interactions.

6. Muscle Mass and Strength Decrease and Contribute to Impairment of Physical Function and Poor Quality of Life in Older Men. Advancing age is associated with a decrease in fat-free mass and an increase in fat mass. Surveys conducted in New Mexico (21) and Minnesota (24) indicate that 22% of men over 60 and 50% of men over 80 have sarcopenia, defined as appendicular skeletal muscle mass less than 2 standard deviations below the mean for healthy, young men. These data provide evidence that sarcopenia is an important public health problem. Sarcopenia is associated with a 4-fold increased risk of disability in the elderly (21). The loss of muscle mass and strength is an

important determinant of the individual's ability to live independently (21, 23, 29), and contributes to increased risk of falls and fractures (151-158), dependency, and poor quality of life (23). Aging-associated sarcopenia is due to many causes including androgen deficiency, abnormalities in the GH/IGF-I axis, decreased energy intake and physical activity, and chronic illness (20, 29, 31, 37, 159). Of these, age-related decrease in testosterone levels has been well documented, is potentially reversible, and is the focus of this proposal.

7. Testosterone Replacement Increases Muscle Mass and Strength in Androgen-Deficient, Young and Older Men, but the Effects of Androgen Replacement on Physical Function in Older Men have not been Well Studied. Spontaneous and experimentally-induced androgen deficiency in young men, is associated with decreased lean body mass, and increased fat mass (56, 57). Conversely, testosterone replacement of young, hypogondal men increases fat-free mass (56-58, 86, 160, 161), muscle size, and muscle strength (58, 162). Two long-term, placebo-controlled studies have shown that testosterone treatment increases fat-free mass and decreases fat mass in older men with low normal testosterone levels (11, 163). In HIV-infected men with low testosterone levels (160), testosterone replacement increases maximal voluntary strength. Although testosterone replacement increases fat-free mass and maximal voluntary strength, we do not know if testosterone improves physical function. Most previous studies of testosterone replacement in older men did not examine changes in physical function. The few studies that did examine this issue suffered from methodological problems in the measurements of physical function. We believe a major reason for the failure to demonstrate improvements in physical function is that the **measures of physical function used in previous studies were relatively insensitive and "threshold-dependent"**. The widely used tasks such as 0.625 m stair climb, standing up from a chair, and 20 meter walk require only a small fraction of an individual's maximal voluntary strength. In most healthy, older men, the baseline maximal voluntary strength is far higher than the threshold below which these measures would detect impairment. Given the low relative intensity of the tasks used, these men neither show impairment in these threshold-dependent, measures of physical function at baseline, nor would one expect performance on these tasks to show improvement during testosterone administration. One unique aspect of the proposed study is that we will use **threshold-independent measures of physical function that require near-maximal strength of critical muscle groups** such as the quadriceps. Because testosterone improves maximal voluntary leg strength, it would be expected to improve these threshold-independent measures of physical function (**Preliminary Data**). Another unique aspect of our study is that we will not only measure physical function by objective tests, we will also assess **perceptions of physical function**, by validated instruments.

8. Testosterone and Sexual Function in Older Men. Sexual function in men is a multi-component process that includes central mechanisms for regulation of sexual desire and arousability, and local mechanisms for penile tumescence, orgasm, and ejaculation. Primary effects of testosterone are on sexual interest and motivation (164). Testosterone replacement of young, hypogonadal men improves a range of sexual behaviors including frequency of sexual activity, sexual daydreams and thoughts, feelings of sexual desire (50, 165-167), spontaneous erections, episodes of nocturnal penile tumescence, and duration of erections in response to visual erotic stimuli (168). In male mammals, relatively low normal testosterone levels can maintain sexual function (169, 170). Testosterone regulates nitric oxide synthase activity in the cavernosal smooth muscle, and it is possible that achievement of optimal penile rigidity might require physiologic testosterone levels (171). The orgasm and ejaculation are androgen-independent. In middle-aged and older men, erectile dysfunction and androgen deficiency are two independently distributed, disorders that sometimes co-exist in the same patient. Eight to ten percent of men with erectile dysfunction have low testosterone levels (48, 172-176). The prevalence of low testosterone levels is not significantly different between men with impotence and those without impotence (173). Testosterone administration does not improve sexual function in men with normal testosterone levels (177). Many, but not all, older men with low testosterone levels, experience improvements in their libido and overall sexual activity with androgen replacement (2, 178-180). The response to testosterone supplementation in this group of men is variable (2, 178-180) because of the co-existence of other disorders such as diabetes mellitus, hypertension, cardiovascular disease, and psychogenic factors. A meta-analysis (180) concluded that testosterone administration is associated with greater improvements in sexual function compared to placebo treatment in men with sexual dysfunction and low testosterone levels. However, previous studies of testosterone replacement in older men have been flawed because of failure to include a placebo-control group, small sample sizes, selection of men with erectile dysfunction associated with multiple systemic illnesses that may attenuate response to testosterone therapy, and inclusion of men with normal testosterone levels. In this study, we will select relatively healthy older men with low testosterone levels, exclude those with systemic diseases, and conduct a placebo-controlled, randomized trial. In addition to the assessments of sexual desire and activity, we will also evaluate testosterone effects on marital interactions, which might improve due to an increase in sexual desire and sense of well being, independent of the change in erectile function.

7. **Effects of Testosterone Replacement on Cognitive Functioning are Poorly Understood.**

a. Relevance of Testosterone in Neural Functioning. The majority of research on the effects of steroidal hormones on brain function has focused on the role of estrogen. Classical estrogen signaling occurs through nuclear receptors, but estrogens also exert non-transcriptional effects (181, 182),(183). Estrogen receptors co-localize on forebrain magnocellular cholinergic neurons and on locus ceruleus neurons (184). The density of apical dendritic spines of CA1 pyramidal neurons decreases after ovariectomy in the rat, an effect prevented by estradiol (185). The mechanisms involved in the neuroprotective and neurotrophic effects of estrogen are not understood, but involve interactions (186) with nerve growth factor (187), brain-derived neurotrophic factor (188), insulin-like growth factor-I (189) and fibroblast growth factor (190). Estrogen can act directly at neurotransmitter complexes or ion channels (191), exhibit anti-oxidant effects in brain, reduce neuronal death after exposure to prooxidant and ß-amyloid (192)**,** enhance expression of the neuroprotective *bcl*-2 family (193), and improve cerebral blood flow (194)**. Testosterone is aromatised to estrogen in the brain, and some effects of testosterone might be mediated through its conversion to estradiol**. Androgen receptors are expressed in the brain (195), and androgen effects on organization of the brain during development (196) are likely mediated through androgen receptor (197, 198). Androgens increase neurite arborization, facilitating intercellular communication (199). Testosterone also has nongenomic effects, and affects serotonin, dopamine, acetylcholine (200), and calcium signaling (201)**.**

b. Testosterone and Cognitive Functioning. Androgens effects on cognitive function are domain-specific. For instance, observations that men outperform women in a variety of visuo-spatial skills suggest that androgens enhance visuospatial skills (202). Janowsky et al. (203) tested verbal and visual memory, spatial cognition, motor speed and cognitive flexibility in a group of older men who received 3 months of testosterone supplementation. Testosterone replacement was associated with a significant improvement in spatial cognition only. Serum testosterone levels were not significantly correlated with spatial performance, but estradiol levels showed a significant inverse relationship with spatial performance suggesting that estradiol may inhibit spatial ability. In San Bushmen (204), testosterone, but not estradiol, levels correlate with better spatial ability and worse verbal fluency. Circulating levels of dihydrotestosterone, a metabolite of testosterone, positively correlated with verbal fluency. Barrett-Conner**,** et al (205) found association between total and bioavailable testosterone levels, and global cognitive functioning and mental control, but not with visuospatial skills. Other studies (206, 207) have reported a curvilinear relationship between androgen levels and spatial ability such that high testosterone females and low testosterone males show the best performance. Several small clinical trials in elderly hypogonadal men have provided conflicting results. Sih (10) et al found no effect, while Herbst, et al (208) reported an effect. Testosterone also enhances verbal fluency. Hypogonadal men performed worse on tests of verbal fluency than eugonadal men, and showed improvement after testosterone replacement (164). In transsexual males, administration of anti-androgen and estrogen, prior to surgery for gender reassignment, decreased anger and aggression proneness, sexual arousability, and spatial skills, and increased verbal fluency ability. Conversely, testosterone administration to females decreased verbal fluency and increased spatial skills (209, 210).

In summary, the literature on testosterone and cognition is highly equivocal. The inconsistency in findings cannot be interpreted as evidence that there is no effect. Rather methodological problems appear to limit the generalizabilty of results. Limitations of previous studies, that we will overcome in the proposed study, include limited sample sizes with heterogeneous, poorly defined samples; the use of a variety of neuropsychological tests, some that lack prior psychometric validation; and inconsistent methods and the use of differing protocols in clinical trials.

8. The Role of Aromatization in Mediating Testosterone Effects. Testosterone is the predominant androgen secreted by the testis that is converted to two active metabolites, estradiol 17 β and 5-α DHT. Testosterone serves both as a hormone and a prohormone. The prevalent belief is that aromatization of testosterone to estradiol is required for mediating its effects on bone resorption (211-213), sexual differentiation of the brain, some subdomains of cognitive function (203), plasma lipids (63), gonadotropin suppression (214-218), and atherosclerosis (110). Although estrogen inhibits bone resorption markers, nonaromatizable androgens have also been shown to affect bone resorption (212, 213). Non-aromatizable androgens produce a greater reduction in plasma HDL-cholesterol than aromatizable androgens (63). Testosterone retards atherosclerosis progression in a mouse model of atherosclerosis through its conversion to estradiol in the vessel wall (110).

9. Physiologic Testosterone Replacement by the Testosterone Gel. Testosterone gel is a hydroalcoholic gel that contains 1% testosterone and provides continuous transdermal delivery of testosterone for 24-hours (162, 219). A daily application of 7.5 g of gel delivers 75 mg of testosterone daily to the skin. Approximately 10% of the applied testosterone is absorbed across the skin during the 24-hour period (162, 219). Daily applications of the 75-mg dose maintains serum testosterone in the mid-range for young men (162, 219). Testosterone levels are uniform over the 24-hour dosing interval and consistent from day-to-day. Average testosterone concentration associated with the 75-mg dose at steady state is ~700 ng/dl, and with the 50-mg dose 566 ng/dL (162, 219). The circulating levels of estradiol and DHT are in the normal range during treatment with 50- to 100-mg doses. Studies of up to 6-month

duration report significant gains in lean body mass, a reduction in body fat, improvements in sexual function, mood, and general health perceptions in hypogonadal men treated with 50 to 100-mg of testosterone gel (162, 219). The skin tolerability of the gel is excellent; only 4% of men treated with the gel experienced skin irritation. The excellent skin tolerability, once a day application, and ease of application should enhance patient accrual and retention.

D. PRELIMINARY DATA

The objective of this section is to present data from antecedent NIH grants, "Androgenic Steroids, Muscle Strength, and Protein Synthesis (1RO1DK45211, S. Bhasin, PI); "Testosterone Replacement, Resistance Exercise, and HIV-Wasting (1RO1DK49296, S. Bhasin, PI); "Sarcopenia: Testosterone Dose Response in Older Men, (1RO1AG14369, S. Bhasin, PI)"; the EPAT Study (Howard Hodis, PI); the Women's Memory Study (Galen Buckwalter, co-PI); and the Baltimore Longitudinal Aging Study (Mitchell Harman). These data demonstrate that all the techniques to be used in this project are already operational, and that we have access to and substantial experience in recruiting large number of subjects, old and young, for clinical research studies. These data also demonstrate that:

1. When followed longitudinally, men demonstrate an age-related decline in serum testosterone levels that is independent of other health factors (Harman et al, J Clin Endocrinol Metab 2001)
2. Data from an ongoing study of the effects of estrogen replacement in postmenopausal women demonstrate the experience of the investigating team and the ability of CCA-IMT to detect longitudinal changes in atherosclerosis progression (Hodis et al, manuscript is in the Appendix);
3. Electron Beam Tomography (MDCT) can be a useful, non-invasive tool for demonstrating intervention-induced changes in rates of atherosclerosis progression (Budoff et al, Am J Cardiol 2000; Manuscript is in Appendix);
4. Health-related Quality of Life declines with advancing age;
5. Physiologic testosterone replacement increases fat-free mass, muscle size, and maximal voluntary strength in androgen-deficient men (Bhasin et al, JCEM 1997)
6. Testosterone replacement increases fat-free mass and muscle strength, but not specific tension in HIV-infected men with weight loss and low testosterone levels (Bhasin et al, JAMA 2000);
7. Data from a previous study demonstrate that changes in sexual function as assessed by international index of erectile function are associated with demonstrable changes in HRQOL (Paige et al, manuscript under review);
8. Data from a Women's Memory Study document the expertise of the investigating team in studying hormone effects on cognitive function (1RO1AG14745; Galen Buckwalter, co-I, work in progress).

1. Longitudinal Changes in Total and Free Testosterone Levels with Advancing Age: The Baltimore Longitudinal Aging Study (Harman et al, Paper in J Clin Endocrinol Metab is in Appendix). Cross-sectional studies have shown that total and free testosterone levels are lower in older men than younger men. However, there has been a paucity of longitudinal data on the age-related changes in testosterone levels. The extent to which decline in testosterone levels is the result of the aging process *per se*, as opposed to chronic illness, and other age-related factors remains controversial. The frequency with which aging leads to low testosterone levels consistent with hypogonadism has not been defined. We measured total testosterone and SHBG levels in 890 men in the Baltimore Longitudinal Aging Study (BLSA). Using a mixed effect model, we found independent effects of aging to reduce testosterone levels. After compensating for the date effect, we observed significant, independent, age-invariant, longitudinal effects of age on both total testosterone and free testosterone index (free testosterone index = Total testosterone/SHBG) with an average change of –0.124 nM/L/year and –0.0049 nM SHBG/year. The percentage of men with testosterone levels in the hypogonadal range was 20% in men over 60, 30% in men over 70, and 50% in men over 80. Our observations of health factor independent, age-related longitudinal decreases in testosterone and free testosterone index, resulting in high frequency of hypogonadal values, suggest that further investigation of testosterone replacement in older men with low testosterone levels are justified.

2. **Hormone Replacement and Atherosclerosis Imaging Studies (Howard Hodis, PI).** The investigating team has substantial experience in the area of hormone replacement, atherosclerosis imaging and women's cardiovascular health for the past 5 years. The studies such as WELL-HART (a randomized, double-blind, placebo-controlled, serial coronary angiographic/carotid ultrasonographic trial of hormone replacement therapy (HRT) in postmenopausal women with established coronary artery disease), and EPAT (a randomized, double-blind, placebo-controlled, carotid artery ultrasound trial designed to test the effects of estrogen replacement therapy (ERT) on the progression of subclinical atherosclerosis in postmenopausal women without preexisting cardiovascular disease) illustrate our experience in hormone replacement arterial imaging trials. EPAT has been completed and WELL-HART is in its last year of randomized treatment (80, 220) (Table 1).

Estrogen in the Prevention of Atherosclerosis Trial (EPAT, Howard Hodis, PI, 1RO1AG18798). EPAT was a randomized, double-blind, placebo-controlled, carotid artery ultrasound trial designed to test whether micronized 17β-estradiol (1 mg daily) versus placebo reduces progression of subclinical atherosclerosis (carotid IMT) in healthy

postmenopausal women without preexisting cardiovascular disease with LDL-cholesterol levels of at least 130 mg/dL. After randomization, subjects were treated for 2 years with unopposed ERT or placebo and with lipid-lowering medication (primarily HMG-CoA reductase inhibitors) if needed, to maintain LDL-cholesterol levels <160 mg/dL. All subjects received dietary counseling according to step II AHA dietary recommendations. Carotid artery ultrasonography was performed at baseline (2 visits) and every 6 months on trial. Mean age of the 221 subjects randomized to EPAT was 62.2 years (range 46 to 80 years). The primary end point was the rate of change in the distal CCA far wall IMT in computer processed B-mode ultrasonograms (80, 111, 112, 132, 220).

Table 1 Comparison of CCA IMT by Treatment Group in the EPAT Study*

Variable	Placebo N	Placebo Mean (SD)	Estrogen N	Estrogen Mean (SD)	P value
Overall cohort:					
Baseline IMT (mm)	102	0.774 (0.14)	97	0.7520 (0.11)	.48
Rate of IMT change (mm/year)	102	0.0047 (0.021)	97	-0.0007 (0.016)	.04

***Intent to treat analysis; subjects with baseline and ≥1 on-trial IMT measurement.**

Baseline CCA IMT was not significantly different between the ERT- and placebo-treatment groups (Table 1). There was continued progression of subclinical atherosclerosis (positive rate of change in CCA IMT) in the placebo-treated group, whereas in the ERT-treated group there was a significant reduction in the progression of atherosclerosis (negative rate of change in CCA IMT). The difference in the progression rates between the ERT- and placebo-treated groups was significantly different ($p<0.04$).

3. **Our Expertise in Evaluating Reproducibility of Electron Beam Tomography and Its Ability to Assess the Rate of Progression of Coronary Calcium (Paper by Budoff et al in Am J Cardiol is in the Appendix).** Limited reproducibility of calcium scores has been cited as a potential problem for longitudinal measurements of plaque burden (221-224). Previous studies have reported mean reproducibility of 14-38% using scanning algorithms that are no longer up to date. Until recently, limitations of image acquisition (number of slices obtainable during one breath hold) and suboptimal ECG gating led to higher inter-scan variability. We have carefully evaluated multiple ECG gating and breath hold strategies to minimize variability on coronary scanning by MDCT (223, 225-227). The most common trigger time employed during MDCT is 80% R-R interval (225). We have performed several studies (228, 229) that have demonstrated that coronary motion is at its nadir in early diastole (30-50% of the R-R interval). By imaging in the early cardiac cycle, at approx. 40% of the R-R interval instead of the conventional 80% of the R-R interval, CAC score can be significantly reduced (230).

To determine the rate of progression of atherosclerosis using coronary calcium scores derived from MDCT, we evaluated 299 asymptomatic persons (227 men and 72 women) who underwent two consecutive MDCT scans at least 12 months apart (147). The average change in calcium score for the entire group was 33.2±9.2%/year. The treated group receiving statins demonstrated an average increase in calcium scores of 15 ± 8%/year compared with the 39 + 12% for the untreated patients ($p < 0.001$). Among the 60 patients for statin monotherapy, 37% had a decrease in the calcium score from baseline to follow-up scan. The relative increase in calcium scores did not vary significantly by gender or risk factors, with the exception of statin-treated hypercholesterolemic subjects. Scores of zero on the initial scan predicted a low likelihood of significant calcium deposits on repeat scanning. Treatment with a statin was associated with a 61% reduction in the rate of coronary calcium progression. Twenty patients demonstrated regression of MDCT calcification in the treated group, significantly more than in the untreated group (30% vs 6%, $P<0.05$; (147). The study demonstrates the usefulness of MDCT in assessing the efficacy of interventions to retard progression of atherosclerosis, non-invasively, over periods of greater than 12 months. In separate studies, change of coronary calcium scores was associated with marked increase in the risk of cardiac events.

4. Changes in Health-Related Quality of Life with Advancing Age (Data provided by Ron Hays, Ph.D., Rand Corporation). Dr. Hays and his colleagues at Rand Corporation, who developed the original Rand-36 measure of health-related quality of life, have generated the normative ranges for different subdomains of health-related quality of life men and women in different age-ranges. There is an age-related decline on overall health-related quality of life scores, although some subdomains show a greater age-related change than others. The greatest magnitude of decline has been observed in physical function and medical component scores.

Table 2. Age-Related Changes in Physical Function and Medical Component Scores (Ware et al, SF-36 Health Survey Manual)

N	Age	Physical Function Score	Medical Component Score

71	18-24	53.5	50.9
199	29-34	55.0	48.9
239	35-44	53.0	51.0
145	45-54	50.4	51.0
105	55-64	46.9	51.6
293	65+	42.0	52.5

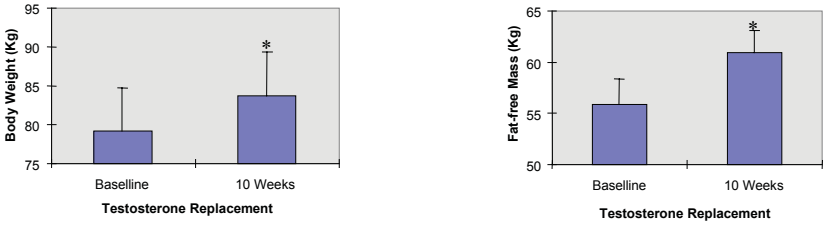
5. The Use of International Index of Erectile Function and HRQOL Instruments for Demonstrating Improvements in Emotional Well-Being and Relationships Among Users of Sildenafil (Paige et al, manuscript under review). Erectile dysfunction can have negative effects on health-related quality of life as it effectively limits choices for sexual interaction and satisfaction and can potentially lead to emotional distress and marital discord. This study assessed the associations of sildenafil use with erectile function, relationship with sexual partner, functional status, and emotional well-being in men with erectile dysfunction. Subjects were recruited from a university hospital urology and internal medicine clinic, and from university-affiliated community primary care clinics. Change scores were calculated from the International Index of Erectile Function; the marital interaction scale from the Cancer Rehabilitation Evaluation System Short Form (CARES-SF); the 5-item emotional well-being scale from the SF-36, and the SF-12. The mean age of the respondents was 60.5 years (range 36-80). 53% were married; 62% had a 4-year college degree; mean household income was $75,000. With the use of sildenafil, there was a statistically significant improvement in scores for erectile and sexual function ($p<0.001$), relationship with sexual partner ($p=0.007$), and emotional well-being ($p<0.001$). 38% of respondents indicated that sildenafil had definitely improved the quality of their life; and 29% of respondents indicated that sildenafil had definitely improved their relationship with their partner. In a multivariate model, improvements in erectile function and relationship with partner were each correlated with improvement in emotional well-being ($R^2=0.20$, $p<0.001$). Users of sildenafil reported dramatic improvements in erectile and sexual functioning. These improvements were associated with positive changes in emotional well-being and relationship with one's sexual partner.

6. Testosterone Increases Fat-Free Mass, Muscle Size and Strength in Healthy, Hypogonadal Men (Bhasin et al., JCEM 1997). We examined the effects of replacement doses of testosterone on fat-free mass, muscle size and strength in healthy, young, hypogonadal men before and after 10 weeks of treatment with 100-mg testosterone enanthate weekly (37). Body weight increased by 4.5±0.6 kg (P=0.005). Fat-free mass, estimated from underwater weight, increased by 5.0±0.8 kg (P=0.004) (Figure 1); body fat did not significantly change. Significant increases in muscle strength were also noted after treatment (143±20 vs. 213±24 lb., P< 0.001). These results demonstrate that replacement doses of testosterone augment fat-free mass, muscle size and strength in young, hypogonadal men. Others have reported similar data on the effects of testosterone replacement in androgen-deficient men.

Figure 1. Effects of Testosterone Replacement on Body Weight and Fat–Free Mass in Hypogonadal Men

Mean (± SEM) body weight, and fat-free mass before (Baseline) and after 10 weeks of weekly IM 100 mg testosterone enanthate in 7 hypogonadal men. * $p < 0.005$ *vs* baseline

7. Testosterone Replacement Increase Muscle Strength and Fat-Free Mass in Androgen-Deficient, Human



Immunodeficiency Virus-Infected Men (Paper in JAMA is in the Appendix). We determined the effects of testosterone replacement, with or without a program of resistance exercise, on muscle strength and body composition in androgen-deficient, HIV-infected men with weight loss (160). This was a placebo-controlled, double–blind, randomized, clinical trial in which HIV-infected men with serum testosterone less than 300 ng/dl, and weight loss of 5% or more in the previous six months were randomly assigned to one of four groups: placebo, no exercise; testosterone, no exercise; placebo plus exercise; or testosterone plus exercise. Placebo or 100–mg testosterone enanthate were given IM weekly for 16 weeks. The exercise program consisted of supervised, individualized (based on each subject's initial 1-RM) progressive strength training 3 times per week for 16 weeks. The energy and protein intake were standardized at the outset to 36 Kcal/kg/day and 1.2 g/kg/day, respectively.

We paid particular attention to minimizing the learning effect by having the men come back to the Exercise Laboratory on two or more occasions until they were familiar with the equipment and technique thus achieving stability of measurement prior to initiating treatment. Muscle strength in five exercises was measured using the 1-RM

method. In the placebo alone group, muscle strength did not change in any of the five exercises (-0.3 to -4.0%). This indicates that this strategy was effective in minimizing the influence of the learning effect. Men treated with testosterone alone, exercise alone, or combined testosterone/exercise, experienced significant increases in maximum voluntary muscle strength in the leg press (+22 to 30%), leg curls (+18 to 36%), bench press (+19 to 33%), and latissimus dorsi pulldowns (+17 to 33%) exercises. The gains in strength in all the exercises were greater in men receiving testosterone, or exercise alone than in those receiving placebo alone. The change in leg press strength was correlated with change in muscle volume (R=0.44, P=0.003) and change in fat-free mass (R=0.55, P<0.001).

Average lean body mass increased by 2.3 kg (P=0.004) and 2.6 kg (P<0.001), respectively in men who received testosterone alone or testosterone and exercise, but did not change in men receiving placebo alone (0.9 kg, P=0.21). There was a greater increase in thigh muscle volume in men receiving testosterone alone (40 cm^3, P<0.001), exercise alone (62 cm^3, P=0.003) than in men receiving placebo alone (5 cm3, P=0.70). This study demonstrated that when the confounding influence of the learning effect can be minimized and appropriate androgen-responsive measures of muscle strength are used, testosterone replacement is safe and effective in increasing maximal voluntary strength in HIV-infected men with low testosterone levels.

Table 3. Maximum Voluntary Muscle Strength (kg) in the Leg Press* Exercise

	No Exercise		**Exercise**	
	Placebo	**Testosterone**	**Placebo**	**Testosterone**
Baseline	259 (±20)	252 (±31)	260 (±19)	255 (±23)
Week 16	264 (±33)	292 (±30)	332 (±21)	310 (±28)
Change from baseline	+5 (±16)	+40 (±15)	+72 (±14)^	+55 (±10)^
P vs. zero change	0.766	0.021	<0.001	<0.001

Change in effort-dependent maximal strength in the leg press exercises measured by the 1-RM method. The data are mean (±SEM).^ Denotes significant difference P<0.05 vs. placebo in the no exercise group; * overall ANOVA P=0.015;** overall ANOVA P=0.004.

Testosterone and resistance exercise were each associated with significant increases in 1-RM strength and muscle volume. However, specific tension increased only in the groups receiving resistance exercise, but not in men receiving testosterone alone. Our data suggest that testosterone administration will likely improve measures of physical function that are dependent on maximal voluntary strength, while resistance exercise training may have additional beneficial effects on physical function by improving the quality of muscle function. Conversely, measures of physical function that do not require maximal or near-maximal strength may not demonstrate improvements with testosterone administration. Commonly used measures of physical function such as 0.6 meter stair climbing, 20-meter walk, and the sit-to-stand transition require only a fraction of the maximal voluntary strength and do not show impairment until muscle strength declines below a threshold strength that is substantially lower than the maximal voluntary strength. Therefore, it is not surprising that studies of testosterone administration in healthy older men whose baseline strength is above the threshold at which these measures show impairment have failed to demonstrate changes in these threshold-dependent measures of functional performance. We hypothesize that testosterone treatment will augment performance in measures of physical function, such as stair climbing power using the Margaria power test (103), load carry test, and a quarter mile walk test (104), that allow the subjects to exert maximal or near-maximal strength above this threshold and are, therefore, not susceptible to the threshold effect. In this study, we have emphasized the use of measures of physical function that are less susceptible to threshold effect.

8. The Women's Memory Study. Dr. Buckwalter is co-Investigator on AG-14745 (PI: DB Petitti: *Alzheimer's Disease and Estrogen Replacement*); this report demonstrates his extensive experience in cognitive screening and in-depth neuropsychological evaluation. For this study of the association between the use of hormone replacement therapy (HRT) and dementia, 6,923 women, > 75 years of age and members of Kaiser Permanente for at least seven years, were randomly selected from Kaiser Permanente's database based on whether they had used HRT during the past seven years. Of these, 236 were deceased, 99 were unable to speak English, and 243 were too debilitated to complete a phone interview. An additional 2,098 declined to participate. The average age of 3,681 participants was 78.8 (± 3.3) years; 489 (13.3%) did not finish high school, 1012 (27.6%) were high school graduates, 1350 (36.7%) had completed some college work, and 822 (22.4%) had completed at least a baccalaureate degree. All participants received a computer-assisted, Telephone Interview of Cognitive Status-modified (TICSm). Individuals who scored below criteria had a family member interviewed as well as a medical chart review. Diagnostic classification is underway using this information. A sub-goal of this project is to determine if there are differences in the neuropsychological performance of women who have been long-term users of HRT and women who are not using HRT. One hundred and five women who were confirmed estrogen users were randomly selected from the pool of

participants and matched for age and education with an equal number of non-users. A total of 106 women agreed to participate in this neuropsychological testing. All were tested at their homes with a two-hour battery of tests that evaluated verbal and non-verbal memory, attention, working memory, visual perceptual ability and other relevant cognitive domains. The battery of tests used is very similar to the one proposed for the present study. Given the unambiguous identification of hormone use status through pharmacy records and the rigorous sampling procedures, this study will provide one of the most definitive information on the effects of estrogen use on cognitive function.

9. **Recruitment Experience.** The investigating team has successfully recruited large numbers of subjects for several funded projects from sources that will also be used in the proposed study. Dr. Joaquin recruited 824 older men and women (mean age 72) from Senior Multi-purpose Community Centers for a Community Outreach Geriatric Health Screening Project during a 3-year period (231). Dr. Buckwalter recruited 3,681 women, > 75 years of age by screening Kaiser Permanente database. Dr. Bhasin recruited 110 older men for two androgen-studies over a 12-month period, and 150 HIV-infected men for a study of androgen-deficiency in HIV-infected men. Dr. Hodis recruited 224 women for a study of the effects of hormone replacement on atherosclerosis progression. Dr. Paige recruited over 150 men for a study of the effects of sildenafil on HRQOL in men with erectile dysfunction. We will recruit men from 14 Multi-Purpose Senior Centers in Los Angeles, the Beach Cities Health District, Kaiser Permanente, and by direct advertising in radio and local newspapers. In Phoenix, Dr. Harman, an architect of the Baltimore Longitudinal Aging Study, has 25 years of experience in conducting clinical trials in older men. Kronos Aging Institute specializes in providing care to the healthy elderly, and the Kronos TeleHealth Call Center has effectively used 800 numbers for recruitment in multi-center clinical trials for a number of research organizations.

D. STUDY DESIGN

1. Sample Size Estimate

We estimate that approximately 125 men will be needed in each of the two treatment groups to test the proposed hypotheses. We base this test on the following considerations:

- One-way analysis of variance comparing the change from baseline to end-of-treatment between the three treatment groups in the outcome variable
- Type I error rate of 0.05
- Power 80%
- 25% cumulative drop out rate over the 5-year period based on published experience (2, 11)

For the rates of atherosclerosis progression, we have computed the sample size required to detect a treatment effect size comparable to that found in an estrogen trial in postmenopausal women (EPAT Trial). For the current sample size projections, we assume a similar standard deviation (SD) of 0.02 mm/year and a difference in atherosclerosis progression rate between testosterone and placebo groups of 0.008 mm/year. At a significance level of 0.05 and about 80% power, we will require approximately 100 subjects per study group to achieve a treatment effect size comparable to that found in EPAT. The treatment effect size detectable with this given sample size is far smaller than that found in previous lipid-lowering trials, CLAS and MARS, which yielded treatment effect sizes of around 1.4. Assuming an approximate cumulative 25% dropout, the proposed sample size of 125 men in each group gives us 80% power. Therefore, we will enroll a total of 250 men. In our previous study (143), the yearly rate of change in calcium scores was 33% in asymptomatic persons, with a standard deviation of 12%. We assume that the common standard deviation is 35 points per year of calcium score and that 100 men will complete the study in each arm. If the rate of change in coronary calcium scores were similar in placebo treated-men to that reported in the previous study, we have greater than 90% power to detect a 25% difference in the rate of change of calcium scores in either direction between the placebo and testosterone groups (e.g., the difference between 33% increase from baseline in calcium scores for one group vs 24% change in the other). These calculations are based on our previous study (143), and another previous assessment of the effects of statins on coronary artery calcification by MDCT (144). The annual rate of change in calcium scores in older men would likely be greater than that observed in the previous study. If the standard deviation of change were similar, then we would have even greater power to detect a 25% difference between placebo and testosterone groups.

A sample size of 100 men in each of the two treatment groups will also provide 85% power to detect clinically meaningful changes from baseline scores between the three groups in subdomains of physical function, general health perceptions, emotional well being and energy/fatigue. We recognize that there is uncertainty about what is a clinically meaningful change in health-related quality of life. While some investigators have argued that any change in HRQOL is meaningful, we posit that a 5 point difference between the two treatment groups would be clinically significant because previous studies using the SF-36 instrument have revealed a 5-8 point decrease in various subdomains of HRQOL in older men, as compared to younger men. Assuming a standard deviation of about 12 points, we will have better than 85% power to detect this difference.

We estimated the number of men that would be needed in order to detect differences between testosterone and placebo users that are as large or larger than the difference between men 75+ years of age and men 50 years of age for the Boston Naming Test, the test of logical memory, and the Animals test with a power of 0.95, alpha=0.01, two-tailed test. The following table shows the mean values for each of the tests for individuals 75+ years of age and the mean values for those 50-59 years of age that have been obtained in prior studies and reported in Spreen and Strauss, 1991, the difference in means between men 75+ and men 50-59, and the number of subjects needed in each group to detect this difference in means with a power of at least 0.85, alpha equal to 0.01.

Test	75+ Mean (SD)	50-59 Mean	Difference	N per group needed to detect
Boston Naming Test	51.7 (6.2)	56.7	-5.0	34
Animals	15.1 (4.3)	18.6	-3.5	34
Logical Memory				
Immediate	19.1 (6.7)	23.6	-4.5	48
Delayed	15.3 (7.6)	20.1	-4.4	64

Therefore, the proposed samples size of 100 men in each group should give us more than 95% power to detect clinically meaningful treatment effects.

For leg press strength, we assume that the difference of interest in the change in leg press strength during the three-year treatment period is 20±55 kg, which is a modest treatment effect size (effect size, $f = 20/55 = 0.36$ SD units). This assumption is based on our previous study of testosterone replacement in androgen-deficient HIV-infected men (Bhasin et al, JAMA) in which an actual difference in change of 35±55 kg in leg press strength was observed during 16 weeks of testosterone treatment of young, HIV-infected, androgen-deficient men. We posit that the older men such as those recruited for the proposed study might not exhibit as much of a change in response to testosterone treatment as young, hypogonadal men. We assert that a 20 kg increase in leg press strength should be clinically significant because Fiatarone et al. (232)demonstrated that a 10 kg increase in leg press strength was associated with significant improvements in physical function, as assessed by stair climbing power, walking speed, and time for sit-to-stand transition. A sample size of 100 men in each of the two treatment arms gives us 80% power to detect a 20 kg difference in change in leg press strength between the groups.

For physical function, we have used the data of Fiatarone et al. (232). In that study, resistance exercise in frail elderly was associated with 11.1±22.5 W increase in stair climbing power, while the no-exercise group experienced a 2.5 W decrease. If testosterone is associated with a similar magnitude of change, 120 men in each group will allow us to detect differences of this magnitude between groups in stair climbing power with >90% power, at alpha=0.05.

For leg power, we only have variance data at one time point. In a sample of older men (mean age 73), the mean and SD for leg power were 149 and 53 watts, respectively. Assuming a correlation of 0.5 between repeated measurements, a sample size of 100 men in each group will allow us to detect a 20 W difference in the change in leg power between the groups with 80% power.

Regarding FSIVGT, although there have been no large scale randomized clinical trials of the effects of testosterone replacement on insulin sensitivity in older men, a few small studies have revealed a large effect size (approx. 1.0 SD units). A sample size of 42 men in each of the two treatment arms would give us sufficient power to detect this effect size of change in insulin sensitivity index with greater than 90% power. Therefore, we assert that 50 men in each of two arms (total of 100 men) would provide sufficient power to detect meaningful changes in insulin sensitivity index by FSIVGT.

2. Subjects

Inclusion criteria:

a) Community-dwelling men ≥ 60 years old in good health with at least 8 years of education
b) Total testosterone < 400 ng/dL and > 100 ng/dL. Men found to have serum testosterone less than 100 ng/dL would be referred to their physicians for evaluation of androgen-deficiency. Men with free testosterone level less than 50 pg/mL, by the equilibrium dialysis method, will also be eligible.
c) Able to give informed consent, and pass screening test for possible dementia:

Exclusion criteria:

a) Use of testosterone, other androgens including DHEA and androstendione, or rhGH in the preceding year.
b) Total testosterone less than 100 ng/dL. These individuals with severe hypogonadism will undergo further evaluation and possible treatment by their personal physician.

c) Current alcohol or drug dependence (Alcohol Use Disorders Identification Test [AUDIT] score ≥ 8) or androgenic steroid use (T, anabolic steroids, over-the-counter steroids such as androstendione and dehydroepiadrosterone [DHEA].
d) Diseases or medications known to affect gonadal function.
e) Anticonvulsant or glucocorticoid (equivalent to prednisone 20 mg daily for > 2 weeks) therapy.
f) Prostate cancer, breast cancer or other cancers that may limit life expectancy to < 5 years.
g) Limiting neuromuscular, joint or bone disease, or history of stroke with residual neurological defect.
h) Evidence for any neurological condition that would impact cognitive functioning including epilepsy, multiple sclerosis, HIV, Parkinson's disease, stroke, or other focal lesion
i) DSM-IV criteria for an Axis I psychiatric disorder within the past year; use of psychotropic medication for at least six months, or dementia (Telephone Interview for Cognitive Status-modified score < 31).
j) Severe symptoms of BPH (American Urological Association [AUA] symptom index score of > 21), prostate nodule or induration on digital rectal examination (DRE), and prostate specific antigen (PSA) > 4 unless there has been a negative transrectal biopsy within 3 months.
k) Limiting heart disease (unstable angina, NY class III or IV congestive heart failure) or myocardial infarction (MI) within 3 months of entry.

A cardiopulmonary exercise stress EKG test will be performed in order to identify those who might develop ischemic EKG changes during the course of incremental exercise and therefore might be at risk for developing cardiovascular complications during the course of muscle performance and physical function tests. Those men who develop significant ST-T segment changes or significant BP changes during the exercise test will NOT undergo tests of muscle performance or physical function. These men may continue to participate in the study and undergo other evaluations, as approved in this protocol. These participants will be advised of the EKG changes and referred to their primary care providers for further evaluation.

l) Serum ALT and AST > 3 x upper limit of normal), or serum creatinine > 2.5 mg/dL.
m) Hemoglobin (Hb)-A1c > 9.0% (normal < 6.4%).
n) Hematocrit > 48%.
o) Untreated thyroid disease.
p) Uncontrolled hypertension (systolic blood pressure (BP) > 160 or diastolic BP > 100 mm Hg).
q) Body mass index (BMI) > 35 kg/m^2.
r) Untreated severe obstructive sleep apnea, as determined by Berlin's questionnaire.

3. Treatment Protocol

a. Design. The study will be a randomized, placebo-controlled, balanced-design, double-blind study of healthy, community-dwelling older men. In this **5-year** study, enrollment will take place during the first 21 months, and all men will be **treated for three years**.

b. Randomization and Stratification. Subjects who meet the eligibility criteria for the main study will be randomly assigned to one of two treatment groups:

Group I: Placebo gel
Group 2: Testosterone gel

Subjects will be stratified by age (605-75 and >75) and site before randomization into the two treatment groups. In addition, we will balance the groups with respect to baseline IMT (less than 0.075 mm and greater than 0.075 mm).

4. Intervention/Treatment.

ii. Testosterone. The subjects will receive either 7.5 g testosterone gel to achieve a nominal delivery of 75 mg testosterone daily or placebo gel. The goal will be to achieve serum testosterone concentrations between 500-900 ng/dL in testosterone-treated men. Two weeks after starting the testosterone or placebo gel, serum total testosterone level will be measured 4 hours after gel application. If the serum testosterone concentration in a testosterone-treated individual is greater than 900 ng/dL, the dose will be decreased from 75 mg to 50 mg daily. If on the other hand, serum testosterone concentration is less than 500 ng/dL, testosterone dose will be increased from 75 mg to 100 mg daily. At the same time, the placebo dosage will be adjusted in the same direction in another subject by an unblinded observer.

10. Outcome Measures

a**. Measurement o Atherosclerosis Progression**

i. We will measure the rate of change in distal CCA far wall IMT in computer image processed B-mode ultrasonograms, and

ii. Rate of change in total coronary calcium scores by MDCT

b. Cardiovascular risk factors.

i. Plasma lipids: total plasma cholesterol and triglycerides, LDL-Cholesterol, HDL-Cholesterol, and VLDL-Cholesterol determined by the precipitation method;

ii. Apolipoproteins AI (apoAI), ApoB, and ApoCIII by electroimmunoassay

iii. The fractionation of lipoprotein AI (Lp-AI) and lipoprotein AI:AII will be performed by immunoaffinity chromatography. The fractionation of ApoB-containing lipoprotein families into cholesterol-rich (Lp-B) and triglyceride-rich lipoprotein B:C (Lp-B:C), lipoprotein B:C:E (Lp-B:C:E), and lipoprotein AII:B:C:D:E (Lp-AII:B:C:D:E or Lp-AII:B complex) will be measured by sequential immunoaffinity chromatography.

iv. Inflammation sensitive markers: high sensitivity C-reactive protein, and fibrinogen.

v. Visceral fat cross-sectional area will be measured by MDCT

vi. In 100 men, insulin sensitivity will be measured by FSIVGT using the Bergman Minimal Model

c. Health-Related Quality of Life and Its Major Determinants. HRQOL will be assessed by using a validated instrument, developed and pre-tested by Dr. Ron Hays in focus groups in older men with low testosterone levels. We will also objectively measure three important determinants of HRQOL, namely, muscle mass and physical function, sexual function and cognitive function, as described below.

d. Tests of Muscle Mass, Performance and Physical Function

i. We will assess whole body fat-free mass and fat mass, and appendicular muscle mass by DEXA scan.

ii. Maximal voluntary leg and bench press strength will be measured using the 1-repetition maximum method (1RM). These exercises were chosen because they represent the large muscle groups of the upper and lower extremities and have been shown to be androgen responsive. We will measure power of the knee extensors using Bassey's leg rig.

iii. We will use the following measures of physical function: stair-climbing power using the Margaria Power test; Walking speed over 400-meters on a track; 100-meter timed walk with 15% load carry.

e. Sexual Function and Marital Interaction. Sexual function including sexual desire will be assessed using the International Index of Erectile Function (IIEF), and the marital interaction by using the Cancer Rehabilitation System Short Form (CARES-SF).

f. Assessment of Cognitive Function. The battery selected for this study utilizes many tests used in the Women's Memory Study, under the direction of Dr. Buckwalter. These tests have been shown to detect gender- (233, 234) as well as estrogen effects (233, 235). We will characterize a range of cognitive functions with emphasis on domains reported to be affected by testosterone. Given the evidence that testosterone affects spatial and fluency skills, we emphasize these domains but given the failure of previous studies to effectively evaluate a range of domains, we include a comprehensive battery of standardized neuropsychological tests that includes Boston Naming Test, Verbal (category and phonemic) fluency, California Verbal Learning Test, FACES, Paragraph recall (Logical Memory), immediate and delayed recall, Digit and Visual Memory Spans, Judgment of Line Orientation, and Trails A and B.

g. Hormone Levels. We will measure total and free testosterone levels as markers of androgen bioavailability, estradiol levels, and SHBG and LH as markers of androgen action.

6. Experimental Protocol. The study will consist of a screening phase, a 2-week control period, and a 3-year treatment phase. Each visit will be on the indicated day ± 14 days.

Consent and Screening

The protocol will be explained to the interested subjects and an informed written consent will be obtained. The subjects will undergo a detailed history and physical examination. Blood will be drawn for blood counts and chemistries, PSA and serum testosterone levels to assess eligibility. Subjects who meet all other eligibility criteria will undergo mini mental status examination to exclude dementia. Subjects will be given AUA, Berlin sleep apenia questionnaire, AUDIT, Geriatric Depression Scale.

Control Period (Days -14 to 0)

Baseline Studies in the Clinical Research Center (CRC). The subjects will come to the CRC at the end of the control period for baseline studies. During this visit the following procedures will be performed:

1. Physical examination (including prostate, breast, and genital exams)
2. Height, weight (with minimal clothing and on the same scale)
3. Exercise Electrocardiogram (ECG) stress test
4. The CCA-IMT by B-mode ultrasound, and coronary calcium scores by MDCT.
5. Abdominal fat by MDCT scan
6. Assessment of body composition by DEXA

7. Serum hormone studies (total and free testosterone, LH, SHBG, and estradiol levels)
8. Insulin sensitivity by the modified, frequently sampled intravenous glucose tolerance test.(only 100 men will undergo FSIVGT)
9. Safety measures (CPK, PSA, hemoglobin and chemistries, AST, ALT and alkaline phosphatase; AUA symptom score; sleep apnea questionnaire).
10. Measurements of muscle performance and physical function.
11. Health-related quality of life by the pre-tested HRQOL instrument.
12. Sexual function and marital interaction by International Index of Erectile Function, and the CARES-SF questionnaire.
13. Plasma lipids, apolipoproteins, lipoprotein particles, and inflammation sensitive markers.
14. Neuropsychological tests of cognitive function.

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15. EKG to be performed.

5. Schedule of Events for the Study

Test	Baseline	2 week	3 mo.	6 mo.	9 mo.	12 mo.	15 mo.	18 mo.	21 mo.	24 mo.	27 mo.	30 mo.	33 mo.	36 mo:	37 mo
Consent & Screening	X														
Randomization	X														
Testosterone/Placebo Gel	←													→	
Compliance Checks		X	X	X		X		X		X		X		X	
Body Weight & Vitals	X		X	X	X	X	X	X	X	X	X	X	X	X	
History & Physical Exam (H&P)	X			X		X		X		X		X		X	
CBC, chem., PSA levels	X		X	X		X		X		X		X		X	
Exercise ECG/stress test	X														
CCA-IMT (ultrasound)	X X			X		X		X		X		X		X	X
MDCT-CAC	X							X						X	
Hormones	X	X		X				X						X	
Plasma lipids	X			X				X						X	
Insulin Sensitivity by FSIVGT(100 men)	X			X										X	
HRQOL/Geriatric Depression Scale	X			X				X						X	
Sexual Function and Marital Interaction	X			X				X						X	
Neuropsychological Tests of Cognitive Function	X			X				X						X	
FFM, Muscle performance and Physical Function (if applicable)	XX			XX				XX						XX	
Adverse Experiences, AUA, BERLIN SLEEP APNEA FORMS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
EKG		X				X				X				X	
AUDIT & Mini Mental State Questionnaire	X														
DEXA Scan	X			X				X						X	

Treatment period

Testosterone/Placebo Gel Application and Dose Adjustment. After randomization, the subjects will be advised to apply daily 7.5 g of either testosterone gel containing 75-mg testosterone or placebo gel. The research staff will provide to the men verbal and written instructions for proper gel application, and for minimizing the risk of transfer to the sexual partner. During week 2, the application sites will be examined for skin reaction; the technique for gel application reinforced, and serum testosterone levels measured. If serum testosterone levels are less than 500 ng/dL, the dose will be increased to 100-mg daily; and if levels are >900 ng/dL, the dose will be decreased to 50-mg daily. To

maintain blinding, a designated unblinded physician will make the dose adjustments, and a subject in the placebo group will have his dose adjusted in the same manner. This strategy has worked well in other testosterone studies.

Outpatient Visit to the Clinical Research Center During Months 3, 9, 12, 15, 21, 24, 27, 30, and 33. The following procedures will be performed during these visits:

1. Weight on the same scale, with minimal clothing every 3 months
2. Physical examination including a digital rectal examination will be performed every 6 months
3. Assessment of drug compliance by gel counts and medication logs, and dispensing of study drug every three months
4. Adverse experiences will be recorded every 3 months. The application site will be examined for skin reactions. The AUA prostate symptom score and sleep apnea scores using Berlin's questionnaire will be recorded.
5. Safety measures (CPK, PSA, complete blood counts and chemistries, AST, ALT and alkaline phosphatase) will be assessed during months 3, 6, 12, 18, 24, 30 and 36.
6. EKG to be done at 12, 24, and 36.

Detailed Clinical Research Center Visit During Months 6, 18 and 36. On these occasions, the subjects will come to the CRC for 3 days. The following procedures will be performed:

1. Physical examination (including prostate, breast, and genital exams)
2. Weight on the same scale with minimal clothing
3. Assessment of body composition by DEXA
4. Serum hormone studies (total and free testosterone, LH, SHBG, and estradiol) 4-12 hours after gel application.
5. For 100 men only, insulin sensitivity by the modified, frequently sampled intravenous glucose tolerance test (FSIGT) at 6 and **36 months** only, using the Bergman Minimal Model, and visceral fat by MDCT scan at **18 and 36** months.
6. Safety measures (CPK, PSA, complete blood counts and chemistries, AST, ALT and alkaline phosphatase)
7. Measurements of muscle performance and physical function.
8. Health-related quality of life by the SF-36 instrument and Geriatric Depression Scale.
9. Sexual function and marital interaction will be assessed by International Index of Erectile Function, and the CARES-SF questionnaire. Cognitive function will be assessed by previously validated instruments.
10. Neuropsychological tests of cognitive function will be administered during months 6, 18, and 36.
11. Plasma lipids, apolipoproteins, lipoprotein particles, and inflammation sensitive markers will be measured during months 6, 18, and 36.

Assessment of Atherosclerosis by CCA-IMT and MDCT

1. The CCA-IMT will be measured by B-mode ultrasound **every 6 months**. One additional CCA-IMT measurement will be performed a month after the completion of treatment.
2. Coronary calcium scores will be assessed by MDCT during **months 18 and 36** only.

E. METHODS

1. Data Analysis and Statistical Methods

a. Interim Monitoring. The study will be monitored by the Data Safety Monitoring Board (DSMB), an independent group that will periodically review the results in order to assess safety. No interim efficacy assessment is planned.

b. Statistical Analyses

i. Descriptive Analyses. We will calculate descriptive measures for each of the variables. Means, medians, standard deviations, range, minima, maxima, and interquartile ranges will be computed for all continuous variables. Frequencies will be calculated for categorical variables.

Prior to analysis, the outcome variables will be analyzed to determine if they meet the assumptions of normal distribution. It is possible that some variables may display non-normal or skewed distribution; in that case, appropriate transformations may be applied. If the variable remains non-normal in its distribution even after appropriate transformation, we will use nonparametric tests.

ii. Baseline Comparability. We will use summary statistics and graphical techniques, such as boxplots, to compare the baseline characteristics of treatment groups. Pre-treatment values will be compared between the placebo- and testosterone-groups using Analysis of Variance (ANOVA), Kruskal-Wallace tests or Chi-square tests, as appropriate, to determine if the groups are balanced with respect to baseline characteristics.

iii. Compliance Analysis. Subject's compliance with treatment will be assessed by the number of gel packages and tablets used, expressed as a percent of the total number of gel packages or tablets that should have been used during treatment. Percent compliance will be averaged across subjects within each treatment group to obtain group means.

iv. Testing Specific Hypotheses. All analyses will use an intent-to-treat approach. The primary outcome variable is the rate of atherosclerosis progression. We will calculate per-subject rate of change in the right distal CCA far wall IMT and in total CAC score by MDCT. The rate of change in the right distal CCA far wall IMT will be computed for each subject by fitting a regression line of IMT on years since baseline. The estimated slope of the regression line will be used as that subject's IMT rate of change. For CAC, the 18 month and 3 year rates of change, calculated as (post-treatment score – baseline score)/years of treatment will be treated as separate dependent variables in the analysis of variance. Analyses of trial end points will be performed on the intent-to-treat sample, which will be defined as all randomized subjects who had a baseline and at least 1 follow-up carotid IMT measurement taken after randomization. Statistical assumptions of the planned analyses will be verified. Alternative analyses will be performed if the assumptions of the planned analyses are not justified. Key assumptions that will be verified are: 1) normality of error terms in the model; 2) homogeneity of variances between treatment groups; and, 3) deviations from linearity in the regression of carotid IMT measurements against time in-study. These data will also be subjected to a repeated measures analysis of variance.

The effects of on-trial end point variables other than treatment modality on the per-subject rate of change in the right distal CCA far wall IMT will be assessed. The parameters to be tested will include laboratory data (lipids, total testosterone, estradiol and SHBG levels, etc.), clinical variables (blood pressure, body mass), and lifestyle variables (smoking). Baseline and on-trial values of these data will be collected in order to address questions of clinical significance within each treatment group such as: 1) which baseline characteristics have differential effectiveness on the rate of change in IMT or CAC score; and, 2) what is the role of changes in these variables on the rate of change in IMT and CAC score. These analyses will utilize multiple linear regression with the annual rate of change of the right distal CCA far wall IMT or CAC score being the dependent variable. Analyses will be conducted in the combined sample with a covariate included to control for treatment group. Interaction terms with treatment group will be introduced to test whether relationships between independent variables and the trial end point are of equal magnitude between the study groups. Experiment-wise error rates in these analyses will be controlled. Change from baseline to end-of-treatment in plasma concentrations of lipids, lipoproteins, apolipoproteins, lipoprotein particles, and inflammation sensitive markers, insulin sensitivity index, and visceral fat area in the three treatment groups will be compared by using repeated measures ANOVA and analysis of covariance. Paired t-tests will be used to determine if the values at the end of treatment differ from baseline within each treatment group.

As secondary, exploratory analyses, we will assess if change in plasma lipids, lipoproteins, apolipoproteins, and lipoprotein particles correlate with change in serum testosterone levels. We will also fit regression models on the change in each outcome measure with terms for group, baseline testosterone, and the group by baseline testosterone interaction in order to assess if treatment works to a greater or lesser extent in men with low testosterone levels.

We will examine whether changes in CCA IMT and CAC scores correlate with changes in circulating levels of lipids, lipoproteins, apolipoproteins, lipoprotein particles, and inflammation markers. If testosterone treatment does change the rate of atherosclerosis progression, it would be of interest to know whether changes in CCA IMT or CAC scores correlate with changes in these markers of cardiovascular risk. For these variables, we will generate the Pearson correlation coefficients. Scatter plots will be used to assess assumptions of linearity and homoscedasticity.

A secondary outcome variable is change from baseline in health-related quality of life scores. Means and standard errors will be calculated for each of the domains of HRQOL, the IIEF, and the marital interaction scale at baseline and for each interval. The significance of these differences will be estimated using two-way repeated measures analysis of variance with interaction, with time as the within groups factor and treatment as the between groups factor. The repeated measures analysis of covariance will also be used for appropriate covariates. Posttest contrasts will be used for pairwise comparison of groups. Similar models will be used to analyze changes in leg press strength, power, and measures of physical function. Student-Newman-Keuls approach will be used to assess group differences. In -secondary analyses, the Student Newman Keuls test will also be applied to change from baseline to 6 months and from baseline to 18 months, and from baseline to 3 years.

As secondary, exploratory analyses, we will assess if changes in health-related quality of life, physical, sexual and cognitive functions correlate with change in testosterone levels. We will also fit regression models on the change in these outcome variables with terms for group, baseline testosterone, and the group by baseline testosterone interaction in order to assess if treatment works to a greater or lesser extent in men with low testosterone levels.

We will examine whether changes in HRQOL correlate with changes in objective measures of physical, sexual and cognitive functions. We will determine if the changes in scores for the physical function subdomain of the HRQOL instrument correlate with changes in objective measures of physical function. For these variables, we will generate Pearson correlation coefficients. Scatter plots will be used to assess assumptions of linearity.

We will examine whether changes in muscle strength correlate with changes in the measures of physical function (400-meter walk, Margaria stair climbing power). If testosterone does improve muscle strength, and also improves

physical function, it would be of interest to know whether changes in muscle strength correlate with changes in physical function. We are interested in knowing whether changes in leg press strength and physical function correlate with changes in lean body mass. For these variables, we will generate the Pearson correlation coefficients.

For measures of cognitive performance, differences between baseline performance and performance after the clinical trial will be tested with a two-way repeated measures ANCOVA, controlling for baseline performance and age and education, and other potential confounders. Additional tests on the association between circulating levels of testosterone and estradiol will be conducted with correlation and multiple regression models.

2. Ultrasonographic End Point Measurement (See Appendix for Quality Control Procedures)

a. Carotid Ultrasound Image Acquisition. B-mode carotid artery images for IMT measurements will be acquired with a high resolution ultrasound imager using a linear array 7.5 MHz probe. Electrocardiogram (ECG), external time code information and ultrasound images will be simultaneously recorded with a videotape recorder. For image acquisition, subjects will be placed supine and positioned in a 45 degree molded head block to present the optimal angle for ultrasound examination. Using B-mode, the right CCA is imaged in cross section and the scan head moved laterally until the jugular vein and CCA are stacked with the former above the latter. In this position, the central image line passes along the common diameter of both vessels. The scan head is then rotated around the central image line 90 degrees maintaining the jugular vein stacked above the CCA while obtaining a longitudinal view of both vessels. In this longitudinal view, the CCA far wall is horizontal. The proximal portion of the carotid bulb is included in all images as a reference point for standardization of IMT measurements. Stacking the jugular vein and CCA determines a repeatable probe angle, which allows the same portion of the wall to be imaged at each examination (236), and decreases measurement variability (112). Images are acquired from the carotid bulb and internal carotid artery, but emphasis of ultrasound imaging is on the distal centimeter of the CCA because least variability occurs in this area (237). The far wall is used for statistical purposes since measurement of near wall thickness is less accurate (238).

Each ultrasound scan is recorded on tape and processed images are stored on jazz disks. The ultrasound power, echo detector gain and dynamic range are recorded to establish identical conditions for serial examinations. This establishes a standardized instrument setup for all tests within a subject. A copy of each individual's baseline image is used as a guide to match the vascular and surrounding soft tissue structures for follow-up examinations and reproducing probe angulation. The brightness and contrast settings of the image display are checked daily and standardized. These techniques have significantly reduced measurement variability between scans (112).

b. Equipment Certification. The ultrasound machine used in this study will be calibrated by using a standardized phantom. Accurate ultrasound imager calibration information is necessary to calibrate the computerized carotid IMT analytical software program. If the calibration of an ultrasound imager is inaccurate, this problem will be rectified by a service technician and the accuracy of calibration will be retested before the imager is certified.

c. Ultrasonographer Training. Ultrasonographers will be trained at the USC Atherosclerosis Research Unit (ARU) core imaging facility. Carotid anatomy is reviewed and excellent to poor quality IMT images are reviewed. The pitfalls leading to poor images are demonstrated. Ultrasonographers are trained on volunteers that range from regular to more difficult cases to improve scanning skills. In particular, the principals of vessel stacking and baseline image reproducibility are emphasized and the standardization procedures for equipment set-up and recording of image information are taught. The ultrasonographer are certified if they follow the standardization protocol and are able to obtain carotid images that yield IMT measurements with a variability of 4% or less with repeated imaging.

d. Quality Control (See Appendix for detailed procedures). Initially, images are sent to the core imaging center on a daily basis for evaluation and if corrective procedures are needed, the information is sent back to the ultrasonographer within 48 hours. Once established, ongoing image quality and standardization procedures are monitored at the core imaging center. The images are checked for the system information, including input power, echo detector gain, and dynamic range values to ensure that identical conditions are used for each follow-up examinations. The ultrasound image is assessed for quality, including brightness and clarity of the lumen-intima and media-adventitia boundaries as well inclusion of structures such as the bulb and stacking of the jugular vein. All follow-up images are compared with baseline image to ensure reproducibility of probe angulation and proper image acquisition. If deterioration of image quality occurs, the ultrasonographer is retrained in the image acquisition procedures. To monitor operation of the system, a phantom is regularly scanned. The images obtained from the phantom are measured with our IMT assessment software; any change in system performance is investigated.

Variability of carotid IMT acquisition will be monitored throughout the study. Variability will be determined from the repeat baseline ultrasound scans obtained at screening and at month 0 approx. 1 to 2 weeks apart. The subjects will have a repeat ultrasound examination 1 to 2 weeks following the end of study scan. The repeat ultrasound at baseline and as subjects complete the trial are a part of our quality control procedures. The absolute difference in IMT between the repeat ultrasounds will be charted over time to monitor possible longitudinal drift in image

acquisition. The absolute IMT difference in each repeat ultrasound will also be compared to the mean absolute difference in IMT obtained in our prior reproducibility studies using these methods. Any absolute difference greater than 0.04 mm (twice the standard deviation of the mean absolute difference) will be initially checked for suitability of image processing. If image processing is found to be optimal, the difference will then be discussed with the ultrasonographer. Repeated instances of such outliers will indicate the need for ultrasonographer retraining. The charts of longitudinal variability in ultrasound measurements will be summarized by operator in a monthly report.

e. Computer Analysis of IMT. Our computer system and software program to analyze carotid ultrasound images utilizes automated rather than manual boundary detection to locate the lumen-intima and media-adventitia echo boundaries at sub-pixel resolution (112). To measure CCA wall thickness and lumen size, B-mode ultrasound images are recorded on SVHS video tape and processed off-line with an image processing system consisting of a PC computer with a video digitizer board interfaced to a Sony SVO 9500MD video tape system. Using this system, both half-frames of the interlaced video image are merged, viewed, and digitized, thus improving the resolution of the overall method 2-fold compared with methods that rely on still mode single-field VCR's. The ECG signal is used to standardize all IMT measurements at the same point in the cardiac cycle; namely at the R wave. Methods successfully used in our previous studies will be adapted for this proposed trial.

f. Reproducibility of CCA IMT Measurements. In the many studies that we have conducted, intra- and inter-sonographer coefficient of variation (CV) of carotid IMT acquisition and measurement is less than 4%(112). In addition, in a recent trial of estrogen replacement in postmenopausal women, the standard deviation (SD) of change was 0.02 mm/year, which included both between and within subject variability.

3. **MDCT**. The MDCT studies will be performed at both sites with an Imatron C-150XL Ultrafast CT scanner in the high-resolution volume mode, using 100 ms exposure time, as described. EKG-triggering will synchronize the images in the same point in diastole, corresponding to 40% of R-R interval.
    a. **Measurement of Coronary Calcium.** Proximal coronary arteries will be visualized without contrast, and at least 30 consecutive images will be obtained at 3 mm intervals. Coronary calcium will be defined as a plaque of at least 3 contiguous pixels (area 1.02 mm2) with a density of >130 Hounsfield units. The lesion score will be calculated by multiplying the lesion area by a density factor derived from the maximal Hounsfield unit within this area. A total calcium score will be determined by summing the individual lesion scores from each of the 4 anatomic sites (left main, left anterior descending, circumflex, and right coronary) using the volumetric method. Density, Agatston score, Volumetric score and number of lesions within the entire coronary tree will be assessed in each participant at each measure. Furthermore, quantification of mitral, aortic and aortic valve calcification will also be performed in each MDCT scan. A single experienced investigator, Dr. Budoff, blinded to the group assignment or subject identity, will interpret all the scans using commercially available software (Neo Imagery Technologies, City of Industry, CA), and inter-reader variability will be assessed with a second reader in 5% of cases, and similarly, 5% of cases will be re-read to assess for intra-reader variability. (**See Appendix for Standardization and Quality Control Procedures**).
    b. **Measurement of Intra-abdominal/Visceral Fat:** Abdominal adipose tissue areas will be measured by MDCT. This technique allows for precise measurement of the size of the adipose tissue depots located in the abdominal cavity (omental, or intra-abdominal fat) and in the abdominal subcutaneous compartment. Subjects are examined in the supine position with both arms stretched above the head. The scan is performed at the L4 to L5 vertebral level using a scout image of the body to establish the precise scanning position. Cross-sectional images will be analyzed with the computer interface of the scanner. Intra-abdominal adipose tissue area is quantified by delineating the intra-abdominal cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body. After the coronary artery scanning, a single CT slice will be obtained at the level of the umbilicus to assess intra-abdominal and subcutaneous fat distribution.[i,ii] The abdominal cavity is outlined on the computer screen, and the numbers of pixels appearing as fat (densities between -130 and 0 Hounsfield units) inside the abdominal cavity, as well as in the whole slice, are calculated. The amount of extra-abdominal (subcutaneous) fat is calculated by subtracting intra-abdominal fat from total abdominal fat. This method limits the radiation exposure, and the results are highly correlated with total abdominal fat.[ii] Abdominal height was determined as the distance between 2 parallel horizontal lines touching the most posterior and most anterior edges of the abdomen.

3. Lipid, Lipoproteins, Apolipoproteins, Lipoprotein Particles, and Inflammation Sensitive Markers

a. Lipids, Lipoproteins. Total plasma cholesterol, TG, and HDL-C levels will be determined by enzymatic assays and standardized to the CDC using the Lipid Research Clinic protocol (239). HDL-C will be measured after precipitation of the apoprotein B-containing lipoproteins (LDL and VLDL) in whole plasma by heparin-manganese

chloride (240). Very low density lipoprotein cholesterol (VLDL-C) will be assumed to equal one-fifth of the plasma triglyceride level and LDL-C will be calculated using the Freidewald equation (241): LDL-C=TC-(TG/5+HDL-C).

b. Apolipoproteins. Plasma samples for apolipoprotein analysis will be preserved by addition of 10 [illegible] caproic acid-thimerosal solution per 1 ml of plasma. Apolipoproteins AI (apoAI) (242), ApoB (243), ApoCIII (244), and ApoE (245) will be determined by electroimmunoassay. ApoCIII will be measured in whole plasma and in heparin-Mn^{++} supernates (ApoCIII-HS) and precipitates (ApoCIII-HP) and the ratio of ApoCIII-HS to ApoCIII-HP will be calculated (246). ApoCIII-HS approximates the quantity of ApoCIII in HDL, whereas ApoCIII-HP approximates that in VLDL plus LDL. Heparin-Mn^{++} precipitation of lipoproteins ("VLDL+LDL") will be carried out by a modified procedure (247) of Burstein et al. (240) and the precipitated lipoproteins will be dissolved in 10% $NaHCO_3$ for measurement of apoCIII-HP.

c. Lipoprotein Particles. The fractionation of lipoprotein AI (Lp-AI) and lipoprotein AI:AII (Lp-AI:AII) will be performed by immunoaffinity chromatography (248). The retained and unretained fractions will be concentrated and levels of Lp-AI and Lp-AI:AII particles determined on basis of plasma ApoAI values and the distribution of ApoAI in retained and unretained fractions. The ApoB-containing lipoprotein families consist of cholesterol ester-rich lipoprotein B (Lp-B) and triglyceride-rich lipoprotein B:C (Lp-B:C), lipoprotein B:C:E (Lp-B:C:E), and lipoprotein AII:B:C:D:E (Lp-AII:B:C:D:E or LP-AII:B complex) (247, 249). The fractionation of these lipoprotein families will be performed by sequential immunoaffinity chromatography of Apo B-containing lipoproteins (heparin-Mn^{++} precipitates) on immunosorbers with antisera to ApoAII, ApoE, and ApoCIII, respectively (249-251). Alternatively, aliquots of Apo B-containing lipoproteins will be chromatographed on these three immunosorbers. The ApoAII-retained fraction contains Lp-AII:B complex, ApoCIII-unretained fraction contains Lp-B, ApoCIII-retained fraction after subtraction of ApoE-retained fraction consists of Lp-B:C, and Apo E-retained fraction after subtraction of Apo AII-retained fraction contains LP-B:C:E. Concentrations of all ApoB-containing lipoproteins will be expressed in terms of ApoB (mg/dL). The percentage of ApoB in discrete ApoB-containing lipoprotein families will be calculated from the sum of ApoB concentrations of these lipoprotein families. ApoB will be measured by electroimmunoassay. The separation of cholesterol ester-rich Lp-B particles from all triglyceride-rich lipoproteins (termed Lp-B_c) will be performed by immunoaffinity chromatography of whole plasma or ApoB-containing lipoproteins (heparin-Mn^{++}-precipitate) on an anti-ApoCIII-immunosorber. The levels of the two classes of ApoB-containing lipoproteins will be expressed in terms of their ApoB content, and corrected for recoveries.

4. **Inflammatory Markers.** C-reactive protein is measured by a colorimetric immunoassay validated in our laboratory (252); its CV is 5.6%. The method is highly correlated with other methods (253). The expected normal range is 0.1 to 5.5 µg/ml. The assay for **Interleukin-6** is a quantitative sandwich immunoassay using monoclonal antibodies against IL-6 (R&D Systems). The CV is less than 10%. The **fibrinogen assay** uses a clotting assay with bovine thrombin on an ST4 coagulation instrument (Diagnostica Stago). The CV is < 5%.

5. Health-Related Quality of Life. To assess whether testosterone replacement in older men with low serum testosterone affects emotional well-being, mental health and physical functioning, subjects (both placebo and experimental groups) in this study will complete both general and disease-specific measures included in a questionnaire. The Rand 36-Item Health Survey 2.0 (SF-36) will be used to assess general HRQOL(254). The SF-36 measures eight domains of the quality of life: physical function, bodily pain, vitality, role limitations due to physical problems, general health perceptions, emotional well-being, social function, and role limitations due to emotional problems. It also includes a single item that provides an indication of perceived changed in health. The eight domains are scored separately from 0 to 100 with higher scores representing better HRQOL. It can also be scored to yield two orthogonal factor-based summary scores for physical and mental health. Patient scores at baseline will be compared with age and gender matched population controls presented in the SF-36 2.0 manual.

7. Assessment of Sexual Function. We will assess erectile and sexual function using the International Index of Erectile Function (IIEF), a validated, 15-item measure that is able to detect treatment-related changes in men with erectile dysfunction(255). The five domains of IIEF include erectile function, orgasmic function, sexual desire, intercourse desire, and overall sexual satisfaction. Internal consistency reliability (Cronbach's alpha) of the total scale was 0.90 and four-week test-retest reliability 0.82. Adequate construct validity for the IIEF was established by comparing clinical patients with age-matched controls(255). Dr. Paige used this instrument in a recent study to measure the impact of sildenafil on health-related quality of life among men with erectile dysfunction(256). The marital interaction scale from the Cancer Rehabilitation Evaluation System Short Form (CARES-SF) will be used to assess each patient's relationship with his sexual partner(257). The scale includes a set of items for men in a significant relationship and an alternate set of items for those not in relationships. The CARES-SF has been validated in a variety of settings and is scored from 0 to 4 with *lower* scores representing *better* interaction.

8. Body Composition Analysis by DEXA Scanning. The limitations of indirect anthropometric indices and bioelectrical impedance for body composition have been discussed (25, 258). Appendicular lean and fat mass

content, percent of fat mass, total body muscle mass can all be analyzed from a single DEXA scan (25, 259-261). The precision, reproducibility, and the ease with which DEXA scanning can be performed make it attractive in this patient population. The precision of regional body composition using DEXA is less than that for the whole body (260). Appendicular skeletal muscle mass is derived as the sum of the fat-free masses of the arms and legs (261).

8. Evaluation of Muscle Performance and Physical Function. We have selected the dominant muscle groups of the legs and shoulder girdle for the strength and power studies. These muscle groups have been associated with reductions in functional activities (262-264). The functional tests will require significant contributions from these same muscle groups. **Maximal voluntary strength** will be measured in the leg press and bench press exercises by the one repetition maximum (1RM) method. The subjects will undergo 5-10 minutes of warm up followed by one set of 5-10 repetitions of the selected exercise using 40-60% of the estimated maximum. Following a one-minute rest interval, the subjects will perform 3-4 attempts with progressively heavier weights leading up to the 1RM, with each attempt separated by 0.5-1 minute rest. The last successfully completed lift will be recorded as the 1RM. The strength will be reassessed within 2-7 days after the first test. During the second visit, the instructions are reinforced and measurements are repeated. If the measurements are within 5% of the initial measurement, the better of the two measurements is recorded, as previously described from our laboratory. **Leg power** is defined as the rate of force development and, at least in the lower extremity, is strongly related to performance of functional activities in the elderly (181, 265). Aging-associated sarcopenia is due in large part to a loss of the fast twitch, Type II fibers and the coincident decrease in explosive force. We will assess power in the lower extremity by using a specially constructed leg power rig, which has been validated and used safely in people up to 93 years of age. Subjects will perform five to ten trials of unilateral leg and hip extension with the right leg, attempting to generate as much force as possible, accelerating the weighted flywheel from rest. Peak muscle power will also be measured using Keiser Seated Leg Press and Keiser Seated Chest Press with Keiser A-420 electronics designed specifically for this purpose. Resistance equivalent to 40%, 50%, 60%, 70%, and 80% 1-RM will be used for the leg press exercise while resistance equivalent to 30%, 40%, 50%, 60%, and 70% 1-RM will be used for the chest press exercise in order to establish submaximal power and force/velocity curves, as has been previously established in younger individuals[24]. Subjects will be instructed to perform the exercise movement as hard and as fast as possible at the designated resistance for one-repetition. This procedure will be repeated with 30 seconds rest between repetitions until a plateau in power output is achieved. From preliminary trials this requires 4 – 6 repetitions. The highest score achieved (watts) will be taken as the power score for that workload. The procedure will be then repeated following 2 – 4 minutes rest for the next resistance. Loads will be assigned in random order. During all follow-up testing, both the same absolute weight used in the pretests as well as, the newer (relative) weight will be used for testing. The power tests will be repeated within 2-7 days after the first test and duplicated exactly at 6, 18, and 36 months of treatment.

Functional Tests-Measures of muscular strength and power are well correlated with functional activities such as walking and stair climbing. However, as pointed out by Dutta and Hadley (23), they generally do not measure function-specific performance. The extent to which improvements in measures of muscle performance translate into improved physical function has not been firmly established. To help elucidate these relationships, this study proposes to assess three measures of physical function that are not threshold limited. After familiarization and practice, all functional tests will be performed three times during each testing session, interspersed with 3-min rest periods, and will be repeated on a non-consecutive day scheduled within a 7-day period as described for strength testing in order to control for the influence of task familiarization. All functional tests will be repeated at 6, 18, and 36 months.

The *Margaria Stair Climb Test*[25]. In frail elderly, a four step stair climb for time has often been used as a functional index. However, our experience with healthy older men has indicated higher levels of function in these men than typically seen in frail elderly. Thus, the less demanding 4 step stair climb does not discriminate as well as a more rigorous Margaria Power Test, , which requires subjects to run up a staircase as fast as possible with time recorded by activation of switchmats on the 8th and 12th stair. We will modify the Margaria test in two ways. First subjects will walk as fast as possible (rather than run) up the 12 step stair case. Second, Subjects will carry a load equivalent to 25% of their baseline body weight. The load will be carried in the arms and held against the chest as if carrying a bag of groceries. In addition to capturing elapsed time, muscular power will be calculated by dividing the time elapsed between switch-mats into the product of the body weight, the vertical distance between switch-mats (m) and the acceleration of gravity (9.8 $m \bullet sec^{-2}$). For times measured between the 4th and 8th step, untrained men exhibit scores in the range of 15-17 watts/kg, with test-retest reliability is 0.85 and coefficients of variation of 2% over a period of 5 weeks[25]. A standard staircase will be used with step rise of $\approx$ 17.0 cm and tread width of $\approx$ 30.0 cm. Switch-mats will be interfaced with a multi-timer (Lafayette Instruments) to provide timing over several step-

segment combinations, e.g., 1st to 4th, 4th to 8th, 8th to 12th and 1st to 12th. The switch-mats will be affixed to the stairs with non-slip adhesive and covered with a non-slip surface. After familiarization, three trials will be given and the best time recorded, with 2 – 4 min rest between trials

Walking & Load Carrying Test – Walking speed is a commonly used functional assessment in the frail elderly [27-30], and most elderly persons have difficulty maintaining an adequate walking speed (1.22 $m \bullet s^{-1}$)to cross a traffic intersection under the protection of the green-light[31]. Typically, short distance walking courses such as 6-20 meters are employed. For the present trial, we propose to increase the walking distance to 50 meters, as this is the average distance to cross a major cross-walk intersections. Subjects will be asked to walk (but not run) as fast as possible along an indoor course. Subjects will start with one foot on a switch-mat and by their own volition, begin walking as fast as possible along a marked an measured course. The markings will delimit the sides of the course so as to help subjects follow a straight path. Large switch-mats will be placed at 25 meters and at 50 meters. Elapsed time will be recorded to the nearest 0.01 sec with a the same multi-timer used in the stair climb. The load carrying task represents common activities of daily living such as carrying groceries, laundry, or objects from place to place. This test will be performed by carrying a canvas shopping bag in each hand filled with total weight equal to 25% of the subject's body weight over the same 50 m course as fast as possible without running.

Lift and Reach Test. The third functional test will evaluate upper extremity muscle strength and endurance. As described by Painter et al[32], the test will be performed by asking the subject will stand facing a table with two shelves above it. One shelf is set at the patient's shoulder height; whereas the other is just above the patient's head. The object to be lifted will weigh 25% of the patient's body weight. The subject will lift the object from the table to the first shelf, to the next shelf, and return to the table top as many times as possible in 1 minute. The weighted object must momentarily touch each surface before it is moved. To ensure compliance, switch-mats will be placed on the three surfaces and interfaced with an amplifier that will emit a tone when a switch-mat is touched. The number of lifts completed is then recorded.

Reaction Time. We will measure reaction time in both upper extremity and lower extremity movements. The lower extremity device employs a simulation of reacting to a need for emergency braking while driving an automobile. After instruction and practice, subjects will be asked to move their right foot from a simulated accelerator pedal to a brake pedal upon presentation of a visual stimulus in the form of a simulated brake light positioned 10 meters away. The "accelerator" pedal is instrumented with a micro switch that activates a digital timer when pressure is removed. The "brake" is similarly instrumented and when pressed, stops the timer. Both reaction time (time from introduction of the stimulus to the beginning of the movement of the foot off the "accelerator") and movement time (time measured from the start of the movement to the end of the movement) will be measured.. To reduce the effects of learning and familiarization, subjects will be given repeated trials, 30 seconds apart, until the reaction time reaches a plateau. At that point, after two minutes rest, three additional trials will be given with the fastest score recorded to the nearest 0.001 sec for both reaction and movement time.

Upper extremity reaction and movement time will be measured with a reaction/movement timer (Bassin Anticipation Timer, Lafayette Instruments, Lafayette, IN). This test evaluates hand-eye coordination and anticipation. The subject is instructed to watch a light as it travels down the runway. They must anticipate the light reaching the target and press a pushbutton to coincide with the arrival of the light at the target. The timer will provide a light stimulus velocity of 3 mph for trials that will include a simple key press using a finger as well as an arm movement to a key press as described by Williams et al[33]. To reduce the effects of learning and familiarization, subjects will be given repeated trials, 30 seconds apart, until the times plateau. At that point, after two minutes rest, three additional trials will be given with the fastest score recorded for both the finder and arm movement patterns. Times will be recorded to the nearest 0.001 sec for both movement patterns.

9. **Neuropsychological Tests of Cognitive Function**

Screening for Possible Dementia. Before randomization, the men will undergo a telephone-administered, cognitive screening test, to identify possible dementia. The Telephone Interview for Cognitive Status (TICS) (266), a brief instrument (10 minutes), consists of 11 items that assess a range of cognitive domains that are affected by dementia. Scores on the telephone instrument correlate highly with scores from the MMSE examination and sensitivity and specificity are 94% and 100%, respectively (276, 277). A modified version (TICSm) (268), that eliminated items difficult to verify in epidemiological studies and added a delayed recall procedure to increase sensitivity to dementia, consists of 12 items with a maximum score of 50. The sensitivity and specificity of TICS and TICSm (268) are essentially similar. The inclusion of a delayed recall procedure may prove crucial in sensitivity to incident dementia

in longitudinal studies. We will use TICSm as the screening instrument for dementia, and men will be categorized into possibly demented and non-demented, based on the recommended cutpoint of 31 (268).

Neuropsychologic Test Battery. The neuropsychologic tests will be administered at baseline and at 18 and 36 months by a trained research assistant. The battery selected for this study utilizes many tests used in the Women's Memory Study, under Dr. Buckwalter's direction. This battery has been shown to detect gender (233, 234) and estrogen effects (233, 235), and characterizes a range of cognitive functions with emphasis on domains reported to be affected by hormonal therapy. Given the evidence that T affects spatial and fluency skills, we emphasize measures of these domains; but given the failure of previous studies to evaluate a range of domains, we include a comprehensive battery of standardized tests. Confrontational naming will be assessed with the widely used Boston Naming Test (269). Verbal fluency will be assessed with the 60 second naming task. Phonemic fluency will be assessed with Controlled Oral Word Association Test (COWA) which instructs subjects to provide as may words as possible beginning with the letters F, A and S. Episodic verbal memory, tested with verbal list learning, is reported to be sensitive to estrogen (235). There will be five learning trials, plus immediate recall, delayed recall, and delayed recognition trials. The California Verbal Learning Test (270) is widely used in elderly subjects. Paragraph recall will be assessed with two paragraphs from the Wechsler Memory Scale Logical Memory Subtest (WMS-III), each providing 46 bits of information for immediate recall. The FACES test from WMS-III assesses visual memory while minimizing the ability to verbally encode the information. The Judgment of Line Orientation test (271) assesses visual perception independent of motor output. Short-term memory will be assessed with verbal and nonverbal memory span tasks. The verbal tasks will use digit span forward and digit span backward from the revised WMS-III. The visual tasks will use the visual memory span forward and memory span backward tasks (WMS-III). The Trail Making Test (Parts A and B) is a complex visual scanning graphomotor task often used to assess executive control processes. Two self-report measures of psychological status will be administered. These instruments take approximately 15 minutes to complete. Mood will be assessed by Geriatric Depression Scale (272). A range of psychological characteristics (depression, obsessive-compulsiveness, hostility, somaticization, paranoid ideation, phobic anxiety, anxiety, and interpersonal sensitivity) will be evaluated with the Symptom Check List - 90 - R (SCL-90-R) (273). Subjects will also complete a neurobehavioral checklist that evaluates functional competence.

A very similar battery takes approximately two hours to administer to women over the age of 75 and we estimate a similar amount for time for these men. The protocol for administration of these tests will allow for frequent breaks.

10. Assessment of Insulin Sensitivity. The "cold" Insulin-Modified Frequently Sampled Intravenous Glucose Tolerance Test (FSIGT) will be used (173-174) to assess insulin sensitivity and glucose disposal. After an overnight fast, subjects will be placed at bed rest and will have two intravenous catheters placed: one in the antecubital area for injection and one in a hand vein for blood sampling. The hand will be warmed to 65F to arterialize the venous blood. After basal blood samples, dextrose (300 mg/kg) will be given intravenously over 1 minute. An intravenous injection of insulin (0.03 Units/kilogram weight) will be given 20 min. after dextrose injection. Plasma samples will be obtained at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, and 240 min. for glucose and insulin levels. Insulin sensitivity will be calculated by computer modeling of the insulin and glucose patterns during the FSIGT, using Bergman Minimal Model (274, 275), as described in our previous papers (276).

11. Methods for Hormone and PSA Measurements. Serum testosterone and estradiol levels will be measured by immunoassays established in our laboratory (9, 58, 66, 160, 277, 278). Serum LH and FSH will be measured by specific immunometric assays, (9) with sensitivity of 0.05 mIU/ml of 2nd IRP-hMG for both LH and FSH. The cross-reactivity of other glycoprotein hormones and the free alpha subunit is <1%. Free testosterone levels will be measured by equilibrium dialysis. Estradiol levels will be measured by immunoassay after celite chromatography. Sex-hormone binding globulin will be measured by an immunofluorometric assay (9, 278). Serum PSA levels will be measured using an immunoradiometric assay (9, 278). The range of serum PSA levels in men without prostate disease is 0-4 ug/L.

12. Quality Control and Standardization of Procedures and Equipment (See Appendices for Detailed Procedures). DXA machines at the sites will be calibrated daily by using a soft-tissue phantom. Dr. Storer will instruct exercise technicians in the procedures for quality control and exercise testing. He will visit the facilities every year to re-certify the exercise technicians and verify quality control procedures. The tests of muscle performance and physical function will be performed by using the same equipment at both sites. Dr. Storer will train the personnel in the proper use of the equipment. He will instruct the personnel in the proper procedures for the conduct of these tests. He will review the results and procedures to assure that reproducibility is optimal. We will calibrate the exercise equipment twice a year. The procedures for standardization and quality control in MDCT and CCA-IMT are described in the Appendix.

F. UNIQUE FEATURES OF THIS PROPOSAL THAT WILL HELP MINIMIZE THE IMPACT OF POTENTIAL CONFOUNDING FACTORS.

1.Careful Selection of Subjects. We will select subjects who are androgen-deficient in order to maximize the chances of finding an androgen effect. Studies (11) that included older men with normal testosterone levels failed to find an unequivocal increase in muscle strength, sexual, or cognitive function. The studies of men with unequivocally low testosterone levels have demonstrated significant increases in muscle mass and strength (58, 160).

2. Standardization of Procedures and Equipment, and Quality Control (See Appendix for Detailed Procedures). We recognize the critical importance of standardizing procedures and rigorous quality control to the success of any multi-site study. We have gone to great lengths to establish standardized procedures, and assuring the availability of similar equipment and software at the two sites.

3. Careful Selection of Testosterone Dose. The increments in testosterone levels above baseline in some previous studies, especially those that used a patch for replacement, were relatively small (11, 12, 203, 279). The peak testosterone concentrations in these studies were in the low normal range. In the proposed study, we will use a dose that will unequivocally raise serum testosterone concentrations into the mid-normal range for healthy young men.

4. **Selection of Measures of Physical Function that are Androgen-Responsive, Important for Activities of Daily Living, and Require Near-Maximal Strength.** Previous studies of androgen administration in older men (11) have failed to show improvements in measures of physical function because the measures used in those studies required only a fraction of maximal voluntary strength, and the baseline strength of healthy, older men exceeds the threshold below which these measures will show impairment. We have included in this study measures of physical function that require near-maximal effort and are threshold-independent. For instance, instead of the 0.625 m stair climb, we propose to use the Margaria stair climbing test; instead of the 20-m walk, we propose to use a quarter-mile walk.

5. **Assessment of Body Composition.** We recognize that various methods for assessing body composition may not necessarily measure the same body compartments (280). The algorithms used to compute fat-free mass and fat mass are based on empirically derived equations that may not uniformly apply to individuals of all races and genders. We recognize that methods for body composition assessment such as DEXA are susceptible to changes in body water. However, we have already demonstrated in our previous study (58, 160) that apparent increase in fat-free mass during testosterone administration is not due to water retention over and above that associated with protein accretion.

6. **Minimizing the Potential Confounding Influence of Learning Effect.** We recognize that many participants in this study will not have had prior weight lifting experience, and may be particularly susceptible to the learning effect. Therefore, we will instruct the subjects in proper use of the equipment and exercise routines, and to return to the Exercise Facility on more than one occasion prior to initiating treatment in order to assure that stability of measurement has been achieved. We found these procedures to be very effective in minimizing the confounding influence of learning effect in our previous study of HIV-infected men (160).

7. **Focus on Those Subdomains of Health-Related Quality of Life that are Likely Androgen-Responsive.** There are no data on the effects of testosterone replacement on health-related quality of life in older men. We recognize that only some subdomains of the HRQOL might be androgen-responsive. For instance, testosterone replacement of older men with low testosterone levels has been shown to improve perceptions of physical function (11). Similarly, in HIV-infected patients, gains in fat-free mass are associated with improvements in perceptions of physical function (281). Therefore, we posit that testosterone-induced gains in muscle mass, strength, and physical function will be associated with significant improvements in perceptions of physical function. Additionally, we will assess sexual function, marital interactions, and cognitive function that also affect quality of life.

8. **The Integrated Multi-Disciplinary Nature of the Investigating Team.** This project brings together investigators from several pertinent disciplines. Dr. Bhasin is an expert in androgen biology, muscle physiology, and anabolic effects of androgens. He is the Principal Investigator on two NIH-funded and one FDA-funded studies to examine the anabolic effects of testosterone on protein synthesis in young and older men, and men and women with HIV wasting syndrome. Dr. Harman is a gerontologist with 25 years of experience in epidemiological and interventional studies of older men and women. He was one of the architects of the Baltimore Longitudinal Aging Study. Dr. Hodis has been a pioneer in the use of CCA-IMT for the assessment of atherosclerosis in longitudinal studies. Dr. Budoff is an expert in the use of MDCT for determining coronary calcium. The MDCT Reading Center at Harbor-UCLA is the reading center for four NIH studies including the Multi-Ethnic Study of Atherosclerosis (MESA), CARDIA, South Bay Heart Watch, and EDIC. Dr. Budoff is performing several longitudinal studies tracking atherosclerosis in patients with ESRD, diabetes, and spinal cord injury, as well as progression studies under the influence of drugs such as ACE inhibitors. Dr. Storer is an exercise physiologist with expertise in assessment of muscle performance and physical function. His insight into the measurement of muscle function has been the key to the success of our previous studies. Dr. Buckwalter is an expert in neuropsychological testing and hormone effects on cognition. Dr. Lee a biostatistician with many years of experience in the conduct and analysis of clinical trials. He developed the procedures for randomization and data analysis and estimated the sample size and power. Dr. Ron Hays is an expert in the assessment of quality of life; he developed and validated the instruments for assessment of perceptions of physical function. Dr.

Neil Paige is an expert in assessment of sexual function and marital intimacy. These investigators, who have worked together for many years to successfully complete several major NIH-funded projects.

TIME TABLE

It will take the initial 3 months to develop the case report forms, protocol manuals, and for other organizational details. Recruitment will take place within the first 21 months. Therefore, the data analysis will take place in the last 3 months of the 5th study year. We recognize that this is a tight timeline and have discussed this issue explicitly with the staff at NHBLI. They are aware of the tight time line, but were reluctant at the very outset to allow us to propose more than a five-year budget. However, in view of the substantial amount of organizational effort that has already gone into standardizing the equipment and procedures for assessment of physical function, neuro-cognitive function, MDCT-CAC, and CCA-IMT measurements, and recruitment, we anticipate a short initial lead time. Pre-existing collaborations between Drs. Budoff and Hecht, and Drs. Bhasin and Harman should facilitate rapid take-off.

E. HUMAN SUBJECTS

1. Human Subjects Involvement

Human subjects participation will consist of a two-week screening and evaluation period and a three-year treatment period. There will be two inpatient visits to the Clinical Research Center at baseline at which time a number of evaluations will be performed.

2. Study Population

Two hundred and fifty community dwelling men, 60 years of age or older with baseline testosterone levels between 100 and 400 ng/dL will be recruited for this study if they meet the Inclusion and Exclusion criteria. Subjects of all ethnic groups will have an equal opportunity to participate if they meet the eligibility criteria.

On the Exclusion of Women and Children from This Study

Women will not be included in this study because testosterone supplementation at the dose proposed will have unacceptable virilizing side effects in women. However, we are investigating the effects of physiologic testosterone replacement in women in separate ongoing studies.

Children are not being included in this study because the specific objective of this study is to determine the effects of testosterone replacement on atherosclerosis progression in older men.

3. Specimens Obtained

Venous blood and urine samples will be obtained from the subjects specifically for this research. The patient's medical records may be reviewed (with consent) to ascertain eligibility for this study.

4. Subject Recruitment

In Los Angeles, the men who meet the eligibility criteria will be recruited from the South Bay and South Central region of Los Angeles County. In addition, we have access to several large multi-purpose senior centers that provide meals and day care services to over 25,000 seniors. In Phoenix, AZ, we will recruit subjects by direct advertising, from senior centers, and by the use of an 800 site, operated by the Kronos Tele-Health Call Center. In Boston University Medical Center, we will recruit from the BUMC health care facilities as well as free living, community dwelling individuals responding to local advertising.

Subjects will enter the study only after they have signed an informed consent, approved by the Institutional Review Boards (IRB) at both sites and after all questions have been answered. Consent will be obtained by one of the investigators or by the Research Manager. The protocol and consent form for this study have been submitted to the IRB for approval.

5. Potential Risks

The potential risks of this study include the risks of blood drawing, assessment of muscle strength and physical function, CCA-IMT, MDCT scan, questionnaires, and testosterone treatment. The risks of venous blood sampling include some pain, bleeding or a bruise where the needle was inserted. Serious complications such as blood clots or infections are very rare when proper precautions are taken.

Assessment of muscle strength and physical function may cause shortness of breath, leg fatigue, and muscle soreness, particularly in individuals who do not have prior weight lifting experience. In individuals with pre-existing heart disease, exercise can precipitate chest pain; this risk will be minimized by pre-screening the subjects and monitoring their heart rate and blood pressure during the course of the testing.

> "A cardiopulmonary exercise stress EKG test will be performed in order to identify those who might develop ischemic EKG changes during the course of incremental exercise and therefore might be at risk for developing cardiovascular complications during the course of muscle performance and physical function tests. Those men who develop significant ST-T segment changes or significant BP changes during the exercise test will NOT undergo tests of muscle performance or physical function. These men may continue to participate in the study and undergo other evaluations, as approved in this protocol. These participants will be advised of the EKG changes and referred to their primary care providers for further evaluation."

The potential side effects of testosterone in men include weight gain, acne, leg edema, breast tenderness and enlargement, adverse effects on the lipid profile, sleep apnea, and a small reversible increase in hemoglobin. These side effects are rare when the dose is limited to that necessary for physiologic replacement. Serious side effects such as hepatotoxicity and hepatic neoplasms, anecdotally identified with oral administration of high dose, long-term administration of 17-alpha-alkylated derivatives of testosterone in athletes, have not been observed in men or women when testosterone is administered parenterally or transdermally in physiologic replacement doses.

Testosterone supplementation increases hemoglobin by stimulating erythropoietin and by its direct effects on stem cells in the bone marrow. The increment in most men with low testosterone levels is modest, and is clinically not significant. However, in some men, particularly older men who are smokers, the increase in hemoglobin might be greater. Therefore, we will monitor hemoglobin levels during the course of the study, and increments in hemoglobin levels above 52% will warrant reduction in testosterone dose, referral to a specialist for evaluation and treatment including therapeutic phlebotomy.

Testosterone administration is absolutely contraindicated in men with prostate cancer. Therefore, men with history of prostate cancer are not eligible for this study.

The risk of prostate cancer in older men receiving testosterone supplementation is unknown. There is agreement that testosterone supplementation does not cause prostate cancer. However, the concern is that many older men harbor microscopic foci of cancer in their prostate and we do not know whether testosterone supplementation could cause a subclinical prostate cancer to grow and become clinically overt. In addition, intensive monitoring for prostate cancer by frequent PSA levels and digital rectal examination during the course of the study could cause some sublinical prostate cancers that would have otherwise remained undetectable to become diagnosed. This risk is inherent in any clinical research study that requires intensive monitoring. In two previous studies of testosterone replacement in older men (Snyder et al, 1999; Tenover 2000), the number of men found to have prostate cancers during the three-year treatment period was not significantly different between the placebo and testosterone-treated men. In fact, in these two studies, the replacement doses of testosterone were administrated to men for three years with a very low incidence of significant side effects. Testosterone may also cause the prostate gland to grow larger and this may cause problems in some men.

The topical use of androgel is associated with a small incidence of skin irritation; in most instances, the irritation abates with the change of application site. In a previous study, the incidence of skin irritation with the use of the testosterone gel was substantially lower than that associated with the use of the non-genital patch. There is also the potential for transfer of testosterone to sexual partner or to children who might come in close skin-to-skin contact with the individual. The subjects will be advised about this possibility and warned to avoid close contact of the site of application with another individual. An instruction sheet, prepared by the manufacturer to minimize the risk of transfer, will be provided to each individual.

The participants will be closely monitored with serial measurement of lipid profile, liver function tests, hematocrit, physical examination, PSA levels, and will receive serial digital rectal examinations.

The risks of carotid ultrasound are minimal and include some discomfort.

MDCT entails exposure to X-radiation. While no amount of radiation is considered completely safe, the radiation exposure from an MDCT scan is less than a rad and within the range of exposure considered acceptable.

6. Procedures for Protecting Against Potential Risks

The risks of blood drawing will be minimized in that only experienced practitioners (licensed physicians and clinical research nurses) will perform phlebotomy. Risks of injury from participating in exercise testing will be minimized by several safety measures. The subjects will be instructed in proper technique and the risks minimized by careful supervision. Patients will be screened with ECG stress testing to rule out cardiac ischemia and arrhythmias. This test will be done in the presence of an experienced physician. The likelihood of musculoskeletal injury from exercise testing will be minimized by providing a warm-up period proceeding each exercise session including warm-up stretching exercises. Further, an experienced exercise trainer will oversee all exercise testing sessions.

The risks of testosterone replacement therapy will be minimized by close monitoring with serial physical examinations, liver function tests, complete blood counts, digital rectal examinations and lipid profiles.

There is also potential for transfer of testosterone to sexual partner or to children who might come in close skin-to-skin contact with the individual. The subjects will be advised about this possibility and warned to avoid close contact of the site of application with another individual. An instruction sheet, prepared by the manufacturer to minimize the risk of transfer will be provided to each individual.

The potential risks involved in the Frequently Sampled Intravenous Glucose Tolerance (FSIGT) test will be minimized by calculating in advance of the procedure, the doses for both the glucose and the insulin to be administered An attending physician working with the research team will review the doses and sign the orders the day before the procedure occurs.

Data Safety Monitoring Board

In accordance with our discussions with Dr. Patrice Nickens of National Heart Lung and Blood Institute, we will establish a Data Safety Monitoring Board to oversee the progress of these studies. Appropriate amounts of moneys have been budgeted to cover the costs associated with the operation of the Data Safety Monitoring Board.

A Data Safety Monitoring Board consisting of four nationally recognized experts in Geriatrics (Thomas T. Yoshikawa, MD, Professor of Medicine, UCLA School of Medicine, Chair, Department of Medicine, Charles Drew University, and Editor-in-Chief, Journal of American Geriatrics Society) and atherosclerotic heart disease (William French, M.D., Professor of Medicine, UCLA School of Medicine, Chief, Interventional Cardiology, Harbor-UCLA Medical Center) will be established.

Once the study is initiated, the Data Safety and Monitoring Board will assume the oversight of the study and will monitor the randomization and recruitment, the progress of the studies, compliance with the protocol, and subject safety. The Data Safety and Monitoring Board has the authority to determine whether the trial should be terminated prematurely for safety reasons.

A designated unblinded Local Safety Monitoring Physician at each site will have access to the safety laboratory studies and will manage participants that develop significant adverse effects according to the guidelines independent of the local investigators who will remain blinded.

Procedures for Handling Specific Adverse Events

a. If severe symptoms of benign prostatic hypertrophy develop during the course of the study, as indicated by an AUA symptom score of 21 or greater, the unblinded physician will review medications, and obtain urinalysis and urine culture. If a urinary tract infection is detected, it will be treated with appropriate antibiotics. Medications such as anti-histamines and decongestants that might exacerbate symptoms will be discontinued. If severe symptoms persist even after treatment of urinary tract infection and discontinuation of offending medication, or if no urinary tract infection or offending medication is identified, the treatment will be discontinued and the patient will be referred to an urologist for evaluation and possible treatment of benign prostatic hypertrophy. The number of men who develop severe symptoms, and the number of men requiring surgical treatment for benign prostatic hypertrophy will be recorded. Because the study uses an intent-to-treat analytic strategy, the subjects will continue to be followed in the study and have all other measurements performed on schedule.

If men develop acute urinary retention, they will be referred to an urologist for evaluation and treatment. The evaluation of men who develop acute urinary retention might include placement of a Foley catheter, urine culture, and a review of medication. If a urinary tract infection is detected, it will be treated with appropriate antibiotics, and any offending medication such as antihistamines and anti-cholinergics will be discontinued. Foley catheter will be discontinued after one week. The number of men who develop acute urinary retention and require prostate surgery (e.g., TURP, TULIP, TUNA, stent placement, etc.) will be recorded. The study drug (placebo or testosterone) will be discontinued until the subject has had an urologic evaluation and the acute retention has resolved. However, study related measurements would be continued because of the intent-to-treat study design.

b. Increments in PSA. A significant increase in serum PSA level, defined as an increment of 1.4 ng/ml between any two visits, or a PSA velocity of greater than 0.75 ng/ml/year for a period of greater than two years will be verified by repeating the PSA level, and a digital rectal examination, urinalysis, and urine culture will be performed. Carter et al have reported that a PSA velocity, defined as the annual rate of change of PSA, of greater than 0.75 ng/ml/year is more likely to be associated with a prostate cancer and less likely with a benign prostatic disease. Similarly, although there is considerable test-retest variability in serum PSA measurements, data from Merck's PLESS study of finasteride in men with benign prostatic hypertrophy demonstrated that the 95% confidence interval of change in PSA levels between two measurements performed three to six months apart is 1.4 ng/ml. Therefore, a change of greater than 1.4 ng/ml between any two measurements, should be verified by repeating, and if this change persists, then it would warrant evaluation. If a urinary tract infection or prostatitis is found, it will be treated with appropriate antibiotics. If PSA levels remains elevated one month after discontinuation of antibiotics, the study treatment (placebo or testosterone) will be discontinued and the subject will be referred to an urologist for further evaluation and possible biopsy.

c. If a subject is found to have an abnormality on digital rectal examination, study drug (placebo or testosterone) will be discontinued and the subject referred to an urologist for evaluation and possible prostate biopsy. If the biopsy does not demonstrate prostate cancer, then the treatment will be resumed. Regardless, the subjects will continue to undergo study-related measurements.

d. Increase in hematocrit. If hematocrit rises above 52%, the designated local unblinded physician will recheck the hematocrit, and measure serum testosterone level, and reduce the testosterone dose. At the same time, the dose of

placebo will also be reduced by the unblinded physician in another subject to maintain blinding. Hematocrit will be monitored until it has returned to less than 52%. If it persists over 52% despite a reduction in testosterone dose, the subject will be referred to a subspecialist for evaluation for an underlying cause of erythrocytosis. If no other apparent cause is found, therapeutic phlebotomy will be performed to bring the hematocrit below 52%. Some men might need periodic therapeutic phlebotomy to maintain their hematocrit below 52%. If they are unwilling to undergo periodic phlebotomy, the drug treatment (placebo or testosterone) will be discontinued, but they will continue be followed.

e. Severe Obstructive Sleep Apnea. If symptoms of severe sleep apnea develop during the course of the study, as assessed by Berlin's questionnaire, the study treatment (placebo or testosterone) will be discontinued and the subject will be referred to a pulmonologist for further evaluation and treatment. Subjects who develop mild to moderate symptoms of sleep apnea during the course of the study will be referred to a pulmonologist for further evaluation, but will continue study drug administration unless symptoms demonstrate progressive worsening or the pulmonologist recommends discontinuation of treatment.

f. Other Intercurrent Illnesses. It is possible that some men will develop intercurrent illnesses unrelated to study drug administration. In this case, we will record the nature of the illness, and the study drug will be continued. The subjects and the investigators will remain blinded inspite of these intercurrent illnesses.

7. Risk Benefit Analysis

These studies will provide novel, invaluable information that will help us determine whether older men with low testosterone levels should receive testosterone replacement. The study will help define the risks and benefits of testosterone supplementation in older men. The risks of this study are low to moderate. Thus the risk to benefit ratio is felt to be favorable.

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