Effect of Weight Loss on Prostate Cancer Pathology
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1. RATIONALE

Statement of Problem: Obesity is an epidemic, a major public health concern, and is a significant risk factor for progression and mortality from prostate cancer. Research from our group and others suggest that weight loss interventions consisting of exercise and reduction in dietary fat may play an important role in the prevention of prostate cancer progression (secondary prevention).

Objectives: In this project we seek to establish the potential efficacy of weight loss for preventing the progression of prostate cancer by evaluating the effect of a 4-9-week weight loss intervention on the proliferation and apoptosis of prostate cancer tissue from radical prostatectomy specimens. We also seek to validate intermediate serum biomarkers that reflect or influence the weight loss-induced changes in prostate cancer tissue and which can be applied in future large-scale intervention studies aimed at secondary prostate cancer prevention in prostate cancer survivors.

Based on the background and preliminary data presented in this proposal **we hypothesize** that:

(1) Overweight and obese men that lose weight over a 4-9-week period prior to radical prostatectomy will have decreased proliferation and increased apoptosis of prostate cancer tissue relative to men that do not achieve weight loss, and that prostate cancer tissue obtained from men with weight loss will show evidence of a decreased tone of the local IGF axis including reduced phosphorylation of the IGF1 receptor, as well as lower levels of IGF-1 protein levels relative to men that do not lose weight.

(2) Serum from men that lose weight will decrease the proliferation and increase the apoptosis of LNCaP cells in ex-vivo bioassays to a greater degree than serum obtained from men that do not lose weight, and that serum IGF-I levels will also fall with weight loss while IGFBP-1 levels will rise.

(3) The weight loss-induced changes in serum ex-vivo bioassay results and serum IGF-1 & IGFBP-1 levels will directly correlate with the proliferative, apoptotic, and IGF axis changes seen in prostate cancer tissue. Specifically, we expect that: (a) Increased weight loss will correlate with a fall in serum IGF-I and a rise in serum IGFBP-1 as well as with a reduction in the ex-vivo proliferation bioassay and increase in ex-vivo apoptosis bioassay. (b) Increased weight loss will correlate with increased apoptosis staining and decreased Ki67 staining in the prostate cancer tissue. (c) The post-intervention change in the serum ex-vivo proliferation and apoptosis bioassays and serum IGF-I and IGFBP-1 levels will directly

correlate with the change in tissue proliferation and apoptosis making these markers clinical tools for secondary prevention prostate cancer weight loss trials.

Objective 1. To evaluate if weight loss prior to radical prostatectomy in overweight and obese men results in antiproliferative and pro-apoptotic effects on prostate cancer tissue histopathology. Overweight and obese men that have elected to undergo radical prostatectomy will be randomized to a control group (no weight loss) vs. a 4-9-week weight loss program followed by radical prostatectomy. Proliferation (Ki67) and apoptosis (TUNEL) of the prostate cancer tissue will be compared between the groups. We will also compare the proliferation and apoptosis indices between the baseline prostate needle biopsy specimens and corresponding radical prostatectomy specimens. In addition, prostate cancer tissue IGF-axis RNA and protein levels (IGF-1, total and phospho-IGF-1 receptor, and down-stream molecules), will be compared between the weight loss and control groups.

Objective 2. To evaluate if weight loss prior to radical prostatectomy in overweight and obese men impacts on the circulating levels of IGF-related analytes and on the Ex-vivo mitogenic and apoptotic activity of patient sera on LNCaP cell lines. The ex-vivo mitogenic and apoptosis bioassay in LNCaP cells will be performed on pre and post sera from the Control Group – No Weight Lossand Weight Loss Group at baseline and prior to radical prostatectomy. In addition we will compare baseline and post-intervention serum IGF-I and IGFBP-1 levels by ELISA.

Objective 3. To determine if the weight loss-induced changes in the serum biomarkers/bioassays from Objective 2 correlate with prostate tissue histo-marker changes in Objective 1. This analysis will assess if these serum biomarkers can therefore be used as surrogate biomarkers for future large-scale secondary prevention trials in overweight and obese prostate cancer survivors to monitor the success of weight loss intervention. For example, if the degree of LNCaP apoptosis in the ex-vivo apoptosis bioassay correlates with apoptosis in prostate cancer tissue, then the ex-vivo apoptosis bioassay may serve as a useful intermediate bioassay in large-scale trials to monitor the potential efficacy of weight loss in delaying prostate cancer progression or similarly, IGF-I or IGFBP-1 may prove to be the most predictive circulating indicator of prostate tumor apoptosis.

Long-Term Objectives: Our long term objective is to establish weight loss as an effective treatment to slow prostate cancer progression (secondary prevention) in overweight and obese prostate cancer survivors. If our presently proposed trial is found to be positive, indicating that weight loss results in improvements in the proliferative and apoptotic indices in malignant epithelium, our next trial (**intermediate goal**) will be a large scale, multi-site study evaluating if weight loss impacts on PSA doubling time in men with a rising PSA after primary therapy for prostate cancer. Given that PSA doubling time is becoming established as a surrogate for prostate cancer survival, results from this larger scale trial will determine the need for weight loss strategies in overweight and obese prostate cancer survivors.

2. BACKGROUND AND SIGNIFICANCE

Introduction: Obesity is an epidemic, a major public health concern, and is a significant risk factor for progression and mortality from prostate cancer. Research from our group and others suggest that weight loss interventions consisting of exercise and reduction in dietary fat may play an important role in the prevention of prostate cancer progression (secondary prevention). For example, our group demonstrated that a diet and exercise weight loss intervention in obese

men reduced the serum IGF-1 and increased serum IGFBP-1, factors known to affect prostate cancer progression in experimental systems. We have also developed unique ex-vivo bio-assays that indicated that sera from obese men that underwent a weight loss intervention had anti-mitogenic and pro-apoptotic effects on prostate cancer cells *in vitro*, and this was a result of the above mentioned changes in IGF axis parameters. In addition, our group demonstrated that feeding mice a low-fat diet resulted in decreased prostate tumor growth, increased tumor apoptosis, decreased tumor proliferation (ki67 staining), and decreased serum and tumor IGF-1 protein levels. Although these studies suggest a weight loss intervention may potentially impact on human prostate cancer progression, studies directly evaluating the effect of weight loss on prostate cancer histology in humans have yet to be performed. Furthermore, intermediate serum biomarkers to monitor the success of weight loss interventions on prostate cancer progression have not been established. To this end, we are now proposing a prospective randomized trial to evaluate if diet and exercise-induced weight loss in overweight and obese men for 4-9 weeks prior to radical prostatectomy results in decreased proliferation and increased apoptosis in prostate cancer tissue. We also propose to study whether weight loss prior to radical prostatectomy impacts on the expression of IGF-axis-related proteins in prostate cancer tissue and on the circulating levels of IGF-1 and IGFBP-1 in patient sera. Our ultimate goals are to determine if weight loss can potentially affect prostate cancer progression through affecting proliferation and apoptosis of prostate cancer tissue in humans, and to establish serum biomarkers for future large-scale secondary prevention trials in overweight and obese prostate cancer survivors to monitor the success of weight loss interventions.

Data presented herein outlines the strong evidence linking obesity and prostate cancer progression. The effect of obesity, weight loss, and exercise on the hormonal milieu including the IGF axis proteins, and the potential impact on prostate cancer progression is outlined in the background section of the research protocol.

Obesity and Prostate Cancer Progression: Prior studies examining the relationship between adult body mass index (BMI) and risk of developing prostate cancer have been mixed with several large studies showing a positive association[1-3] and others showing no association.[4, 5] Of the studies that found a significant association between increased BMI and risk of developing prostate cancer, several found that the risk for developing advanced disease was even stronger than the risk of developing prostate cancer in general.[1, 3] Visceral obesity may play an important role in the link between obesity and development of prostate cancer. One study among men in China found that men in the highest quartile of waist-to-hip ratio had an almost 3-fold increased risk for developing prostate cancer.[6]

While the relationship between obesity and prostate cancer risk is unclear, the relationship between obesity and progression and mortality from prostate cancer is well established.[1, 7-9] Two recent studies, one of which was from our group, utilized large multi-institutional multi-racial databases to address whether increased BMI was associated with higher biochemical failure rates following radical prostatectomy.[10, 11] Both studies concluded that obese men were more likely to have higher grade disease, and obesity was an independent predictor of prostate cancer recurrence following radical prostatectomy. Interestingly, both studies found that black men were more likely to be obese, which may explain, in part, the higher mortality from prostate cancer among black men.[12] In regards to obesity and prostate cancer mortality, two large prospective studies deserve particular attention. In 1959 and again in 1982, the American Cancer Society enrolled a cohort of patients for longitudinal studies on cancer, known as the Cancer Prevention Study (CPS) I and II, respectively. Men were then followed for 13 years in CPS-I and 14 years in CPS-II. Together these studies followed a total of 816,268 men during which time there were 5,212 prostate cancer deaths. Both CPS-I and CPS-II reported that

obese men (BMI >30 kg/m^2) were significantly more likely to die from prostate cancer with a 27% increased risk of prostate cancer death from CPS-I and a 21% increased risk of death from CPS-II.[8] More details regarding CPS-II were recently published which showed that severely obese men (BMI >35 kg/m^2) were at even greater risk of dying from prostate cancer with a 34% higher risk of prostate cancer death relative to normal weight men.[9] The data linking obesity with prostate cancer progression and mortality supports the urgent need for further studies evaluating weight loss interventions and prostate cancer progression.

Hormones, Obesity and Prostate Cancer Progression

Sex Hormones and Leptin: Studies on obesity and prostate cancer are complicated by the fact that obesity is not only associated with excess body fat, but also altered serum levels of numerous hormones including testosterone, estrogen, insulin, insulin-like growth factor-I (IGF-I), and leptin, all of which have to some degree been linked to prostate cancer. Whereas androgens,[13, 14] estrogens,[15] leptin,[16, 17] and adipokines[18] such as adiponectin may play an important role in prostate cancer progression in obese men, these hormones and growth factors will not be discussed here as the focus of our proposal and our preliminary data points towards the IGF axis proteins.

Insulin: Obesity is associated with insulin resistance and non-insulin dependent diabetes mellitus.[19] It has been hypothesized that insulin resistance may be related to prostate cancer development.[20] For example, Hsing et al. found that insulin resistance, diabetes, and elevated insulin/glucose levels increased prostate cancer risk.[21, 22] Other studies, however found no association or decreased prostate cancer risk in men with diabetes.[23, 24] Given the amount of conflicting data in the literature it is difficult to determine the true impact of insulin resistance and or diabetes on prostate cancer development or progression.

IGF-I and IGF Binding Proteins: IGF-I is a peptide growth factor and a potent mitogen for the growth of androgen responsive and androgen independent human prostate cancer cell lines.[25] IGF-1 plays a pivotal role in regulating cell proliferation, differentiation, and apoptosis.[26] Epidemiological investigations have found a positive correlation between elevated serum IGF-I levels and the risk of developing prostate cancer.[27-29] Tissue levels of IGF-1 appear to be a critically important factor during initiation and progression of prostate cancer.[26, 30] Animal studies have demonstrated that caloric restriction decreases growth of prostate tumors and decreases serum IGF-1 levels.[31, 32] The activity of IGF-1 is modulated by high-affinity IGF-binding proteins (IGFBP's 1–6).[33] Circulating levels of IGFBPs-1 and –2 vary in response to nutritional status and in response to changes in energy metabolism.[26] This is particularly true for IGFBP-1, which is perhaps the most dramatically regulated serum protein in response to diet, with levels changing up to fifty-fold in response to fasting.[34] In the energy-restricted state, which is strongly protective against many forms of tumors including prostate cancer, circulating levels of IGFBP-1 are increased, and to a lesser degree IGFBP –2 are also increased.[30, 35, 36] Overeating and obesity, on the other hand, lead to hyperinsulinemia and decreased levels of IGFBP-1, although the effect of insulin on serum IGFBP-2 levels in humans is less clear.[26, 37, 38] Serum IGFBP-1 levels have also been shown to increase following low-fat diet and exercise interventions, probably due, in part, to changes in serum insulin.[38]

Dietary Fat and Prostate Cancer Progression: The mechanisms through which dietary fat may impact on prostate cancer progression (and conversely decreasing dietary fat may prevent prostate cancer progression) are multiple and include increased energy intake, inflammatory pathways, alteration of sex steroid hormones, and effects on serum and tissue growth factors.[31, 41-43] Epidemiological studies have demonstrated a significant positive association between

dietary fat intake and the risk of developing advanced prostate cancer.[45, 46] For example, Fradet et al. prospectively monitored dietary fat intake, prostate cancer progression and survival in 384 men diagnosed with prostate cancer between 1990 and 1992. They found that increased consumption of saturated fat was associated with an increased risk of dying from prostate cancer.[47] Other epidemiologic studies have failed to observe a correlation between dietary fat intake and overall risk of prostate cancer, but importantly, no studies have shown an increased risk of prostate cancer with a low-fat diet.[48, 49]

Exercise and Prostate Cancer: In a recent review of the literature on physical activity and the risk for prostate cancer, Thune and Furberg found that 14 of 28 epidemiological studies reported that increased occupational or leisure-time activity reduced the risk for prostate cancer by 10-70 percent.[50] Only four studies reported the opposite relationship. The majority of epidemiologic data supports the association between more intensive exercise and lower prostate cancer risk.[50] Barnard et al. recently examined serum from men that attended the Adult Fitness Program at the University of Nevada Las Vegas for at least 10 years and compared prostate cancer relevant biomarkers with an age matched group of overweight men with a sedentary lifestyle.[51] Results of prostate cancer biomarkers were more favorable in the exercise group including decreased serum IGF-I levels, increased serum IGFBP-1 levels, and decreased serum-stimulated growth of a human prostate cancer cell line (LNCaP) in vitro when cultured in media containing patient sera.[51] There is a great need for further studies examining the effects of exercise on serum and tissue biomarkers in prostate cancer patients.

Preclinical Studies in Energy Restriction and Prostate Cancer: Several recent preclinical studies support the role of caloric restriction for preventing the development and progression of prostate cancer. Mukherjee et al. utilized two transplantable prostate tumor models: (1) androgen-dependent Dunning R3327-H adenocarcinoma in rats and (2) androgen-sensitive LNCaP human carcinoma in severe combined immunodeficient mice. They found that caloric restriction reduced tumor growth in both models relative to animals fed ad libitum, and increased apoptosis was found in tumors from the energy restricted groups relative to the ad libitum groups.[31] Boileau et al. induced prostate tumors in rats by subjecting them to N-methyl-N-nitrosourea and testosterone (a well established model for prostate cancer), and found a reduced proportion of mice developed prostate tumors in the caloric restriction group relative to an ad libitum fed group.[52] Likewise, Suttie et al. found decreased development of prostate cancer in TRAMP mice that were restricted in calories (relative to an ad libitum group), and tumors from the restricted group had reduced tumor grade.[53] In summary, pre-clinical models of energy restriction strongly support the conduct of a prospective randomized trial in men evaluating weight loss and biologically relevant tumor markers.

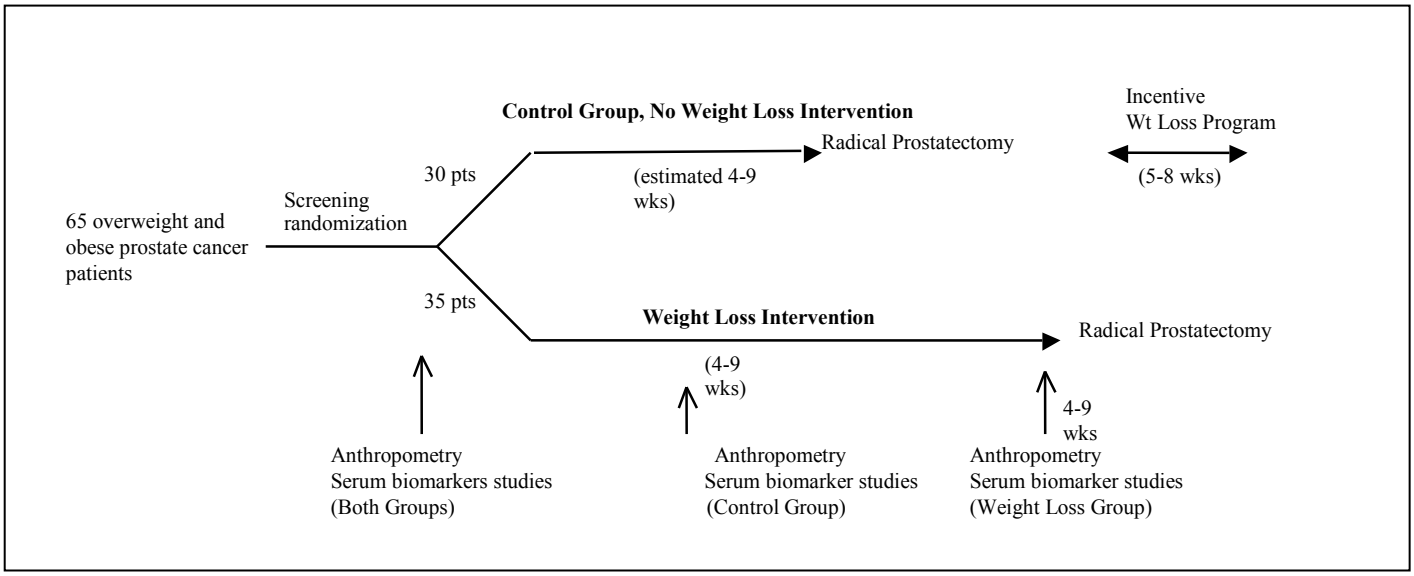
Significance: If we find that the proposed weight loss intervention increases apoptosis and/or decreases proliferation in malignant epithelium, this will form the basis for immediate initiation of larger scale trials evaluating weight loss for prostate cancer survivors. In our experience, the most likely design for this type of larger trial in the future would be to enroll men with a progressively rising PSA following primary therapy for prostate cancer with the aim of evaluating if weight loss can decrease the PSA doubling time over 1-year. Emerging data from our group and others suggests that PSA doubling time directly relates to mortality in men with prostate cancer and therefore represents an important surrogate for future large scale trials.[54,55] Our research team has direct experience with this large scale experimental design as we are presently conducting a multicenter prospective randomized trial of this nature testing a botanical dietary supplement juice from pomegranates. In the event that the proposed weight loss trial demonstrates an effect, we have extensive collaborative relationships in place to make feasible

a larger scale definitive trial in the future evaluating weight loss in men with rising PSA levels as described above.

Relevance to Veteran's Health: The proposed research is directly relevant to Veteran's health. Given that the majority of the VA population is aging males, prostate cancer remains a significant health concern for our patients. Prostate cancer is second to skin malignancies in incidence of prostate cancer in the United States and is the second leading cause of male cancer deaths.[56] Obesity is also a major health concern among Veterans. Research from our group found approximately 70% of veterans with prostate cancer that underwent radical Prostatectomy were overweight or obese. Given the sometimes long and protracted course of prostate cancer, if weight loss is ultimately found to decrease prostate cancer progression, this will be an important non-toxic intervention for patients to consider that have elected watchful waiting, or as an adjuvant to other primary therapies.

3. WORK PROPOSED

Overview: The study design for the present proposal is illustrated below. To obtain adequate power to detect a difference in prostate tumor apoptosis between the groups, the trial will require 30 patients per group. Based on our prior experience with the weight loss intervention we will be using in this trial, we are assuming a 15% dropout rate in the Weight Loss Group. Therefore, we are adding an additional 5 subjects to the Weight Loss Group to bring the total in this group to 35 and the total in the overall trial to 65.



Screening and Recruitment of Subjects - Minority Participation: Subjects will be recruited for the present trial from the VA Medical Center, Greater Los Angeles Health Care System. Dr. Aronson conducts the urologic oncology clinic at the VA in which 130 new prostate cancer cases are diagnosed per year. Subjects with a new diagnosis of prostate cancer at the VA are offered standard therapies as well as the opportunity to participate in clinical trials. Subjects expressing interest in the weight loss trial will be given the consent form to review and the phone number of Dr. Aronson's research coordinator. Given the high prevalence of overweight and obese subjects (up to 70 % at our VA[10]), and given the high number of radical prostatectomy procedures performed at our VA (40/year), we anticipate no problem meeting our

recruitment goal of 65 subjects over 5-years. As well, given that 40-50% of men that undergo radical prostatectomy at the VA are AAM, we anticipate 40-50% of men in our clinical trials will be AAM.[62] This is extremely important given the increased risk of obesity in AAM and the undue burden of increased morbidity and mortality of prostate cancer in AAM. For men randomized to the weight loss intervention group, the 4-9 week delay in radical prostatectomy has been shown not to increase the risk of biochemical recurrence.[63] However, we will not enter patients in this trial with a high risk of having advanced disease (Gleason Grade > 4+4) to avoid the potential risk (albeit extremely low) for progression during the weight loss intervention.

Inclusion Criteria:

1. Subject is overweight or obese (BMI > 25 kg/m^2)
2. Patient with pathologically confirmed adenocarcinoma of the prostate and has elected to undergo radical prostatectomy
3. Able to adhere to physical activity intervention (able to walk for 10 minutes without rest)
4. Able to come to the VA CRC for study visits over 4-9 weeks (necessary if the subject is randomized to the weight loss intervention group)
5. Patient agrees to stop taking dietary or vitamin supplements (Lycopene***, Vitamin E, selenium, genistein, fish oil) or herbal supplements (e.g., saw palmetto, PC-SPES) prior to starting the dietary intervention.

Exclusion Criteria:

1. Gleason grade of prostate cancer > 4+5.
2. History of ever receiving androgen deprivation therapy or antiandrogen therapy
3. History of receiving finasteride* in the past 9 months
4. Patient receiving insulin** for diabetes mellitus
5. Current use of weight loss medications
6. Significant co-morbidities (i.e. cardiac, pulmonary, liver disease, ongoing alcohol/drug abuse)
7. Cardiac pacemaker

* Androgen deprivation therapy: Leuprolide, Goserelin. Antiandrogen therapy: Flutamide, Bicalutamide

** Changes in insulin levels may impact on the proposed biomarkers (IGF-1, IGFBP-1)

*** Lycopene has previously been shown to impact on apoptosis in prostate cancer tissue[64]

Clinical Trial Flow Chart:

Activity	**Screen wk -1 to 0**	**baseline**	**wk 1**	**wk 2**	**wk 3**	**wk 4**	**wk 5**	**wk 6**	**wk 7**	**wk 8**	**wk 9**	**surgery**
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	
Obtain Informed Consent	✔											
Review Inclusion/Exclusion	✔											
Medical Hx & Phys Exam	✔											
Concomitant Medications	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	
Waist-to-Hip measurement[3]	✔	✔		✔		✔					✔	
Weight	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
Give subject 3-Day Food Diary to complete		✔	✔	✔	✔	✔	✔	✔	✔	✔		
Randomization	✔											
DEXA Scan	✔										✔	
Collect 3-Day Food Diary		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
Serum Biomarkers/Bioassays[1]	✔										✔	
Weight loss Intervention Visits[2]		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
Assess adverse events	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
Tissue collection for biomarkers												✔

Footnotes:

1. Fasting serum biomarkers/bioassays to be studied are IGF-1 and IGFBP-1 and the ex-vivo mitogenic and apoptotic activity of subject serum on LNCaP cells. Additional fasting serum studies will be insulin, PSA, and lipids.
2. The No Weight Loss Group has the same Baseline Visit procedures and Final Visit procedures (within 3-days prior to surgery) as the Weight Loss Group: Dexa Scan, fasting serum biomarkers, anthropometrics, and a 3-Day Food Diary.
3. At week 9 or Final Waist/Hip Measurement will be at the Final Visit Prior to Surgery.

Study Procedures: Subjects will have their first study visit at the VA Greater Los Angeles Healthcare System Clinical Research Center (CRC) which will include subject consent and review of the entry criteria. Once consent is obtained, a medical history and physical exam will be performed including height, weight, and measurement of waist to hip ratio (anthropometrics). The urologist's DRE and clinical stage will be recorded from the subject's urology note, and prostate needle biopsy pathology including Gleason grade and percent of cores with cancer will be recorded. A fasting serum specimen will be collected for biomarker studies. A DEXA scan will be performed (to determine lean body mass to calculate caloric intake for the weight loss program) and to determine percent body fat. The Subject will then return home and during the next week will keep a 3-Day Food Diary (Appendix 2). Subject's will be randomized within 3-days of their initial visit and contacted at home with the results of the randomization.

Randomization: If a subject meets all eligibility criteria, the following steps will occur:

1. The study coordinator will FAX the subject's initials, study ID, and date of visit to the junior statistician (to be named).
2. Within 24 hours the statistician will inform the research coordinator of the randomization result (weight loss vs. No Weight Loss Group) and date of randomization. The research coordinator will inform the study subject of the results and schedule subsequent study visits.
3. The randomization will use a permuted block design with 5 blocks of size 13 (7 weight-loss and 6 no weight-loss.

Control – No Weight Loss Intervention Group: After randomization to the control group, these men will proceed with radical prostatectomy as scheduled. They will have one additional visit to the VA CRC within 3 days prior to radical prostatectomy for measurement of weight, height, and waist to hip ratio, fasting blood will be collected for biomarkers and a DEXA Scan. During the week prior to the radical prostatectomy a 3-Day Food Record will be completed as well. We are doing this second visit in the control group to monitor for possible weight loss that these subjects may initiate on their own, although we do not anticipate this occurring to any significant degree. Subjects in the Control group will be given the phone number of the study coordinator to schedule their optional 5-8-week weight loss program after they have recovered from their surgery. This optional weight loss program is given as an incentive for their participation in the trial.

Weight Loss Intervention Group: Subjects randomized to the Weight Loss Group will receive an appointment to the VA CRC to meet with the research study coordinator and the study dietician to initiate the weight loss intervention. Subjects will receive one of two standard structured energy-restricted meal plans (1200, 1500, 1800 Kcal/day) using meal replacements and portion-controlled foods. Prescribed plans will be based upon resting energy expenditure and lean body mass as determined by DEXA scanning. The goal of the meal plan is to provide a total calorie intake incorporating 500-800 Calorie deficits per day. Since men will be provided with exercise regimens as well, they should experience a 1 to 2 pound weight loss per week during the weight loss intervention. Based on our experience in prior trials, we anticipate subjects will lose 4-6% of their body weight over 5-8-weeks as a result of the weight loss intervention.

Subjects will have weekly visits with the study dietician until the time of the radical prostatectomy. Dr. Li or her associates will oversee each of these visits with the study dietician. The subject will receive instruction on recipes, ideas and places for food shopping, education on healthy food preparation, and preparation of meal replacements. Recommended diets will

contain 20-25% energy from fat, 15-20% from protein and 50-65% from carbohydrates largely from fruits and vegetables with some whole grains. Fiber recommendations will be a total of 25 grams/day from fruits, vegetables, legumes, and high fiber cereals.

Subjects will be counseled by Dr. Li or her associates on performing 1 hr of exercise/day including aerobic, resistance, and flexibility activities. As an exercise incentive, subjects will be given a pedometer and exercise an log (Appendix 3) to complete and results will be reviewed with the dietitian at each visit. Subjects will be weighed at each visit and waist to hip ratio will be measured at the Screening or Baseline, Weeks 2, 4 and 9. Following the 4-9-week weight loss intervention and within 3 days prior to radical prostatectomy, weight and waist to hip ratio will again be measured, fasting serum will be collected for biomarkers, and a DEXA Scan will be performed to assess the change in body fat and lean body mass as a result of the intervention. In addition, during this week subjects will also complete a 3-day Food Diary to assess dietary and caloric intake to bring with them for their final study visit prior to surgery.

At each study visit, we will go over concomitant medications, adverse events and we will assess subjects for fatigue as well as the ability to perform their usual daily tasks (functional ability). If subjects have significant loss of strength or daily function, we will take them off protocol and have them re-evaluated in the preoperative clearance clinic at the West Los Angeles VA to make sure they can safely undergo radical prostatectomy.

Post-Prostatectomy Follow-up: Subjects in the Weight Loss Group will have scheduled visits at 3 months and 9 months post-op to assess for adverse events, and to measure body weight to determine if subjects maintain the weight loss achieved during the pre-operative intervention. If they are unable to come to the VA, this visit can take place over the phone. This will be the same for the Control subjects.

Radical Prostatectomy–Tissue Collection: At the time of prostatectomy, frozen tissue blocks of benign prostate tissue and prostate carcinoma will be obtained immediately following surgery. Frozen sections will be evaluated by a pathologist to ensure the quality of the specimens and verify if the specimen represents benign or malignant tissue. Fresh tissue will be embedded in OCT (polyvinyl alcohol and polyethylene glycol), 'snap frozen', and stored at -80^{o}. The remainder of the tissue will be fixed in 10% neutral buffered formalin and embedded in paraffin for routine histology and immunohistochemistry studies. The amount of fresh tissue obtained will vary depending on the size of the carcinoma, but our experience has shown that at least one 1.0 x 0.5 x 1.0 cm tissue block representative of the carcinoma, and a similar sized block of benign prostate tissue can be retrieved from most prostatectomy specimens. At the time of radical prostatectomy a sample of fat from the anterior surface of the prostate, a sample of subcutaneous fat and fat from the inner abdominal wall will be collected. Half of the tissue will be flash frozen and used for genomic and proteomic analysis. The other half will be cultured at 37 degrees centigrade in M199 media and the conditioned media will be harvested and used to quantify secreted inflammatory cytokines such as IL8 and IL6.

Serum and Plasma Studies-Overview: Serum insulin, Free and Total Testosterone, Estradiol, Leptin, IGF-1, IGFBP-1, PSA, lipids, ex-vivo mitogenic bioassay, and the ex-vivo apoptosis assay will be performed at baseline and prior to radical prostatectomy in all subject in the Weight Loss Group and control group. We are measuring PSA levels given that PSA represents an established tumor marker of disease progression in prostate cancer and weight loss may potentially decrease the PSA. PSA levels will also be measured in subjects in the control and Weight Loss Groups post-operatively every 6-months (for the 10-year study duration) as is routine in our GU Oncology clinic at the VA. White blood cells will be collected from the buffy

coat, RNA extracted, and PCR arrays will be performed to quantitate inflammatory cytokines and chemokines. Subject weights will also be recorded at these visits.

Ex-Vivo Bioassays: The ex-vivo mitogenic and apoptosis bioassay will be performed in the laboratory of Dr. Aronson. These bioassays are designed to test the presence of serum factors affecting the proliferation and apoptosis of androgen dependent (LNCaP) prostate cancer cells by substituting FBS in the medium with human serum collected before and after an intervention.

Rationale for prostate cancer tissue analysis: Weight-loss may affect prostate cancer through numerous mechanisms involving both primary and secondary effects (through changes in serum IGF molecules or other factors). These changes may influence the expression of mRNA of key molecules, the levels of proteins expressed in cancer cells, or the phosphorylation/activation state of key growth and survival signaling cascades. Our primary goal in this project is to identify proliferative and apoptotic changes in prostate cancer tissue in response to weight loss. We will also focus our studies based on our prior observations in preclinical xenograft models that showed diet-induced changes in IGF1 gene expression and protein levels, and reduction in the phosphorylation of the IGF-1 receptor in prostate cancer tissue. We will follow these with additional related studies of down-stream survival molecules such as Akt/phospho-Akt, activated caspase-3, BCL2, and BAX. Initial findings will guide us regarding the performance of these secondary outcome measures.

Immunostaining and Interpretation: Immunostaining of paraffin embedded pretreatment prostate needle biopsy specimens and malignant tissue from radical prostatectomy specimens will be performed using ki67 monoclonal antibodies (DAKO) and a TUNEL assay (BioVision). The cancer tissue with the highest Gleason grade will be analyzed in a blinded fashion by counting 300 cancer cells per slide. Our main objective (Objective 1 of this proposal) will be to compare the percent of cells staining positive for ki67 and TUNEL between the Control Group-No Weight Loss and Weight Loss Group (controlling for Gleason grade). We will also compare the percent of cells staining for ki67 and TUNEL in the prostate needle biopsy specimen relative to the prostatectomy tissue (also matching for Gleason grade). In addition, we will perform correlation analysis to determine if weight loss correlates with proliferation and apoptosis. We hypothesize that with increasing weight loss there will be decreased ki67 staining and an increasing percentage of cells with TUNEL staining. To further evaluate the effect of weight-loss on prostate tumor staining of the IGF signaling cascade we will analyze immunostaining of phospho-IGF-1R (using a Cell Signaling antibody) and phospho-Akt (Cell Signaling). We will also evaluate for earlier stages of apoptosis as measured by activated caspase-3 (Oncogene). If we detect changes in apoptosis, we will also stain for BAX and BCL2 using antibodies from R&D. If we detect changes in proliferation as measured by ki67 staining, we will also stain for Mcm-2 (which has been shown to be a prognostically important prostate cancer proliferation marker) using antibodies from R&D.[66]

Prostate Cancer tissue RNA and Protein Analysis: For Western analysis, equal amounts of protein from the tumor tissue lysates will be evaluated by 8% SDS-PAGE analysis. Beta Actin antibodies (Cayman Chemicals, MI) will be used for loading control. Membranes will be developed using the ECL system (Amersham). Primary antibodies to total and phosphorylated IGF1 receptor (Cell Signaling) will be used. As a secondary aim, if we find that weight loss significantly impacts on the IGF-1 axis proteins as hypothesized above, we will perform analysis of total and phosphorylated Akt (Cell Signaling) which is known to be activated downstream from the IGF-1 receptor. In the event that increased apoptosis is observed in the experimental Weight Loss Group, we will also immunoblot for BAX and BCL2 using R&D antibodies.

Quantitative real time PCR analysis (QRT-PCR): Total RNA from the frozen tissue will be extracted using the RNeasy kit (Qiagen) and checked for its purity and total RNA content. QRT-PCR will be performed on all samples to determine the expression of IGF-1, the apoptosis-related genes (BCL2, BAX) and the housekeeping gene Beta-actin. cDNA will be synthesized using the First Strand cDNA Synthesis kit for RT-PCR (Roche). Gene-specific primers and probes were designed and synthesized by the as previously published and are not listed here. PCR will be performed (in triplicates) in a LightCycler thermal cycler (Roche) using previously published conditions and amplification programs. A negative control without a cDNA template will be run simultaneously with every assay. Standard curves will be obtained using serial dilutions of the beta-globulin gene (DNA Control Kit; Roche). The concentration of each gene product will be determined on the basis of a kinetic approach using the lightcycler software (Roche) and normalized for *ACTB*. When comparing gene expression and protein levels between the groups, we will match the Gleason grade of the tissue. All of the above methods are fully operational in our laboratory.

Proteinomics/Genomics: We plan to further study the molecular nature of the weight loss-induced changes in the tumor proteome through gene array and proteomic approaches coupled with confirmatory protein and RNA analyses.

Statistical Analysis

Statistical Design: The statistical design of this study is a randomized repeated measures utilizing random mixed block sizes known only to the statistician constructing the randomization list to assign subjects to two study arms in a ratio of 7:6, weight loss to no weight loss.

Sample Size Determination: An evaluable sample of at least 60 subjects (no weight loss=30, weight loss=30) is expected to provide 80% power to detect an effect size of 0.74 using a two group t-test with a 0.050 two-sided significance when comparing the apoptotic index (primary outcome variable) in the radical prostatectomy malignant epithelium between the weight loss and no Weight Loss Group. The apoptotic index will be measured in the malignant epithelium with the highest Gleason grade. To account for a post-randomization attrition rate of up to 15% in the Weight Loss Group, a total of 35 subjects will be randomized to the Weight Loss Group, thus bringing the total sample size to 65 subjects. Kim et al. observed an effect size of 1.74 on apoptotic index using a nutritional intervention in a similar study.[64]

Data Analysis: Data analyses will be performed on randomized subjects who complete the study. All parameters measured will be summarized descriptively. The number of subjects, mean, standard error of the mean, median and min-max values will be presented for continuous parameters and the number and percentage of subjects in each category will be presented for categorical parameters. No imputation of missing data is planned. Demographic and baseline characteristics will be summarized using descriptive statistics overall and by treatment group to assess baseline comparability. Treatment groups will be compared using the appropriate nonparametric tests for categorical parameters and analysis of variance models for continuous parameters.

Primary Outcome Variable: The primary outcome variable is apoptotic index of the highest Gleason grade malignant epithelium in the radical prostatectomy specimen. The primary objective is to compare the mean apoptotic index between the Weight Loss Group and the Control Group-No Weight Loss. The two independent sample t-test will be used to compare the index between the groups. If the index is non-normally distributed we will use the Wilcoxon rank sum test. Next, linear regression models will be constructed to test whether the treatment effect

is significant after controlling for Gleason grade, stage and any demographic factors that are significantly different between the groups.

Secondary Outcome Variables: The secondary outcome variables are: the proliferative index in prostate cancer epithelium obtained from the radical prostatectomy specimen, the change in apoptotic and proliferative indices of malignant epithelium between the diagnostic prostate needle biopsy and corresponding radical prostatectomy, the change (baseline vs post-intervention) in serum IGF-related analytes: IGF-1 and IGFBP-1, the ex-vivo mitogenic and apoptotic activity of patient sera on LNCaP cells, and the change in body weight and percent body fat by DEXA over 4-9-weeks.

Secondary Statistical Analyses: The statistical methods for these analyses will be analysis of variance (ANOVA), linear regression, and correlation analysis. ANOVA will be used to determine if the serum biomarkers (IGF-I, IGFBP-1, proliferation and apoptosis bioassays) change over the course of time. These models will include a term for time, treatment (weight-loss versus no weight loss) and the time by treatment interaction effect. We will also construct ANOVA models for weight and body fat over time to determine if the treatment by time effect (intervention effect) is significant. Next, linear regression models will be constructed for each biomarker to assess the factors that predict change in that marker. Predictors will include actual weight loss (percent change), group, grade, PSA, percent body fat, and demographic factors. These models will allow us to assess the direct effect of weight loss on each of the serum biomarkers and allow for the assessment of whether weight change or intervention is the primary factor predicting change in the biomarkers. This assessment is required due to the fact that the intervention will have varying effects on weight based on the amount of subject compliance with the weight loss regime. Regression models will also be constructed to compare the groups with respect to the pre-prostatectomy biomarkers and the tissue markers and the between group tissue markers (apoptosis, Ki67 staining). A separate model will be constructed for each marker including the predictors of group, grade, PSA, percent body fat and demographic factors. Next, we will construct regression models to determine if the biomarkers, or change in biomarkers, affect the post-op pathology. Ordinal regression will be used for post-op pathology. These models will include terms for the demographic factors and for each of the biomarkers. Since there are a large number of biomarkers we will first construct single biomarker models which will include the covariates for each outcome and then combine together all significant markers into multiple regression models. Finally, we will use the Spearman correlation to test for correlations between pre-prostatectomy biomarkers and the tissue markers. This analysis will specifically address **Objective 3** of the proposal, that being to determine if a correlation exists between serum biomarkers and tissue biomarkers. The Spearman correlation is a non-parametric rank based correlation measure that does not depend on the assumption that the markers are normally distributed.

Subgroup Analyses: Subgroup analyses of parameters will be performed according to stratification by PSA level, previous prostate cancer treatment, tumor stage, and by Gleason score. Clinically relevant demographic factors, baseline characteristics, and medical history will be examined for differences in the primary efficacy parameter.

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