# Genistein Supplementation to Mitigate Cardiometabolic Dysfunction in Patients Undergoing Androgen Deprivation Therapy for Prostate Cancer

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**1. TITLE:** Genistein Supplementation to Mitigate Cardiometabolic Dysfunction in Patients Undergoing Androgen Deprivation Therapy for Prostate Cancer

**Alternate Title:** GeniPro (Genistein Supplementation to Mitigate Cardiometabolic Dysfunction in Patients Undergoing Androgen Deprivation Therapy for Prostate Cancer)

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**2. EXTERNAL COLLABORATORS:** There are no external collaborators.

**3. PRECIS/ABSTRACT**

Androgen deprivation therapy (ADT) is a widely used treatment for prostate cancer (PCa). Despite its benefits in improving survival in men with advanced PCa, ADT itself is associated with increased metabolic syndrome, diabetes, and cardiovascular disease (CVD) risk. The rapid and sustained decrease in serum testosterone levels induced by ADT results in an increase in body fat, dyslipidemia, insulin resistance, and arterial stiffness—all associated with CVD risk and many of which are evident within 3 months of ADT initiation. *Unfortunately, few studies have been conducted to investigate therapies that may prevent or mitigate ADT-induced cardiometabolic dysfunction in patients with PCa. We hypothesize that genistein, a naturally-occurring soy isoflavone that functions as a selective estrogen receptor modulator, will be beneficial in promoting cardiometabolic health in men with PCa undergoing ADT.* Dietary intake of soy products has long been known to correlate with indexes of metabolic health, and experimental and animal studies have shown genistein to improve insulin sensitivity and lipid metabolism through several mechanisms of action. Clinical trials, primarily in postmenopausal women, suggest that oral genistein administration decreases fasting lipid and insulin concentrations. Co-Investigator, Dr. Omer Kucuk, has shown that genistein supplementation decreases serum total cholesterol levels in men with PCa. Here, we propose a pilot, randomized, double blind, placebo-controlled trial of daily oral genistein (60 mg/d) with comprehensive cardiometabolic profile testing in 24 men initiating ADT for PCa.

**4. INTRODUCTION and BACKGROUND:**

Prostate cancer (PCa) is the most commonly diagnosed non-skin cancer in men, with an estimated prevalence of >2,700,000 men in the U.S[1]. Androgen deprivation therapy (ADT), the chemical or surgical inhibition of testosterone and other androgens to minimize prostate cancer cell growth, is among the most common treatment modalities for PCa[2]. ADT has established benefits in improving survival in patients with advanced PCa[2,3]. Although evidence does not support the use of ADT for low-risk, localized PCa, utilization rates in this sub-set remain high[4,5]. Given the ubiquitous roles of androgens in physiological homeostasis, the implications of widespread ADT use are significant. Adverse effects of androgen deprivation include adverse body composition and cardiometabolic changes, such as increases in body fat, decreases in muscle mass, insulin resistance, and serum lipid alterations[6-16], which could have negative long-lasting effects on quality of life and morbidity in PCa survivors, especially if coupled with age-related metabolic alterations. Changes in cardiometabolic risk factors, such as insulin resistance and arterial stiffness have been observed as early as 12 weeks[7-10,13,17]. Recent meta-analyses

investigating ADT morbidity have indicated a 75% increased risk for metabolic syndrome[18], 36% increased risk for diabetes[18], and a 38% increased risk for CVD[19]. Unfortunately, only a limited number of studies have been conducted to investigate therapies that may ameliorate the adverse metabolic effects of ADT[20]. These studies have primarily been exercise-based interventions, and, although inducing some beneficial changes in body composition and physical fitness, have generally not been effective at changing metabolic parameters[20,21]. *Additional therapies that may prevent or mitigate ADT-induced cardiometabolic dysfunction in patients with PCa are critically needed.*

**Genistein, a naturally-occuring soy isoflavone, may be a viable alternative for improving cardiometabolic health in men requiring ADT.** Dietary intake of soy products has long been known to correlate with reduced chronic disease risk, including PCa[22,23]. The beneficial effects of soy products have been attributed to isoflavones, phytochemicals (or phytoestrogens) that are structurally similar to 17β-estradiol and function as selective estrogen receptor modulators[24]. Genistein is the most biologically active isoflavone, whose molecular actions, among others, include modulating the expression of genes involved in cell survival, cell cycle, and apoptosis[24]. Several clinical studies, including one by Co-Investigator and Mentor, Dr. Omer Kucuk, have described the efficacy and safety of genistein/soy in decreasing or stabilizing serum and tissue prostate specific antigen in patients with PCa[25-27]. *The beneficial effects of genistein also extend to cardiometabolic health.* Meta-analyses of controlled, clinical trials confirm the insulin sensitizing and plasma lipid lowering effects of soy products in non-cancer populations[28-30]. Experimental studies have shown genistein to improve cardiometabolic health through several mechanisms of action, including promoting activation of peroxisome-proliferator activated receptors (PPAR) and 5'-adenosine-monophosphate-activated protein kinase (AMPK), important for insulin action and lipid homeostasis[31,32]; and regulation of HMG-CoA reductase and acyl-CoA:cholesterol acyltransferase, important for cholesterol synthesis and esterification[33]. In men with PCa, Dr. Kucuk has shown that genistein supplementation significantly decreases serum total cholesterol levels over a period of several weeks (**Fig. 1**)[25]. *Given the metabolic changes that occur following ADT initiation in men with PCa and the beneficial metabolic effects of genistein in other populations, we hypothesize that genistein will be beneficial in reducing insulin resistance and cardiovascular dysfunction, in men with PCa undergoing ADT.*

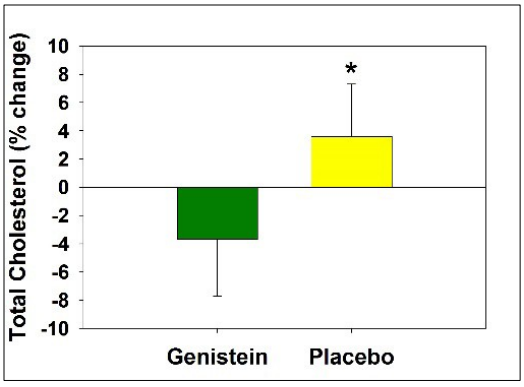


**Fig 1. Decrease in blood total cholesterol following genistein supplementation[24].** N = 47 men with prostate cancer were randomized to 30 mg genistein daily or placebo for 3-6 wks prior to prostatectomy. *$P < 0.05$

Genistein is a safe, natural product with long-known therapeutic benefits. Previous clinical trials investigating genistein's metabolic effects have primarily focused on postmenopausal women[30,34-36]. Only one previously published study has investigated the cardiometabolic effects of soy isoflavones in men receiving ADT[37], although patients had already been on ADT for a minimum of 12 months. *This proposed study will provide the first data in determining if genistein supplementation initiated at the start of ADT therapy is beneficial in mitigating the cardiometabolic adverse effects of ADT in men with PCa.* Furthermore, the majority of previous studies have primarily relied on crude measurements of cardiometabolic outcomes derived from

fasting plasma concentrations, such as fasting insulin and glucose and fasting lipids. *Here, we propose a panel of comprehensive, dynamic tests that will provide more physiological insight into the cardiometabolic effects of genistein in PCa.*

**5. OBJECTIVES**

**Specific Aim 1): Assess indexes of insulin dynamics (insulin sensitivity and secretion) determined from an oral glucose tolerance test before and 12 weeks after a daily genistein or placebo supplement.** The primary outcome will be the Matsuda index of whole-body insulin sensitivity. β-cell insulin secretion capacity will be assessed with the Insulinogenic Index. Additional indexes of insulin resistance and secretion derived from fasting and post-challenge measurements of plasma glucose, insulin, and C-peptide will be assessed. *We hypothesize that a) insulin sensitivity and secretion will decline following initiation of ADT; and b) supplementation with genistein will improve insulin sensitivity and secretion compared to placebo.*

**Specific Aim 2): Assess, in Aim 1 subjects, measures of vascular function before and 12 weeks after a daily genistein or placebo supplement.** Arterial stiffness will be assessed with augmentation index and pulse wave velocity measured by applanation tonometry. Vascular endothelial function will be assessed with flow-mediated dilation (FMD) measured by ultrasound. Additional CVD-related outcomes will be a fasting comprehensive serum lipid profile. *We hypothesize that a) arterial stiffness will increase and FMD will decrease following initiation of ADT, and b) supplementation with genistein will improve these key measures of vascular health compared to placebo.*

This proposed clinical trial of genistein supplementation is a novel approach that may help to alleviate major adverse metabolic comorbidities associated with ADT in men with prostate cancer and help to ensure a healthy, extended quality of life.

**Aim 1 rationale:** Testosterone influences insulin sensitivity, independently of its effects on body composition, in a variety of insulin target tissues (e.g., muscle, liver, adipose), with molecular mechanisms similar to those of genistein, including PPAR activation and AMPK signaling[38,39]. The majority of clinical trials investigating genistein effects on insulin resistance have used the homeostatic model assessment of insulin resistance (HOMA-IR) and/or fasting insulin as outcomes[30,34-36] _ENREF_33, which do not take into account the post-challenge tissue response to glucose[40] and may more reflect hepatic, as opposed to whole-body, insulin resistance[41]. Experimental data have also shown genistein to promote glucose-stimulated insulin secretion by pancreatic β-cells[42-44], although few, if any, clinical trials have verified such an effect. *Use of an OGTT in this pilot study will allow for the assessment of the dynamic metabolic changes that occur following a glucose challenge and thus, provide a better indication of the effects of genistein on whole-body insulin sensitivity, as well as insulin secretion.*

**Aim 2 rationale:** In addition to alterations in plasma lipids, low testosterone is associated with other predictors of cardiovascular risk in non-cancer populations, including flow-mediated dilation (FMD) and arterial stiffness, as shown by Co-Investigators Dr. Umpierrez[45] and Dr. Quyyumi[46], respectively, and others[47-51]. However, the handful of studies that have investigated the effects of ADT on these additional vascular outcomes have provided conflicting results[8,9,13,14]. As with androgens, genistein has been shown (primarily in postmenopausal women) to influence cardiovascular function independently of changes in plasma lipids, including increased FMD[34,36,52,53] and reduction in arterial stiffness[54,55]. Genistein's lipid-

independent effects on vascular function may be mediated by a reduction in vascular injury via inhibition of thrombosis and platelet activation and aggregation[56] and/or activation of nitric oxide signaling pathways[52,57]. *In this study of men undergoing ADT, we will assess the effects of genistein with outcomes that represent a variety of potential mechanisms associated with cardiovascular risk.*

## 6. STUDY DESIGN AND METHODS:

**Overall Study Design:** This will be a pilot double-blind, randomized, placebo-controlled trial of daily oral genistein in 24 men initiating ADT for PCa, as detailed in Fig. 2. Metabolic testing will be performed following an overnight fast in the Emory University Hospital Clinical Research Network (CRN) of the Atlanta Clinical and Translational Science Institute (ACTSI) before initiation of ADT and study drug, and will be repeated 12 weeks after baseline testing.

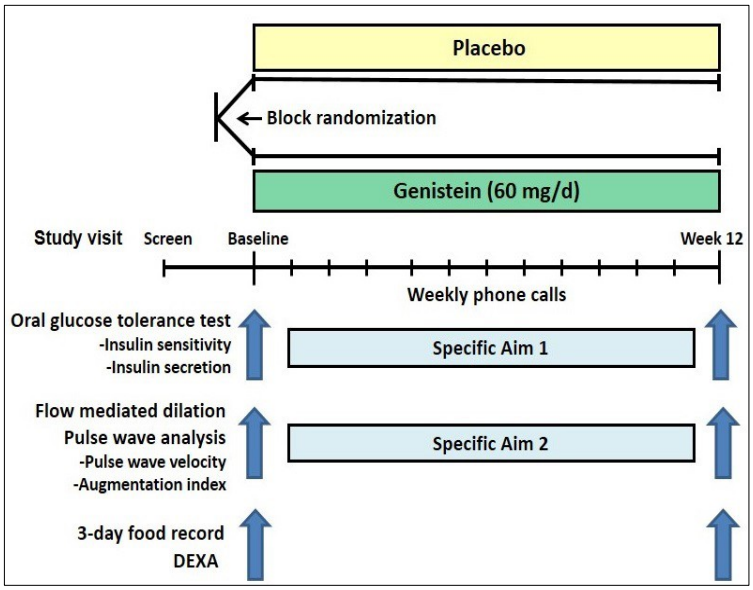


**Fig. 2. Study Schema.** Subjects will ingest 60 mg daily oral genistein or matching placebo for 12 weeks. Cardiometabolic testing will be performed following an overnight fast before initiation of ADT and study drug, and will be repeated 12 weeks after baseline testing. The primary endpoint will be whole-body insulin sensitivity derived from an oral glucose tolerance test (Specific Aim 1). Vascular function will be assessed with applanation tonometry and ultrasound (Specific Aim 2). Dietary intake and changes in body composition will also be assessed.

**Study initiation and baseline assessments:** All patients will receive standard counseling for diet and exercise by their oncology care team. Patients will be asked to withhold from dietary supplements containing genistein or soy. If subject are consuming dietary supplements with genistein or soy, they will undergo a 2 week washout period prior to study initiation. Patient who are taking a multivitamin or dietary supplements without soy or genistein may continue with these vitamins and supplements during the study period. Baseline study assessments (procedures described below) will occur at the Emory CRN on the same day (morning) they initiate ADT therapy and study drug. Participants will be required to be fasted for at least 12 hours. Participants will be offered a standard meal (not research related) following the study visit procedures.

**Randomization/Intervention:** Subjects will be block randomized to either 60 mg/day oral genistein (30 mg Bonistein™ taken twice daily in the form of two I-Cool® tablets daily, provided by DSM Nutritional Products, Ltd., Basel Switzerland) or matching placebo.

**Follow-up:** Subjects will receive weekly reminders via phone call to enhance compliance and monitor for potential adverse events. Compliance will be assessed with pill counts returned at the end of study and plasma genistein concentrations. The 3-month study visit will be scheduled to coincide with the subject's standard of care follow-up visit. Three-month assessments will be the same as baseline assessments.

**Procedures to be performed** (all for research purposes only. See Table 1 for specific data collection information)**:**

1. **Medical examination:** Medical history and medications will be reviewed by a member of the research team, and a physical exam will be completed by a licensed physician.
2. **Urine collection:** Urine will be collected for measurement of urinary oxidative stress markers (e.g., F2-isoprostanes). The clean catch method will be used to collect urine.
3. **Anthropometric measurements:** Height and weight will be measured without shoes. Height will be measured with a manual stadiometer. Weight will be measured with a digital scale. Body mass index (BMI) will be calculated from height and weight.
4. **Flow mediated dilation (FMD):** We will determine endothelium-dependent FMD of the brachial arteries using two-dimensional ultrasound images[46]. Briefly, the brachial artery of the non-dominant arm will be imaged using a high-resolution 10-MHz ultrasound transducer. Supra-systolic pressures will be used to produce 5 min of ischemia in the forearm. Upon reflow, imaging will be performed to measure FMD. Arterial diameter will be measured using validated software (Medical Imaging Applications, Coralville, Iowa). The end point will be % change in diameter in response to reactive hyperemia. FMD is calculated as (post-ischemia diameter-baseline diameter)/baseline diameter x 100.
   **Arterial stiffness:** Arterial stiffness will be assessed as pulse wave velocity (PWV) and radial pulse wave analysis measured noninvasively using the SphygmoCor® Pulse Wave Velocity system (PWV Medical, NSW, Australia)[46]. In brief, peripheral pressure waveforms will be recorded from the radial artery at the wrist, using applanation tonometry with a high-fidelity micromanometer. After 20 sequential waveforms have been acquired, a validated generalized transfer function will be used to generate the corresponding central aortic pressure waveform. Augmentation index (AIx) and pulse pressure amplification are derived and AIx normalized for heart rate of 75 bpm (AIx75). Carotid-femoral artery PWV will be determined using transcutaneous Doppler flow velocity recordings simultaneously over the common carotid artery and the femoral artery.
5. **Oral glucose tolerance testing (OGTT):** The OGTT will consist of a 75g glucose challenge with repeat blood sampling minutes by trained nurses in the CRN. This test will occur after the above vascular function tests. To perform the test, a flexible intravenous catheter will be placed in the antecubital space of the left arm. At time “zero", a 75 g dose of oral glucose will be provided. Subjects will be required to consume the glucose within 5 minutes of administration. Blood will be drawn at baseline, prior to the initiation of glucose consumption. Subsequent blood samples will be drawn at 10, 20, 30, 60, 90, and 120 min following initiation of glucose ingestion.
   a. **Indexes of insulin sensitivity/insulin resistance:** The primary outcome will be the whole-body insulin sensitivity index (ISI) assessed by the method of Matsuda and DeFronzo[58]. This index has been validated against the gold-standard euglycemic clamp technique, and represents a composite of both hepatic and peripheral tissue sensitivity to insulin. It is calculated as: 10,000 ÷ square root of [(fasting glucose x fasting insulin) x (mean OGTT glucose concentration x mean OGTT insulin concentration)]. HOMA-IR will be determined as a secondary measure of insulin resistance and calculated as: [fasting insulin (μU/ml) × fasting glucose (mmol/l)] ÷ 22.5 [40].

b. **Indexes of insulin secretion:** β-cell insulin secretion capacity will be assessed with the Insulinogenic Index and calculated as: $\Delta Insulin_{(t30-t0)}$/ $\Delta Glucose_{(t30-t0)}$ [59]. Additional indexes of insulin secretion will include fasting insulin and HOMA-β% calculated as: [20 x fasting insulin (μU/ml)] ÷ [fasting glucose (mmol/l) – 3.5] [40], and fasting and post-glucose challenge C-peptide/glucose ratios[60].

6. **Blood sampling:** Up to approximately 43 ml of fasted blood will be collected from a peripheral vein. Blood will be drawn for determination of circulating glucose, insulin, c-peptide, lipids, metabolomics, redox biomarkers, genistein, and collection of mononuclear cells. Epigenetic markers and/or telomere length may also be measured in the buffy coat or plasma. At 10, 20, 30, 60, and 90 minutes following oral glucose ingestion, approximately 7 ml blood will be drawn for determination of circulating glucose, insulin, and C-peptide. Two hours after glucose ingestion, approximately 16 ml blood will be drawn for determination of glucose, insulin, C-peptide, redox biomarkers, and metabolomics. A total of 85 ml blood will be drawn in one study visit.
7. **Handgrip strength:** A small, handheld dynamometer will be used to measure grip strength. Participants will be asked to squeeze the instrument as hard as they can for 3 seconds, which will measure the pounds of force applied.
8. **Body composition analysis:** DEXA will be used to estimate total and regional body composition, which will include body fat, lean body mass, and bone mineral content/density. Participants will be asked to lie still on a scanning table with their arms at their sides for approximately 15-20 minutes. Body composition may also be assessed using bioelectrical impendence analysis (BIA); for this procedure participants will lie still on a table for <5 minutes with electrodes placed on hands and feet. Waist and hip circumference will also be measured with a measuring tape to assess fat distribution.
9. **Three-day food records:** Participants will be asked to complete a food diary for three days (2 weekdays and 1 weekend day) where they will record everything they eat and drink, including supplements. They will be given food records prior to their study visit and asked to return completed food records on the day of their visit. Alternatively, participants may choose to email or mail study investigators the completed food record.
10. **Questionnaires:** Participants will be asked to complete a questionnaire assessing their physical activity over the past 7 days. Participants will be asked to complete two questionnaires assessing quality of life issues, the EPIC – Expanded Prostate Cancer Index Composite, and the RAND 36-Item Short Form Survey Instrument (SF-36).

Potential Risks/Discomforts:

The risk of participating in this study is minimal.

a. **Genistein:** There are few reported side effects of this dietary supplement. Some studies have reported gastrointestinal (GI) effects. A previous study in men with PCa supplementing 450 mg (7.5 x the dose proposed by the current study) reported loose stools as a side effect (de Vere White RW 2010 Nutr Cancer). Another study in postmenopausal women supplementing a similar dose as ours (54 mg/day) reported GI symptoms such as epigastric pain, abdominal pain, dyspepsia, and constipation (Marini H 2007 Ann Intern Med), although other similar studies in postmenopausal women

reported no difference in side effects from those taking placebo (Atteritano 2014 J Clin Endocrinol Metab, Squadrito 2013 J Clin Endocrinol Metab, Irace 2013 Eur J Clin Invest).

b. **Venipuncture:** There is some minor discomfort and risk of mild bruising during venipuncture. Standard sterile techniques will be used during phlebotomy; thus, infection is unlikely. Disposable pre-sterilized needles and syringes will be used for all blood drawing in this study; needles and syringes will not be reused.
c. **DEXA:** DEXA involves exposure to small amounts of radiation. The radiation dose is equal to or less than the amount of background radiation received in a round-trip flight from New York to Los Angeles or the natural environmental radiation the average person receives in the United States annually. The risk from radiation exposure of this magnitude is considered to be negligible when compared to everyday risks.
d. **BIA:** The BIA machine passes a weak electrical current through electrodes attached to hands and feet. Participants will not feel anything. There are no known risks associated with the procedure, although participants who have pacemakers or other implantable electronic devices will be excluded from this procedure (but may participate in other procedures)
e. **Flow-mediated dilation**: This test requires use of a blood pressure cuff which may cause some mild discomfort during cuff inflation. There will be a period of time after the test when a trained technician will monitor for any unusual reactions to the testing. There are no risks or discomforts caused by applanation tonometry.
f. **Other:** The fixed timing of procedures may be inconvenient to some subjects. Additional risks will be associated with confidentiality issues surrounding the collection/recording of data, but steps will be taken to minimize these risks, as described in the DSMP.

**Potential Benefits:**

a. **Direct:** Participants will be compensated a total stipend of $100 for their time and inconvenience. Subjects will also receive a comprehensive history and physical examination by a physician and gain detailed information about their habitual dietary nutrient intake and body composition by a registered dietitian, as well as results of their OGTT test and blood lipid panel. Some of these tests are not a part of routine care for patients, thus subjects may obtain further information regarding their nutritional health. Participants will be encouraged to share the results with their healthcare providers. There may be no specific health benefit to patients' participation in the study.
b. I**ndirect:** The long-term goal of this study is to identify intervention strategies to minimize adverse cardiometabolic health consequences associated with ADT therapy. If our hypothesis is correct, genistein would be an inexpensive, easily obtainable phytochemical that could be supplemented in this population.
c. The anticipated benefits of the proposed research study outweigh the potential risks to participating subjects. The risks incurred are minimal relative to the potential benefit of finding an effective therapy to reduce the adverse effects of ADT therapy.

**Data Collection:** Table 1 summarizes the data to be collected. Specimens to be collected at baseline and follow-up will include blood and urine. Participants will be asked questions

regarding their contact information, social history (such as education, marital status, employment status), and medical history (such as smoking, sun exposure, alcohol consumption, supplement use) from participants to be recorded in case report forms. Relevant information from the patients' medical record will be collected by investigators or study coordinators and include data needed to complete or verify the self-reported information; demographics, routine clinical laboratory markers (such as albumin, calcium, creatinine, 25(OH)D, vitamins, and minerals), cancer diagnosis and medical history glucose tolerance, medication and supplement intake.

**Table 1. Data to be collected and source**

| Data Item | Source | Visit |
|---|---|---|
| -Vitals (e.g, blood pressure, pulse, temperature) | Medical Exam | All |
| -Demographic Information | Self-report; Electronic Medical Record | Baseline |
| -Medical history (e.g., cancer dx, , microbiology, standard clinical laboratories) | Self-report; Electronic Medical Record | All |
| -Social history (e.g., education, marital status, employment status) | Self-report; Electronic Medical Record | Baseline |
| -Dietary supplement and medication intake | Self-report; Electronic Medical Record | All |
| -Glucose tolerance | Oral glucose tolerance test | All |
| -Plasma glucose, plasma insulin, plasma c-peptide | Oral glucose tolerance test | All |
| -Plasma metabolomics | Blood draw | All |
| -Plasma aminothiols (GSH, GSSG, Cys, CySS) | Blood draw | All |
| -Mononuclear cells | Blood draw | All |
| -Epigenetic markers and telomere length | Blood draw | All |
| -Plasma lipids | Blood draw | All |
| -Plasma genistein levels | Blood draw | All |
| -Urinary markers of oxidative stress | Spot urine collection | All |
| -Handgrip strength | Hand-held dynamometer | All |
| -Whole body and regional body composition | Dual energy X-ray absorptiometry | All |
| -Body composition and total body water | Bioelectrical impedance analysis (BIA) | All |
| -Waist and hip circumference | Measuring tape | All |
| -FMD, Flow Mediated Dilation | Ultrasound | All |
| -Arterial Stiffness, PMV and Augmentation Index | Applanation tonometry | All |
| -Height, weight, BMI | Manual stadiometer and digital scale | All |
| -Dietary intake | Three-day food record | All |

| | | |
|---|---|---|
| -7-day physical activity (PA) | International PA Questionnaire | All |
| -Qualilty of life (with Prostate Cancer) | EPIC | All |
| -Quality of life (overall health) | RAND (SF-36) | All |

**Randomization and Blinding:** Subjects will be block randomized and stratified to either 60 mg/day oral genistein (30 mg Bonistein™ taken twice daily in the form of two I-Cool® tablets daily, provided by DSM Nutritional Products, Ltd., Basel Switzerland) or matching placebo. The Investigational Drug Pharmacy will provide the randomization scheme.

**7. ARCHIVED SPECIMENS:** Specimens that remain after completion of the study will be stored for future studies beyond the scope of the current study only if subjects provide written informed consent to grant long term storage of samples. Any stored samples will be de-identified with a specific code whose identity can only be accessed by authorized study personnel appointed by the PI.

**8. COMMUNITY PARTICIPATION:** Aside from participation in the study, this proposed study will not involve the community in the design or conduct of the study as the hypothesis is still in its infancy and would require larger confirmatory studies. Results of the research will be shared via publication in the scientific literature. Should our hypothesis prove correct, we will see additional funding for larger, confirmatory studies. If larger studies agree with the hypothesis, future studies will consider community-based research to impart the knowledge within the PCa community.

**9. PARTICIPANT SELECTION:**

**Sample size:** A total of 24 male adults with prostate cancer who will undergo ADT therapy will be recruited. As this is a pilot study, we have not accounted for expected refusals or withdrawals.

**Inclusion criteria:** 1) Medical indication for ADT via luteinizing hormone-releasing hormone (LHRH) analog ± oral anti-androgen, 2) diagnosis of prostate cancer, 4) ECOG performance status ≤ 2, 5) life expectancy > 6 months, and 6) ability to provide informed consent

**Exclusion criteria:** 1) Transmural myocardial infarction, unstable angina, or congestive heart failure requiring hospitalization within the last 6 months, 2) acute coronary event within the past month, 3) chronic liver disease, 4) current use of cytotoxic or immunosuppressive drugs, 5) chronic glucocorticoid or acute glucocorticoid or other synthetic steroid intake within the last month, 6) chronic diarrhea or malabsorptive diseases (e.g., Crohn's disease), 7) stage 5 chronic kidney disease or need for hemodialysis, 8) supplemental oxygen dependency, 9) brain metastasis, 10) severe cognitive dysfunction impairing ability to provide informed consent or consume study drug, 11) dysphagia or requirement for artificial feeding, 12) surgery or hospitalization within the last month, 13) chemotherapy or radiation therapy within the last 60

days, 14) type 1 or type 2 diabetes, 15) HIV/AIDS, 16) hx of organ transplant, and 17) ECOG performance status > 2

**Subject recruitment plan:** Potential subjects will be recruited among patients seen by Dr. Omer Kucuk and his Genitourinary Oncology group at the Emory, St. Joseph's, and Grady campuses.

**Screening for eligibility:** Patients will be pre-screened for eligibility using the Emory University Hospital (EUH) electronic medical record, and eligible subjects will be approached during their clinic visit. Potential study subjects will be identified among patients seen by Dr. Omer Kucuk and his Genitourinary Oncology group at the Emory, St. Joseph's, and Grady campuses. Dr. Kucuk and his multidisciplinary oncology group initiate ADT therapy in approximately 8 patients per month. A study investigator or a study coordinator will conduct screening for eligibility among patients for whom ADT therapy is indicated.

**Study withdrawal:** Participants may withdraw from the study at any time. Automatic withdrawal occurs when a participant moves away, refuses to take study drug, or refuses assessments for Specific Aim 1.

## 10. INFORMED CONSENT PROCESS:

A written and signed informed consent will be obtained in person in a private area. After identification of a person as eligible for the study, approved study personnel will explain the study in detail in the consent form as well as verbally. Potential participants will be informed of the purpose of the study, the study protocol, and the routine and potential risks associated with the study procedures. All risks, costs, and benefits will be discussed. Participants will be assured of their right to withdraw at any time without prejudice to their care. They will be assured of confidentiality in maintaining records and reporting of results. Any additional information regarding questions or concerns from potential or confirmed study participants will be provided.

We will request a partial HIPAA waiver to be able to access the medical record for screening.

The consent process will take place during a patient's clinic visit. Potential study participants will have the option of taking the blank, unsigned consent forms home to consider prior to signing the consent forms, in which case patients who decide to participate will complete a verbal consent by telephone and will complete the full, written consent process during their study visit (prior to any procedures). Participants will have ample time to call the study team with questions/concerns and make an informed decision about their participation in the study.

An approved study designee will provide verbal and written consent. The study is discussed thoroughly with each potential participant, and he/she will be encouraged to ask questions regarding the study and his/her participation. If the potential participant decides to enroll, he/she will sign the written informed consent, as will the study team member conducting informed consent. A copy of the signed consent form will be provided to enrolled study participants.

Subjects must be able to read, write and understand the consent documents. Study personnel will subjectively assess the capacity to give informed consent through the use of questions to ensure understanding and comprehension of the study. Only adults ages 18 yrs and older will participate in this study.

If indicated (e.g., if a patient decides to take forms home to think about it), a verbal consent by telephone will be obtained to account for any study activities that occur prior to the first study visit. These include a 3-day food record, a wash-out period (if applicable), and fasting before the first visit. These procedures will be thoroughly explained by phone, and the study personnel conducting the consent by telephone will sign the verbal consent form.

**11. INCIDENTAL FINDINGS:** Subjects will be receiving a DEXA and FMD scan for research purposes only. The research does not require health professionals to read the scans. The researchers are not qualified to interpret the images for healthcare purposes, and the scans will not be used for clinical or diagnostic purposes. If the researchers have a question about something on the scan, the subject will be told and the subject will have an option to have the scan sent to a qualified health professional for review and any further medical treatment. The DEXA procedure generates a report that includes bone mineral density (as well as body fat and lean body mass); however, it does not include medical interpretation. This report will be given to participants, and they will be encouraged to share this information with their healthcare providers in making a diagnosis about the state of his/her bones, body fat, and muscle mass.

Subjects will also undergo oral glucose tolerance testing, as well as blood lipid and blood pressure testing. This information will be made available to the subjects and their physicians if desired. In addition, with the subject's permission, we will notify the subject's primary physician of findings that may have medical importance, such as abnormalities in blood pressure and blood tests. We will not provide medical care for the potential abnormalities but we will refer the subjects to their own physicians. In absence of a primary physician, we will assist the subjects to find appropriate referrals.

**12. COMPENSATION:** Participants will be paid a total of $100 for completion of the 2 study visits ($50 per visit), in the form of cash. Participants will be paid upon the completion of each visit. Participants will only be paid for visits in which they participated. There will be no compensation or reimbursement for travel, parking or other expenses.

**13. STATISTICAL ANALYSIS:**

Descriptive statistics will be performed on all variables. The normality of distributions will be examined on continuous variables, and log-transformations performed as needed. All statistical analyses will be 2-sided with an alpha of 0.05. We will use mixed-model repeated measures ANOVA to determine if there are any effects of time, treatment, or group-by-treatment interactions on insulin sensitivity, insulin secretion, vascular function measures, or body composition. Biostatistician, Kirk Easley, MS, will be consulted, as needed and using de-identified data, for statistical analyses.

The primary endpoint for this study will be whole-body ISI. Sample size calculations were based on the only available study investigating changes in whole-body ISI in PCa patients receiving ADT[17]. Assuming a decline in whole-body ISI on average of 2.12 units over 3 months in the control group, on average no change in whole-body ISI in the genistein group, and an estimated pooled standard deviation in each group of approximately 1.77 units, a sample size of 12 PCa patients per treatment group (**24 subjects total**) will ensure 80% statistical power to detect a treatment difference of 2.12 units if the true difference between treatments is 2.12 units (two-sided two-sample equal-variance t-test and 5% significance level). Assuming a pooled standard deviation of 2.26% for FMD[8] and 9.11 m/s for PWV[13] in men with PCa undergoing ADT, we will have 80% power to detect a statistically significant difference of 2.7% and 10.9 m/s between treatment groups for FMD and PWV, respectively.

It is possible that we have overestimated the true difference in outcomes between treatment groups in sample size calculations. However, this pilot clinical trial will provide important estimates of variability (within-subject and between-subject standard deviation) for the primary outcome. The estimates of variability, essential components for sample size calculations, will be valuable to power future longitudinal clinical studies. Due to the small sample sizes, results from this study will focus on the magnitude of the differences for each outcome, consistency of findings and clinical significance. Additionally, a sample size of 12 subjects per group has been proposed as being adequate for pilot clinical trials and providing useful data on precision about the mean difference and standard deviation for an outcome[61].

## 14. DATA AND SAFETY MONITORING PLAN:

The study will be conducted under the Winship Data and Safety Monitoring Plan (DSMP). For all study procedures, trained clinical research study personnel will be with a participant at all times to monitor patient safety. Adverse events are expected to be uncommon and to not pose more than minimal risk to the study participants. With the exception of blood draws, participants may chose not to perform any of the procedures described in the consent form or to cancel operations during a procedure without affecting their standing in the study or their medical care team.

**Data and Safety Monitoring Committee:** The Data and Safety Monitoring Committee (DSMC) of the Winship Cancer Institute will provide oversight for the conduct of this study. The DSMC functions independently within Winship Cancer Institute to conduct internal monitoring functions to ensure that research being conducted by Winship Cancer Institute Investigators produces high-quality scientific data in a manner consistent with good clinical practice (GCP) and appropriate regulations that govern clinical research. Depending on the risk level of the protocol, the DSMC review may occur every 6 months or annually. For studies deemed High Risk, initial study monitoring will occur within 6 months from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. For studies deemed Moderate Risk, initial study monitoring will occur within 1 year from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. Subsequent monitoring will occur in routine intervals per the Winship Data and Safety Monitoring Plan (DSMP).

The DSMC will review pertinent aspects of the study to assess subject safety, compliance with the protocol, data collection, and risk-benefit ratio. Specifically, the Winship Cancer Institute Internal Monitors assigned to the DSMC may verify informed consent, eligibility, data entry,

accuracy and availability of source documents, AEs/SAEs, and essential regulatory documents. Following the monitoring review, monitors will provide a preliminary report of monitoring findings to the PI and other pertinent individuals involved in the conduct of the study. The PI is required to address and respond to all the deficiencies noted in the preliminary report. Prior to the completion of the final summary report, monitors will discuss the preliminary report responses with the PI and other team members (when appropriate). A final monitoring summary report will then be prepared by the monitor. Final DSMC review will include the final monitoring summary report with corresponding PI response, submitted CAPA (when applicable), PI Summary statement, and available aggregate toxicity and safety data.

The DSMC will render a recommendation and rating based on the overall trial conduct. The PI is responsible for ensuring that instances of egregious data insufficiencies are reported to the IRB. Continuing Review submissions will include the DSMC recommendation letter. Should any revisions be made to the protocol-specific monitoring plan after initial DSMC approval, the PI will be responsible for notifying the DSMC of such changes. The Committee reserves the right to conduct additional audits if necessary.

**Frequency of data analysis:** Blood samples will be analyzed on a quarterly basis for clinically relevant items such as blood glucose and lipids. Other blood or urine analytes will be measured in a single batch at the end of the study. The body composition analysis provide immediate results. Vascular function studies and food records will be analyzed on a quarterly basis. All items will be analyzed in a blinded fashion. Due to the blinded nature of the study, statistical analyses will not be performed until all participants have completed the study.

**Adverse events reporting:**

The Emory University IRB Reportable Events Guidelines for adverse events (AEs) and serious adverse events (SAE's) will be followed and timely reporting to the IRB will occur yearly and as needed. Any SAEs will be reported to the IND sponsor promptly and to the IRB within 10 days of the event if it is (a) an unanticipated problem involving risk to participants or others, (b) a death that is possibly, probably, or definitely related to the research, (c) an anticipated event occurring with a greater frequency, duration, or severity than what is described in the protocol related documents, or (d) any other information that suggests the research places participants or others at greater risk of harm than was previously known. A reportable events log will be maintained by the PI and reported annually to the IRB (or more frequently if required.)

The clinical course of study participants will be monitored for adverse events on a weekly basis through the use of participant reminder calls and assessment of medical records. All AE's, including those related to study procedures and/or those related to the study drug, will be recorded throughout the study period and significant clinical events are recorded as narrative data in the case report forms. Weekly phone calls will occur for the 12 weeks participants are in the study, weekly electronic medical record review will occur throughout the study and until 30 days after the last dose of study drug is taken. For the purposes of study reporting, all deaths, unexpected serious adverse events (SAEs) and any AEs potentially related to study participation or study drug (genistein) and occurring following consent and until 30 days after the last dose of study drug, are reported using an AE form in the subject's binder and sent to the Emory IRB and the DSMB (per the final DSMB charter). Ongoing SAEs and AEs will be

followed until resolved. New onset events beginning more than 30 days after the last dose of study drug will not be collected unless the co-investigator, Dr. Omer Kucuk, MD deems that the event is study-related (e.g. late onset drug toxicity). Adverse event data are compiled and reported to the DSMB.

All adverse events will be assessed by the investigator as to their severity and attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol). Any AE that is reported to either the PI or the study team by a study subject or by medical staff caring for the subject and which meets the criteria will be documented as such.
Adverse events will also be further labeled as “expected” AEs (which are predefined and listed as potential risks in the informed consent), “unanticipated” AEs, and serious adverse events. Serious adverse events (SAE) are predefined as: any experience that suggests a significant hazard, such as events which: a) are fatal, b) are life threatening, c) result in permanent disability, d) require inpatient hospitalization, or e) involve cancer, a congenital anomaly, or drug overdose.

Written IND safety reports will be submitted to the FDA by the IND sponsor, for serious, unexpected suspected adverse reactions within 15 calendar days of learning of its occurrence. If the event is fatal or is deemed to be life threatening, the report will be made within 7 calendar days. The IND sponsor will also make an assessment of whether the event constitutes an unanticipated problem posing risks to subjects or others (UP). This assessment will be provided to the Emory University IRB, which, in turn will make a final determination. If the Emory IRB determines an event is a UP it will notify the appropriate regulatory agencies and institutional officials.

**Study team oversight:** The study PI, Dr. Alvarez, will review all data collection forms on a quarterly basis for completeness and accuracy of the data as well as protocol compliance. The Emory-provided “Investigator Self-Monitoring Tool” will be completed approximately every 6 months and signed by a member of the study team and the PI. The PI and research coordinator will meet on a weekly basis (and as needed) to discuss study updates, issues, and progress.

**Training on study procedures:** An in-service will be conducted with all active members of the research team that are directly involved in day-to-day study procedures prior to the study start date and when significant protocol changes occur. A copy of the most recent protocol will be maintained in the study regulatory binder with signatures of all active study team members. Documentation of training will be maintained with dates and signatures.

**Additional Protections Against Risk:**

**All study procedures:** A licensed physician will complete a medical history and physical examination before study procedures. Experienced personnel will perform all procedures. Since the proposed tests are not inherently hazardous, hazard is likely to occur only as the result of impaired participant confidence or sudden unwillingness to complete a test. To avoid this possibility, study personnel will thoroughly explain all tests to potential participants prior to them signing the consent form. Potential participants will have the opportunity to see all test equipment and facilities before giving consent or undergoing testing. If any adverse event was to arise during the course of testing, a study physician will be on call for support. A qualified physician will have primary responsibility for outpatient care. If a participant suffers from claustrophobia or other distress during a procedure, the procedure will be discontinued. The

participant will then be managed in the Emory ACTSI outpatient CRN, which, if applicable or warranted, have immediate access to EKG monitoring, cardiac resuscitation equipment and is located in close proximity to the Emory University Hospital, respectively. Every effort will be taken to prevent injury or distress that may result from this study.

**Subject identification and screening:** Our strict inclusion and exclusion criteria for entry will minimize potential risks.

**Blood sampling:** Experienced nurses will perform blood draws and heparin lock insertion procedures using aseptic techniques. Disposable pre-sterilized needles and syringes will be used for all blood drawing in this study; needles and syringes will not be reused. Participants will be asked to stay hydrated with water the day before the study visit. Discomforts associated with venipuncture are rapidly reversible.

**Flow-mediated dilation:** There will be a period of time after the test when a trained technician will monitor for any unusual reactions to the testing.

**Genistein supplements:** Subjects will receive weekly phone calls, text, or encrypted email to monitor for potential adverse events. We will screen specifically for GI symptoms, as well as any signs of allergic reaction (rash, shortness of breath). If a participant reports any symptoms potentially related to the study drug, the PI will consult with study physicians (Drs. Ziegler and/or Kucuk) who will follow-up with the participant by phone to determine the clinical severity of the symptoms. Based on the severity of symptoms, the study physicians will decide whether to continue the study drug as prescribed, lower the dose to 30 mg/day, or discontinue completely. Regardless of decision on study drug, subjects will continue to be followed and follow-up procedures performed as per study protocol.

**Confidentiality:** Only Emory institutional review board (IRB)-approved study personnel will have access to individually identifiable information about human subjects. Confidentiality will be assured by the use of subject codes rather than personal identifiers. All subject records will be kept in locked file cabinets or password-protected electronic files and will be accessible only to the PI and the investigative team. After the study is completed, all data will be kept according to NIH regulations in a locked file.

**Stopping rules:** The Winship DSMB will make a recommendation to the investigator and sponsor whether or not the study should be stopped based on adverse events. Individual participants will be able to withdraw at any point in the study and for any reason.

## 15. CONFIDENTIALITY

All participant interactions will be conducted in private settings (either behind closed doors or in individual curtained cubicles.) Only institutional review board (IRB)-approved study personnel will have access to individually identifiable information about human subjects. This may include the study PI (Jessica Alvarez), co-investigators, research coordinators and other approved study personnel. All information and materials will be obtained for research purposes only and the data will be kept in strict confidence for use in this proposed research only. Confidentiality will be assured by the use of subject codes rather than personal identifiers. All subject records will be kept in locked file cabinets in the PI's research office and will be accessible only to the PI and the investigative team. The master list connecting the subject codes to identifying information will be secured in a web-based case report form (RedCap) that is supported for faculty by the ACTSI. All data maintained in the computerized database will be accessible only

with a login and protected password. After the study is completed, all data will be kept in a locked file.

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