GREEN TEA SUPPLEMENTATION LOWERS INSULIN-LIKE GROWTH FACTOR I

IN MEN AT HIGH RISK FOR PROSTATE CANCER

By

Christopher Santo Errante

A THESIS

Presented to the Graduate Programs in Human Nutrition

and the Oregon Health & Science University

School of Medicine

in partial fulfillment of

the requirements for the degree of Master of Science

in

Clinical Nutrition

June 2013

School of Medicine

Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the Master's Thesis of

Christopher Santo Errante

has been approved

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Jackilen Shannon

_____

Melanie Gillingham

_____

Charles Roberts

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LIST OF ABBREVIATIONS

BMI-Body mass index

CaP-Prostate Cancer

DHA-Docosahexaenoic acid

EGCG-Epigallocatechin-3 gallate

EPA-Eicosapentaenoic acid

FO-Fish-oil, or fish-oil supplemented treatment arm

GT-Green tea polyphenol

GTFO-Green tea + fish oil supplemented treatment arm

IGF-I-Insulin-like Growth Factor I

IGF-IR-Insulin-like Growth Factor I Receptor

IGF-IIR-Insulin-like Growth Factor II Receptor

IGFBP-1-6-Insulin-like Growth Factor Binding Proteins 1-6

M6P-Mannose-6-phosphate

MD – Mean difference

NSILA-Nonsuppressible insulin-like activity

OCTRI-Oregon Clinical and Translation Research Institute

OHSU-Oregon Health and Science University

OR-Odds ratio

PP-Double placebo-supplemented treatment arm or placebo

PSA-Prostate-specific antigen

PUFA-Polyunsaturated fatty acids

PVAMC- Portland VA Medical Center-Veterans Association

RIA-Radioimmunoassay

RR-Relative Risk

ACKNOWLEDGEMENTS

ABSTRACT:

GREEN TEA SUPPLEMENTATIO LOWERS INSULIN-LIKE GROWTH FACTOR-I IN MEN AT HIGH RISK FOR PROSTATE CANCER

There is mounting evidence that increased circulating levels of insulin-like growth factor I (IGF-I) are associated with an increased risk of prostate cancer (CaP). Green tea (GT) supplementation significantly reduces circulating IGF-I levels in CaP patients and IGF-I levels and tumor development in the murine TRAMP model of CaP. To determine if GT affects IGF-I levels in men prior to development of CaP, we conducted an analysis of blood samples from a randomized, double-blind, placebo-controlled trial entitled "Catechins and ω-3 fatty acids: impact on fatty acid synthase activity in the prostate. A randomized controlled trial." Men scheduled for repeat prostate biopsy were recruited from three hospitals in Portland, OR, and randomized into one of four treatment arms: placebo(PP), fish oil (FO), GT, or GT/FO. Blood samples were drawn before and after a treatment period of 12-20 weeks. Pre and post-treatment plasma (n=71) and serum (n=3) IGF-I concentrations were determined using commercially available radioimmunoassay kits. There were no significant differences in IGF-I concentrations between the GT (n=13) and placebo groups (n=24) at baseline (p=0.46). Following treatment, IGF-I concentrations were significantly lowered in the GT supplemented group as compared to placebo (p=0.01, mean difference =-24.91, 95%CI -46.30, -3.52). These results were also confirmed with a non-parametric Wilcoxin Rank Sum test (p=0.017). When all four arms were analyzed for interaction using an orthogonal linear contrast, none was found between GT and FO(p=0.13, 2- tailed). In addition, there was no significant difference found between GT and GTFO groups at either pre or post-intervention. These findings suggest that GT supplementation lowered IGF-I and that this effect is independent of any

effect of FO supplementation. Following this, we considered FO an additional placebo and found the overall main effect of GT remained significant when men supplemented with FO were added to the analyses (p=0.03, Wilcoxon rank-sum test). These findings suggest that GT polyphenols lower circulating IGF-I in pre-cancerous men which may, in turn, reduce CaP risk.

CHAPTER I: INTRODUCTION AND SPECIFIC AIMS

In 2011, there were over 240,000 new cases of prostate cancer (CaP) in the United States, and men currently have a 1 in 6 chance of developing CaP in their lifetime. Treatments and screening procedures have substantially improved but CaP morbidity and mortality remains a widespread problem throughout the world. Research focusing on the risk factors for CaP and potential prevention measures could have important implications for men's health. Increased circulating concentrations of the hormone insulin-like growth factor - I (IGF-I) in humans is consistently reported to be associated with an increased risk of CaP. Dietary factors that can alter the concentration of this protein, such as the green tea polyphenol (GT) antioxidants, may be one potential mechanism to reduce CaP risk. The goal of this proposal was to determine if GT supplementation in humans could reduce circulating IGF-I concentrations in men at high risk for CaP.

Evidence that GT reduces IGF-I can be found in animal, cell culture and, more recently, human studies. Oral consumption of GT reduced serum concentrations of IGF-I in transgenic adenocarcinoma mouse prostate (TRAMP) mice. In addition, GT supplementation decreased IGF-I in cultured human hepatocellular carcinoma cells as well as human colorectal cancer cells. There have been two small clinical trials that have shown GT supplementation to lower IGF-I in men already diagnosed with CaP, but only one of those studies reached statistical significance. To date, no studies have been conducted to determine if GT supplementation in men at high risk for CaP can lower blood concentrations of IGF-I. This project is the first to examine the effects of GT supplementation on circulating concentrations of IGF-I in men *specifically* at high risk for CaP. We worked within the context of a recently completed randomized, double

blind, placebo-controlled trial of GT and FO supplementation in men at high risk for CaP.

While our primary aims were to address the effects of GT on IGF-I, the original trial included a FO only and FO plus GT supplemented arm, allowing us also to investigate any effects FO may have on IGF-I, either alone or in combination with GT. A few studies have noted synergism between GT and FO, but not in relation to IGF-I. In addition, a minimum of research has related FO supplementation to IGF-I. While some research in cattle and rats shows FO significantly raises IGF-I compared to control diets, other animal studies have shown inconclusive results. In humans, two clinical trials have shown no significant effect of FO on IGF-I. This is the first chemoprevention study looking at FO supplementation as a modulator of circulating IGF-I in men at high risk for CaP.

Specific Aims:

Specific Aim 1: Determine if GT supplementation versus a placebo lowers circulating IGF-I concentrations in men at high risk for CaP. We used radioimmunoassays to measure IGF-I concentrations before and after GT supplementation in both the supplement and placebo groups.

- We hypothesized that GT supplementation would lower circulating IGF-I concentrations versus placebo in men at risk for CaP.

Specific Aim 2: Determine if age or BMI confound or modify the effect of GT on IGF-I in men at high risk for CaP. This was tested by using analysis of variance (ANOVA).

- We hypothesized that age and BMI would not confound the effect of GT on

IGF-I.

Secondary Aim : Determine if there is any association between circulating IGF-I and prostate specific antigen (PSA) values in men at high risk for CaP.

- We hypothesized that there will be a positive association between IGF-I and PSA in humans at risk for CaP.

Secondary Aim: Determine whether or not FO and GT had either a synergistic or antagonistic effect on IGF-I.

- We hypothesized FO and GT would have neither a synergistic nor an antagonistic effect on IGF-I.

Exploratory Aim 1: Determine if FO supplementation versus a placebo changed IGF-I concentrations in men at high risk for CaP. We used radioimmunoassays to measure IGF-I concentrations before and after FO supplementation in both the supplement and placebo groups.

- We hypothesized that FO supplementation would have no effect on IGF-I concentrations in men at high risk for CaP.

Exploratory Aim 2: If FO has no interaction with GT in the GTFO group, and has no main effect itself on IGF-I, we will consider it a placebo in the GTFO and FO arms. After this we will combine the GTFO and GT arms to make a new GT group, and combine the FO and PP groups to make a new PP group. We will then see if GT lowers IGF-I versus

placebo after combining treatment arms.

- We hypothesized that GT would also lower IGF-I versus placebo after combining treatment arms and considering FO an additional placebo.

In a country with a growing elderly population, the incidence of CaP is likely to remain high. Alternative methods to reduce the risk of CaP and lower the relatively high associated health care costs are greatly needed. GT supplementation is one potential method to lower IGF-I concentrations and thereby lower CaP risk. GT polyphenols are active in a variety of GT beverages. They are also available in concentrated supplement form at a relatively low cost to the consumer and with few, if any, adverse effects. In addition, FO is an increasingly popular supplement because of its purported health benefits and anti-inflammatory properties. Determining if FO has an effect on IGF-I in men at high risk for CaP can provide a new perspective on its usefulness in chemoprevention.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

Prostate Cancer in The United States

It is estimated that prostate cancer (CaP) will account for 29% of all male cancers in the United States (US) in 2012. An estimated new 241,740 new cases of CaP will be diagnosed this year. This puts CaP at the top of the list as the most commonly diagnosed cancer in men. Lung cancer comes at a rather distant second at an estimated 116,470 new cases a year. While deaths from CaP have been decreasing since 2000 they still account for nearly 10% of all male cancer deaths. This year, an estimated 28,170 men will die of CaP.[1]

The chance of a man developing CaP varies remarkably from birth through old age. Every male born in the US has a relatively small probability of developing CaP through age 39. It is roughly 0.01% or 1 in 8,499. This probability skyrockets after a man's 40th birthday. Men between the ages of 40-59 have a 1 in 38 chance of developing CaP, and after their 60th birthday the risk jumps to 1 in 15; after age 70, it rises to 1 in 8. Clearly, CaP is associated with aging. In 2003 more than 70% of CaP cases were in men of retirement age (over 65). The lifetime risk of developing CaP for men is a staggering 1 in 6 or roughly 16%. No other cancer for men or women has a higher prevalence.[1] CaP is a major health care problem in the US that will not be going away soon.

Prostate Cancer Through-out the World

CaP is also prevalent throughout the rest of the world. While the US has the highest rates of CaP in the world, Western countries in general have higher rates than other parts of the world. In addition to the US, Canada, Sweden, Australia and France

were categorized as "high-risk" countries in a recent report on global incidences and mortality of CaP in 15 countries.[2] In the same report, Asian countries, namely Singapore, China, Japan, India, and Hong Kong, were ranked as "low-risk." [2] Despite the differences between Western and Eastern rates of CaP, there were significant increases in CaP throughout the world between 1973 and 1992. Some of the increases since 1986 in Western, "high-risk" countries can be explained by increases in cancer screening using the PSA method.[2] "Low-risk" countries cannot use increased PSA testing to explain their increases in incidence.[2] Other environmental changes such as diet and environment are postulated to be related to the rise in CaP incidence. It has been speculated that increases in dietary fat and decreases in exercise, (especially in highly westernized countries like Japan) can explain marked increases in these historically "low-risk" countries.[2]

Race and ethnicity play a large role in determining CaP risk as well. African Americans have a markedly high incidence as compared to other racial groups. The incidence of CaP in African Americans is 275.3 per 100,000 men compared to 172.9 per 100,000 men for whites. The mortality rates for African Americans are also 2.3 times higher than whites. Hispanics and Asian/Pacific Islanders have notably lower incidences than whites at 127.6 and 107.2 per 100,000 respectively.[3]

Staging, Grading and Progression of Prostate Cancer

The progression of CaP is measured by a few different means[5-6] which can be divided into two categories: stage and grade. The grade of CaP is determined to see how different the malignant tissue is from normal tissues. It also gives physicians an idea of how rapidly the cancer may spread. The "G-scale" of cancer grading is used for a number

of cancers, including CaP, and includes grades 1-4 (G1-G4). This, incidentally, is not the same as the "Gleason" score. In general, G1 grade prostate tumors are slow to spread and are very similar in appearance to normal prostate cells. G3 and G4 grade prostate tumors spread the fastest and look much different than normal prostate cells.[5]

The Gleason score system is used specifically for grading CaP tumors.[5] The Gleason system is divided into 5 specific grading patterns.[5-6] Each pattern is given a score of one through five. The Gleason score itself is calculated by summing the scores of the two most common patterns. Thus Gleason scores range from grades 2-10.[5-6] A tumor of grade 2 is considered of low severity and not likely to spread fast. A tumor with a grade of 10 is considered the most aggressive kind of tumor, expected to spread fast.[6] Gleason grades are determined by examining prostate tissue biopsy samples under a microscope.[5]

CaP staging measures the extent of CaP growth and to what extent it has spread throughout the body. There are four stages of CaP. The lower the stage, the less the cancer has progressed. Stage I CaP is not detectable by digital rectal exam (a common method of cancer screening), has not spread outside of the prostate, and has a G1 grade or a Gleason score of four or less. Stage II is simply of higher grade than stage I and also has not yet spread outside of the prostate. Stage III CaP has spread past the prostate (e.g., to the seminal vesicles) but has not yet spread to the lymph nodes. Finally, stage IV CaP has spread far beyond the seminal vesicles to other parts of the body including but not limited to: the lymph nodes, bones, rectum or bladder.[5]

Prostate-Specific Antigen in Prostate Cancer Diagnosis

Prostate tissue antigens were first discovered in 1960. They were originally called a number of names by different researchers.[7] Seminal antigens were also being investigated for use by forensic scientists and were isolated in 1964.[7] While 1970 is the earliest confirmed report of a prostate-specific antigen (PSA) isolated from prostate tissue, it was not until 1979 that it was purified and introduced to the clinical setting.[7-9] PSA was later also identified in blood in 1980.[7]

After several studies in the 1980s, Killian and colleagues found that not only was PSA the best predictor of CaP progression, it was able to predict progression from the lowest grade 6 months prior.[10] Stamey and colleagues confirmed this but noted that PSA lacked sensitivity because it was also elevated in benign prostate hyperplasia (BPH).[9] In 1986, the Food and Drug Administration (FDA) approved PSA solely for the purpose of treating CaP patients.[8] It wasn't until 1994 that the FDA approved PSA to be used as a diagnostic screening tool. Since then, PSA testing has markedly increased throughout the world. Approximately 40 million PSA tests are estimated to be performed annually, with half of those performed in the US alone.[8]

As already mentioned, PSA has been controversial because of lack of sensitivity.[9] In order to increase sensitivity an initial general cut-off value of 4 ng/mL was proposed to indicate increased CaP risk.[11] When it was determined that even small increases in PSA below 4ng/mL increased CaP risk, age specific cut-off values were proposed. These included an upper limit of >2.5 for men less than 50 years of age.[11]

Just like IGF-I, PSA exists in free and bound form in the blood. Generally, approximately 25% of PSA is found in the free form.[12] Researchers have recently

proposed using the ratio of free to total PSA to improve the specificity of the PSA marker and prevent unneeded biopsies and over-diagnosis.[12]

As mentioned below, PSA can also cleave insulin-like growth factor binding proteins (IGFBPs), which may or may not increase the bioactivity of IGF-I.[13] As increases in IGF-I and PSA are both related to increased risk of CaP, their relationship requires further investigation.[13-19] Once PSA cleaves the IGFBP, the half-life of IGF-I is greatly reduced, and it has less time to bind with the IGF-R.[13] A realistic model for PSA increasing the bioactivity of IGF-I has not been created. This is in part because the bioactivity of IGF-I is rarely studied. Maeda and colleagues, however, were able to show that the cleaving of IGFBP-5 by PSA measurably increased IGF-IR and Akt phosphorylation in cell culture.[14]

It is important to note that researchers have also shown that elevated levels of PSA in CaP patients did not have a significant effect on IGFBP-3 cleavage.[20] They also mentioned that PSA is not the most potent serum protease and that other proteases have a greater effect on IGFBP cleavage.[20]

As IGF-I has been considered a possible marker for CaP[13-19] it has also been researched for its correlation with PSA.[17-22] Only one study was found showing a positive correlation between PSA and IGF-I in men without CaP.[22] It was a weak correlation ($r = 0.14$; $P = 0.006$) and was noted in 367 healthy men.[22] Several studies, however, have shown no significant correlation between PSA and IGF-I.[17-21]

Two studies have also looked at the ability of IGF-I to increase the diagnostic reliability of PSA as a marker for CaP.[12,19] In particular the IGF-I/free-PSA ratio was found to increase the detection of CaP in men with total PSA in the range of 4-

10ng/mL.[12]

Risk Factors for Prostate Cancer

Risk factors for CaP can be divided into two groups: modifiable and non-modifiable risk factors. Modifiable risk factors include environmental and dietary risk factors. Non-modifiable factors include anything from basic heredity to the expression of specific biomarkers. Genetic or hereditary risk factors for cancer in general have only been considered relevant for some 40 years. They are, however, currently considered the most established risk factors for CaP.[4] In fact CaP caused by heredity is diagnosed an average of 6 to seven years earlier than non-hereditary CaP.[4,23]

Environmental/dietary risk factors, while less definitive, are considered quite important because 85% of CaPs are considered to be sporadic and not related to heredity. In 1992, the Johns Hopkins School of Medicine found that 43% of CaPs that are diagnosed at the age of 55 or younger could be attributed to an "autosomal dominant inheritance of a rare yet highly-penetrant high-risk allele (yet to be identified)."[24-25] This may seem like a large percentage, but because so few incidences of CaP are diagnosed before the age of 55, these inherited forms account for only 9% of all CaPs by age 85.[25] In addition, while a number of specific genes (eg.HPC1, HPCX) have been associated with CaP incidence, they are still considered "candidate" genes.[23] Not one has been specifically found to be of clinical significance.[23]

It has been estimated that between 10-15% of men affected by CaP have one or more relatives with the disease.[25] As the number of relatives with CaP increases, a man's risk of developing CaP increases substantially.[23] Having a brother with CaP doubles

one's lifetime risk if the brother's cancer developed after age 60.[4,23] If the brother

developed CaP before age 60 this risk increases to three fold. A similar pattern (1.5 and

2.5 times respectively) was seen if a man's *father* developed CaP before or after age 60.

The greatest, five-fold increase in risk was found in men having three or more male

relatives with CaP.[23]

Dietary risk factors for CaP receive much attention, in part, because they are

considered modifiable risk factors. The previously stated dichotomy between Western

and Eastern incidences of CaP in some ways parallels the dichotomy between Western

and Eastern diets. Studies have shown that Japanese men who moved to the US

developed CaP at higher rates than men in their home country.[26] Western diets are usually

described as higher in total fat as well as higher in animal products (meat and dairy in

particular). Several studies have linked higher fat diets with an increased risk of CaP.[26]

There is, however, some skepticism with the methodology of studies linking CaP with

dietary fat. Often, food frequency questionnaires are used which may be inaccurate. Also

the study populations are often far too heterogeneous. A recent meta-analysis analyzing

29 of such studies found a great inconsistency in the quality of diet history instruments

being used. They did find a small significant association between CaP and total fat

consumption when data was pooled. They also noted many of the studies did not

adequately adjust for confounding variables. Inconsistent quality may have also been due

to the lack of nutrition experts on review committees who are more familiar with

metabolic pathways of fat storage. The authors maintained that the research looking at

CaP and fat intake still remains unclear.[27]

Other dietary risks are being studied in relation to CaP. Low circulating levels of selenium were found to correlate with increased risk of CaP in a recent meta-analysis which analyzed the findings of 20 selected epidemiological studies.[28] High-dairy diets are also speculated to increase CaP risk in some studies.[29-32,33] Three meta-analyses have found positive associations with dairy consumption and CaP.[29-31] One proposed mechanism suggests high-dairy diets may cause higher-blood calcium levels that, in turn, promote CaP. A study investigating calcium intake and CaP risk found that intakes above 2000mg a day increased risk for "total, advanced and metastatic CaP."[31] Another proposed route for calcium's effect on CaP risk is through IGF-I.[32] A dietary intervention study found that men who had high intakes of low-fat milk (and thus calcium) had a 10% increase in plasma IGF-I concentrations. Researchers postulate this increase in IGF-I could indirectly increase their risk for CaP.[33] However, these suggestions have been contradicted by a meta-analysis that found no such association between dairy consumption and CaP risk.[33]

There are also a few protective factors associated with CaP risk. The most notable one to date is the phytochemical compound lycopene. In a 2002 prospective study, it was found that consumption of lycopene (most commonly found in tomato products) is associated with a significant reduction in risk of CaP.[34] A meta-analysis of 21 studies also supports this inverse association.[35] Even more recently a systematic review in 2012 found 8 clinical trials looking at lycopene and CaP.[36] After one meta-analysis within the review, the authors found that participants receiving lycopene supplementation had significantly lower PSA versus placebo (MD = −1.58, 95%CI −2.61, −0.55). In a second meta-analysis of four randomized controlled trials the authors failed to find a significant

change in CaP incidence related to lycopene supplementation.[36]

A number of studies have also evaluated the association between consumption of soy phytoestrogens and CaP risk.[37-39] Two recent meta-analyses found increased intake of soy phytoestrogens to be correlated with decreased CaP risk.[37-38] The phytochemicals in GT (polyphenol antioxidants) have also been implicated in decreasing the risk of CaP. GT and CaP will be more thoroughly discussed below.[40]

A number of hormones, including IGF-I, are being investigated for their potential role in CaP development.[23] Krala and colleagues said it well that hormones "lie on the borderline of environmental and genetic factors."[23] In this, they mean that both genetic and environmental factors can alter hormone levels to varying degrees. As this study focusses specifically on IGF-I and because increased IGF-I has been shown to be associated with increased CaP risk, we will now explore the entire IGF-axis and its origins.

The Somatomedin Hypothesis

The discovery of IGF-I cannot be discussed without at least mentioning what has come to be called "the somatomedin hypothesis." In 1957, scientists noted that growth-hormone (GH) alone could not account for the sulfation of mucopolysaccharides in the cartilage of rats.[41] It was later discovered that this mediating sulfation-factor also stimulated DNA and protein synthesis.[42-43] Before the sulfation factor's role was fully understood, its name was changed to "somatomedin" due to its multiple roles as a mediator of somatotropin activity. Later on, somatomedin was further divided into somatomedins A and C.[44]

At the same time as the somatomedins were originally being studied, another group of researchers, including E.R. Froesch and A. Jakob, had published a series of papers regarding nonsuppressible insulin-like activity (NSILAs).[45-50] NSILAs were compounds isolated from serum which imitated the effects of insulin but were not affected by anti-insulin injections.[50] In 1978, one of the NSILAs was eventually named insulin-like growth factor I (IGF-I) and was found to have 48% structural homology with insulin.[51] A similar compound with different observed function was called insulin-like growth factor II (IGF-II).[51] Following the complete amino-acid sequencing of somatomedin C, it was found to be the same exact compound as IGF-I. The latter name is now customarily used. The IGF family is, however, still often referred to collectively as the "somatomedins."[50,52]

The "somatomedin hypothesis" is still referred to as an explanation of the IGF/GH axis. As recently as 2007, changes were proposed to the hypothesis indicating antagonistic actions of GH on IGF-I and vice versa. This was deemed the "augmentative/counteractive hypothesis." While GH stimulates gluconeogenesis and lipolysis, IGF-I tends to counteract this effect due to its insulin-like role. At the same time, IGF-I stimulates protein synthesis, augmenting the effects of GH. The proposed change to the hypothesis seeks to emphasize that IGF-I helps to reduce what the authors call the "diabetogenic effect" of GH. Other current investigations include additional sites of IGF-I production besides the liver; the effects of GH on bone growth; and the locations of GH receptors throughout the body. The IGF-I system as it is known today is described in greater detail in the next section.[53]

The Insulin-Like Growth Factor Axis

The IGF axis is essential for the growth and development of nearly all body tissues.[54-55] At the same time, it also plays a definitive role in the proliferation of cancerous tissue.[55-56] The axis is made up of a number of different players. IGF-I and II are the primary ligands; the IGF-I and the IGF-II/mannose-6-phosphate receptors (IGF-IR and IGF-IIR) facilitate the effects of the ligands; and the IGF binding proteins 1-6 (IGFBPs 1-6) increase the half-life of the IGF ligands. Insulin has also been considered part of the axis, although its actions are not dependent on GH.[54-55]

Insulin, which is produced by the pancreatic beta cells, originates as proinsulin and is processed to insulin and C peptide. IGF-I and II are peptide hormones produced mainly by the liver;[57] however, they are also produced locally in a number of other tissues.[54] These include, but are not limited to, bone, kidney, and lungs.[54] Generally, the IGF peptide hormones circulate at levels approximately 100 times higher than other peptide hormones.[58] The hepatic production of these two peptides is regulated by circulating GH. Unlike, GH, IGF-I remains stable throughout the day.[50]

Any factors that may compromise the liver, including severe malnutrition and cachexia may decrease circulating concentrations of IGF-I and II.[59-62] In fact, IGF-I has been shown to markedly decrease after a 5-day fast and subsequently restored after re-introduction of nutrition.[59] Factors that affect circulating concentrations of GH also affect circulating levels of the IGF peptides. This includes disruptions in the release of GH-releasing hormone (GHRH) or the effectiveness of GH receptors.[50]

The IGF-IR is a tyrosine kinase receptor similar to the insulin receptor (IR) in that they are both $\alpha_2 \beta_2$-heterotetramers. IGF-IRs are expressed by nearly all cells throughout

the body. IGF-IRs have a very low affinity for insulin and likewise IRs do not readily bind with IGF-I.[63-64] At pharmacological doses, injected IGF-I can causes hypoglycemia in humans.[50] This is due to the fact that at such high doses, IGF-I has a much greater probability of activating IRs. In addition to ligand-specific receptors, the existence of hybrid receptors in varying concentrations has been discovered.[54] These receptors are in the form of dimerized αβ-half-receptors; the IGF-IR half-receptor combining with the IR half-receptor. Compared to IGF-I, insulin has reduced affinity for hybrid receptors. Although difficult to determine, it is now believed that in many cases, there may be a higher percentage of hybrid receptors present on the cell surface than IR or IGF-IRs[50,54]

The IGFBPs or binding proteins play a major role in regulating the actions and concentrations of the IGF-I and II peptides.[50,54] They extend the half-life of circulating IGFs by preventing them from circulating freely. Un-bound, or free IGF-I has a half-life of only a few minutes.[50] When bound to IGFBP-3 this is extended to between 12-15 hours.[54] It has been estimated that 80% of IGF-I in circulation is bound to IGFBP-3 with the addition of an acid-labile subunit.[50] For this reason, IGFBP-3 is the most important binding protein related to circulating IGF-I concentrations. The remaining 5 IGFBPs are not nearly as saturated as IGFBP-3, though they play a role in binding some of the remaining circulating IGFs not bound to IGFBP-3.[55] IGFBP-5, while a very small player also can form a ternary complex with the acid-labile subunit and IGF-I and II[54]. IGFBP-5 also has a 10-100 greater affinity for IGF-II. IGFBP-1 and 2 have much lower half-lives of 90 minutes and have less effect on IGF-I.[54] Lastly, IGFBP-5 and 6 provide little influence on the IGF axis due to their low concentrations and are not considered significant players.[54]

A particular potent modulating factor of IGFBP concentrations are the proteases that cleave some of the lower molecular weight IGFBPs.[54,66-67] There are three groups of these proteases – plasmin, gamma nerve growth factor and "Kallikrein-like serine proteases" which include PSA.[59] The cleaving of the IGFBPs increases levels of free IGFs and makes them more available to react with receptors.[59,67] In disease states such as CaP where PSA levels are elevated, IGFBP-3 could theoretically be cleaved at a higher rate. However, a study in 2003 noted that in patients with CaP, PSA and other proteases related to the prostate did not significantly reduce IGFBP-3 concentrations compared with men who did not have CaP.[20]

IGF-II reacts comparably with both IGF-IR and the isoform of the IR called IR-A.[59,66] What is called the IGF-IIR is actually identical to the mannose-6-phosphate receptor (M6P).[59,66] The two are usually referred to as IGF-IIR/M6P. IGF-IIR, unlike IGF-IR is not a tyrosine kinase receptor. It is rather a single glycoprotein that spans the cell membrane and actually has a much longer extracellular domain than IGF-IR.[59] The current understanding of the role of IGF-IIR is that it is mostly a regulator of IGF-II levels in the blood. IGF-IIR in some ways plays a similar role as the IGFBPs, keeping IGF-II levels from being elevated.[59]

Other Variables Affecting IGF-I Concentrations

There are a number of other factors that have been shown to affect IGF-I concentrations in humans. Age is perhaps the most important factor as IGF-I concentrations vary throughout the lifetime.[68-69] After a peak at puberty, IGF-I declines steadily with age.[68-69] Other factors include protein intake, kilocalorie intake, BMI,

alcohol intake, and dairy intake.[70-75] Of the dietary factors listed, protein intake or more significantly, lack thereof can cause the greatest variations in IGF-I. Low-protein diets, vegan diets, and malnutrition can lower IGF-I.[71,76] IGF-I is generally otherwise stable with adequate nutrition.[71] Dairy intake may have some influence, but research is limited.[72-73]

Age-specific reference values have been determined using a variety of assays.[68] A more recent study in 2010 computed reference values using an Immulite assay.[77] In adults serum IGF-I is highest between the ages of 25-29, near 200ng/mL. It then steadily declines until 40 in women, where it is stable until age 60. In men, it continues to decline throughout life. Men over age 80 have nearly half the IGF-I concentrations they did in their 20s.[77]

Serum IGF-I was shown to be 19% higher (p<.0001) in 8-year-old boys who increased their intake of skim milk (n=12) rather than meat (n=12). In addition, a 12-week intervention in women aged 55-85 saw the milk-supplemented group (n=101) increase IGF-I by 10% (p<.001) versus a control group (n=104) that saw no increase.[78] A more recent study of younger women averaging 36.8 years found no difference in IGF-I concentrations between women supplementing yogurt before and after exercise (n=15) versus supplementing a sucrose beverage (n=14).[79]

The Role of IGF-I in Prostate Cancer

Research continues to accumulate demonstrating associations between IGF-I and cancer development. As previously mentioned, IGF-I plays a necessary role in the proliferation of cancerous cells and tumorogenesis.[54] In addition, IGF-I is necessary for

the growth of the prostate gland itself.[54] While IGF-I itself is not secreted locally by prostate tissue cells, IGF-IRs are secreted by both the stromal and epithelial cells of the prostate.[54] In fact, IGF-IR is expressed in a number of cancer cell lines and is activated by normal concentrations of circulating IGF-I.[54]

Experiments by several researchers in mice point to IGF-I concentrations having a direct impact on tumor growth and cancer development.[13,81-85] In mice specifically bred to have reduced GH and thus IGF-I, mammary tumors were significantly delayed compared to controls after exposure to a potent carcinogen.[83] Inhibiting or blocking IGF-IR has been shown to slow cell proliferation.[13,84] IGF-IR antibodies have also been tested, however IGF-I levels seem to increase in response to lowered IGF-IR activity, reducing the effectiveness of the antibodies.[13]

The underlying hypothesis to this day is simply that CaP cells that spend a long period of time in a relatively high IGF-I environment may simply have an increased chance of developing into malignant cells. This is thought to be the case because IGF-I signaling generally prolongs cell life and reduces apoptosis. In light of the fact that so many factors play a role in the IGF-axis and that there are so many individual variations in IGF-I levels among the general populace, developing a complete picture is rather difficult. Nevertheless, researchers think that even small reductions in circulating IGF-I may play a role in reducing CaP risk.[13,54]

Numerous studies have looked at the relationship of the IGF axis and CaP development.[80,85-88] Elevated serum and plasma levels of IGF-I have been shown to increase the risk for CaP.[80, 85-88] A study by Chan and colleagues found such an association and is considered one of the key studies relating circulating IGF-I levels to

CaP risk.[80] They conducted a nested case-control study that used men who had been participating in the Physicians Health Study. The Physicians Health Study involved 14,916 participants who provided, among other things, samples of plasma. Out of these participants, 152 cases were identified. 152 controls were matched to each case. IGF-I, IGF-II and IGFBP-3 concentrations were measured in all participants. Paired t-tests were used to look at the mean concentrations between both groups. The average concentration of IGF-I in the case group (269.4 ng/mL) was significantly higher (p=0.03) than the control group (248.9 ng/mL). Concentrations of IGF-II and IGFBP-3 did not differ between the case and control groups. It was also found that men in the highest quartile of IGF-I concentrations had a relative risk (RR) of 2.4 compared to the lower quartile; this relationship was found to be statistically significant. IGF-II was not found to have an association with CaP risk. IGFBP-3 was significantly inversely associated with CaP risk after controlling for IGF-I concentrations. They explained this by considering that higher IGFBP-3 levels allowed less free circulating IGF-I. There was also no significant difference in the observed associations between low-grade versus high-grade CaPs. The study speculated that targeting IGF-I levels as a treatment for CaP may very well lead to a more effective treatment measure for the disease.[80]

In a population-based case-control study by Wolk and colleagues, in Sweden, IGF-I and IGFBP-3 concentrations were also measured in relation to CaP risk.[87] The study involved recruiting men under the age of 80 who had recently been diagnosed with CaP. The case subjects were then matched with controls within 10-year age groups. Controls were thoroughly examined through rectal exams for prostate nodules. Controls with suspected nodules had prostate biopsies taken to confirm whether or not they had

any sign of CaP. Blood samples were then drawn to determine serum IGF-I and IGFBP-3 levels. T-tests were performed to compare the two groups. Altogether 210 CaP cases and 224 control subjects were analyzed. Results similar to the aforementioned Chan study were obtained. The mean IGF-I concentration in the case group was 158.4 ng/mL and the mean in the control group was 147.4 ng/mL. This difference of 11 ng/mL was found to be statistically significant, with a p-value of 0.02. The study found an increasing odds ratio (OR) for CaP associated with increases in serum IGF-I. There was no statistically significant association found between IGFBP-3 and CaP risk. Another important finding was that the IGF-I/CaP correlation was weaker in men over age 70. This could possibly be due to men over age 70 generally having lower circulating levels of IGF-I. The study concluded that IGF-I levels are a significant risk factor for CaP in men under age 70.[87]

A case-control study nested within the Northern Sweden Health and Disease Cohort Study was conducted to measure plasma concentrations of IGF-I, IGFBP-I, IGFBP-2, IGFBP-3, and insulin in 149 men with a diagnosis of CaP as well as 298 controls.[88] Case subjects' blood samples had been taken between 1 month and 10 years before their cancer diagnosis. Control subjects were randomly chosen from original cohort members and were matched by age at a rate of 2 controls per case subject. A variety of statistical analyses were performed to compare the two groups. Like the two previous studies mentioned, IGF-I levels were significantly higher in case subjects than controls. In addition, in this particular study, IGFBP-3 levels were also significantly higher in cases than controls. Case group values for IGFBPs 1 and 2 as well as insulin were not significantly different from control group values. Because blood samples in this study were taken before cancer diagnosis there is a greater chance that blood levels of

IGF values were less affected by metabolic changes stemming from the presence of CaP itself.[88]

This nested case-control study was later extended to include 281 men who had been diagnosed with CaP after recruitment into the original cohort. In this extension, 560 matched controls were used. The study found similar results and through logistic regression analyses demonstrated significant increased ORs for developing CaP when serum IGF-I concentrations were elevated, especially for men who were younger than 59 years when they were recruited. This study supported other studies suggesting that IGF-I concentrations are a more significant risk factor for CaP in men of relatively younger ages.[88]

While these studies found generally weak associations between IGFBP-3 and CaP risk, one study did find a positive association. A case-cohort study performed by Severi and colleagues, found that increased concentrations of IGFBP-3 at baseline were associated with an increased risk of CaP. Incidentally, they did not find an association between high baseline concentrations of IGF-I and increased risk for CaP.[89] A very large meta-analysis also found that associations between IGFBP-3 and risk for CaP have generally been inconclusive with IGFBP-3 levels only correlating to an inverse relationship with CaP risk in retrospective studies.[90] Despite these inconclusive findings, IGFBP-3, due to its necessary association with IGF-I, may play a significant role in the development of CaP.

IGFBP-3 has also been found to have an IGF-independent role in the inhibition of cell proliferation and the stimulation of apoptosis in a variety of cells.[91-95] There have been several studies relating these effects of IGFBP-3 to specific mechanisms in CaP

growth.[91-95] In one such study, Peng and colleagues analyzed LNCaP cells treated with synthetic androgen R1881 by itself or combined with calcitriol and observed how it affected IGFBP-3 expression and cell proliferation. The authors found that when LNCaP cells were treated with high amounts of R1881, calcitriol-stimulated expression of IGBP-3 increased. This, in turn, increased inhibition of cell proliferation through an IGFBP-3-p21/p27 mechanism.[91]

It has also been postulated that the molar ratio of IGF-I to IGFBP-3 in the blood may play a role in CaP risk. A study by Li and colleagues identified 408 men who had been diagnosed with CaP before the age of 73 and matched them with 437 sibling controls. Serum IGF-I and IGFBP-3 were measured and correlated with various measures of disease "aggressiveness" at the time of diagnosis. Among these were PSA, tumor stage, and Gleason score. Investigators found that a higher IGF-I to IGFBP-3 ratio was associated with an increase in CaP risk, more so for men with a weak family history.[96]

A case-control study that took place in China found similar results. 128 newly diagnosed CaP cases were matched with 307 randomly selected controls in Shanghai, China. Epidemiological risk factors were obtained from participants using a questionnaire after they were selected. Serum IGF-I and IGFBP-3 were obtained from all participants, though only samples obtained 1 day or more before treatment were used in the analyses. Of the samples analyzed, it was found that men in the highest quartile for IGF-I to IGFBP-3 ratio had a risk for CaP that was 2.5 times greater than the lowest quartile.[97]

The association between IGF-I and CaP growth has also been demonstrated in an animal model.[98] A study by Kaplan and colleagues showed that TRAMP mice had increased expression of IGF-I mRNA during CaP progression. Serum IGF-I levels were

"precociously elevated" in TRAMP mice during early stages of cancer progression. Lastly, Torring and colleagues demonstrated that treating rats with IGF-I for 3 to 7 days caused significant growth of rat prostates and seminal vesicles.[98]

Green Tea and Chemoprevention

Green tea (GT) has been widely studied in recent years for its role in cancer prevention.[99] Tea in general is the second most commonly consumed beverage worldwide. All tea comes from the *camellia sinensis* plant and is processed in a variety of ways to produce black, green and oolong tea. The specific compounds in GT that have been speculated to have chemoprotective effects are a group of catechins, a type polyphenol antioxidant. This group is aptly named "GT polyphenols" or sometimes "GT catechins." It is made up of four compounds: epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate, epigallocatechin, and epicatechin. Of these four, the most abundant in green tea is epigallocatechin-3-gallate, which accounts for 62% of the total polyphenol content in GT. Of the three aforementioned teas, GT has the highest concentration of polyphenol antioxidants. This is mainly because GT does not undergo the fermentation process that is used to produce black and oolong teas.[100]

GT and cancer prevention has been investigated in various animal models, cell-cultures, cohort studies, and case-control studies. Epidemiological studies tend to provide conflicting evidence, which may be due to multiple uncontrolled confounders. that also contribute to cancer such as alcohol consumption and cigarette smoking. Generally, cohort studies have not shown a relationship between GT consumption and cancer risk.[101]

One key cohort study in Japan that examined 8,552 men and women on their

lifestyle activities did find that increased GT consumption seemed to delay the onset of cancer. It specifically found that women who drank more than 10 cups of GT a day, on average developed cancer 9 years later than those who drank 3 or less cups per day (p=0.01).[102] The study produced less promising results for men. Authors believed this may have been due to the fact that cigarette consumption in males increased with GT consumption. In addition, a greater percentage of males smoked cigarettes than females.[103]

Case-control studies have produced the most promising evidence for the chemopreventive potential of GT. Among these studies, cancers of the stomach, pancreas, colon, prostate and breast have all stood out as having inverse relationships with GT consumption. GT intake was found to be positively associated with esophageal cancer, but this was likely due to tea that was consumed at scalding hot temperatures.[101]

Two case-control studies found significant decreases in the risk of pancreatic cancer with GT consumption.[103-104] A study by Kono and colleagues observed 139 cases with gastric cancer and compared them to 2,574 hospital controls and 278 controls from the community. A decrease in risk for gastric cancer was found with drinking 10 or more cups of GT a day.[105] Three studies from China confirmed these results.[106-108] Studies have also been found showing an inverse relationship between GT consumption in breast, prostate, colon and rectal cancers.[101] The relationship between GT and CaP will be elaborated on in further detail below.

Green Tea and Prostate Cancer

Because CaP usually develops slowly over an extended period of time, it is in

many ways a good candidate for chemoprevention.[109] The effects of GT on CaP risk and progression has been studied more thoroughly in the last decade or so. Research shows that GT may be useful for both those at high risk of CaP and those who have already been diagnosed.[109] GT is a low-risk, inexpensive, and widely available product that is available in a variety of forms. Consumed by itself, it is calorie-free and a good source of hydration. These factors, in addition to its potential biologic activity have made it an attractive potential chemopreventive agent to researchers and consumers alike.[109]

So far there have been several epidemiological studies relating GT to CaP risk. Most of them have reported a significant decrease in CaP risk with increased GT consumption. Both case-control and cohort studies have shown inverse relationships between intake of GT and risk of CaP.[110] A recent meta-analysis that analyzed 13 such studies concluded that green, but not black tea may have a protective role in CaP.[110] A few clinical trials have been performed that investigated the strength of GT catechins as a treatment or preventive agent for CaP. Results from these trials have been modest at best. Two trials analyzing biomarkers for CaP risk are discussed later in the section titled "GT and its Effect on IGF-I and IGFBP-3 in CaP."[111-112]

One of the more recent cohort studies to date, by Kurahashi and colleagues examined 404 men who had been part of the Japan Public Health Center-based Prospective Study. The original cohort study had 49,920 men who had filled out questionnaires that, among other things, estimated GT consumption. This was one of the first studies that correlated GT intake with different stages of CaP. It was found that GT consumption was not associated with the risk of localized CaP. It did find, however, that there was a dose-dependent, inverse relationship with GT intake and risk of advanced

CaP. Men who drank 5 or more cups of GT per day had an inverse risk (0.52, 95% CI: 0.28, 0.96) of advanced CaP compared to men who drank one or less.[113]

A cohort study by Kikuchi and colleagues looked at GT consumption and CaP risk within the Ohsaki Cohort Study. The Ohsaki study originally included 26,481 men aged 40-79. All men were asked to fill out a food questionnaire, which, among other things, had three categories for GT consumption: 1-2 cups, 3-4 cups or 5 or more cups a day. Multivariate hazard ratios were used to analyze the incidence of CaP versus tea intake. Study authors found no association between GT consumption and CaP risk.[114]

A case control study by Jian and colleagues that took place in Hangzhou, southeast China examined 130 men with "histologically confirmed adenocarcinoma of the prostate." 274 age-matched inpatient controls were also enrolled. An in-person interview was administered to subjects that assessed amount of tea consumed per day using a variety of questions. CaP risk versus tea intake was assessed using multivariate logistic regression, which adjusted for a variety of risk factors. The odds ratio as compared to non-tea drinkers was low for those drinking tea for over 40 years 0.12 (95% CI _ 0.06–0.26) and even lower for those who consumed 1.5 kg of tea leaves per year. 0.09 (95% CI _ 0.04–0.21). This study was notable in that it was the first to analyze CaP risk versus frequency, duration, and quantity of GT consumed. The authors concluded that increases in all three factors can lead to a lower risk of adenocarcinoma of the prostate.[115]

Sonoda and colleagues conducted a case control study that sought to determine the relationship between traditional Japanese diet and risk for CaP. 140 men between the ages of 59-73 years were enrolled and age matched with 140 hospital controls. Cancer

stages ranged from I (n=2) to IV (n=16) with most the cases falling in between. Daily

intakes of foods and beverages for the past 5 years were determined using a validated

semi-quantitative food frequency questionnaire. ORs were calculated for CaP risk versus

GT consumption in quartiles. The highest quartile of consumption (10 or more cups a

day) yielded an OR of 0.67 (CI=95%). Authors noted this modest effect but found it was

not statistically significant.[116]

Green Tea and its Effect on IGF-I and IGFBP-3 in Prostate Cancer

GT polyphenols and EGCG have both been shown to affect IGF-I and IGFBP-3

concentrations in several studies.[111-112,117-121] There have been two such human studies,

published in the last three years. Both of them involved the administration of the GT

supplement pill Polyphenon E to patients already diagnosed with CaP.[111-112] Similar

treatment studies have been conducted in mice.[119-120] Lastly, cell culture studies have

been conducting relating GT to IGF-I and IGFBP-3.[117-118]

In 2011, a randomized, double-blind, placebo-controlled trial tested the effects of

Polyphenon E supplementation on men who had biopsy-confirmed CaP. All 50 men

enrolled in the study had scheduled a prostatectomy. They were randomized to receive

either the GT supplement or a placebo for the 3-6 weeks before their surgery. Among

other markers, serum IGF-I and IGFBP-3 were measured in pre- and post-treatment

blood samples. IGF-I concentrations were lowered more in the treatment group than in

the placebo group (-6.90 ± 20.97 vs. -1.20 ± 21.82 ng/mL, $P = 0.53$). Though this

decrease was not statistically significant, a greater proportion of treatment subjects had

decreases in IGF-I than subjects receiving the placebo. Similar results were found for

increases in IGFBP-3, though not statistically significant (20.38 ± 289.3 vs. -74.76 ± 238.11 ng/mL, P = 0.24). Subjects in the treatment group had greater increases in IGFBP-3; in addition, a greater proportion of these subjects had increases in IGFBP-3. Lastly, the IGF-I/IGFBP-3 ratio was more greatly lowered in the GT treated group, without showing statistical significance. Study authors found the results promising, but felt that among other limitations, the treatment period may have been inadequate for the desired effects on chosen end-points.[112]

An open-label, single-arm, two-stage phase II clinical trial was conducted in 2009 with a similar population. Polyphenon E supplementation was provided to all participants prior to their radical prostatectomy. A variety of biomarkers were measured, among these were serum concentrations of IGF-I and IGFBP-3. Researchers found concentrations of both markers were significantly reduced in the participants. They also found that 14 out of 18 of men experiencing a drop in PSA concentrations also had a drop in IGF-I and 17 of them had a drop in IGFBP-3. The median dosing period for this study was 34.5 days.[111]

The most significant animal study to date that relates GT's effects to serum levels of IGF-I and IGFBP-3 was an experimental study conducted by Gupta and colleagues of the effect of GT on TRAMP mice.[119] 100% of TRAMP mice are expected to develop CaP at the onset of puberty. They are used primarily because the development and progression of their CaP closely resembles the development and progression of CaP in humans. The study involved two experimental groups of 8-week old TRAMP mice. Each group was divided into an experimental group and a control group. The mice in the experimental group were fed a 0.01% GT solution for 24 weeks while the control group was fed tap

water for the same period of time. The concentration of GT solution was equivalent to a human dose of 6 cups of GT per day, which is relatively achievable.[119] Five mouse prostates in each group were monitored for growth at 20 and 30 weeks. The GU apparatus as well as the prostates were removed from the mice for various tests and examinations.[119] MRI scans showed significantly less growth in the prostates of treated mice. The scans also demonstrated that all the untreated mice developed cancer while only 30-40% of the treated mice developed tumors by the end of the of the treatment period. It was also demonstrated that the treated group suffered no cancer metastases while the untreated group suffered metastases in the lymph, lungs, liver and bones.[119]

Serum IGF-I and IGFBP-3 concentrations were also measured in both groups. IGF-I concentrations were significantly lower in the GT treated group compared to the untreated groups. It was also found that serum IGFBP-3 concentrations were significantly higher in the treated versus untreated groups.[119]

It also appears that the timing of the GT supplementation in the course of CaP development may influence its effectiveness. A study by Adhami and colleagues, also using TRAMP mice, supplemented GT at various ages that would correspond to different stages of cancer development. The GT was initiated at 6 weeks (normal prostate), 12 weeks, 18 weeks, and 28 weeks. (Typically TRAMP mice have normal prostates at 6 weeks and at 28 weeks have differentiated adenocarcinoma with metastases.) A feeding regimen similar to the study by Gupta was used where GT was given three days a week in the treatment group. It was similar to four to six cups of GT per day in humans. At 32 weeks of age, serum IGF-I and IGFBP-3 were measured in rats from each treatment group. It was found that IGF-I concentrations were most significantly affected in the

group that started supplementation at 6 weeks. Inhibition of GT was progressively less marked the later the supplementation was started. IGFBP-3 concentrations trended similarly. The largest increase of IGFBP-3 was seen in the treatment group started earliest. Lastly, IGF-I in the prostate epithelial cells was also lowest in the group started at 6 weeks.[120]

In a follow-up study, the same group repeated the experiments and instead of measuring serum concentrations of IGF-I and IGFBP-3, they measured their respective concentrations in the dorso-lateral prostate tissue of the mice. The study found that GT supplementation significantly reduced prostatic IGF-I levels and significantly increased prostatic IGFBP-3 levels in the mice.[121]

Another study by Harper and colleagues investigated the supplementation of EGCG alone and its effects on the development of CaP in TRAMP mice. The authors found that EGCG inhibited early stage CaP in the mice, but not later stage CaP.[122] They demonstrated similar finding to the previously mentioned study by Gupta and colleagues.[119] They found that EGCG reduced cell proliferation, induced apoptosis and also decreased IGF-I levels in the ventral prostate of the TRAMP mice.[122]

Fish Oil Supplementation and Prostate Cancer Risk

Research showing relationships between fish oil (FO) consumption and CaP is limited.[123-127] A few epidemiological studies have looked at the relationship between fish intake, FO intake or EPA/DHA (primary components of fish oil) intake and CaP risk.[125,127] Fish intake itself is not necessarily an indicator of EPA/DHA intake as not all fish are a rich source. An ecologic study did find that Alaskan natives, who have been

found to have relatively high blood levels of EPA/DHA, had a much lower incidence of CaP.[128] Other studies have looked directly at serum plasma fatty acid concentrations of EPA/DHA and risk for CaP.[127] These include cohort, case-control and nested case-control studies. Generally, it has been found that neither fish consumption nor intake of EPA/DHA are associated with any decrease in CaP risk. There are some exceptions, but they are few.[127,129]

The most comprehensive meta-analysis so far by Brouwer looked at studies relating alpha-linolenic acid (ALA) to CaP risk as well as studies relating EPA to CaP. Eight observational studies (5 prospective cohorts, 3 case-control) that focused on either EPA intake or blood levels were combined.[130] An RR of 0.90 (95%CI0.81–1.01) was obtained for all studies. A RR of 0.91 was obtained for prospective studies only (95%CI 0.80–1.03). The same procedure was applied to 7 observational studies looking at either DHA intake or blood levels, and CaP. A similar RR of 0.91 (95% CI 0.81–1.04) was found for DHA and CaP. A similar, more recent meta-analysis that analyzed EPA and DHA separately confirmed these results with a RR of 1.03 (95% CI: 0.973, 1.096; $P = 0.2780$).[130]

Fish Oil Supplementation and IGF-I

The effect of FO consumption on IGF-I, if any, has not been well described. Research tends to be conflicting and is not often related to chemoprevention. A med-line search found no reviews relating these two variables. Of the original studies that have, a fair portion of them have been conducted on animals.[131-133] Sporadic human studies have generally looked at specific age groups or have only been applicable to specific disease

states.[134-139] While FO may be able to restore IGF-I in acute disease states, it has had no significant effect thus far in clinical trials.[136,138]

Animal studies suggest FO may increase IGF-I, but evidence is limited. Lactating dairy cows were supplemented with flax-seed, palmitic acid (control), conjugated linoleic acid, or FO for 15 days. The FO and flaxseed groups were combined into a "n-3 supplemented" group for select analyses. Compared to controls, the n-3 supplemented group had significantly higher plasma IGF-I concentrations (84.6 ng/mL vs. 73.4 ng/mL, $P < 0.03$).[132] A similar study of beef heifers supplemented with progressively higher doses of FO supported these results. Heifers on the highest level of FO supplementation demonstrated significantly higher plasma IGF-I concentrations when compared with the non-supplemented control group (p<.05).[131]

A very similar study in sows with varying fat supplementation (palm, fish oil etc.) measured IGF-I but did not report statistical analyses to show if IGF-I differed between groups.[133] Only one study using pigs reported a slight increase (p<0.10) in IGF-I after a diet of 7% FO for three weeks compared to 7% corn oil.[134] Multiple rat studies have been conducted at varying lengths of FO supplementation.[135]

Four different studies in highly specific populations looked at FO supplementation in adolescent boys (13-15 years)[140], formula-fed infants,[135] Chron's patients[137] and severe burn patients.[61] Adolescent boys (n=78) were randomized to eat either a control bread or a bread fortified with high-DHA FO.[140] After 16 weeks of daily bread consumption, IGF-I levels were significantly correlated with erythrocyte DHA levels ($\beta = 0.24$, $P = 0.03$).[140] A 3-month long, 2x2 study on infants (n=83) investigated the effect of protein intake on IGF-I. Authors found no effect from including FO in one

of the four treatment arms.[135] Chron's patients, who have been found to have decreased IGF-I saw increases in the hormone after either omega-6 or EPA/DHA regimen. A difference of effect was not noted between the two regimens.[137] Finally, the study on burn patients found that FO present in intravenous (IV) nutrition support allowed for the most rapid recovery of IGF-I levels.[61]

In the realm of clinical trials, one study originally investigated FO and lycopene's respective effects on COX-2 and IGF-I gene expression in men at high risk for CaP. [143] In a follow-up study, the same authors measured post-intervention IGF-I gene expression in both groups. While they found a 1.5-fold decrease in IGF-I gene expression in the FO group, it was not statistically significant.[139]

A recent Phase II trial was the first to investigate FO's effect on IGF-I in men scheduled for prostatectomy due to CaP. After 4-6 weeks on a low-fat diet with 5g/day FO or a traditional western diet there was no difference in serum IGF-I between groups.[138]

Lastly an ongoing two-year trial of women at high risk for breast cancer reported preliminary findings on the effects of Lovaza, Raloxifene (Ral), or Ral plus Lovaza (4g esterified FO/day) on IGF-I. After one year Lovaza had no effect on circulating IGF-I levels.[136]

Interaction Between Fish Oil and Green Tea Polyphenol Supplementation

Almost no research has looked into the interaction between GT and FO supplements when taken concomitantly. A handful of studies note a synergistic effect between GT and FO.[144-147] None of them relate to IGF-I or the IGF-axis. The most

noteworthy was a study by Giunta and colleagues which was able to show that FO

increased the bioavailability of EGCG ($P < 0.001$) in the brain tissues and plasma. They

also found that FO and EGCG had a significant synergistic effect on inhibiting beta-

amyloid plaque formation in the brain ($P < 0.001$).[144]

A study by Bose and colleagues found that $Apc^{Min/+}$ mice who were supplemented

with both FO and EGCG had a significant reduction in the number of tumors formed

versus an un-supplemented control group. Even more interesting was the fact that the

treatment group had significant reductions in Akt phosphorylation in the tumors, which

may have resulted in increased tumor cell death.[147]

Yoshino and colleagues found adding GT to the diets of mice being fed FO

increased the hypolipaemic effects of the FO and decreased the lipid peroxidation

associated with the FO ingestion. The effect was observed after 21 days.[145]

Lastly, Shirai and colleagues fed mice low and high-fat diets for three months

with and without the addition of GT extract. The low-fat diet contained lard while the

high-fat diet contained FO. After three months they found that GT extract with FO

improved plasma lipid markers in the mice. They also noted that no negative

consequences of combining GT and FO were detected.[146]

Pharmokinetics of Green Tea Polyphenols

Despite the popularity of GT polyphenols as a potential chemopreventive agent

and nutritional supplement, their metabolic availability somewhat eludes researchers. It is

well established that GT polyphenols are absorbed in the gastrointestinal (GI) tract.[148-151]

In fact, the measurement of plasma catechins is a validated method for confirming GT

polyphenol absorption.[150] In addition, urine metabolites of the various GT catechins are often measured in order to estimate a picture of bioavailability.[148-149] What is not fully understood is the extent of variability in absorption, metabolism and distribution in humans.

(Epi)catechins and (epi)gallocatechins are both absorbed but their respective bioavailabilities are different.[152] Studies in both animals and humans seem to indicate that (epi)catechins are the better absorbed of the two.[153-156] This is notable because EGCG, considered to be the more biologically active of the catechins, seems to have the least bioavailability. Metabolites of (epi)catechins are more prevalent in the urine following GT administration.[152] Some research also suggests that the bioactivity of EGCG is further reduced as a considerably large portion of it is sequestered into bile following absorption.[152]

While research suggests that nearly 70% of GT catechins pass unabsorbed into the colon, colonic bacteria may play a significant role in breaking them down. This may lead to colonic absorption of other metabolites, including phenolic compounds. Colonic absorption of these metabolites may actually exceed those absorbed in the small bowel.[157]

Catechins in their free form are generally found in relatively small amounts in the plasma compared to conjugated forms.[156] This is due to the fact that even prior to absorption a large portion of catechins are metabolized by enzymes in the brush border.[157] Free catechins that are absorbed undergo rapid detoxification through glucuronidation, sulfation, and methylation. Much of these reactions take place in the liver and kidney. After conjugation, GT catechins are less bioactive.[150]

GT catechins are rapidly cleared from circulation as well. In humans, research

suggests that plasma catechins peak within 1.5 – 2.5 hours and are undetectable after only

24 hours.[156] In addition, while larger doses elicit higher plasma concentrations of

catechins, this dose dependent effect tapers off at oral doses of 4.5 g.[156]

The exact tissue penetration of GT catechins after absorption is not well

understood. It seems that (epi)gallocatechins are in the highest concentration in intestinal

tissue, while (epi)catechins are highest in kidney tissue.[156] Tissue penetration into the

prostate has been proven in both mice and humans.[153-154] While prostate tissues levels are

low and measured in pmol/g of prostate tissue, unconjugated forms of GT catechins have

been found to penetrate prostate tissue.[154] Prostate tissue does, however, contain enzymes

which methylate and reduce the bioactivity of these unconjugated catechins.[154] Catechins

seem comparably less available to mammary tissues.[155] Of all tissues, catechins are most

available to GI tissue. This includes the mouth, stomach, esophagus and intestinal tract.[152]

As catechins often encounter these tissues before conjugation their chemopreventive

effects are more pronounced. This is especially true in the colon as the large majority of

ingested catechins are transported into the colon. Further research is needed to show the

relative effectiveness of absorbed catechins in various organ systems.[152]

The absorption and effectiveness of GT catechins may also be affected by the

inclusion of additional dietary constituents.[158-160] Some research suggests that milk may

slightly inhibit the absorption of (epi)gallocatechins and enhance the absorption of

(epi)catechins.[158,160] Other research suggests that any absorptive inhibition by milk is

negligible.[159] While milk proteins may bind to catechins, simulated digestion by the

addition of proteases allowed a greater recovery of unbound catechins.[159]

Summary

Data in animals linking GT, lowered IGF-I concentrations and lowered CaP incidence is provocative. Little data linking GT to the IGF-axis and CaP incidence is available in humans. In addition, research characterizing FO's effect on IGF-I is sparse. This study examined the relationship of GT supplements, and CaP in a group of men at high risk for CaP randomly assigned to GT supplements or placebo. It also sought to determine what effect, if any, FO had on circulating IGF-I.

CHAPTER 3: Preliminary Data/Study Design

General Sub-Study Design

A sub-analysis of serum and plasma blood samples was conducted as part of a randomized controlled trial titled: "Catechins and ω-3 Fatty Acids Impact on Fatty Acid Synthase Activity in the Prostate: A Randomized Controlled Trial." This clinical trial was conducted by Dr. Jackilen Shannon at Oregon Health and Science University (OHSU). The purpose of our sub-analysis was to determine the impact of GT supplementation on circulating levels of IGF-I in men considered at high risk for CaP.

Study Design

Study participation was voluntary with informed consent papers signed by all interested, eligible participants. Recruited participants were randomized into four different treatment arms, in 2 x 2 design. Subjects assigned to Group 1 received FO and GT capsules. Subjects assigned to Group 2 received FO placebos and GT capsules. Group 3 subjects received FO capsules and GT placebos. Finally, group 4 received FO placebos and GT placebos. Figures 1 and 2 illustrate the basic design and subject assignment.

Figure 1- Original Treatment Arm Proposal

39
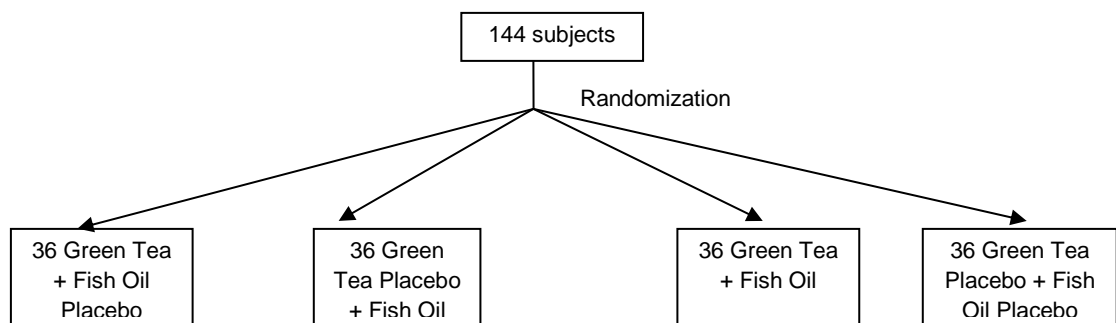
Figure 2- Treatment Diagram/Pill Assignment

		Fish Oil Arm (# of tablets/day)	
		Yes	No
	Yes	3 Fish Oil / 2 Green Tea GROUP 1	3 FO Placebo/ 2 Green Tea GROUP 2
Green Tea (GT) Arm (# of tablets/ day)	No	3 Fish Oil/ 2 GT Placebo GROUP 3	3 FO Placebo/ 2 GT Placebo GROUP 4

Supplementation Procedure

Study supplements were stored at the Portland VA Medical Center (PVAMC) and
OHSU research pharmacies and distributed in one-month quantities to the study
coordinator. The supplement capsules for both GT and FO were distributed in two
separate containers. Containers were labeled with the dosing instructions, subject name,
the protocol number, and the subject ID number. The study coordinator was in charge of
distributing the treatment or placebo and was blinded to the type of capsules being
provided.

Upon receipt of the subject-signed consent form (via fax), the PVAMC and
OHSU research pharmacies reviewed each subject's medical record for pharmaceutical
interactions, contraindications and known allergies (see Table 1). Dr. Mark Garzotto or
Dr. Tomasz Beer was responsible for prescribing the study supplements to the subjects
after they had consented to participate in the study.

Table 1: Supplement Contraindications

Supplement	Drug Interactions	Contraindications	Allergies
FO Supplement	For both supplements: antithrombotic medications such as warfarin, Coumadin, Aggrenox, enoxaparin, abciximab, clopidogrel, Plavix and heparin	Hemophilia, van Willebrand's disease or other blood disorders (except when the subject is evaluated by a hematologist who determines that fish oil supplementation is not contraindicated).	Any fish allergy
GT Supplement		Total bilirubin greater than institutional limit of normal	Allergies to green tea

After contraindications were assessed during the final eligibility screen, the VA or OHSU research pharmacies dispensed a month's supply of FO/GT/placebo to each of the subjects. Supplements were shipped to the subjects in 30 day supplies until the treatment period was over. In some instances, subjects picked up the supplements themselves from the coordinator. During the 2nd and 3rd visits, any unused study supplements were returned to the respective pharmacy (VA or OHSU) using pre-paid mailers. On the date of the subject's repeat biopsy (either the 4th or 5th visit), the subject was expected to bring any unused study supplements to the biopsy appointment. All subjects received supplementation for 12-14 weeks, unless they chose to withdraw or they were found to have an unacceptable toxicity related to the supplements. Unacceptable toxicity was determined on a case-by-case basis by a clinician. Treatment also could have been extended for up to 20 weeks due to any delay in the repeat prostate biopsy (as long as the delay is not due to study treatment).

Compliance

Compliance was assessed by pill count. Subjects in all four treatment arms who took ≥80% of their prescribed pills were considered compliant. The number of pills taken per day was recorded by each participant in their own individual supplement diary. Subjects returned their supplement diaries to the study coordinator at the final visit (repeat biopsy date). Any subject that refused to take the supplements they were given were removed from the trial. Plasma concentrations of both GT and RBC ω-3 PUFA were measured across all four treatment arms to relate pill count to specific changes in these biomarkers. Changes in plasma concentrations of GT or PUFA were not, however, considered criteria for compliance.

Supplement Composition

GT capsules each contained 300mg of extracted GT. Subjects received 2 capsules per day of either GT or GT placebos. (Figure 2) GT placebos contained dicalcium phosphate with a food grade coloring substance. Fish oil supplement capsules contained 0.5 grams of ethyl esters of *doco*sahexaenoic acid (*DHA*) and eicosapentaenoic acid (EPA). In the respective FO groups, one capsule was taken three times daily (Figure 2) and provided a total of 1.9g EPA+DHA with an EPA/DHA ratio of 1:1. Both these capsules and their corresponding olive oil placebos were opaque brown gel caps.

Participant Recruitment

Study participants were men recruited from PVAMC, OHSU and Kaiser Permanente Northwest (KPNW) urology clinics. Figure 3 illustrates the recruitment flow for the study. Any men who had been scheduled for a repeat prostate biopsy following

their initial biopsy were eligible to participate. Participants needed to have a negative initial biopsy but still be considered high risk due to their physician suspecting occult cancer. It was assumed that the mean age of participants would be around 60 years, however any subject who was 21 years or older and fit all other inclusion criteria was eligible to participate. Any men who fit the above criteria were contacted by the study coordinator. If men were interested in taking part in the study, a consent form was sent to them. If after reading the consent form, they were still interested, a visit 1 appointment was set-up for them.

Figure 3 – Recruitment Flow Chart
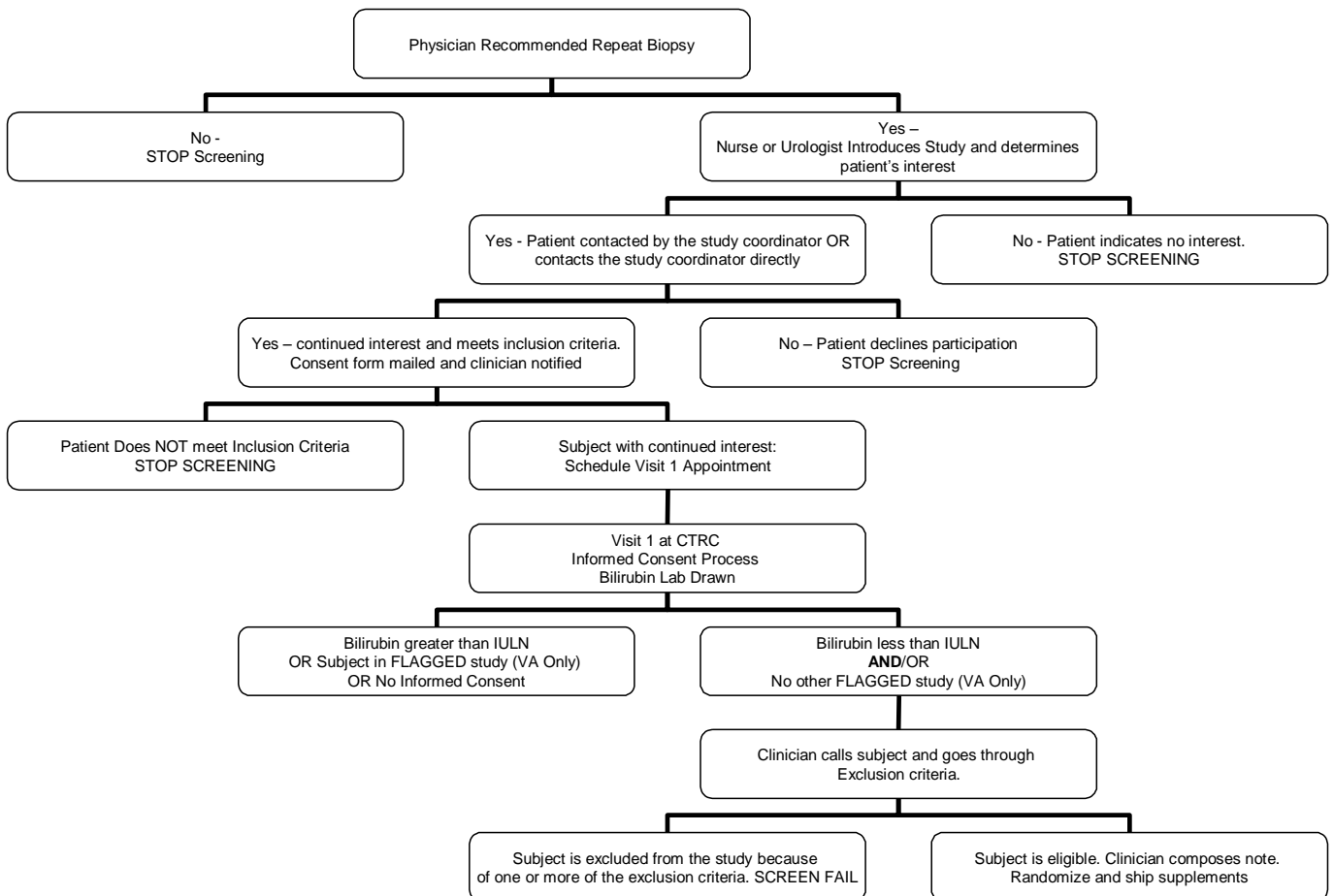
At visit 1, the study coordinator completed the informed consent process. Study subjects had a chance to learn about the goals of the study, the study process, and any potential benefits and risks associated with the study. If subjects remained interested, they signed an IRB approved consent form.

Following the consent form completion a diet history questionnaire and a risk factor questionnaire were administered to each potential subject. The coordinator also completed a brief survey that asked for any information regarding changes in the subjects' diets, medication use or medication dosage and to the intake of dietary supplements and/or herbal remedies in the time period following their initial visit. This questionnaire was used with each participating subject on a monthly basis. In addition to these questionnaires an outpatient specimen collection form (including vitals) was completed and each subject was questioned about any concerns that would contraindicate phlebotomy. If there were not contraindications, a Clinical and Translational Research Center (CTRC) research staff member performed blood draws to test for baseline RBC fatty acid levels, serum osteocalcin and baseline total bilirubin. Spot urine samples were collected to measure markers for bone turnover.

Serum bilirubin was tested prior to treatment because GT supplementation is metabolized in the liver and any underlying liver dysfunction may have impacted the safety of GT supplement use. If the total bilirubin concentration was within the institution limits of normal, the subject underwent a final screen by the clinician. During this screen, the physician discussed all the exclusion criteria with the subject (see table 2 for inclusion and exclusion criteria).

Inclusion criteria were simple. A subject was eligible if they were 21 years or older and had signed the informed consent form. Exclusion criteria were a bit more complicated. Any definitive invasive CaP on the initial biopsy automatically excluded the subject. In addition, if there was any significant active medical illness that the clinician believed would preclude treatment, the subject was excluded. Other exclusion factors included history of ventricular tachycardia or ventricular fibrillation; use of warfarin or need for therapeutic anticoagulation at time of biopsy or at any time during the trial; allergy or sensitivity to green tea, FO, or olive oil; history of hemophilia, van Willebrand's disease or other bleeding disorder; total bilirubin greater than respective institutions upper limit; and any VA subject that was part of a 'flagged' high risk study. If subject had a history of a bleeding disorder, they were allowed to participate if they were evaluated by a hematologist and were determined fit for FO supplementation. Subjects who reported any use of FO greater than 1 gram per day or green tea supplement use within 30 days before day 1 of treatment study were excluded. Also subjects taking less than or equal to 1 gram of FO per day had to agree to suspend use before the beginning of the trial.

If subjects met all eligibility criteria they were randomized into one of four treatment groups (see figure 2). A biostatistician determined the treatment group assignment based on a randomization protocol blocked by age (<65 years, ≥ 65 years). The randomization list was generated prior to the start of the trial. For a general overview of the timeline of events, see table 2.

Table 2: Inclusion/Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
a. At least 21 years of age b. Signed consent form c. Recommended for repeat biopsy by clinician.	a. Definitive invasive CaP on initial biopsy b. Significant active medical illness that in the opinion of the clinician would preclude protocol treatment. c. History of ventricular tachycardia or ventricular fibrillation d. Subject reported use of fish oil (greater than 1 gram per day) or green tea supplement within 30 days before Day 1 of study treatment a. Subject reported use of fish oil ≤ 1 gram per day and unwilling to discontinue use for the duration of the trial e. Use of warfarin or need for therapeutic anticoagulation at time of biopsy or at any time during the course of the trial. f. Subject reported allergy or sensitivity to fish oil, olive oil or green tea g. Subject reported history of hemophilia, van Willebrand's disease or other bleeding disorder, except when the subject is evaluated by a hematologist who determines that fish oil supplementation is not contraindicated. h. Total bilirubin greater than institutional upper limit of normal i. VA subjects may not be a part of another 'flagged' high risk study as noted, in red, on the cover sheet of subjects' VISTA/CPRS electronic medical record.

Table 3: Treatment Schedule for All Participants

Treatment Schedule	Screening	Baseline Visit	Visit 2 Phone Visit	Visit 3 Phone Visit	Visit 4 or 5 (Repeat Biopsy)	Visit 5 or 6 (follow-up)
Initial Biopsy & Recommend for Repeat Biopsy	X					
Informed Consent	X	X				
Inclusion Exclusion Criteria	X	X				
Bilirubin Test		X			X	
Randomization		X				
Frozen Plasma, Serum & Urine		X			X	
Vital Signs (height, weight, blood pressure)		X			X	
Diet & Risk Factor Questionnaire		X				
Study Supplement &/or Placebo		X	X	X	X	
Fish Oil & GT Adverse Effects		X	X	X	X	X
Change to Diet/ Rx/ Suppl/ Herbal Remedies Questionnaire		X	X	X	X	x
Repeat Biopsy and PSA (clinical care);					X	

Blood Sampling

Fasting blood samples were taken from all study participants at the first and final study visits. One 5-mL tube of blood was analyzed for normal total bilirubin, the results of which were provided to the respective research pharmacy prior to dispensing the study supplements. Two 10-mL fasting blood samples were obtained for OHSU participants by the CTRC nursing staff at pre- and post-intervention; for VA participants by the CTRC nursing staff pre-intervention and by the VA lab post-intervention; for KPNW participants by the CTRC nursing staff at pre-intervention and by the KTNW lab post-intervention. One 10-mL tube of blood was processed to allow for analyses of RBC fatty-acids and other plasma nutrients; the other 10-mL specimen was drawn into a gold-topped serum separator tube and was processed to allow for analyses of serum osteocalcin and separation of peripheral blood mononuclear cells. The blood specimens were processed by the CTRC core lab and stored in a -80 degree freezer. Blood specimens were shipped to the OHSU pharmacokinetics core lab and analyzed by Dennis Koop for fatty acid composition.

PSA, Gleason Score and Cancer Diagnosis

PSA testing was a normal part of subject's clinical care and was obtained at initial screening as well as within 72 hours of repeat biopsy. If cancer was diagnosed at repeat biopsy it was given a Gleason score by the attending physician.

Obtaining Plasma and Serum Blood Samples for Radioimmunoassay

While we originally intended to use serum for obtaining circulating IGF-I concentrations, it was found that there were more matched (pre/post) pairs of plasma samples available than serum from the original study. Paired plasma samples were not available for all 86 participants. In three cases where plasma samples were not available, paired serum samples were obtained. Altogether, 72 pre-post plasma samples were available in addition to the three serum samples. As the commercially available radioimmunoassay test kits measure both serum and plasma IGF-I, the three serum samples were included in the analyses. This brought the total number of paired samples obtained up to 75. Frozen samples were obtained from the CTRC core lab and transported in dry ice to the OCTRI core lab freezer. It should be noted that plasma and serum IGF-I concentration are shown to be virtually equivalent and have near perfect correlation at r=0.96.[65] Therefore, the use of serum and plasma should not skew the results in any way.

CHAPTER 4: METHODOLOGY

IGF-I Radioimmunoassay

Two Radioimmunoassay kits were purchased from IBL-America. All State, Federal and OHSU radiation safety precautions were followed during both assay sessions.

Plasma (n=72) and serum(n=3) IGF-I concentrations were measured in all participants using both pre- and post- treatment blood samples. To ensure each participant's pre- and post- samples were run on the same standard curve, they were kept on the same assay. Samples were assayed in duplicate.

All conical, non-capped, assay tubes were labeled in duplicate. In addition to unknown samples, assay tubes included the following standards: total counts, non-specific binding (NSB), standards ranging from $0 - 10$ ng/ml IGF-1, and a high and low quality control.

At the beginning of each assay, samples were thawed on ice. Lyophilized standards were reconstituted with dilution buffer, warmed to room temperature and mixed. All samples and controls were diluted 1:101 per the manufacturer's recommendation.

Samples and standards were added to the assay tubes. Next 100 µL of the first Antibody (reagent B) was added to the appropriate tubes, turning them blue. 100 µL of the radioactive tracer (reagent C) was then added to all the tubes (including total counts), turning tubes 3 and up violet. Following this, all assay tubes were vortexed.

All tubes were then covered in biofilm and placed in plastic basins, in an ice-water bath and covered with aluminum foil. Basins were placed in a 4 degree C refrigerator for approximately two and a half days (over the weekend).

The following Monday, 500µL of the appropriately mixed precipitating buffer was added to all tubes except the total counts. Tubes were than vortexed and incubated for one hour in a 4 degree C cooler. Following this, 1mL ice-cold deionized water was added to all tubes. All tubes except total counts were then centrifuged at 3400 rpms for 40 minutes at 2 degrees C. In order to ensure accuracy and prevent pellets from being compromised, Pasteur pipettes were used to aspirate the supernatant. Supernatant was aspirated from each tube, one at a time, leaving 1mm of supernatant above each pellet. 4 of the assay tubes required re-centrifuging after pellets partly dissociated during the aspiration procedure. When this occurred, supernatant was not removed, preventing any part of the pellet from being removed. Following repeat centrifuging, pellets were reformed and supernatant was successfully aspirated.

Tubes were counted over a three minute period on a Packard Cobra II 5 channel gamma counter. The counter used a sodium iodide crystal and was rated at 58.5%. Data from the gamma counter was uploaded into ImmunoFit EIA/RIA from Beckman instruments. The software is specifically designed to compute RIA results. A semi-logarithmic curve was generated from the standards. The software than computed values for controls and unknowns and they were transferred to excel for further analysis.

Statistical Analyses

Explanation of Analyses by Treatment Arms:

We conducted two separate analyses to investigate the effects of GT on circulating IGF-I.

The first analysis addressed our primary aim and considered only the GT (n=13) and PP

(n=24) treatment arms as the GT supplemented and PP supplemented treatment groups.

The second analysis was contingent on our secondary aims, and was run after we found

we could consider FO an additional placebo to increase sample size. We added the GTFO

(n=13) group to the GT (n=13) group to make a combined GT-supplemented group of

n=26. Likewise we added the FO (n=24) group to the PP group (n=24) to make a

combined PP supplemented group of n=48. This is further elucidated in the results

section. It should be known that from now on "separate treatment arms" refers to the

original four treatment arms. "Combined treatment arms" refers to GT/GTFO and FO/PP

combined arms. Sample sizes are also indicated to help differentiate the separate

treatment arm analyses from the combined analyses.

Descriptive Analyses

Pre- and post-treatment plasma (n=71) and serum (n=3) concentrations of IGF-I

were measured. In addition pre-post differences for IGF-I were calculated for each

subject. Pre- and post-PSA values, which were on file in the subjects' respective

hospitals, were also obtained. Pre-post differences in PSA values were calculated. Age

and BMI at entry were obtained. Post-treatment age and BMI was not available. All data,

initially on a master Excel spreadsheet was transferred to STATA version 12 for further

analysis. Descriptive analyses were conducted to determine the pre and post-intervention

range, mean and standard error of the mean for IGF-I and PSA. Pre-post differences were also calculated as an absolute difference in concentrations for each of the primary outcome measures (IGF-I and PSA). Descriptive analyses were also applied to individual treatment groups. This includes both separate (GT,PP,FO,GTFO) and combined (GT/GTFO and FO/PP) treatment arms.

Primary Analyses

All analyses were conducted using an intent to treat approach. T-tests were performed to determine if there was a statistical difference in IGF-I concentrations between the PP and GT groups at pre-intervention, post-intervention and in pre to post-intervention change. A p-value of 0.05 or less was considered statistically significant. T-tests were applied to both separate and combined treatment arms. In addition, Wilcoxon Rank-Sum tests were later applied in the same manner as the t-tests to verify findings. Wilcoxon non-parametric tests have been used previously by similar trials where end-points had non-normal distributions.

Secondary Analyses

Pearson's correlation coefficients were run to determine any correlation between IGF-I and PSA at baseline or post-intervention. In addition pre-post differences between IGF-I and PSA were checked for correlation. Partial correlations were used for post-intervention and pre-post change analyses in order to consider any effect that GT may have had on the correlation between IGF-I and PSA. A p-value of 0.05 or less was considered a statistically significant correlation.

Analysis of variance (ANOVA) was conducted to determine the amount of

variability in IGF-I that could be explained by supplementation with GT after accounting

for the possible confounders: age and BMI. ANOVA was applied only to the separate

treatment arms. A p-value of 0.05 or less was considered statistically significant.

To address any synergistic or antagonistic effect between GT and FO in the

GTFO group we used an orthogonal linear contrast.

Exploratory Analyses

To address our first exploratory aim we conducted t-tests to determine if there

was a statistical difference in IGF-I concentrations between the placebo and FO groups at

pre-intervention, post-intervention and in pre to post-intervention change. A p-value of

0.05 or less was considered statistically significant.

After deciding that we could consider FO supplementation as additional PP, we

combined treatment arms (as mentioned previously) and performed the same t-tests and

Wilcoxon tests on them in the manner originally mentioned in the primary analyses

section for the *separate arms*.

Table 4: Outcome Variables Measured for Selected Groupings

All Participants n=74	GT n=13	GTFO n=13	GT/GTFO* n=26	PP n=24	FO n=24	PP/FO* n=48
Baseline IGF-I			Baseline PSA		Age at entry	
Post-Treatment IGF-I			Post-treatment PSA		BMI at entry	
Pre-post Change in IGF-I			Pre-post Change PSA		Total pill counts	
			CaP Dx (if applicable)		Treatment Duration	

*Indicates combined treatment arms

CHAPTER 5: RESULTS

Descriptive Analyses

Descriptive Statistics: General Participant Characteristics

A total of 74 male participants were included in the final statistical analyses. Participant characteristics are displayed in Table 5. All pre-post differences were calculated by subtracting pre-values from post-values. This was to simply illustrate decreases and increases in an easier to read format.

The mean age of the participants at first biopsy was 62.70 ± 0.73 (from here on in given as the mean ± standard error of the mean; $\bar{x} \pm SEM$), with a range of 50 to 78 years (see Figure 4). The mean body mass index (BMI) at first biopsy was 28.47 ± 0.50 with a range of 21.6 to 43.7 (see appendix E for an explanation of BMI).

Number of treatment days and pill counts were assessed in all participants for compliance. Mean pill count for all participants was 277.46 ± 7.99 with a range of 57 to 540. Mean treatment days was 106.12 ± 2.81. This translates to 15.16 weeks. Only 5 participants had treatment periods less than planned minimum of 12 weeks. Of these 5 participants, 3 made it to 11 weeks and only 2 stopped treatment at 4 and 5 weeks respectively.

While all participants enrolled initially had a negative diagnosis of CaP, some did have a positive diagnosis at repeat biopsy. As previously, mentioned the repeat biopsy occurred within 72 hours post-treatment. Of the 74 men analyzed in this study 13 had a diagnosis of CaP. An additional 12 had a diagnosis of prostatic intraepithelial neoplasia (PIN). PIN is precancerous or carcinoma in situ. The remaining 49 men maintained a benign status post-treatment.

Gleason score was also measured in all 13 men positive for CaP. Of these men 1

had a score of 0; 10 a score of 6; and 2 a score of 7. When CaP incidence was analyzed

by separate treatment arms, only the GT arm had 0 diagnoses. The GTFO arm had 15%

incidence, while the PP and FO groups had 21% and 25% respectively. Incidences of PIN

were found in all arms: 4% in PP, 15% in GT, 31% in GTFO, and 21% in FO. When

groups were combined GT+GTFO had 8% incidences of CaP; and 23% of PIN. PP+FO

had 23% incidences of CaP and 12% of PIN.

Table 5 – Characteristics for All Participants (n=74)

Participant Characteristics			
Baseline			
	N	**Mean±SEM**	**Range**
Age at Dx* (years)	74	62.70±0.73	50- 78
BMI*	74	28.47±0.50	21.6- 43.7
IGF-I (ng/mL)	74	152.92±4.79	82.71 - 279.6
PSA (ng/mL)	74	6.20±0.43	0.13- 19.4
Post-Treatment			
IGF-I (ng/mL)	74	166.99±6.73	74.59- 338.7
PSA (ng/mL)	72*	7.14±1.07	0.18- 73.27
Pre-Post Differences			
IGF-I (ng/mL)	74	14.07±4.72	-84.2- 146.7
PSA (ng/mL)	72*	0.93±1.02	-12.3- 67.84

*PSA values were not available for 2 participants post-treatment

Figure 4- Age and BMI distributions for All Participants

Age Distribution for All Participants(n=74)

BMI for All Participants
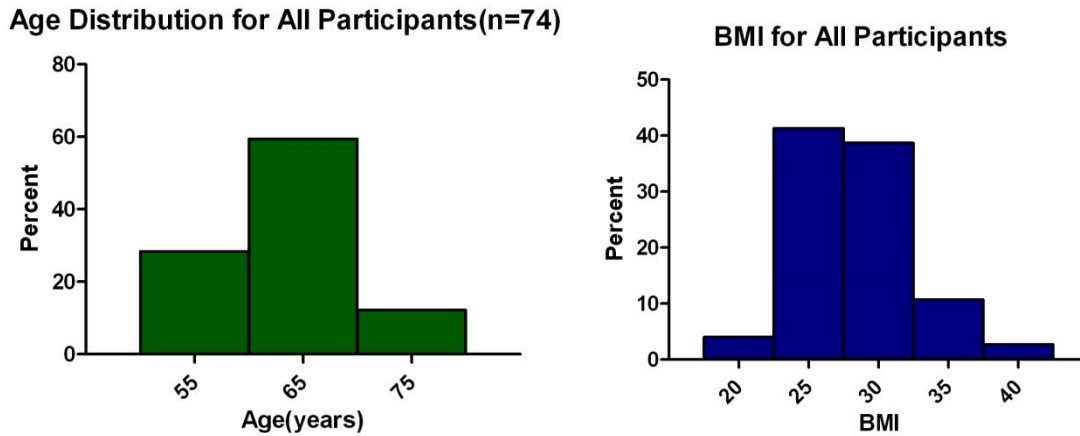
Missing Data

   As previously mentioned post-treatment PSA values were missing for two participants. Also of the 75 paired samples originally tested, one pair was removed from the final analysis because the pre-treatment IGF-I value was not obtained in the initial assay. While the sample was re-run in the second assay, because of the additional freeze-thaw cycle it was left out because sample degradation could not be ruled out.

Descriptive Statistics: IGF-I Concentrations

   Mean IGF-I concentration for all 74 participants at baseline was 152.92 ±4.79 ng/mL and ranged from 82.70 to 279.6 ng/mL. Mean IGF-I post treatment was 166.99±6.73 ng/mL and ranged from 74.59 to 348.7 ng/mL. This represents a mean increase of 14.07±4.72 ng/mL.

   Figure 6 demonstrates pre-post mean IGF-I concentrations divided by treatment arm. For the initial statistical analysis only GT (n=13) and PP (n=24) treatment arms were considered. In the GT arm, mean IGF-I decreased from 143.07±9.37 ng/mL at

baseline to 135.48±9.72 ng/mL post-treatment. This represents a mean difference of -7.59±5.30 ng/mL. The PP treatment arm saw an increase in IGF-I from 152.79±8.85 ng/mL at baseline to 170.12±11.86 ng/mL post-treatment. This is a difference of 17.32±9.09 ng/mL.

While the FO and GTFO groups were not included in the first statistical analysis, the IGF-I concentrations are also listed in tables 7-8. Both the FO and GTFO groups saw increases in IGF-I from baseline to post treatment. The GTFO group increased from 150.65±13.59 to 165.18±17.32 ng/mL; a difference of 14.53±12.77 ng/mL. The FO group increased from 159.62±8.07 to 181.92±12.54 ng/mL; a difference of 22.3±8.20 ng/mL.

For the combined analysis, the GTFO group was incorporated into the GT group and the FO group incorporated into the PP group. With combined means, the GT+GTFO group saw a slight increase in mean IGF-I from baseline to post-treatment of 146.86±8.12 to 150.833±7.13 ng/mL (see table 10). The combined FO+PP group saw a larger increase of 156.21±5.95 to 176.02±8.58 ng/mL (see table 11).

Statistics: PSA Measurements

While analyzing pre-post differences in PSA was not one of our aims, the descriptive data was included for the reader's interest. PSA was measured in all 74 participants at baseline.. At post-treatment only 72 measurements were attainable. Mean baseline PSA was 6.20 ± 0.43 ng/mL and ranged from 0.13 to 19.4 ng/mL. Post-treatment mean PSA was 7.14±1.07 ng/mL and ranged from 0.18 to 73.27 ng/mL. This represents a mean pre-post of 0.93±1.01 ng/mL (see table 5).

For the first analysis of separate treatment arms, PSA was considered for both GT and PP arms. Mean PSA in the GT arm decreased from 6.11±0.46 to 5.92±0.74 ng/mL with an average difference of -0.14±0.71 Mean PSA in the PP arm increased from 7.26±0.91 to 7.41±1.32 ng/mL with a mean difference of 0.08±0.79 ng/mL (see tables 6, 9).

For the second analysis, PSA was considered for combined treatment arms. Mean PSA in the GT+GTFO arm increased from 5.50±0.43 to 8.73±2.76 with a mean difference of 3.78±2.74. The PP+FO arm decreased from 6.58 ±0.61 to 6.29±0.75 ng/mL with a mean difference of -0.32±0.51 ng/mL (see tables 10-11).

Specific Aim 1

Separate Treatment Arms GT (n=13) vs. PP (n=24)

For the first set of primary statistical analyses the GT group and PP groups were analyzed (see Table 12). To address the primary aim to determine if GT supplementation lowered IGF-I in men at high risk for CaP two sample t-tests for equality of means were performed using STATA. Prior to each t-test a two-sample variance equality test was performed. If variances were found to be unequal, t-tests were run with unequal variance using Satterthwaithe's degrees of freedom. Mean IGF-I concentrations will continue to be expressed as mean± standard error of the mean ($\bar{x}$±SD) unless otherwise noted. All t-tests will be of equal variance unless specifically noted. See tables 12 and 13 for all 95% confidence intervals (CI). All p-values are one-tailed unless otherwise noted. Two-tailed p-values can also be found for all t-tests in these tables but will only be mentioned in the text if they are relevant. Paired t-tests were also performed for GT and PP groups looking

the significance of pre-post changes separately. These can be found in table 12, however, they are not relevant to any of the hypotheses.

No significant difference in IGF-I was found between GT and PP at baseline (t=-0.07, p = 0.22) Post-treatment IGF, however, was found to be significantly different between GT and PP groups (t=-1.96, p=0.029)

Finally, to test the hypothesis for aim 1, differences in pre-post IGF-I were compared between GT and PP groups. A two-sample variance comparison test found unequal variances. After running a two-sample t-test IGF-I concentrations were found to be significantly reduced in the GT supplemented group compared to the PP group (t=-2.37, p=0.01). A two-tailed significance between GT and PP groups was also found (p=0.02). The 95% CI for this test was (-46.3, -3.52). This suggests that in the target population a mean difference of -46.3 to -3.52 ng/mL IGF-I can be expected 95% of the time between the pre-post differences of GT and PP supplemented groups. Therefore, we will reject the null hypothesis that GT does not lower IGF-I versus PP.

After visually assessing the individual changes in the GT and PP groups (see figures 16-19), we noted a few extreme increases in IGF-I in the PP group that were over 100 ng/mL. We decided these values clearly could have skewed the mean and thus the results of the t-tests. We also noted a non-symmetric distribution in the PP group. To test for normality in both group we applied the Shapiro-Wilk test to pre-, post-, and pre-post changes for both groups. All three distributions in the GT group were found to be normal (p = 0.28, 0.61 and 0.91 respectively). All three distributions in the PP group were found to violate normality (p = 0.02, 0.007, and 0.0003 respectively). After careful consideration, the non-parametric Wilcoxon rank-sum test was employed to verify the

latter findings of the t-tests. While the distributions of the pre-post changes were fairly

symmetric in both the GT and PP groups, the separate pre- and post- distributions were

not symmetric in all cases. Wilcoxon rank-sum tests, in this type of case are desired

because they measure whether or not there is a distribution shift in the treatment group.

Extreme values do not skew the results because they are ranked. In our case, a one-sided

hypothesis was employed. The null hypothesis was that the GT distribution was equal to

the PP distribution. The one-sided alternative hypothesis was that the GT distribution

would be less than the PP distribution or "shifted" to the left. Two-sided significance was

noted but not required for our criteria.

After running the Wilcoxon rank-sum test to compare pre-post changes between

the GT (n=13) and PP (n=24) groups a significant difference was found between the two

groups. The pre-post changes were significantly lower in the GT group compared to

placebo (p=0.017). Two-sided significance was also obtained at p=0.03.

Table 12- Table of T-test Performed on IGF-I Concentrations for GT (n=13) and PP (n=24) groups

Groups Being Tested	t-test for equality of means (α=.05)								
	Variance Test (α=.05)	Mean Diff	Std. Error Diff	t	Df	p-value 1- tail	p-value 2-tail	95% Confidence Interval of the Difference	
								Lower	Upper
GT vs PP: Baseline	Equal	-9.73	13.89	-0.07	35	0.22	0.49	-37.92	18.47
GT vs PP: Post-treatment	Equal	-34.63	17.69	-1.96	35	0.03	0.06	-70.55	1.28
GT vs PP Post-Pre	Un-equal	-24.91	6.44	-2.37	33.8	0.01	0.02	-46.3	-3.52
GT-pre vs GT-post (paired)	n/a	-7.59	5.3	1.43	12	0.09	0.18	-19.14	3.97
PP-pre vs PP-post (paired)	n/a	17.32	9.09	-1.91	23	0.04	0.07	-1.48	36.12

Figure 9 – Initial Analysis: GT vs. PP
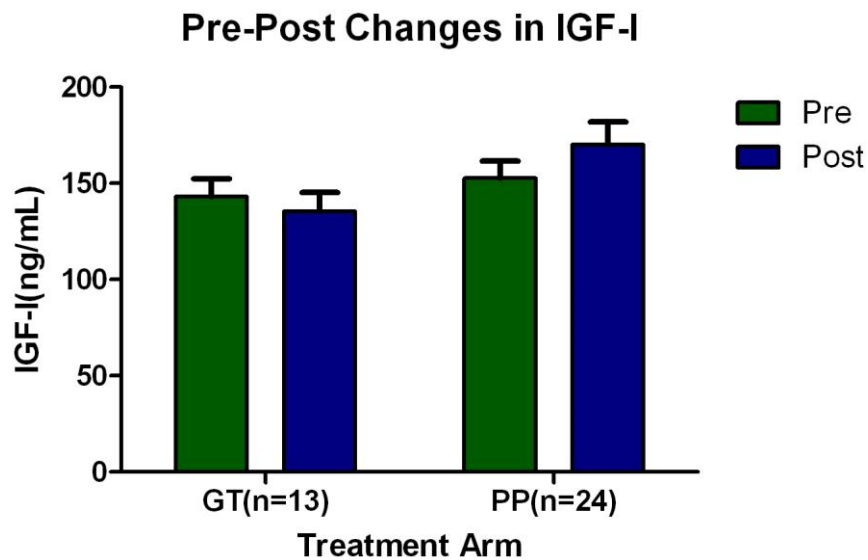
Specific Aim 2

Variability in IGF-I Due to GT Supplementation

To measure the variability in IGF-I due to GT supplementation both one- and two-way analyses of variance (ANOVA) were employed (see Table 13). Post-treatment IGF-I concentrations were the response variable. One-way ANOVA was used to assess variability due to GT supplementation alone. Two-way ANOVA was employed to test for any interaction between age and treatment group or BMI and treatment group. After interactions were not found, two-way ANOVA was then used to see if age or BMI confounded the effect of GT on IGF-I, both independently or when both were added to the model. These tests were also repeated using the combined treatment arm model that included the GTFO and FO arms (see exploratory aim 2 for explanation of combined treatment arms).

The first ANOVA looked at the GT and PP groups. Post-treatment IGF-I concentrations for both groups (n=37) were combined into a single response variable. A second categorical variable was created to denote treatment arm. IGF-I concentrations were assessed for normality using the Shapiro-Wilk test on both GT and PP groups (alpha≥0.05 was considered normal). While normality was met in the GT group (p=0.61), it was not for the PP group (p=0.007). Next using the ladder-of-powers (gladder) command in STATA we looked at possible transformations for the IGF-I variable. The logarithmic transformation was chosen since it was the most symmetric and least skewed. A new transformed variable was thus created, called logIGF-I. The Shapiro-Wilk test was once again tested and normality was met for both GT (p=0.47) and PP (p=0.41) groups. Homoscedasticity was checked using the Bartlett's test for equal variances. Using the

logIGF-I variable, both GT and PP groups were found to have equal variances (chi^2= 0.2, p=0.66). Next a one-way ANOVA was run using logIGF-I. A significant difference in post-treatment IGF-I was found between GT and PP (F=4.26, p=0.047). This was expected as a similar difference was found earlier using a t-test (see table 12).

Age and BMI were both converted from continuous to categorical variables. Previous literature has already divided normal IGF-I levels into specific age categories. These already established ranges by Blum and Breier were used for age categories in this model.[163] Participant ages ranged from 50-78 so the following categories were used: 50-59; 60-69; 70-79; and >80. Normality was found for all categories except 70-79. This violation was disregarded because of the small sample size (n=4) of the category.

Likewise, BMI was divided into several categories, using the complete World Health Organization (WHO) BMI scale. BMI categories were <18.5 (underweight); ≥18.5 to <25 (normal weight); ≥25 to <30 (overweight); ≥30 to <35 (stage I obesity); ≥35 to 40 (stage II obesity); and ≥40 (stage III obesity). Normality was assessed and met for each category using the Shapiro-Wilk test.

Next using the logIGF-I variable a one-way ANOVA was run with only age as independent variable. No significant differences in mean logIGF-I concentrations were found between age categories. (F=1.46, p=0.25) A two-way ANOVA also demonstrated that age was not a significant effect variable when included in the full model with logIGF-I and treatment arm (F=1.50, p=0.24).

Following this an interaction variable was created to look for interaction between age and treatment arm. Under the full model the interaction term was found not to have a significant effect (F=0.1, p=0.90).

Since no interaction was found age was also checked to see if it was a confounding variable and should be included in the model. To do this crude (without age) and adjusted (with age) models were compared. This was accomplished using the *predict estmean, xb* command. First the unadjusted cell means were computed. Then the adjusted cell means were computed assuming age was in the model. A difference of 10% or more between crude and adjusted cell means was considered sufficient to keep the confounder in the model. As mentioned by Hosmer and Lemeshaw, 10% is a commonly used cut-off for confounding.[162] When crude and unadjusted cell means were compared, there was less than 10% difference in all cases. This suggested age was not a confounder .[162]

The same procedure was applied to the variable BMI. The continuous BMI variable was changed into a categorical variable using the established BMI categories. Normality was checked and was not violated in any of the BMI categories. Using two-way ANOVA, neither BMI (F=0.10, p=0.91) nor BMI's interaction with treatment arm (F=0.52, p=0.5993) were significant effects on the mean response.

Since no interaction was found BMI was also checked to see if it was a confounding variable and should be included in the model. To do this, crude (without BMI) and adjusted (with BMI) models were compared. First the unadjusted cell means were computed. Then the adjusted cell means were computed assuming BMI was in the model. When adjusted and unadjusted cell means were compared there was less than 10% difference between them in all cases, suggesting BMI was not a confounder.

The final model was thus a one-way ANOVA incorporating only the logIGF-I and treatment arm variables (see table 13). As mentioned above, a significant difference between factor level means (GT and PP) was found (.047). Final R^2 value was 0.11 using

the regression output provided by STATA's *anova* command. Thus, in conclusion, 11%

of the variability in IGF-I can be explained by GT supplementation. It should be noted

that for the purposes of this thesis, ANOVA procedure was not performed for the

combined treatment arms. Below we have listed the final ANOVA model with the

required assumptions listed, as well as the ANOVA table (table 13).

ANOVA model: $y_{ij} = \mu_i + e_{ij}$ $i = 1\ldots37$ $j = 1, 2$

Assumptions:

1. Each probability distribution of the dependent variable (logIGF-I) at each

    factor level (GT and PP) is normal.

2. Each probability distribution of the dependent variable (logIGF-I) at each

    factor level (GT and PP) has the same variance.

3.   Random samples have been selected from each of 37 populations.

    That is, the responses of each factor level are random selections

    from the corresponding probability distribution and are independent

    of the responses for any other factor level.

Table 13 – One-way ANOVA Table for post-treatment IGF-I by Separate

Treatment Arms (GT and PP)

Root MSE = 0.299 $R^2 = .11$

Source	Partial SS	Df	MS	F	Prob >F
Between Groups	0.38	1	0.38	4.26	0.046
Within Groups	0.38	35	0.09		
Total	3.52	36			

Secondary Aim - Association between IGF-I and PSA

Our secondary aim was to see if there was an association between circulating concentrations of IGF-I and PSA (see figures 6-8). To accomplish this, we used both pairwise and partial correlation tests in STATA. For correlations between baseline IGF-I and PSA we used simple pairwise correlations. Due to the hypothesized effect for green tea supplementation we used partial correlations for post-treatment and pre-post change of IGF-I and PSA. Partial correlations were conducted under two models. The first was conducted with dummy variables for the separate treatment arms (GT,PP,FO,GTFO). The second was conducted after combining treatment arms and assuming FO was an additional placebo. Dummy variables were not required as the combined arms variable was binary.

It is important to note baseline PSA values were obtained for all 74 participants. Post-treatment PSA values were obtained for only 72 participants. Thus correlations in pre-post differences are also only measured for 72 participants.

A weak, yet significant negative correlation (see figure 9) was found between IGF-I and PSA at baseline (r= -0.2408, p=0.038). All correlations between post-treatment IGF-I and PSA as well as correlations between pre-post changes in IGF-I and PSA were positive, but non-significant. At post-treatment, the partial correlations adjusted for separate treatment arms and combined treatment arms were (r=0.11, p=0.34); and (r=0.13, p=0.28). Pre-post difference partial correlations were similar at (r=0.12=0.33) and (r=0.15, p=0.22) after adjusting for separate and combined arms respectively. A scatter plot is pictured (figure 10), showing the correlations between post-treatment IGF-I and PSA after stratification by treatment arm. Please see the "additional figures" section

for the additional stratified scatter plots.

**Figure 10 – Scatterplot, IGF-I vs. PSA at Baseline**
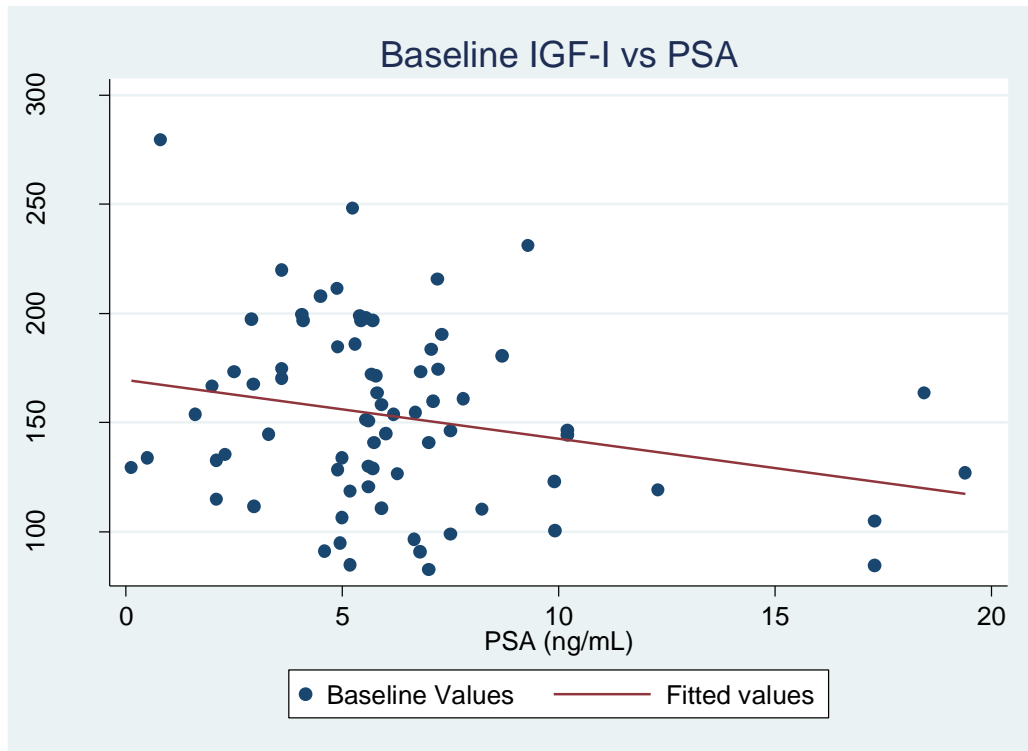
Figure 11 – Post –treatment IGF-I vs. PSA – Stratified by Treatment Arm
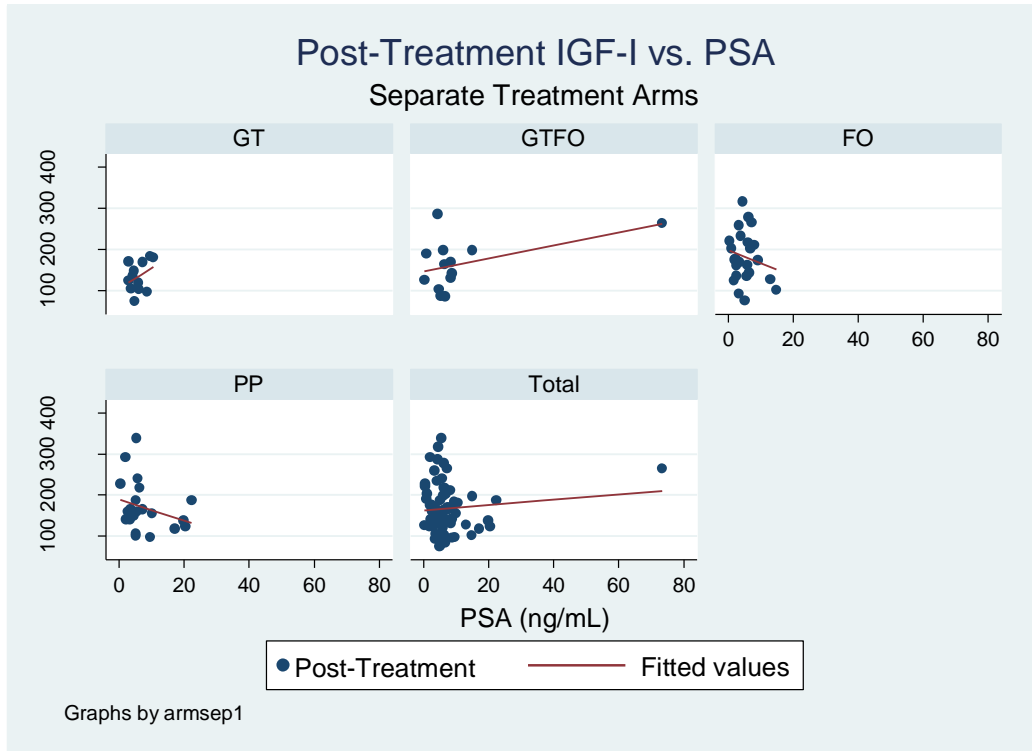
Secondary Aim: Interaction between FO and GT Supplementation

As this study included a GT+FO treatment arm, we ran analyses to determine if FO and GT had either a synergistic or antagonistic effect on IGF-I in this group. A basic one-way ANOVA was combined with an orthogonal linear contrast using STATA. The outcome variable was pre-post differences and the grouping variable designated treatment arm. The *lincom* command on STATA requires that group variances are pooled. As the GT arm had significantly different variance compared to the other three arms, log transformation was employed allow for variance pooling. After running a one-way ANOVA, an orthogonal contrast was run to determine if interaction existed between FO and GT. Table 14 below illustrates the coefficients used to run the linear contrast.

Table 14: Coefficients for Linear Contrast

Contrast	PP	FO	GT	GTFO
Interaction of GT and FO	-1	+1	+1	-1

* $\mu_{1=\text{GT}};\ \mu_{2=\text{GTFO}};\ \mu_{3=\text{FO}};\ \mu_{4=\text{PP}}$

After running the linear contrast, no interaction was found between GT and FO

(p>|t| = 0.20). Therefore GT and FO have neither a synergistic nor an antagonistic effect

on IGF-I. The results are illustrated below.

Table 15: Linear Contrast Test Results

H_0: $\mu_1 - \mu_2 + \mu_3 - \mu_4 = 0$ $\qquad$ H_A: $\mu_1 - \mu_2 + \mu_3 - \mu_4 \neq 0$

Coef.	SEM	t	P>|t|	95% Confidence Interval
-0.13	0.10	-1.29	0.20	{-0.32, 0.07}

Exploratory Aim 1: FO's influence on IGF-I Concentrations

For our first exploratory aim, we looked at whether or not FO supplementation

had any significant influence on IGF-I concentrations versus placebo. This aim also

served to test whether or not the FO treatment arm could be considered equivalent and

effectively combined with the PP treatment arm (see exploratory Aim 2). To do this we

simply compared the pre-post differences in IGF-I between the FO and PP groups. Just as

in our primary analyses, we also compared baseline and post-treatment differences

between the two groups. For each case two-sample t-tests were performed.

At baseline, no significant difference in IGF-I concentrations was found between

the FO and PP groups (t=0.57, p=0.57). After the treatment period, there was still no

significant difference between groups (t=0.68, p=0.50). Finally, pre-post changes in IGF-

I in the FO supplemented group were not different than placebo (t=0.41, p=0.69) As we

did not expect a change in IGF-I in either direction, these p-values reported are two-

sided. See Table 16 below and Figure 12 on the next page.

Table 16- Table of T-test Performed on IGF-I Concentrations for FO (n=24) and PP (n=24) groups

Groups Being Tested	Variance Test (α=.05)	t-test for equality of means (α=.05)							
		Mean Diff	Std. Error Diff	t	Df	p-value 1 tail	p-value 2 tail	95% Confidence Interval of the Difference	
								Lower	Upper
FO vs PP: Baseline	Equal	6.83	11.98	0.57	46	0.7142	0.5715	-17.28	30.94
FO vs PP: Post-	Equal	11.81	17.26	0.68	46	0.7513	0.4974	-22.94	46.55
FO vs PP Pre-post	Equal	4.98	12.24	0.41	46	0.657	0.686	-19.66	29.62
FO-pre vs FO-post (paired)	n/a	-22.3	8.2	-2.72	23	0.0061	0.0122	-39.27	-5.33
PP-pre vs PP-post (paired)	n/a	-17.32	9.09	-1.91	23	0.0347	0.0693	-36.12	1.48

Figure 12 – Exploratory Analysis - Fish oil vs. Placebo
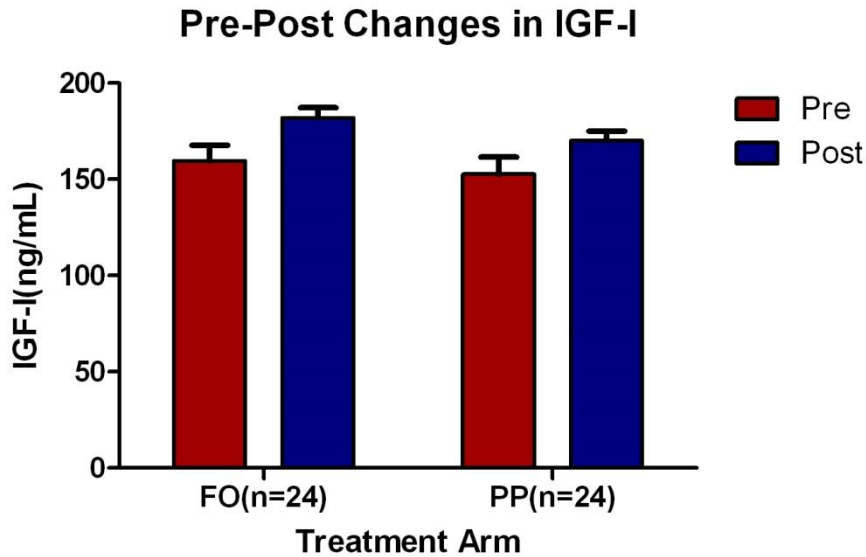
Exploratory Aim 2: Combined Treatment Arms

This aim was dependent on the previous two aims. We previously found that no interaction existed between GT and FO in this study. We also determined that FO had no main effect on IGF-I versus placebo. After running these tests we decided we could consider FO an additional placebo and combine treatment arms to check for the main effect of GT with a larger sample size. Once again, the GT group (n=13) was combined with the GTFO group (n=13) to make a new GT group (n=26). Likewise the PP group (n=24) was combined with the FO group (n=24) to make a new PP group (n=48). The results of the statistical analyses between these two groups are displayed below.

*Combined Treatment Arms- GT+GTFO (n=26) vs. FO+PP (n=48)*

After determining that we could consider FO as an additional placebo, the same t-tests were performed after adding the FO supplemented groups to the GT and PP arms

(see Table 17). Two-sample mean comparison tests between the new GT and PP groups

was conducted with equal variances. At baseline, there was no two-sided (p=0.36) or one

sided (p=0.18) difference between the two treatment groups. After supplementation there

was a significant, one-sided difference between the two groups (p=0.03). After

comparing pre-post changes between the two groups, GT supplementation was found

again, to significantly lower IGF-I concentrations versus placebo (t=-1.67, p=.0495). The

95% CI for this test was (-35.83, 3.15). This suggests that a difference of -35.83 to 3.15

ng/mL IGF-I can be expected 95% of the time between pre-post differences of the GT

and PP groups in the target population. Therefore, we will again reject the null hypothesis

that GT does not lower IGF-I versus PP. A table of the t-tests performed on the combined

treatment groups can be found below.

Table 17- T-test Performed on IGF-I Concentrations for combined groups

GT group (n=26) = GT + GTFO   PP group (n=48) = PP + FO

Groups Being Tested	Variance Test (α=.05)	Mean Diff	Std. Error Diff	t	Df	p-value 1 tail	p-value 2 tail	95% Confidence Interval of the Difference	
								Lower	Upper
GT vs PP: Baseline	Equal	-9.35	10.05	-0.93	72	0.18	0.36	-29.38	10.69
GT vs PP: Post-	Equal	-25.69	13.87	-1.85	72	0.03	0.068	-53.33	1.95
GT vs PP Pre-post	Equal	-16.34	9.78	-1.671	72	0.0495	0.099	-35.83	3.15
GT-pre vs GT-post (paired)	n/a	3.47	7.13	0.49	25	0.32	0.63	-11.2	18.5
PP-pre vs PP-post (paired)	n/a	19.81	6.07	-3.27	47	0.001	0.002	7.61	32.02

The column header "t-test for equality of means (α=.05)" spans the Variance Test through 95% Confidence Interval columns.

Figure 13: Combined Analysis: GT vs. PP
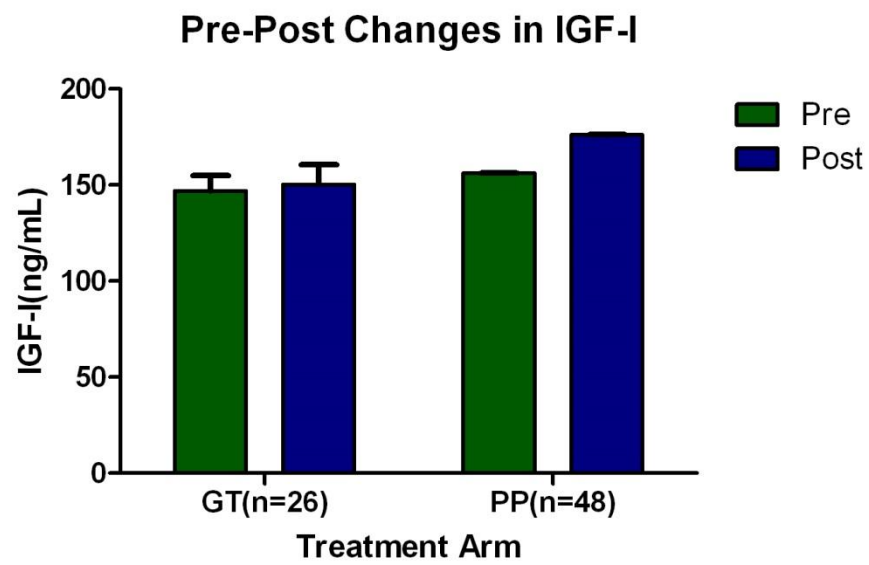
CHAPTER 6: DISCUSSION

Our study demonstrated that GT supplementation lowers IGF-I in men at high risk for CaP. It is both the first placebo-controlled study demonstrating such an effect and the first study investigating the ability of GT to lower IGF-I in men *specifically* at high risk for CaP. We demonstrated this effect in two separate analyses. First, we simply compared the GT only supplemented group to the PP. Both one-and two-tailed differences were demonstrated between the two groups. Neither age nor BMI were found to confound this effect. After demonstrating that FO had no effect on IGF-I in FO arm, and that there was no interaction between FO and GT we combined them into the GT and PP arms respectively. We once again found the lowering effect of GT on IGF-I to be significant compared to PP in the combined arms. As only two other clinical trials have looked at GT's effect on IGF-I in humans we hope these results will fuel more research on GT's effect on the IGF-axis in humans.

The two previous trials covering GT's effect on IGF-I in humans looked at men who were already scheduled for prostatectomy. While these trials provided rationale for conducting this study, they were not conducted on men who could potentially use GT to help reduce CaP risk or hold off CaP development. Our trial had a substantially longer treatment period and was in a population that could practically use GT as a chemopreventive supplement. With an average treatment period of 15.2 weeks,[111-112] it was several times longer than those of Nguyen and McLarty whose ran for an average of 4[112] and 4.9 weeks[111] respectively. Our study was also unique in that we were able to note which participants developed CaP during the treatment period and which treatment group they were assigned too (see appendix D). The GT only supplemented group had no CaP

diagnoses during the trial. In addition the GTFO group had only 2 CaP diagnoses or an incidence rate of 15% for the group. This was a lower percent incidence than both the FO and PP groups. While CaP development is rather complex, these findings are very encouraging for the future use of GT supplements in CaP prevention trials.

Another unique aspect of our trial was that it was originally a 2x2 study comparing separate GT, FO, and GTFO groups to a PP group. We were able to take advantage of this original design to investigate the effects of FO on IGF-I. We were the first randomized, double blind placebo controlled trial to investigate the effects of FO supplementation on IGF-I in men specifically at risk for CaP. Two previous chemoprevention trials, one in CaP patients and another in breast cancer patients, found no significant effect of FO on IGF-I. This study confirms those findings and points to FO having little relevance to IGF-I in humans outside an acute care setting.

After combining treatment arms, our study also had a larger total sample size than McLarty or Nguyen.[111-112] The combined GT treated group in our trial had 26 participants and the PP group had 48. Previous trials both had supplement groups of 24; while McLarty did not have a placebo group[111], Nguyen had a placebo group of 24[112]. It is true our study's most significant results were found using a GT group of 13 and a placebo group of 24. At the same time, the combined treatment arms yielded significant one-tailed results with larger supplement (n=26) and placebo groups (n=48).

In comparing our separate analysis (n=37) to our combined analysis(n=74), there are some important differences to take into account. The GT supplemented group in the separate analysis yielded a mean decrease in IGF-I from baseline (-7.59ng/mL). In the combined analysis the GT group had a slight increase in mean IGF-I from baseline

(3.47ng/mL). This increase is due to the fact that the GTFO group itself had larger increase from baseline of 14.53ng/mL. Likewise, the FO group (22.3ng/mL), which had larger increase than the placebo group (17.3ng/mL), increased the mean of the combined FO/PP group(19.8ng/mL). Therefore, while the combined analysis yielded increases in both GT and PP groups the GT had a significantly lower increase than the PP group.

It is possible to speculate as to why there was an increase in the GTFO group. Research has not yet shown FO to significantly increase IGF-I compared to placebo in humans[136,138], however it has in cattle.[131-132] Given what we currently know, it is extremely doubtful FO has the capacity to reduce IGF-I levels and it may have some small (yet undetermined) capacity to raise IGF-I. While FO may have had an antagonistic effect that was not statistically detectable in the GTFO group, for the purposes of this study we were able to consider it a placebo.

Analysis of variance (ANOVA) demonstrated that post-treatment IGF-I values were neither confounded by age nor BMI; nor was there any interaction between either of these variables and treatment arm. This is important because while block randomization would be expected to account for the effects of age and BMI, both variables have been shown to affect IGF-I values. After regression our final ANOVA model found that 11% of the variability in post-treatment IGF-I was explained by GT supplementation. While 11% is a seemingly small influence, when it is considered that higher IGF-I concentrations are associated with increased risk of CaP an 11% influence could be considered quite remarkable. This is also considering our trial demonstrated a lowering effect of GT on IGF-I.

PSA concentrations, which are presently considered a diagnostic risk factor for

CaP, had a significant but weak inverse correlation with IGF-I prior to intervention. This correlation reversed after intervention and was found to be non-significant. After doing partial correlations to adjust for treatment arm, post-treatment IGF-I values were still not still not correlated with post-treatment PSA. A possible reason for the change in correlation from pre- to post treatment could be the fact that the GT supplements lowered IGF-I. GT may have had little effect on PSA. In addition, five studies now have shown no significant correlation between IGF-I and PSA.[17-21]

Limitations

  This study was slightly underpowered. Post-hoc power calculations were done for both separate and combined treatment arm models. The analysis using only GT (n=13) and PP (n=24) in the separate arms model demonstrated 75% power due to the large difference in pre-post changes between the GT and PP groups. To achieve 80% power one additional GT supplemented participant and four additional placebo supplemented participants would have been required. The combined analysis actually had less power at 53% due to a smaller effect of GT on IGF-I and a slightly greater variance in GT group.

  There are also several limitations related specifically to IGF-I metabolism. GH is known to effect IGF-I and was not measured in any of the participants. Any disorder affecting GH levels that was not noted could have affected IGF-I levels. At the same, at baseline, none of the participants were below the expected IGF-I range for their respective age group. A total of only 6 participants were above their expected IGF-I range for their age group. It would still be wise for future studies to screen for any disorders of the hypothalamic-pituitary axis that may adversely or unpredictably affect

IGF-I levels.

It would also be a good idea for researchers to note any other conditions in participants which could affect IGF-I levels such as liver disease or protein/energy malnutrition. Starvation over several days is also known to effect IGF-I. If any patients had fasted for more than a few days prior to either pre- or post-treatment blood draw, this could have reduced IGF-I levels and confounded the results.[61]

While it is postulated that increases in IGF-I may increase risk for CaP[80], increases in total IGF-I may not be indicative of increases in IGF-I bioactivity. As IGF-I increases cell proliferation only by first activating its receptor (or rarely the IR), bioactivity is certainly an important consideration in IGF-I's relationship to CaP development. Our RIA tests measured total IGF-I only. It is also possible to measure free IGF-I using an enzyme-linked immunoabsorbent assay (ELISA) assay. Free IGF-I also does not guarantee any amount of bioactivity. This is especially due to the fact that free IGF-I has a much shorter half-life than bound IGF-I.[54] It is possible to measure IGF-I bioactivity by using the kinase receptor activation (KIRA) assay. Research on its effectiveness is limited. It has been used in a recently published study on IGF-I bioactivity in centenarians.[161]

Our study unfortunately was not able to fund the testing of IGFBP-3. In future studies it would be recommended that IGFBP-3 be measured if possible. This is because the ratio of IGF-I/IGFBP-3 can roughly illustrate the amount of free IGF-I that is in the blood stream and thus paint a more complete picture of how much IGF-I is available to bind to receptors.[13] As previous research has demonstrated a positive association between the IGF-I/IGFBP-3 ratio and CaP risk, the ratio should always be included in the analysis

if both are tested. The measurement of other binding proteins besides IGFBP-3, again would only add to the participants' "IGF profiles." Lastly, because IGF-II is generally several times higher than IGF-I in the bloodstream throughout life, it should be seriously considered, as research points to its tumorigenic effects.[54]

Individual variations in GT absorption and bioavailability also could not be accounted for. Due to the fact that this study found GT lowered IGF-I, any decreases in GT bioavailability in individual participants it seems were not enough to impact the significance of the results. Researchers may wish to monitor for GI disorders which may manifest during the trial and subsequently decrease GT absorption in the GI tract.

Lastly, this author has had previous experience running RIAs on only one other occasion. While intra-assay CVs were well within goal range and the RIA tests were carried out with extreme care, lack of lab experience does increase the probability of technician error. Having IGF-I tested in a commercial lab, while more expensive, may yield slightly more accurate results.

Future Avenues of Research

The amount of supplemented GT in humans has consistently been in the range of 600-800mg per day.[111-112] If possible, study authors may want to investigate dose dependent effects from 200-800mg. It is also important to note that GT supplements should be given at least twice a day for maximum effect considering that the half-life of GT is relatively short.[111]

Fresh infusions of GT from tea leaves contain compounds which are not present in GT supplement capsules.[99] Therefore researchers may also want to design a

study that compares the relative effects of GT supplements to daily green tea infusions calculated to have equivalent antioxidant activity. Not all men who wish to supplement may want to consume GT in the pill form and may prefer drinking liquid tea.

Treatment periods should be at least one month as current literature shows effects on IGF-I in as little as 30 days.[111] Minimum treatment periods of 12 weeks seem reasonable.

So far GT's effect on IGF-I has not been studied in normal healthy adult males. A study comparing the effects of GT on IGF-I between healthy males and males at risk for CaP may yield interesting results. GT may have more or less of a lowering effect on IGF-I in healthy males.

Research Looking at Other Nutritional Factors and IGF-I

FO supplementation should be further investigated for its effect on IGF-I in a manner similar to GT supplement trials. Double-blind, randomized, placebo-controlled trials using varying levels of FO supplementation are recommended. Some research indicates that FO may increase IGF-I[131-132]. While this trial and two others have shown no significant effects, larger trials may demonstrate more significant results. As FO supplements are becoming more and more popular, especially in the elderly, their effects on IGF-I would be worth investigating.

As plasma PUFA's (including EPA and DHA) were measured in this study, it might also be worth measuring any possible associations between EPA, DHA and IGF-I in the select participants for whom these measurements were taken. The same could be done for other fatty acid measures in these participants.

Considering in this study protein, calories, and dairy consumption were measured

using DHQ, measuring their association with IGF-I at baseline is recommended. As

research has suggested dairy or calcium may increase IGF-I its role in IGF-I regulation

should be further studied.[78]

RESEARCH FUNDING

ADDITIONAL TABLES

Table 6: Characteristics for GT (n=13)

Green Tea						
Baseline	**n**	**Mean**	**SEM**	**Min**	**Max**	**Range**
Age (entry)	13	63.92	2.01	50	76	26
BMI (entry)	13	29.4	0.95	24.4	36.6	12.2
IGF-I	13	143.07	9.37	90.92	190.25	99.33
PSA	13	6.11	0.46	2.96	8.7	5.74
Post-Treatment						
IGF-I	13	135.48	9.72	74.59	183.4	108.81
PSA	12	5.92	0.74	2.84	10.5	7.66
Pre-Post Differences						
IGF-I	13	-7.59	5.30	-41.9	34.75	76.65
PSA	12	-0.14	0.72	-5.8	3	8.8

Table 7: Characteristics for GTFO (n=13)

Green tea + Fish oil						
Baseline	**n**	**Mean**	**SEM**	**Min**	**Max**	**Range**
Age (entry)	13	62.54	2.16	50	78	28
BMI (entry)	13	29.37	1.09	24.4	35.8	11.4
IGF-I	13	150.65	13.60	82.71	248.05	165.35
PSA	13	4.88	0.72	0.13	10.2	10.07
Post-Treatment						
IGF-I	13	165.18	17.32	85.75	286.8	201.06
PSA	13	11.33	5.26	0.18	73.27	73.09
Pre-Post Differences						
IGF-I	13	14.53	12.77	-84.2	112.05	196.25
PSA	13	6.44	5.17	-2	67.84	69.84

Table 8: Characteristics for FO (n=24)

Fish Oil						
Baseline	**n**	**Mean**	**SEM**	**Min**	**Max**	**Range**
Age (entry)	24	62.96	1.09	55	72	17
BMI(entry)	24	27.95	1.04	21.6	43.7	22.1
IGF-I	24	159.62	8.07	84.50	219.8	135.31
PSA	24	5.91	0.82	2	17.3	15.3
Post-Treatment						
IGF-I	24	181.92	12.54	76.16	317.2	241.04
PSA	24	5.21	0.71	0.5	14.8	14.3
Pre-Post Differences						
IGF-I	24	22.30	8.20	-50.25	120.4	170.65
PSA	24	-0.70	0.65	-12.3	4.2	16.5

Table 9: Characteristics for PP (n=24)

Placebo						
Baseline	**n**	**Mean**	**SEM**	**Min**	**Max**	**Range**
Age (entry)	24	61.88	1.23	51	78	27
BMI(entry)	24	28.00	0.86	21.6	38.4	16.8
IGF-I	24	152.80	8.85	96.68	279.6	182.92
PSA	24	7.26	0.91	0.8	19.4	18.6
Post-Treatment						
IGF-I	24	170.12	11.86	96.5	338.7	242.2
PSA	23	7.41	1.32	0.46	22.35	21.89
Pre-Post Differences						
IGF-I	24	17.32	9.09	-51.1	146.7	197.8
PSA	23	0.079	0.79	-8.3	9.9	18.2

Table 10: Combined Arms – GT+GTFO (n=26)

Green Tea (GT+GTFO)						
Baseline	**N**	**Mean**	**Std. Error**	**Min**	**Max**	**Range**
Age at Dx*	26	63.23077	1.452115	50	78	28
BMI*	26	29.38462	0.706316	24.4	36.6	12.2
IGF-I	26	146.859	8.124044	82.705	248.05	165.345
PSA	26	5.496923	0.434706	0.13	10.2	10.07
Post-Treatment						
IGF-I	26	150.3313	10.17318	74.59	286.8	212.21
PSA	25	8.7288	2.761462	0.18	73.27	73.09
Pre-Post Differences						
IGF-I	26	3.472308	7.125744	-84.2	112.05	196.25
PSA	25	3.2844	2.743622	-5.8	67.84	73.64

Table 11: Combined Arms FO+PP (n=48)

Placebo (FO + PP)						
Baseline	**N**	**Mean**	**Std. Error**	**Min**	**Max**	**Range**
Age at Dx*	48	62.41667	0.816949	51	78	27
BMI*	48	27.97917	0.667661	21.6	43.7	22.1
IGF-I	48	156.208	5.945823	84.495	279.6	195.105
PSA	48	6.584583	0.614165	0.8	19.4	18.6
Post-Treatment						
IGF-I	48	176.0184	8.581906	76.16	338.7	262.54
PSA	47	6.288298	0.750664	0.46	22.35	21.89
Pre-Post Differences						
IGF-I	48	19.81042	6.066976	-51.1	146.7	197.8
PSA	47	-0.31723	0.505675	-12.3	9.9	22.2

ADDITIONAL FIGURES

Figure 5-   Age Distribution by Separate Treatment Arms
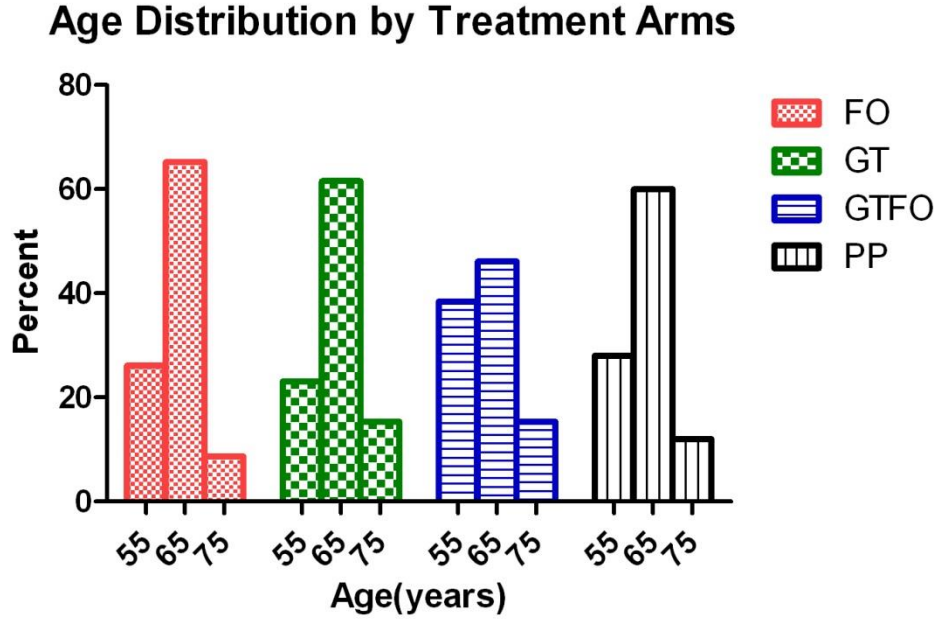
Figure 6 – Age Distribution by Combined Treatment Arms

GT= GT+GTFO   PP = FO+PP
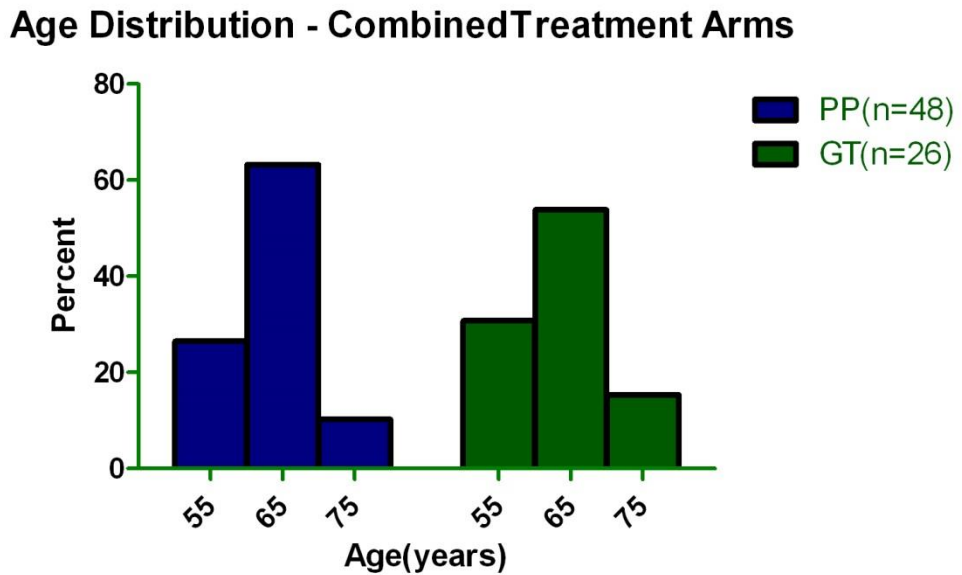
Figure 7: BMI Distribution by Separate Treatment Arms

BMI Distribution by Treatment Arms
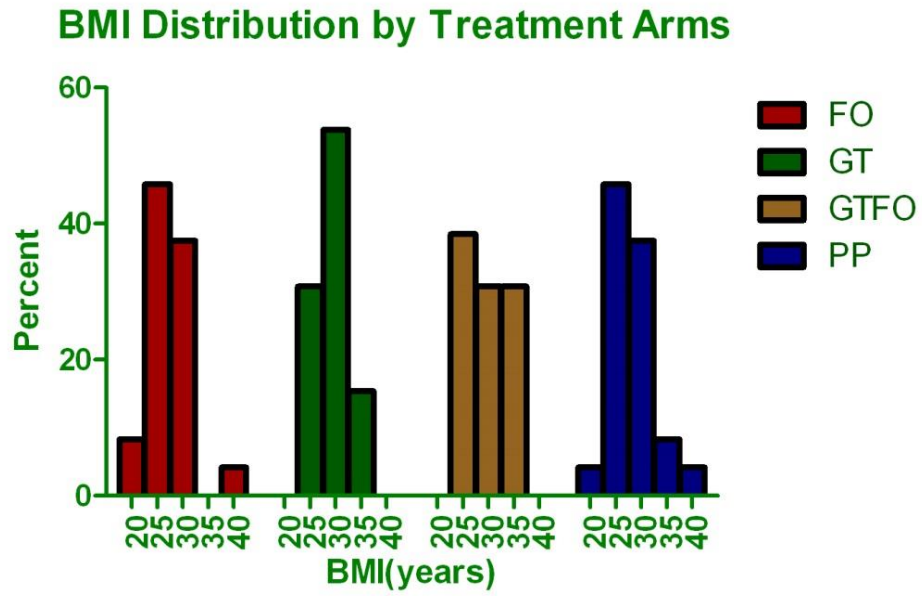
Figure 8: BMI Distribution by Combine Treatment Arms

BMI Distribution - CombinedTreatment Arms
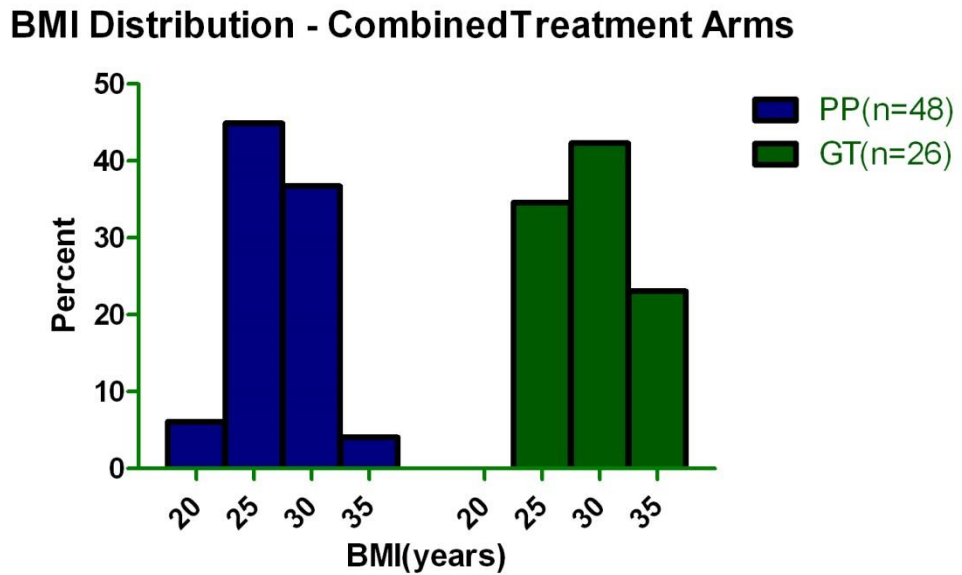
Figure 14: Post-Treatment IGF-I vs. PSA Combined
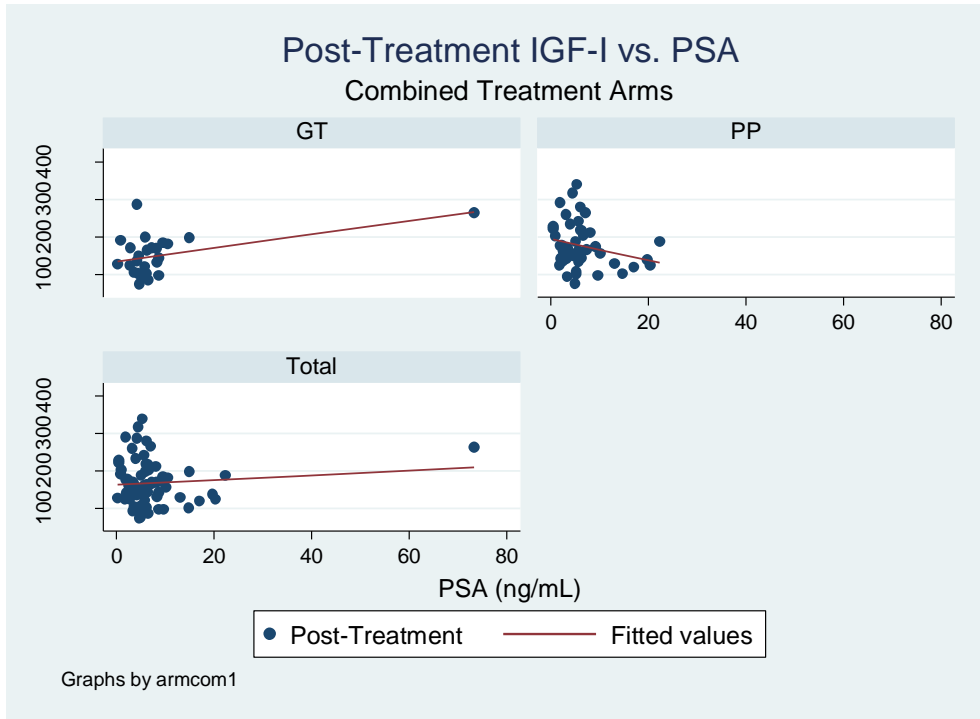
Figure 15: IGF-I vs. PSA Pre-post Changes – Separate Arms
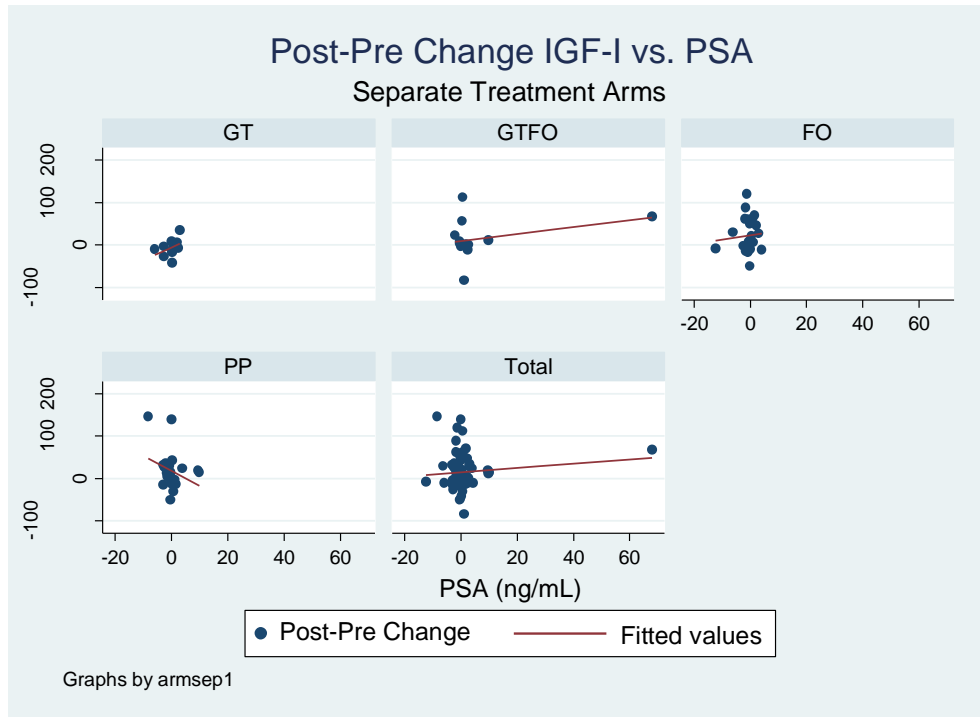
Figure 16: IGF-I vs. PSA Pre-post Changes – Combined Arms
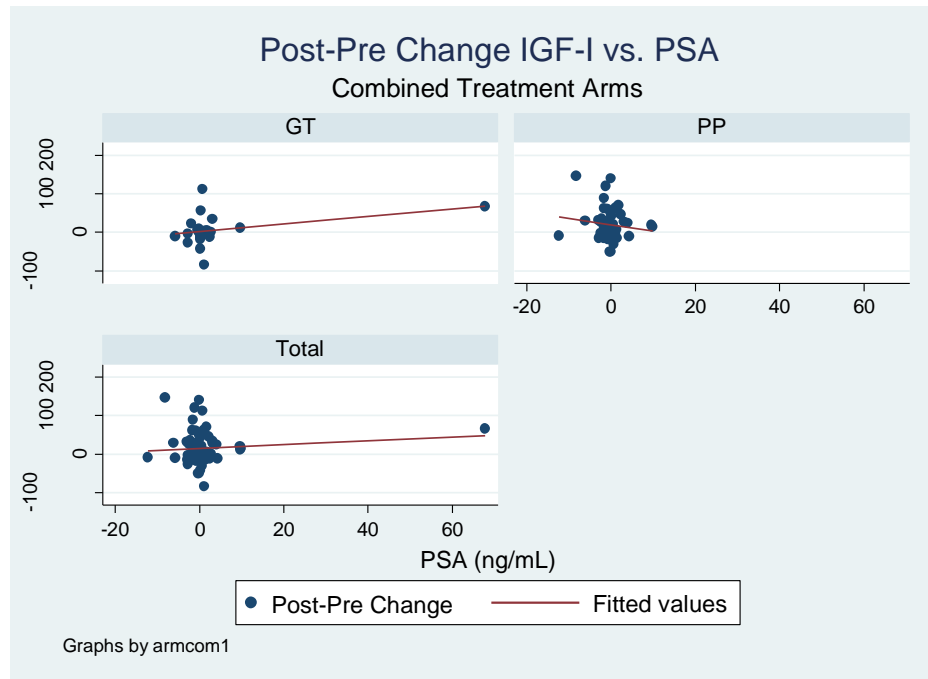
Figure 17: Individual Pre-post Changes in IGF-I, GT Group
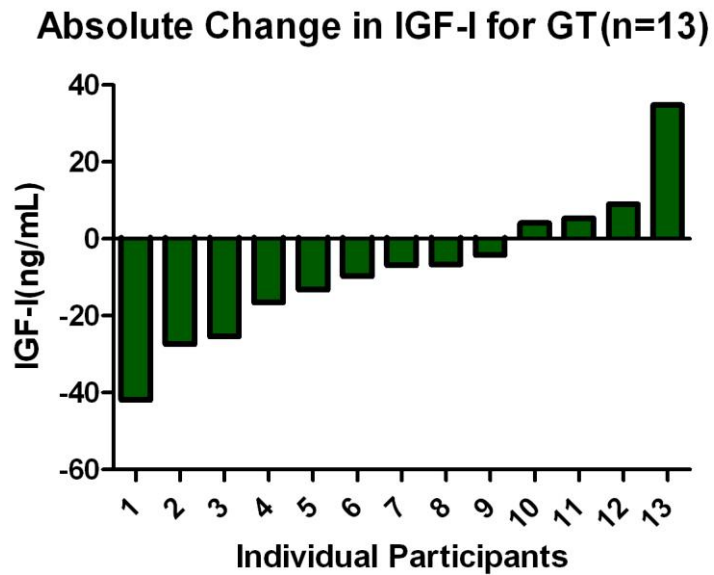
Figure 18: Individual Pre-post Changes in IGF-I, PP Group

Absolute Change in IGF-I for PP(n=24)
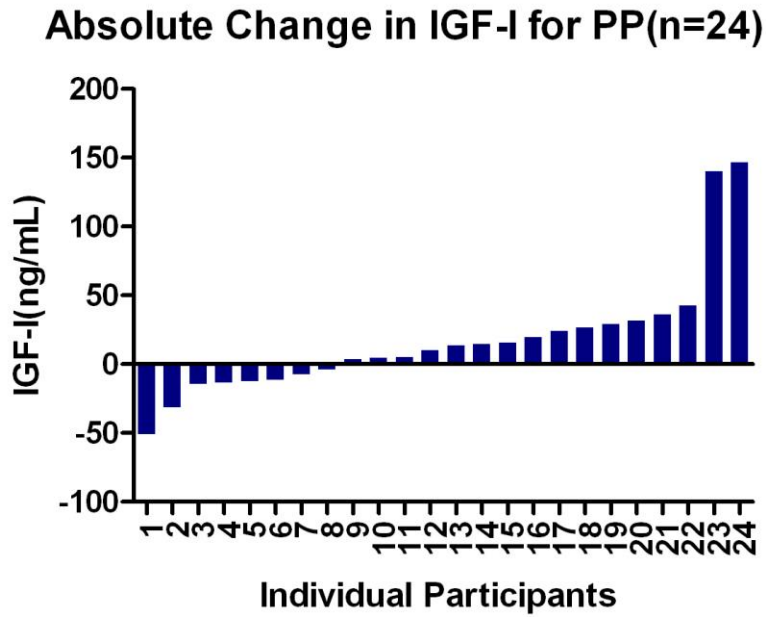
Figure 19: Individual Pre-post Changes in IGF-I, FO Group

Absolute Change in IGF-I for FO(n=24)
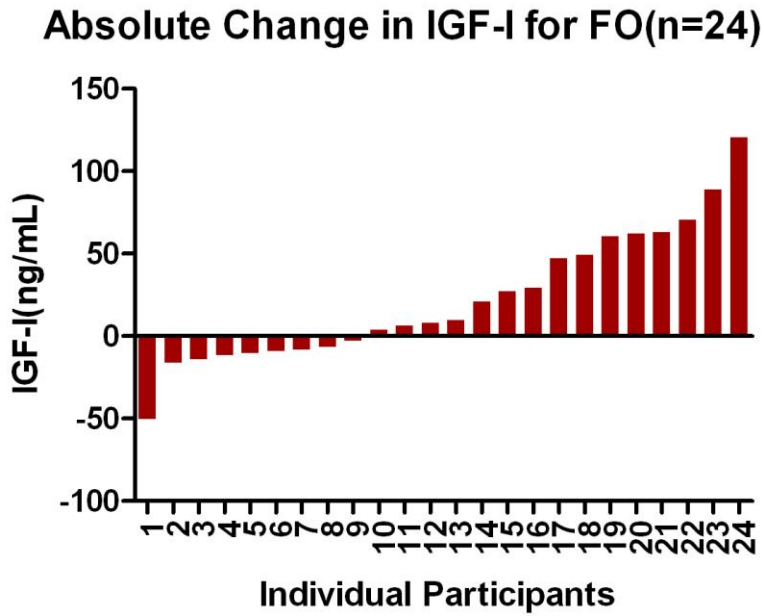
Figure 20: Individual Pre-post Changes in IGF-I, GTFO Group
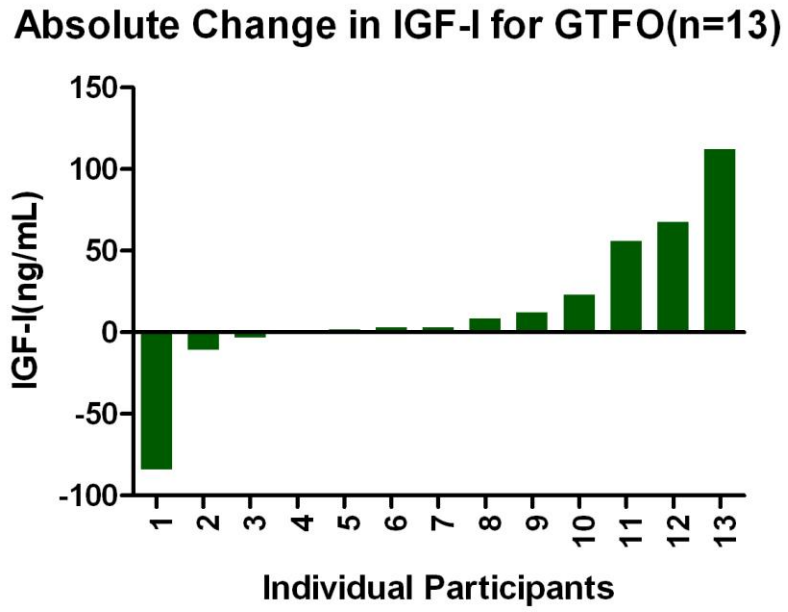
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APPENDIX A: Informed Consent-Most Recent Sample

OHSU **Oregon Health & Science University** Consent Form	MED. REC. NO._____
	NAME _____
IRB#: 1117	BIRTHDATE _____
Protocol Approval Date:	Complete this section
12/23/2010	only if clinical services are provided.

OREGON HEALTH & SCIENCE UNIVERSITY

Consent Form

TITLE: Catechins and ω-3 Fatty Acids Impact on Fatty Acid Synthase Activity in the Prostate: A Randomized Controlled Trial

PRINCIPAL INVESTIGATOR:	Jackilen Shannon, PhD	(503) 220-8262, 57285
		(503) 494-4993
CO-INVESTIGATORS:	Mark Garzotto, MD	(503) 220-8262, 51982
	Tomasz Beer, MD	(503) 494-8534
	Motomi Mori, PhD	(503) 418-1555
	Laura Peters, RN	(503) 220-8262, 57718
	Mark Johnson, RN	(503) 494-4779

Lynn Marshall, ScD	(503) 494-3990
Christopher Amling, MD	(503) 494-0383

RESEARCH STAFF

Wesley Stoller, MA

Paige Farris, MSW

Summer Carter, MSPH, CCRP

Amy Palma, RD, BS

(503) 220-8262, 54931

(503) 220-8262, 54868

(503) 220-8262, 57758

(503) 220-8262, 57280

PURPOSE:

You have been asked to be in this research study because you have had a recent prostate biopsy. Your urologist feels that you may still be at risk of having prostate cancer and has recommended that you have a repeat prostate biopsy. The reason for this research study is to test whether or not a fish oil supplement and a green tea supplement either combined or alone will 1) block excess production and activity of fatty acid synthase or cholesterol and 2) decrease cell growth in prostate tissue with and without cancer.

We want to better understand early changes in prostate cells that may predict whether or not a man will get prostate cancer. One early change is the amount of fatty acids produced in cancer cells. Fatty acid synthase is a protein that controls the amount of fatty acids made. Too much fatty acid synthase has been found in both prostate cancer and pre-cancerous tissue. The amount of cholesterol in the cancerous or pre-cancerous tissue may also have an effect on whether cancer cells live and grow or whether they die. Finally, we will also analyze your diet history for additional dietary compound intake.

We are asking you to provide blood, urine and tissue samples for a blood, urine and tissue bank. Your samples will be analyzed for this study and – if you agree – also stored for use in potential future studies (genetic or non-genetic). We will collect information from your existing medical record throughout the study.

If you choose to be in this study, you will be involved for three to four months. There will be 144 men enrolled in this study between the Portland VA Medical Center Medical Center (PVAMC), Oregon Health & Science University (OHSU) and Kaiser Permanente Northwest Urology Clinics (KPNW).

General Study Information:

This study is a double blind randomized trial. This means neither you nor the investigator will know or can choose whether you get the fish oil or the green tea supplement. You have a 25% chance of being in any one of the 4 groups: fish oil supplement and green tea supplement, fish oil placebo and green tea supplement, fish oil supplement and green tea placebo, or the fish oil placebo and green tea placebo. A placebo is a pill or solution that tastes, looks, and smells like fish oil or green tea, but does not contain the compound we are studying. A placebo is sometimes called a "sugar pill." The study is done this way because sometimes knowing that you are getting the supplement can change the results of the study. If you start having serious side effects to the pills, the investigators can find out which kind of pills you are getting. Please ask the investigator if you have any questions at all about this kind of study.

PROCEDURES:

You can be included in this study if you meet the following:

- You are over the age of 21
- Your clinician has recommended that you undergo a repeat prostate biopsy

If you choose to join this study, this is what will happen:

A biopsy sample and the results of your last biopsy and your repeat biopsy (scheduled in the next three to four months) will be released to the study investigators. If your last biopsy was performed in a place other than OHSU, we will be requesting a signed release of information from you in order to receive your biopsy results from your treating physician.

You will receive a telephone call from (or meet in person with) a clinician to confirm with you that you are eligible to participate in this trial. If it is determined that you are eligible, you will have three visits to the OHSU Clinical and Translational Research Center (CTRC). You will have four visits to the OSHU CTRC if your repeat biopsy date is pushed forward in time for any reason. Your final visit will be at your urology clinic. The following describes what you will do at each visit:

Visit 1 (90 days before your repeat biopsy – 2/2.5 hours long):
You will
- Fast for eight hours before the first visit.
- Fill out a questionnaire about your general health, what you usually eat and whether or not you take vitamins or other supplements.
- Fill out a questionnaire that will give the researchers an idea about your general health in relation to the symptoms that may come from taking the pills we are studying.
- Meet with the CTRC and study staff to measure height, weight, blood pressure and pulse, be asked about your medical history, provide 2½ tablespoons of blood (for total bilirubin test and for research), and a urine sample.

Randomization will occur if your total bilirubin test result is normal. We anticipate your test result will be available before the end of your visit. If this test result is not available, your study supplements will be mailed to you (free of charge).

- You will be given either the fish oil supplement and green tea supplement, fish oil placebo and green tea supplement, fish oil supplement and green tea placebo, or the fish oil placebo and green tea placebo.
- You will receive two bottles at a time; a fish oil/ placebo bottle and a green tea/ placebo bottle. Your supply will last for 1 month.
- You will take one fish oil/placebo pill 3 times a day for a total of 12 weeks.
- You will take one green tea/placebo pill 2 times a day for a total of 12 weeks

Visit 2 (60 days before your repeat biopsy – 15-30 minutes long) AND
Visit 3 (30 days before your repeat biopsy – 15-30 minutes long):

You will
- Fill out a questionnaire about any side effects you may be experiencing.
- Fill out a 2nd questionnaire about changes to 1) the foods you eat, and 2) medications, supplements or herbal remedies you take.
- You will be given 2 refill bottles with enough of your assigned supplement and/or placebo for the next month. If necessary, you may complete these follow-up questionnaires by telephone and we will mail your refills to you.

Biopsy Appointment (at your urology clinic – ½ hour-1 hour long):
You will
- Fast for eight hours before your last visit.
- Fill out a questionnaire about any side effects that you may be experiencing.
- You will fill out a 2nd questionnaire about changes to 1) the foods you eat, and 2) medications, supplements or herbal remedies you take.
- Fill out a short 3rd questionnaire about whether you thought you were getting active capsules or placebo capsules.
- Provide 2½ tablespoons of blood (for total bilirubin test and for research).
- Provide a urine sample at the time of your repeat biopsy. We will store the unused urine.

Your physician will be asked to take two additional biopsy samples (cores) at the time of your repeat biopsy. This will not change the type of procedure or the time involved in the surgery.

Should your repeat biopsy be delayed for reasons unrelated to this study, you will take capsules for 2 additional months or for up to a total of 20 weeks.

Follow-Up Visit (30 Days after your repeat biopsy) – 15-30 minutes long
You will
- Fill out a questionnaire about any side effects you may be experiencing.
- Fill out a 2nd questionnaire about changes to 1) the foods you eat, and 2) medications, supplements or herbal remedies you take.

Additional Biopsy Sample Information:
In these samples, we will look for changes in the amount and activity of fatty acid synthase, and other proteins that make fatty acids. From the two additional samples, we will be looking at the amount of cholesterol and other fatty acids in your prostate cells. The two extra samples taken at your repeat biopsy will be solely for this study and will be stored for future (genetic or non-genetic) studies unless you mark "No future studies" in the SIGNATURES section of this consent form. In the future, samples of your blood, urine, tissue and medical information may be given to researchers as part of the search for a genetic cause of prostate cancer or for other medical conditions. The samples will be labeled as described in the

CONFIDENTIALITY section.

Supplement and Placebo Information:
The supplements used in this study are made from oils and plants found in everyday foods. These supplements are sold over the counter in the United States.

The placebo we are using as a match to the fish oil is made of olive oil. The green tea placebo used in this study is made from dicalcium phosphate and food coloring.

You will keep a daily log of your supplement intake. A calendar will be given to you on your first appointment. The study coordinator will show you how to fill it out. During phone Visit 2 and phone Visit 3, you will be reminded to fill out your diary and to bring it with you to your biopsy appointment.
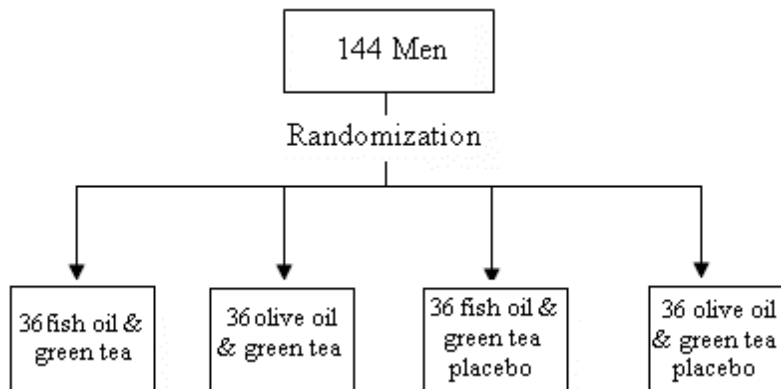
Medical Record Access Information:

We will collect information from your existing medical record throughout the study to review eligibility and to monitor for any changes to your health that may or may not be related to the study supplement.

If you had your first prostate biopsy at a medical facility other than OHSU, we will ask for those records and a sample of that tissue. We will have you fill out a Release

of Records form so we can gather information about your first prostate biopsy from that medical facility.

SUBJECT ACCESS TO INFORMATION:

During the research study, you will not have access to the research data that is collected about you until after the study is completed and the study results have been determined or published. After the study is completed you may request your health information.

RISKS AND DISCOMFORTS:

Prostate biopsy:

The biopsy will be part of your standard medical care. Your physician will describe risks associated with this procedure. We are requesting permission to collect two small additional biopsy samples for research purposes only.

Fish oil supplements:

Physical risk due to the use of fish oil supplements is relatively small. Fish oil supplements thin the blood. There have been no reports of bleeding in other studies of fish oils.

Ordinary fish oil capsules may also cause gas or belching with the odor of fish on the breath. These effects usually do not last long. In this study, we will use an oil supplement that is almost odorless and is highly refined; therefore gastrointestinal effects like gas or belching are expected to be minimal.

Some people have reported diarrhea when starting the oil supplement. If you develop diarrhea that does not go away on its own, the oil supplement will be stopped for several days. Tell your doctor and notify the study coordinator immediately. Your

doctor may have you restart with one capsule per day and slowly increase to the full dose or as much as you can tolerate. If diarrhea continues, the oil supplement will be stopped. A physician will follow your medical progress if you are unable to continue in the study due to such a reaction.

In a study with multiple sites, a randomized trial of subjects with a history of sustained ventricular tachycardia (fast heart rate) or ventricular fibrillation (abnormal heart rhythm) who had an implantable cardioverter defibrillator (ICD) who took fish oil had an increase or decrease in heart activity was found in some patients.

Green tea supplements:

Side effects of green tea supplements usually have been mild and fleeting – similar to the frequency of placebo side effects. Side effects that are less likely to happen are: headache, upset stomach, heartburn, abdominal pain, excess gas, nausea/dizziness and muscle pain. A rare but serious side effect is hepatic (liver) disorders. In 2003, use of a specific green tea supplement (not the one used in this trial) was associated with liver disorders at a frequency of approximately 1 per 100,000 boxes sold over a 12 month period. In most cases, if liver functions are abnormal, they returned to normal after discontinuation of the green tea supplement. Your total bilirubin (a measure of liver function) will be tested before you are randomized to receive a supplement and again at the end of the trial.

Green tea has a small amount of vitamin K. Vitamin K is a natural source for helping your blood clot. It can therefore counteract with blood thinning medications (like Coumadin®). The supplements in the study do not contain enough vitamin K to have significant effects.

Blood draw:

Blood drawing will cause some pain and carries a small risk of bleeding, bruising or infection at the puncture site.

For pregnancy/risk to fetus (For Men):

Neither fish oils nor green tea are known to have any adverse effect in the fetus due to paternal exposure.

Potential drug interactions:

There are several drugs (prescription and non-prescription) that may cause problems when taken with the fish oil or green tea. Measures have been taken to ensure that eligible participants are not taking medications or remedies that may cause interactions. The investigator will carefully review all of the drugs and supplements you are taking before giving you the study drug. If any other health care provider prescribes any new drug(s) for you while you are in this study, please tell the investigator before you take the new drug. You could also have that provider talk to the investigator before prescribing the new drug. In addition, we ask that you not take any **new** over-the-counter drugs or supplements while you are in this study unless you first check with the investigator.

Other:

Information that identifies you will be used in this study and shared with the study sponsor, research staff and non-VA researchers. A breach of confidentiality may result in psychological harm in the form of embarrassment, guilt and/or stress. Breach of confidentiality regarding future genetic testing could potentially impact your family relationships, eligibility for insurance, current or future employment, family plans, immigration status, paternity suits, community standing, stigmatization or future reproduction plans. Other risks to consider include the impact of learning results if no effective therapy exists and on your plans to have children.

Some of the questions included in any of the study's questionnaires (about your diet, risk factors for cancer, family history of cancer, current medications or conditions, etc.) may seem personal or be upsetting to you. You may refuse to answer any question on any questionnaire. If you become so upset by the questions that you appear to need counseling, Dr. Shannon will be notified and you may be referred to your primary care provider for evaluation if necessary.

<u>BENEFITS</u>:

You may or may not personally benefit from being in this study. However, by serving as a subject, you may help us learn how to benefit patients in the future.

ALTERNATIVES:

You may choose not to be in this study. You may also choose to take the capsules even if you choose not to allow us to keep your blood, urine or tissue for future studies.

CONFIDENTIALITY:

A code number will be assigned to you, your questionnaires, and your blood, urine and tissue samples as well as to information about you. Only the investigators listed on this consent form will be able to link the code number to your identifiable information. Other investigators who may receive samples of your blood, urine, tissue or dietary information for future research will be given only the code number, which cannot be used to identify you. Your blood and/or tissue samples will be given to collaborators at Oregon State University and Fred Hutchinson Cancer Research Institute. They will analyze your de-identified samples to determine changes in levels of fatty acids and other lipids.

The information collected for this study will be kept confidential as required by law. The law requires us to keep study records for six years following the end of the study. Study information with identifiers will be maintained until the end of data analyses. At the end of the study, all data, including blood, urine and tissue specimens will be de-identified and retained indefinitely for use in potential future genetic or non-genetic studies.

All other parties including employers, insurance companies, personal physicians and relatives will be refused access to the information or to the samples, unless you provide written permission, or unless we are required by law to do so. Neither your name nor your identity will be used for publication or publicity purposes.

Research records may be reviewed and copied by the Department of Defense, the National Institutes of Health (NIH), and NCCAM (sponsors); the OHSU Clinical and Translational Research Institute (OCTRI); the National Center for Research Resources; OHSU Knight Cancer Institute, the Fred Hutchinson Cancer Research Center; Portland Kaiser Permanente Northwest Institutional Review Board; the Portland Veteran's Administration Medical Center Institutional Review Board, OHSU Institutional Review Board, the Food and Drug Administration (FDA), and the Office for Human Research Protections.

Under Oregon law, suspected child or elder abuse must be reported to appropriate authorities.

FUTURE USE OF BLOOD AND TISSUE:

This research does not include genetic testing. However, in the future, fully de-identified blood specimens may be analyzed in a laboratory by the principal investigator, or other researchers together with the principal investigator, to search for a genetic cause of prostate cancer and/or to investigate how diet may work together with genetics to change a person's risk of developing prostate cancer.

Additionally, the results from your biopsy and a portion of your biopsy sample will be released to the investigators as part of this study. The samples will be labeled as described in the CONFIDENTIALITY section. Only the investigators listed on this consent form will have the ability to link your samples to your identifiable information. Any samples used in future genetic studies will be assigned a code number but links between that number and any information identifying you will be destroyed.

What we do with your samples in the future is up to you. If you do not wish to have your specimens available for future studies, you may still join this study. In this case, we will use your specimens solely for the purpose of completing the current study activities. At the end of data analysis for this study, we will destroy your samples if

you initial next to "No future studies" in the RESEARCH SUBJECT'S RIGHTS section of this consent form.

COSTS:

There will be no cost to you as a participant in this study. You will be reimbursed for mileage to your first study visit, because this visit is not a part of your standard medical care. Even if you choose not to join the study, your mileage will be reimbursed.

LIABILITY:

If you believe you have been injured or harmed while participating in this research and require immediate treatment, contact Dr. Jackilen Shannon at (503) 220-8262, 57285 or Dr. Christopher Amling at the Urology Clinic at (503) 494-4779 during the day OR through the OHSU operator during off-hours at (503) 494-8311 immediately.

It is not the policy of the U.S. Department of Health and Human Services to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, Dr. Jackilen Shannon at (503) 220-8262 ext. 57285. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U. S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663, 2221.

You have not waived your legal rights by signing this form. If you are harmed by the study supplements or study procedures, you will be treated. Oregon Health & Science University does not offer to pay for the cost of the treatment. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

Oregon Health & Science University is also subject to the Oregon Genetic Privacy law (ORS 192.531 through ORS 192.549) and its requirements concerning confidentiality and the legal remedies provided by that law for breach of its requirements. You have not waived your legal rights by signing this form. For clarification on this subject, or if you have further questions, please call the OHSU Research Integrity Office at (503) 494-7887.

PARTICIPATION:

If in the future you decide you no longer want to participate in this research, we will remove your name and any other identifiers from your blood, urine, tissue samples and other information about you, but the material will not be destroyed and we will continue to use if for research.

Dr. Jackilen Shannon (503) 494-4993 or (503) 220-8262 ext. 57285 has offered to answer any other questions you may have about this study. If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887.

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

Your health care provider may be one of the investigators of this research study, and as an investigator, is interested in both your clinical welfare and in the conduct of this study. You do not have to be in any research study offered by your physician.

You may be removed from the study if any of the following occur:

- the investigator stops the study
- the sponsor stops the study
- you develop serious side effects
- you do not follow study instructions
- you do not meet the requirements for taking part in the study
- you are prescribed warfarin (Coumadin®) while in the study.

You will be informed of new findings that may affect you or your wish to continue participation. You may withdraw from this study at any time without loss of any benefits to which you are entitled.

We will give you a copy of this signed form.

Your signature below indicates that you have read this entire form and that you agree to be in this study. Please place your initials next to the statement best representing your level of participation in this trial.

FUTURE STUDIES OKAY

_____ I agree that my urine/blood/tissue samples and other information may be used for this study and stored for possible use in future studies of prostate-related health conditions and/or the genetics of prostate-related health conditions.

PERMISSION NOW:

_____ I agree that my urine/blood/tissue samples with no identifying information may be kept for research about other health problems (genetic or non-genetic). Since no identifying information will be on my urine/blood/tissue samples, I do not need to be contacted in the future for permission.

PERMISSION LATER:

_____ I wish to be re-contacted if my urine/ blood/ tissue samples are considered for further studies (genetic or non-genetic) not listed in this consent form. After the study has been explained, I will then decide if I want my samples to be included in the additional study. I agree that my urine/blood/tissue samples may be kept for research about other health problems (genetic or non-genetic), but I would like to be contacted for permission prior to any future use.

NO FUTURE STUDIES

_____ I agree to participate in this clinical trial of fish oil and prostate cancer risk. My urine/blood/tissue samples and other information may be

stored and used **ONLY** for this study being conducted by Dr. Jackilen Shannon.

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OREGON HEALTH & SCIENCE UNIVERSITY

INSTITUTIONAL REVIEW BOARD

PHONE NUMBER (503) 494-7887
CONSENT/AUTHORIZATION FORM APPROVAL DATE

<div style="border: 2px solid blue;">

Feb. 9, 2011

</div>

Do not sign this form after the
Expiration date of:_ 12-22-2011_____

</div>

Subject Signature: _____Date: _____Time:_____

Person Obtaining Consent: _____Date: _____ Time: _____

APPENDIX B: Treatment Days - Descriptive Stats and ANOVAs

By: Treatment Arm - Separate

	GT (n=13)	GTFO (n=13)	FO (n=24)	PP (n=24)
Mean Days on Treatment ±SD	109.38±18.36	108.85±30.03	99.67±21.39	109.33±26.17
Min	83	28	35	85
Max	147	144	140	210
Range	64	116	105	125

By Treatment Arm- Combined

	GT/GTFO (n=26)	FO/PP (n=48)
Mean Days on Treatment ±SD	109.1154±24.39	104.5±24.15
Min	28	35
Max	147	210
Range	119	175

One-way ANOVA by Treatment Arms- Separate

Source	Partial SS	Df	MS	F	Prob >F
Between Groups	1482.47	3	494.16	0.84	0.4762
Within Groups	41151.44	70	587.88		
Total	46623.91	73			

Equal Variances met: P=0.299   R-Sqaure=.03

Conclusion: No significant differences in treatment days among the four separate treatment arms (p=.4762)

One-way ANOVA by Treatment Arms: Combined

Source	Partial SS	Df	MS	F	Prob >F
Between Groups	359.25	1	359.25	0.61	0.44
Within Groups	42274.65	1	587.14		
Total	42633.91	72			

Equal Variances: met (.96) R-square=0.008

Conclusion: No significant differences in treatment days between the two combined treatment arms (p=.4367)

Appendix C: Pill Counts for Participants, ANOVAs

By treatment arm: Separate

	GT (n=13)	GTFO (n=13)	FO (n=24)	PP (n=24)
Mean Pill Count ±SD	251.31±41.17	282.31±65.74	288.29±49.22	278.17±92.88
Min	180	192	208	57
Max	353	432	404	540
Range	173	240	196	483

By Treatment Arm- Combined

	GT/GTFO (n=26)	FO/PP (n=48)
Mean Pill Count ±SD	266.811±58.24	283.23±73.71
Min	180	57
Max	432	540
Range	252	483

One-way ANOVA by Treatment Arms- Separate

Source	Partial SS	Df	MS	F	Prob >F
Between Groups	12024.55	3	4008.18	0.84	0.47
Within Groups	332697.83	70	4752.83		
Total	344722.78	73	4722.22		

Equal Variances not met: P=0.008   R-Sqaure=.03

Conclusion: No significant differences in pill counts among the four separate treatment arms (p=.4748)

One-way ANOVA by Treatment Arms: Combined

Source	Partial SS	Df	MS	F	Prob >F
Between Groups	4547.86	1	4547.86	.96	.3298
Within Groups	340174.51	1	4724.65		
Total	344722.38	73	4722.22		

Equal Variances: met (.19) R-square=0.01

Conclusion: No significant differences in pill counts between the two combined treatment arms (p=.3298)

Appendix D: Cancer Diagnoses after Repeat Biopsy

IGF-I Concentrations by Cancer Diagnosis

Cancer Dx	N	%	IGF-I (ng/mL)±SEM	
			PRE	**POST**
Benign	49	66.21	144.31±5.31[a]	160.53±8.32
PIN	12	16.22	154.14±10.38	176.81±18.42
Cancerous	13	17.58	184.26 ±13.21[a]	182.27±14.03

[a]**Denotes Significant Difference between groups**

PSA Concentrations by Cancer Diagnosis

Cancer Dx	N	%	PSA (ng/mL)±SEM	
			PRE(n=74)	**POST(n=72)**
Benign	49	66.21	6.21±0.50	7.80±1.59
PIN	12	16.22	6.25±1.16	7.13±1.34
Cancerous	13	17.58	6.14±1.18	4.72±0.73

*Cancer Dx was not found to be a significant predictor of PSA(pre), even after adjustment for age and BMI. (p=0.78)

N-way ANOVA Table for IGF-I (pre) over Age, BMI, Cancer Dx

Source	Partial SS	Df	MS	F	Prob >F
Model	27346.18	8	3418.27	2.30	0.0312
Age at entry	4633.18	2	2316.59	1.56	0.2188
BMI at entry	6129.64	4	1532.41	1.03	0.3990
Cancer Dx	22385.18	2	11192.59	7.52	0.0012
Residual	96783.88	65	1488.98		
Total	124130.059	73	1700.41		

Summary: After considering age and BMI at entry; cancer diagnosis was found to be a significant predictor of mean baseline IGF-I (p=0.001) No interaction was found between: age and BMI; age and cancer dx; BMI and cancer dx. NOTE: This

relationship was not found when mean baseline IGF-I was replaced by mean post-treatment IGF-I. (p=0.7144)

Cancer Dx by Treatment Arm (separate):

	Treatment Arm (n, % of group total)			
Cancer Dx	**GT (n=13)**	**GTFO (n=13)**	**FO (n=24)**	**PP (n=24)**
Benign	11 (85%)	7 (54%)	13 (54%)	18 (75%)
PIN	2 (15%)	4 (31%)	5 (21%)	1 (4%)
Cancerous	0 (0%)	2 (15%)	6 (25%)	5 (21%)

Cancer Dx by Treatment Arm (combined):

	Treatment Arm (n, % of group total)	
Cancer Dx	**GT (n=26)**	**PP (n=48)**
Benign	18 (69%)	31 (65%)
PIN	6 (23%)	6 (12%)
Cancerous	2 (8%)	11 (23%)

APPENDIX E: The Body Mass Index (BMI scale) based on WHO BMI Scale

<18.5 – Underweight
≥18.5 to <25 – Normal weight
≥25 to <30 – Overweight
≥30 to <35 - Obese Category I
≥35 to <40 - Obese Category II
≥40 - Obese Category III

Participant Characteristics by BMI Scale (n=74)

BMI Interval	n	% of Total	Mean IGF-I±SEM Baseline (ng/mL)
<18.5	0	0	n/a
≥18.5 to <25	14	18.91	153.4±15.07
≥25 to <30	39	52.70	156.04±5.97
≥30 to <35	16	21.62	146.99±10.29
≥35 to <40	4	5.41	139.83±14.74
≥40	1	1.36	172.1 (SD n/a)

BMI Table for Separate Treatment Arms:

Treatment Arm	n	Mean BMI±SEM at Entry	BMI Category
GT	13	29.4 ± 0.95	Overweight
GTFO	13	29.37±1.09	Overweight
FO	24	27.95±1.04	Overweight
PP	24	28.00±0.86	Overweight

*ANOVA test - No difference in BMI between treatment arms, p=0.62

BMI Table for Combined Treatment Arms:

Treatment Arm	n	Mean BMI±SEM at Entry	BMI Category
GT	26	29.38 ±0.71	Overweight
GTFO	48	27.98±0.67	Overweight

*ANOVA test - No difference in BMI between treatment arms, p=0.18

APPENDIX F: Distribution by Age Categories

Category 1: ≥50 to <60
Category 2: ≥60 to <70
Category 3: ≥50 to <60
Category 4: >80

IGF-I Concentrations by Age Category (expressed in ng/mL)

Age Category	N	% of Total	Mean±SEM	50th Percentile (observed)	50th Percentile (healthy men)
50-60	21	28.38	163.06±10.16	167.5	169
60-70	44	59.46	149.55±5.85	148.78	161
70-80	9	12.16	145.78±13.43	146.15	98
>80	0	0	n/a	n/a	85

PSA Concentrations by Age Category (expressed in ng/mL)

Age Category	N	% of Total	Mean±SEM	50th Percentile
50-60	21	28.38	4.45±0.52	4.9
60-70	44	59.46	7.05±0.63	5.785
70-80	9	12.16	6.33±0.68	5.9
>80	0	0	n/a	n/a

Cancer Dx by Age Category

Age Category	N	% of Total	Benign	PIN	Cancerous
50-60	21	28.38	14	5	2
60-70	44	59.46	30	4	10
70-80	9	12.16	5	3	1
>80	0	0	n/a	n/a	n/a

Appendix G: Fischer's Exact Test of Proportions between GT and PP groups

Nguyen and colleagues[112] compared the proportion of increases and decreases in IGF-I between the supplemented and placebo groups to see if they were significantly different. This test was replicated using both the separate and combined groups in our study. The following online calculator was used to compute these results:

http://graphpad.com/quickcalcs/contingency1.cfm

Separate Treatment Arms: GT (n=13) and PP (n=24)

	GT	PP	Total
IGF-I Increased	4	16	20
IGF-I Decreased	9	8	17
Total	13	24	37

Fisher's exact test results: The two-tailed P value equals 0.0470

Interpretation: The association between rows (increase/decrease) and columns (supplementation status) is considered to be statistically significant.

Combined Treatment Arms: GT (n=26) and PP (n=48)

	GT	PP	Total
IGF-I Increased	14	31	45
IGF-I Decreased	12	17	29
Total	26	48	74

Fisher's exact test results: The two-tailed P value equals 0.4562

Interpretation: The association between rows (increase/decrease) and columns (supplementation status) is not considered to be statistically significant.

Appendix H: Assay Diagnostics; Assay Specific Insert

Assay	Samples	Unknowns	Average % Difference b/t Duplicates	Intra-Assay CV
1. Session One	75	150	8.6%	6.05%
2. Session Two	102	204	7.8%	5.45%

* Manufacturer's Inter-assay CV: at 50% B/B0 = 5.5%

ASSAY 1: QUALITY CONTROL PARAMETERS

% NSB/T: 1.89
% Bmax/T: 59.12
ED50: 1.6931   +/- 0.17223
MINIMUM DETECTABLE DOSE: 0.03697

FOUR PARAMETER LOGISTIC $[y = (A - D) / (1 + (x/C)^B) + D]$

NO. OF ITERATIONS: 3
Bmax (A): 1.0051 $\pm$ 0.011269
Slope (B): 0.9948 $\pm$ 0.022043
MidPt (C): 1.6817 $\pm$ 0.058987
Bmin (D): -0.0017471 $\pm$ 0.00021725
CORRELATION COEFFICIENT: 0.99921
RMS ERROR: 1.73277
WEIGHTING COEFFICIENTS A0: 0.21208   J: 1.1254

ASSAY 2: QUALITY CONTROL PARAMETERS
% NSB/T: 1.78
%Bmax/T: 56.40
ED50: 1.9344 $\pm$ 0.33161
Minimum Detectable Dose: 0.045 ng/mL

Four parameter Logistic: $[y= (A-D)/1 + (x/C)^B + D]$

No. of Iterations: 3
Bmax   (A):   1.0252 $\pm$ 0.019433
Slope   (B):   1.0182 $\pm$0.040328
Midpt   (C):   1.8494 $\pm$0.10942
Bmin   (D):   -0.0016892 $\pm$0.00038192
Correlation Coefficient: 0.99704
RMS ERROR: 2.93977
WEIGHTING COEFFICIENTS   A0:0.24848   J:1.1004

Assay Specific Insert

Zertifikat / Certificate

human Insulin-like Growth Factor -I RIA, IGF-R21

humaner Insulin-ähnlicher Wachstumsfaktor -I RIA, IGF-R21

Charge / Lot 261112
Haltbarkeit / Expiry 2013-02-11

		Charge/ Lot	Haltbarkeit / Expiry	Bindung / Binding B/B0 [%]	Target Values Zielwerte B/B0 [%]
Unspezifische Bindung / Non specific Binding, %NSB/TC		-	-	1,8	< 5.0
Spezifische Bindung / Specific Binding, %B0/TC		-	-	48	> 30
Komponente / Component					**[ng/mL]**
Standard E	E	250911	2013-09-30	100	0
Standard F	F	250911	2013-09-30	92	0.156
Standard G	G	250911	2013-09-30	87	0.313
Standard H	H	250911	2013-09-30	77	0.625
Standard I	I	250911	2013-09-30	61	1.25
Standard J	J	250911	2013-09-30	45	2.5
Standard K	K	250911	2013-09-30	29	5
Standard L	L	250911	2013-09-30	18	10
Kontrollserum / Control Serum	**M**	**150612**	**2015-06-30**	48	203 (± 41)
Kontrollserum / Control Serum	**N**	**101211**	**2014-12-31**	77	61 (± 12)
Verdünnungspuffer / Dilution Buffer	DB	120612	2014-06-30	-	-
Ansäuerungspuffer / Acidification Buffer	AB	130511	2014-05-31	-	-
Testpuffer / Assay Buffer	A	060812	2014-08-31	-	-
1. Antikörper / 1st Antibody	B	140411	2014-04-30	-	-
Tracer	C	261112	2013-02-11	-	-
Unspezifische Bindung / Non specific Binding	D	240511	2015-05-31		-
2. Antikörper / 2nd Antibody	O	010212	2016-02-29	-	-
Präzipitationsreagenz/ Precipitation Reagent	P	020511	2013-05-31	-	-

Packungsbeilage für weitere Informationen beachten! / Refer to package insert for further information!
Bindungsdaten stellen exemplarische Daten dar und dürfen nicht zur Berechnung verwenden werden / Binding data are exemplary data and cannot be used for calculations

Freigabe der Qualitätskontrolle / Release of Quality Control: 28.11.12

Version 3 100912