DATTA, MRIDUL, Ph.D. Impact of Tomato Juice on Radiation Side Effects and select Inflammatory Mediators in Prostate Cancer Patients undergoing Intensity Modulated Radiation Therapy. (2011)
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This pilot study assessed tolerance of different volumes of processed tomato juice consumed daily and its impact on serum lycopene, selected serum inflammatory mediator levels and radiation-induced side effects in men with localized prostate cancer undergoing radiation therapy. Participants (n = 17) were randomized into control group or one of three intervention groups (4 oz, 8 oz or 12 oz of processed tomato juice daily). Non-Hispanic Whites comprised 71% of study participants. Tumor staging ranged from $T_{1c\text{-}2c}N_0M_0$, with 71% of participant tumors in the $T_{1c}N_0M_0$ stage.

Participants tolerated daily tomato juice supplementation without any adverse gastrointestinal (GI) effects. Serum lycopene decreased in control group participants, while increasing from 0.33±0.11 µg/mL (baseline) to 0.41± 0.12 µg/mL (endpoint) in the intervention group. No correlation between serum and dietary lycopene was detected. Control group participants lost weight, while participants in the intervention groups did not. Not surprisingly, participants exhibited systemic inflammation at baseline. Overtime, increased c-reactive protein (CRP) and interleukin-6 (IL-6) was observed in control group, while decreases in serum CRP, IL-6 and prostaglandin E2 (PGE2) levels were observed in intervention groups ($p>0.05$). No statistically significant within group differences were detected for CRP. Within group differences were statistically

significant for 12 oz group only, when comparing baseline and endpoint with midpoint levels ($p = 0.014$) for IL-6, and when comparing PGE2 baseline levels with midpoint and endpoint ($p = 0.003$).

We observed no statistical correlation between inflammatory markers, cancer characteristics and dietary or serum lycopene, or acute side effects of treatment. Lower performance score was observed in intervention group participants. Daily tomato juice intake appeared to offer a GI protective effect during the first three weeks of treatment. Based on the results of this study, daily consumption of processed tomato juice (at least 8-12 oz) may decrease serum levels of CRP, IL-6 and PGE2; lower performance status score; and offer a protective GI effect during radiotherapy for prostate cancer. This information may assist in improving patient tolerance and minimize acute side-effects of radiation therapy in men with localized prostate cancer undergoing intensity modulated radiation therapy.

IMPACT OF TOMATO JUICE ON RADIATION SIDE EFFECTS AND SELECT INFLAMMATORY MEDIATORS IN PROSTATE CANCER PATIENTS UNDERGOING INTENSITY MODULATED RADIATION THERAPY

By

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A Dissertation Submitted to
the Faculty of The Graduate School at
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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Chronic inflammation is now recognized as one causative mechanism in carcinogenesis (Colotta, Allavena, Sica, Garlanda, & Mantovani, 2009; Mantovani, Allavena, Sica, & Balkwill, 2008), contributing to about 20% of all cancers (De Marzo et al., 2007). The presence of inflammatory cells and mediators such as cytokines and prostaglandins in tumor tissue is considered a "hallmark" of cancer-related inflammation (Mantovani, et al., 2008). In addition to generating reactive oxygen (ROS) and nitrogen species (RNS), inflammation also activates several pro-inflammatory cytokines (such as tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6), IL-1β) which further increase cancer risk by inducing enzymes that produce more ROS/RNS (Ohshima, Tatemichi, & Sawa, 2003). Additionally, cancer treatments, specifically radiation therapy may also induce inflammation (McBride et al., 2004). Researchers have reported upregulation of proinflammatory cytokines (TNF- α, IL-6, IL-1β) during and after treatment, contributing to fatigue (Bower, 2007) and impacting quality of life.

Prostate cancer is the most commonly diagnosed cancer among men ("Cancer Facts and Figures 2010," 2010), and inflammation has been identified as one causative factor (De Marzo, et al., 2007; Wong, Bray, & Ho, 2009). Cell

and animal studies have demonstrated upregulation of several cytokines and inflammatory enzymes in prostate carcinogenesis (Fujita et al., 2002). Researchers have also documented upregulation of several inflammatory mediators during radiation therapy for prostate cancer in men (Christensen et al., 2009; Johnke et al., 2009).

Nutritional modulation for chemoprevention is not a new concept. Consumption of phytonutrients such as lycopene, have been associated with a decreased incidence of prostate cancer in epidemiological studies (Giovannucci, 1999, 2002). Several cell (Hwang & Bowen, 2004; Ivanov et al., 2007; Kanagaraj et al., 2007; L. Kim, Rao, & Rao, 2002) and animal studies (Guttenplan et al., 2001; Konijeti et al., 2010) and clinical trials in men at risk for developing prostate cancer (Bunker et al., 2007; Edinger & Koff, 2006; Mohanty, Saxena, Singh, Goyal, & Arora, 2005; Schwarz et al., 2008) have demonstrated a chemopreventive role of lycopene. Researchers have also evaluated the benefits of lycopene in prostate cancer patients prior to initiating treatment (Jatoi et al., 2007; H. Kim et al., 2003; Kucuk et al., 2001) or after failure of treatment (Clark et al., 2006). Clinical trials have demonstrated a decline in serum prostate specific antigen (PSA) level, tumor bulk, and inflammation, and an increase in serum lycopene levels with lycopene supplementation. However, the impact of lycopene supplementation during radiation treatment for prostate cancer and its impact on biomarkers, physical side effects and quality of life remain unknown.

The *long-term goal* of this project was to increase our understanding of lycopene supplementation during radiotherapy for prostate cancer, to minimize side effects, evaluate biomarker responses, and develop specific guidelines for lycopene intake during radiation therapy. The *objectives* of this project were to assess tolerance of an orally ingested food source of lycopene (tomato juice) during radiotherapy for prostate cancer, impact of lycopene supplementation on serum lycopene levels, radiotherapy-related side effects, and select inflammatory markers. The patient population for this study was newly diagnosed patients with non-metastasized prostate cancer who had not received prior treatment and who were scheduled to receive radiotherapy to the prostate gland (treatment volume to include prostate and seminal vesicles). The *central hypothesis* was that tomato juice supplementation during radiotherapy will increase serum lycopene levels and minimize inflammatory response and radiation therapy-related side effects. The dissertation design is presented in Figure 1.1.

The *rationale* for this project was that knowledge of the impact of lycopene supplementation during radiation treatment in prostate cancer patients will provide important insights about a potential method to reduce side effects associated with radiotherapy in prostate cancer patients. Overall this project had the following three specific aims.

Aim 1

To examine the impact of supplementing three different volumes (4 oz, 8 oz, 12 oz) of tomato juice on serum lycopene levels in men with prostate cancer

undergoing radiation therapy. We also quantified dietary lycopene intake using a validated food frequency questionnaire (National Cancer Institute's Diet History Questionnaire (NCI-DHQ)).

Hypothesis: A dose-dependent response will be observed with tomato juice supplementation, with an increase in serum lycopene levels in the intervention groups. To test this hypothesis, we evaluated the effectiveness of three different doses of tomato juice on serum lycopene levels, which were measured using a liquid chromatography-mass spectrometry (LC-MS) analysis at Dr. Wei Jia's laboratory in Kannapolis, NC. We administered the NCI-DHQ once during this study to determine frequency of consumption of lycopene rich foods among the participants. We also used the NCI-common toxicity criteria (CTC) to evaluate if any participants suffered from gastro-intestinal (nausea, vomiting, heartburn) side effects as a result of tomato juice consumption.

Aim 2

Evaluate serum levels of select inflammatory mediators (c-reactive protein (CRP), prostaglandin E2 (PGE2), TNF-α, and IL-6) in men with prostate cancer prior to initiating radiation therapy and then at midpoint and endpoints along with supplementation of three different volumes of tomato juice.

Hypothesis: A dose-dependent decrease in serum inflammatory biomarkers will be observed with tomato juice supplementation.

To test this hypothesis, we evaluated the impact of three different doses of tomato juice on serum CRP, TNF-α, IL-6 and PGE_2 levels. Traditional enzyme-linked immunosorbent assay (ELISA) tests were conducted to analyze the serum for the presence of CRP, TNF-α, IL-6 and PGE_2 at three different time points.

Aim 3

Evaluate the time of onset and severity of select side effects (urinary frequency and urgency, proctitis, and diarrhea) of radiation therapy in the control group and three treatment groups. We also wanted to evaluate if the cytokine response was predictive of radiation therapy symptoms.

Hypothesis: A dose-dependent response will be observed with tomato juice supplementation, with delayed onset and decreased severity of side effects, and improvement or maintenance of the patient's performance score. To test this hypothesis, we evaluated the effectiveness of three different doses of tomato juice on performance status (measured using the Eastern Cooperative Oncology Group (ECOG) Scoring), the time of onset and severity of urinary frequency and urgency, proctitis, and diarrhea. The symptom severity was measured using the NCI common terminology criteria for adverse events (CTCAE).

The research population (men with prostate cancer undergoing radiation therapy) and phytonutrient (lycopene/tomato juice) supplementation were based on the identified research gaps (Davis et al., 2005). However, the implication of inflammatory mediator involvement was extrapolated from literature reviews of

patients undergoing radiation therapy to the pelvis and lungs. Researchers have since demonstrated serum/plasma cytokine expression in men with prostate cancer undergoing radiation therapy (Christensen, et al., 2009; Johnke, et al., 2009).

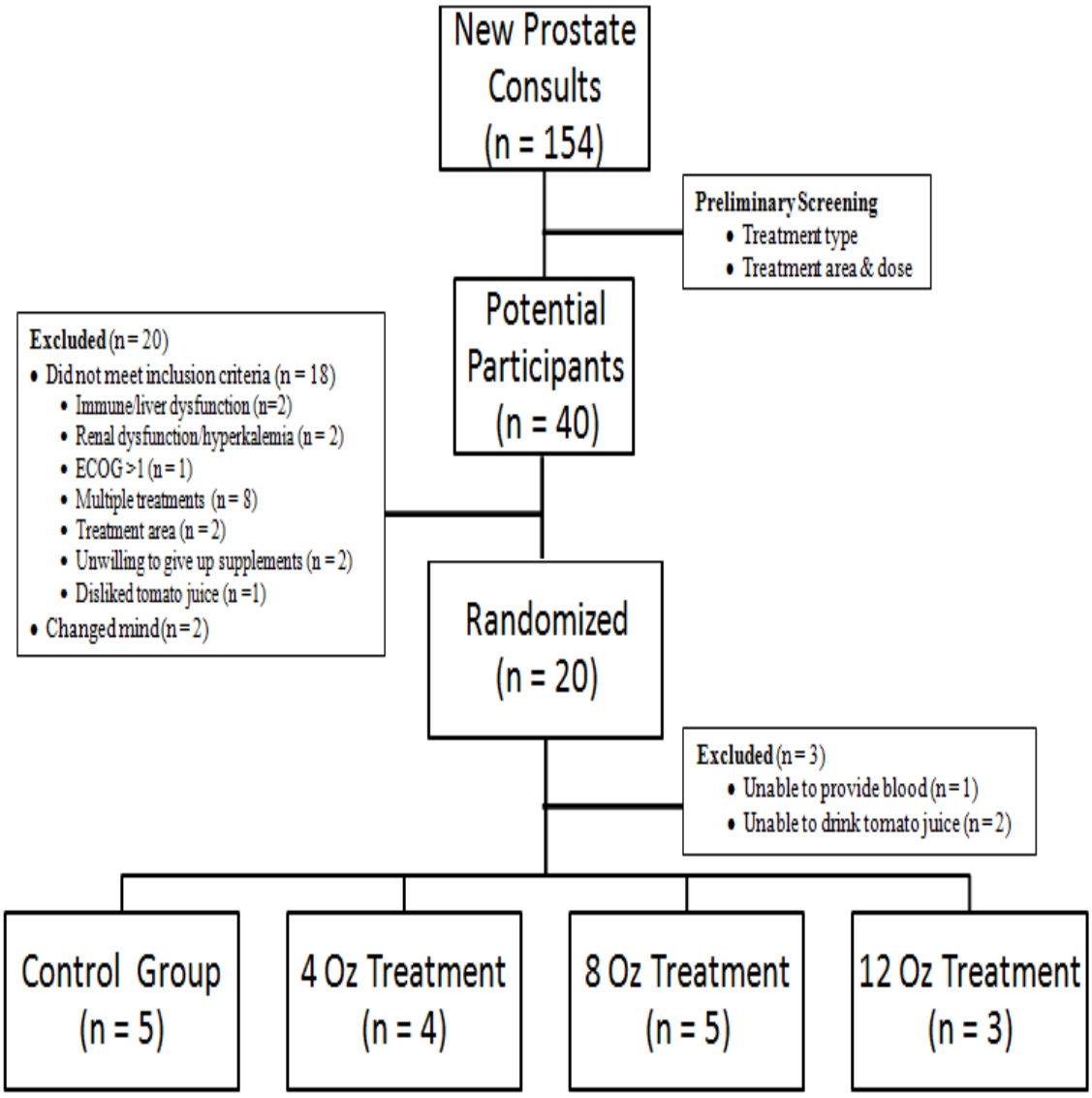

Figure 1.1. Flow diagram representing recruitment and randomization of study participants

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CHAPTER II

REVIEW OF THE LITERATURE

Prostate Cancer

Prostate cancer has the dubious distinction of being the most commonly diagnosed cancer among men in the United States. In 2010, the American Cancer Society projected 217,730 new cases and of these, 6910 new cases were expected in North Carolina. Incidence of prostate cancer is highest among African American men. Prostate cancer is detected by PSA blood test and digital rectal exam, with final confirmation obtained with a prostate biopsy ("Cancer Facts and Figures 2010," 2010). In addition to ethnicity, age and family history (De Marzo et al., 2007), patients with high-grade prostatic intraepithelial neoplasia (HGPIN), genetic variants (such as SNP, CYP3A4, ELAC2, SRD5A2, etc), and elevated biochemical risk factors (e.g., Prostate Specific Antigen (PSA) and/or Insulin like Growth Factor-1) are also considered at a higher risk for developing prostate cancer (Lieberman, 2001). Chronic inflammation has also been implicated in the development of prostate cancer (De Marzo, et al., 2007; Rebbeck et al., 2008).

Inflammation and Prostate Cancer

Inflammation, a natural physiological process, initiated in response to injury as a result of infections (Philip, Rowley, & Schreiber, 2004; Sgambato & Cittadini, 2010) and environmental factors (Grivennikov, Greten, & Karin, 2010), has been implicated in the development of more than 20% of all human cancers, including prostate cancer (Bardia, Platz, Yegnasubramanian, De Marzo, & Nelson, 2009; De Marzo, et al., 2007; Kirschenbaum, Liu, Yao, & Levine, 2001). Epidemiologic, histopathologic and molecular pathological studies have provided evidence to implicate inflammation in prostate carcinogenesis (De Marzo, et al., 2007). Intraprostatic inflammation may be caused by infection, cellular injury, hormonal exposure and/or dietary factors, and direct injury to the prostate epithelium from chronic exposure to these noxious stimuli may lead to proliferative inflammatory atrophy (PIA) or proliferative atrophy (De Marzo, et al., 2007). PIA lesions may transition to PIN, and HGPIN is considered a precursor for prostate carcinogenesis (Wagenlehner et al., 2007). Tissue samples obtained from patients with benign prostate hyperplasia (BPH) have shown the presence of significantly high chronic inflammatory cells and atrophic epithelium indicating the presence of PIA (De Marzo, et al., 2007). A higher risk of developing prostate cancer has been reported in the presence of increased levels of inflammatory cytokines and chemokines in the prostate (Haverkamp, Charbonneau, & Ratliff, 2008). Researchers have demonstrated a decreased risk (21% lower) of developing prostate cancer in current and long-term users of

asprin, further linking inflammation in the development of prostate cancer (Salinas et al., 2010). Tables 2.1 - 2.3 summarize the cell/animal studies and human clinical trials documenting the association of inflammation in prostate pathologies.

Inflammatory Markers:

Key mediators of inflammation that were investigated in this study are briefly reviewed below:

Interleukin-6: Interleukin-6 (IL-6) is a plieotropic pro-inflammatory cytokine produced by leukocytes, fibroblasts and endothelial cells (Lamprecht, Oettl, Schwaberger, Hofmann, & Greilberger, 2007). IL-6 is involved in regulating several key cellular functions such as immune function, acute phase response, inflammation, oncogenesis (Song & Kellum, 2005), proliferation, differentiation, apoptosis and angiogenesis (Culig, Steiner, Bartsch, & Hobisch, 2005). IL-6 is considered a prognostic indicator for prostate cancer development and has been shown to correlate with stages of prostate cancer (Wertz, Siler, & Goralczyk, 2004). While IL-6 is localized to the basal epithelial cells in the normal prostate, in BPH it has been reported to be localized in the stromal and luminal epithelial cells (Royuela et al., 2004). Acting as an autocrine growth factor, IL-6 has been reported to be a key player in the stromal growth in BPH (Kramer, Mitteregger, & Marberger, 2007). Poor outcomes have been reported with elevated IL-6 levels in patients with prostate and other cancers (König, Senge, Allhoff, & König, 2004).

Researchers hypothesize that IL-6 may stimulate the growth of prostate cancer cells and cause progression of the disease through increased IL-6 production (Nakashima et al., 2000). An 18 fold increase in the concentration of IL-6 has been reported in localized prostate cancer compared to normal prostate tissue (Steiner et al., 2004). IL-6 has also been reported to upregulate the expression of COX-2 and PGE2 in a human PIN cell line and has a reciprocal and synergistic effect with COX-2/PGE2 on the growth of human PIN cells (X.-H. Liu et al., 2002). More recently researchers have demonstrated upregulation of IL-6 in men with prostate cancer undergoing radiation therapy (Christensen et al., 2009; Johnke et al., 2009). Diminished IL-6 expression has been reported in prostate tumors with lycopene supplementation (Wertz, et al., 2004). Figure 2.1 illustrates the cascade of events ascribed to IL-6 as discussed by several researchers (Christensen, et al., 2009; Helzlsouer, Erlinger, & Platz, 2006; Kuroda et al., 2007; Nakashima, et al., 2000; Steuber, Helo, & Lilja, 2007; Twillie et al., 1995).

C-Reactive Protein: C-reactive protein (CRP), an acute phase protein (Ford, Liu, Mannino, Giles, & Smith, 2003; Helzlsouer, et al., 2006; Lehrer et al., 2005), is a sensitive, yet non-specific marker of inflammation and is elevated in several diseases and disorders such as obesity, trauma, infection, diabetes and cancer (Walsh, Mahmoud, & Barna, 2003). CRP elevation in cancer patients may signify disease progression or recurrence (Walsh, et al., 2003). Two of the most commonly measured biomarkers of inflammation in prostate cancer are IL-6 and

CRP (Bowen, 2005). CRP levels are inversely associated with circulating levels of lycopene along with other carotenoids, retinoids and antioxidants (Ford, et al., 2003; McMillan et al., 2002).

Tumor Necrosis Factor-α: Tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine, is a paracrine and autocrine mediator (Danilko et al., 2007) of systemic inflammatory and immune reactions, and is produced by leukocytes, endothelia and adipocytes (Lamprecht, et al., 2007). Normal serum values are reported to be between 0-8.1 pg/mL (Akmansu, Unsal, Bora, & Elbeg, 2005). Chronic production of TNF may contribute to tumor promotion, leading to tissue remodeling and stromal development, ultimately contributing to tumor growth and metastasis (Wilson & Balkwill, 2002). TNF- α may trigger the inflammatory process by inducing the acute phase response along with increasing prostaglandin, leukotriene and collegenase synthesis (Huang, Ghai, & Ho, 2004). Acting as a macrophage and neutrophil activating factor, TNF- α triggers the synthesis of interleukin cascade, especially IL-1, IL-6, IL-8 and IL-10 (Danilko, et al., 2007). Loss of androgen responsiveness has been linked with tumor cell TNF production in patients with prostate cancer (Balkwill & Mantovani, 2001).

Prostaglandin: Prostaglandins (PGs) are lipid derivatives and regulate several physiological processes such as immune response, clotting, and platelet aggregation (Dubois et al., 1998; Williams, Mann, & DuBois, 1999). Action of cyclooxygenase (COX) enzyme on arachidonic acid leads to the production of

PGs (Keskek et al., 2006; Smyth, Grosser, Wang, Yu, & FitzGerald, 2009). COX-1 and COX-2 , the two isoforms of COX are involved in PG synthesis (Keskek, et al., 2006). While COX-1 is the constitutive isoform, COX-2 is the inducible form whose expression is induced by proinflammatory cytokines such as IL-1 and TNF-α (Keskek, et al., 2006). PGs (PGE2, PGI2) generated via COX-2 are reported to be immunosuppressive (Williams, et al., 1999) and a key source of inflammation and inflammation related cancers (Smyth, et al., 2009). COX-2 is over expressed in several malignant conditions and may also be induced by radiation and chemotherapies. Therefore, decreasing expression of COX-2 may be potentially important in the prevention and treatment of these malignant conditions (Dorai & Aggarwal, 2004).

PGE_2 is a key player in the immune suppression associated with inflammation and is secreted by several different cells including tumor cells, macrophages and monocytes (Ben-Baruch, 2006). It is synthesized by cyclooxygenase -2 (COX-2), and together they show great potential as targets for cancer therapy (Ben-Baruch, 2006). COX-2 and PGE2 are over-expressed in both PIN and prostate cancer (Kirschenbaum, et al., 2001). Liu et al (2002) demonstrated in a human PIN cell line that COX-2, PGE_2 and IL-6 have a reciprocal and synergistic effect. PGE2 stimulates the release of soluble IL-6 receptor and activates the STAT-3 signaling (X.-H. Liu, et al., 2002).

Radiation Therapy for Prostate Cancer

In prostate cancer, radiotherapy may be used as an independent therapy or in conjunction with surgery, chemotherapy, or hormone therapy (Samant & Gooi, 2005). Radiotherapy is generally administered in small daily doses (fractions), five days a week over a period of several weeks (Samant & Gooi, 2005; Withers, 1992). The number of treatments may vary based on factors such as organ being treated, patient condition, and size of the tumor (personal communication with radiation oncologist BF on September 12, 2007). Conventional radiation therapy has been available since the 1960's and with the advent of technology, treatment dose has increased while the exposure to the normal tissue in close proximity to the tumor has decreased considerably (Duchesne, 2001). Intensity modulated radiation therapy (IMRT) can conform higher dose volumes as close to the tumor as possible (Duchesne, 2001). Despite this specificity of IMRT, non-cancerous tissues still get irradiated and physiological changes noted in patients are related to this low dose exposure (Okunieff, Chen, Maguire, & Huser, 2008). Thus, patients may develop acute or late intestinal radiation toxicity depending on the radiation dose, fractions and treatment time frame (Hovdenak, Fajardo, & Hauer-Jensen, 2000).

Radiation therapy leads to ionization of intracellular water, producing free radicals (Lewanski & Gullick, 2001; Prasad, Cole, Kumar, & Prasad, 2002), which results in DNA strand breaks and plasma membrane damage ultimately leading to cell death (Lewanski & Gullick, 2001). In addition to DNA damage, apoptosis,

necrosis and inactivation of the cell cycle are additional mechanisms through which radiation may cause cell death (Okunieff, et al., 2008). While total body irradiation can have a significant immunosuppressive effect, loco-regional radiation therapy can also have a suppressive effect on the immune system (McBride et al., 2004). Radiation therapy treatment has also been shown to induce inflammation and up-regulate PGE_2 (Milas & Hanson, 1995; Steinauer et al., 2000).

Acute Side Effects During Pelvic Radiation Therapy: Radiation treatment related adverse effects occurring during or within three months of radiation treatment are considered acute and impact a patient's quality of life (Hauer-Jensen, Wang, Boerma, Fu, & Denham, 2007). Acute toxicity is observed frequently in tissues such as the skin and the gastrointestinal (GI) tract, that experience rapid cell proliferation (Mollà & Panés, 2007). Radiation therapy destroys stem cells which prevents the functional cells lost during normal tissue turnover from being replaced (Mollà & Panés, 2007). In addition to the GI toxicities, genitourinary (GU) toxicities are still the most commonly observed treatment related side effects among patients receiving pelvic radiation, and are dependent on the dose and volume of radiation delivered (Hovdenak, et al., 2000; Teh et al., 2004), along with treatment time (Hovdenak et al., 2003) and its process of dissipation through the tissues (Andreyev, 2007).

Andreyev (2007) proposed that radiotherapy induced damage to the blood vessels may lead to ischemia and fibrosis, thus altering GI function by either

worsening pre-existing GI problems or creating new GI dysfunction depending on the affected site. Other researchers have proposed that acute toxicity in the intestines is secondary to damage to the epithelial cells leading to the breakdown of mucosal barrier and causing mucosal inflammation (Hauer-Jensen, et al., 2007). Radiation-induced bowel damage may be evident in > 75% patients receiving pelvic radiotherapy (Cole, Slater, Sokal, & Hawkey, 1993). Cole et. al (1993) demonstrated a significant increase in eicosanoid inflammatory mediators (such as leukotriene B4, thromboxane B2 and PGE_2), implicating them in radiation-induced bowel inflammatory changes in patients receiving pelvis radiotherapy. Table 2.4 summarizes some of the cell and animal research studies reporting the upregulation of COX-2 in radiation induced bowel injury. Figure 2.2 represents the cascade of events reported during radiation therapy (Ben-Baruch, 2006; Cole, et al., 1993; Dorai & Aggarwal, 2004; Dubois, et al., 1998; Kurzrock, 2001; Larsen et al., 2007; Steinauer, et al., 2000; Steuber, et al., 2007; Wang, Bergh, & Damber, 2005; Williams, et al., 1999).

Lycopene

Lycopene is a 40 carbon, lipid soluble, highly conjugated aliphatic hydrocarbon carotenoid responsible for the red color of some fruits and vegetables (Kun, Lule, & Xiao-Lin, 2006; A V Rao & Agarwal, 1999, 2000; A V Rao & Ali, 2007). Lycopene is not synthesized by animals, only microorganisms and plants (Agarwal & Rao, 2000). The molecular formula for lycopene is $C_{40}H_{56}$, its molecular weight is 536.85 Daltons (Kun, et al., 2006; A V Rao & Agarwal,

1999; Shi & Maguer, 2000) and its melting point is 172-175^{0}C (Shi & Maguer, 2000). Lycopene resides primarily within cell membranes and is hydrophobic (Clinton, 1998). It contains 11 conjugated and two nonconjugated double bonds (A. Liu et al., 2006) and has a half life of 2-3 days in the serum (Stahl & Sies, 1992), and reaches maximum serum concentration within 15-48 hours of consuming lycopene rich foods (Gustin et al., 2004; Stahl & Sies, 1992). Lycopene is devoid of vitamin A activity due to the absence of the β-ionone ring (Clinton, 1998; Heber, 2004; Shi & Maguer, 2000).

Lycopene exists in a variety of geometric isomers like all-*trans*, mono-*cis*, and poly-*cis* form (Shi & Maguer, 2000). The *trans* configuration is the most common form of lycopene and is the form found in most plants (Kun, et al., 2006), while in the human plasma 50% lycopene exists as *cis* isomers (A V Rao & Agarwal, 2000; Shi & Maguer, 2000). The *all-trans* isomer of lycopene is the most thermodynamically stable form of the commonly identified isomers –5-*cis*, 7-*cis*, 9-*cis,* 11-*cis*, 13-*cis* and 15-*cis* (Shi & Maguer, 2000). The structural differences among some of the geometrical isomers of lycopene are presented in Figure 2.3.

Food sources of lycopene include tomatoes and tomato products, papaya, pink grapefruit, watermelon, and guava (Clinton, 1998; Kun, et al., 2006; A V Rao & Ali, 2007). Tomato and tomato products contribute more than 85% of dietary lycopene consumed in the North American diet (A V Rao & Agarwal, 2000). Tomato variety and the stage of ripening are key determinants of the lycopene

content of tomatoes (Clinton, 1998; Hadley, Miller, Schwartz, & Clinton, 2002). Tomato products may also contain polyphenols like quercetin, naringenin and chlorogenic acid along with small amounts of glucosinolates, tomatine and cyclolycopene (Bowen, 2005). More than 21 carotenoid pigments have been identified in tomatoes (Shi & Maguer, 2000). Some of these include phytoene, phytofluene, ζ-carotene, γ-carotene, β-carotene, neurosporene (Khachik et al., 2002) and lutein (Shi, Kakuda, & Yeung, 2004). The highest concentration of lycopene is found in the liver, adrenal glands, testes and the prostate (Kun, et al., 2006; Wertz, et al., 2004), while the lowest concentrations are noted in the kidney, lung and ovaries (Kun, et al., 2006). This variation in deposition of lycopene may be indicative of specific mechanisms involved in this process (Bramley, 2000). Tissue lycopene concentration in the prostate is reported to be 0-1.8 nM/gm (Clinton, 1998), and mean plasma lycopene concentrations ranging from 50-900 nM have been reported (Bramley, 2000). Concentration of lycopene in tissues, blood and other body secretions (seminal fluid, breast milk) is dependent on several factors such as food preparation practices, composition of the meal, diet composition, lipoprotein metabolism, and factors affecting lipid metabolism (Clinton, 1998).

Lycopene, a potent antioxidant and anti-inflammatory agent (Huang, et al., 2004; Rafi, Yadav, & Reyes, 2007), protects cells against ROS induced damage (Ford, et al., 2003; Neill & Fleshner, 2006; A V Rao & Shen, 2002). In addition to its role as an antioxidant, lycopene can influence several biological processes to

regulate cell cycle progression, gap junction communication and cytokine and growth factor signaling (Wertz, 2009). Some of the other mechanisms of action for lycopene include induction of phase II enzymes, inhibition of insulin like growth factor 1 (IGF-1) signal transduction, apoptosis induction, inhibition of IL-6 expression, inhibition of androgen activation and signaling, (Wertz, 2009) and modulation of the cyclo-oxygenase pathway (De Stefano et al., 2007; Sengupta, Ghosh, Das, Bhattacharjee, & Bhattacharya, 2006).

Recommended Intake of Lycopene: While researchers have recognized the health benefits of lycopene for some time now, it is still not recognized as an essential nutrient (A V Rao & Rao, 2007). No actual Dietary Reference Intake (DRI) levels currently exist for lycopene. Mean per capita intake of lycopene in the US has been reported to be about 8.2 (Matulka, Hood, & Griffiths, 2004) to 10.9 mg/day (McClain & Bausch, 2003). Clinical trials have been conducted using lycopene supplements (both food-based and pills) ranging from 5-120 mg/day, but a consensus is yet to be reached on the optimal daily dose. While Gustin et al., (2004) recommend daily ingestion of lycopene due to its short half life, they do not advocate more than 30 mg lycopene for chronic consumption.

Bioavailability of Lycopene: Lycopene absorption in humans is reported to be only about 10-30% of amount consumed (A V Rao, Ray, & Rao, 2006; Shi, Qu, Kakuda, Yeung, & Jiang, 2005). Lycopene in raw tomatoes is attached to the membranes which weaken during cooking, making lycopene more available from cooked tomato products (A V Rao & Agarwal, 2000; Weisburger, 2002). In

addition to heat, other factors that affect the bioavailability of lycopene include presence of dietary fiber (Kun, et al., 2006) , and amount and type of fat (Kun, et al., 2006; Lee, Thurnham, & Chopra, 2000; A V Rao & Ali, 2007; Stahl & Sies, 1992). Other factors such as age, gender, hormonal status, smoking, alcohol consumption, body mass and composition may also affect bioavailability of lycopene (A V Rao, et al., 2006; Stahl & Sies, 1992). In addition, dietary components and drugs affecting lipid metabolism may impact serum lycopene concentration (Hadley, et al., 2002). During digestion lycopene is incorporated into micelles and through passive diffusion is absorbed by the intestinal epithelial cells (Shi, et al., 2005). Chylomicrons carry lycopene into the portal circulation, and after being repackaged, lycopene is released into the blood bound to low density lipoprotein (LDL) (Shi, et al., 2005).

Safety/Toxicity Evaluation of Lycopene: Upon reviewing lycopene supplementation studies, McClain and Bausch (2003) reported no systemic toxicity, genotoxicity, or adverse effects on reproductive parameters. No adverse effects were noted in toxicology studies conducted in rats with intakes up to 3 gm/kg/day (Trumbo, 2005). Safety of food based lycopene supplementation in humans has been established by virtue of its unrestricted use (McClain & Bausch, 2003). While no upper tolerable limit has been established for lycopene consumption, synthetic lycopene, lycopene extracts and crystallized lycopene extracts are generally regarded as safe (GRAS) in foods at levels ranging from 0.5 to 7% (Trumbo, 2005). After reviewing published data on lycopene

supplementation, Shao and Hathcock (2006) recommend 75 mg per day as the upper limit for supplementation in humans. Astley and Elliott (2005) propose that 15 mg of lycopene obtained from supplements is equivalent to the addition of approximately 150 gm of lycopene-rich foods in the diet.

Some side effects of lycopene supplementation have been reported in clinical trials. These include nausea and vomiting (Bunker et al., 2007; Jatoi et al., 2007), diarrhea (Clark et al., 2006; Jatoi, et al., 2007), abdominal distention (Jatoi, et al., 2007), flatulence (Edinger & Koff, 2006; Jatoi, et al., 2007), anorexia (Jatoi, et al., 2007) and dyspepsia (Jatoi, et al., 2007). Edinger and Koff (2006) reported heartburn, skin itching and flatulence among some study participants fed 50 grams tomato paste daily for 10 consecutive weeks. Jatoi et al., (2007) also reported occurrence of severe hypotension, prostatic hemorrhage, diarrhea, and anemia. While Jatoi et al., (2007) raise cautious concern about mild adverse reactions to lycopene supplementation, they do not preclude future clinical trials, but instead stress the importance of patient education about any potential side effects.

Prostate Cancer, Inflammation and Lycopene

Epidemiological studies (Giovannucci, 1999, 2002) have documented an inverse relationship between intake of lycopene rich foods and the development of prostate cancer. Several cell (Hwang & Bowen, 2004; Ivanov et al., 2007; Kanagaraj et al., 2007; L. Kim, Rao, & Rao, 2002) and animal studies (Guttenplan et al., 2001; Konijeti et al., 2010), and clinical trials in men at risk for

developing prostate cancer (Bunker, et al., 2007; Edinger & Koff, 2006; Mohanty, Saxena, Singh, Goyal, & Arora, 2005; Schwarz et al., 2008) have demonstrated the chemopreventive role of lycopene. Researchers have also evaluated the benefits of lycopene in prostate cancer patients prior to initiating treatment (Jatoi, et al., 2007; H. Kim et al., 2003; Kucuk et al., 2001) or after failure of treatment (Clark, et al., 2006). Despite the presence of several carotenoids and polyphenols, the chemoprotective effect of tomatoes on prostate cancer has been attributed to lycopene (Hwang & Bowen, 2004). Researchers have even demonstrated a stronger anti-mutagenic effect of food-based lycopene (tomato puree) compared with pure lycopene. This stronger effect was attributed to the likely synergistic effect of all bioactive compounds found in tomatoes (Polívková, Šmerák, Demová, & Houška, 2010). A summary of some of the key trials/studies are provided in Tables 2.5-2.7.

Despite several clinical trials evaluating the role of lycopene in patients with BPH and prostate cancer, data on the efficacy of lycopene supplementation in prostate cancer patients during radiation therapy are lacking. Based on the existing evidence, while some researchers have advocated for the inclusion of tomato extracts/products in prostate cancer treatment and prevention (Guns & Cowell, 2005), others have proposed urgency in evaluating the effectiveness of lycopene supplementation in prostate cancer patients undergoing radiation and androgen oblation therapies (Clinton, 2005; Davis et al., 2005), in order to formulate evidence-based recommendations for this population.

Lycopene Use During Radiation Therapy: Lycopene and other antioxidants have been proposed to resolve free radical induced oxidative damage produced during radiation therapy (Simone, Simone, Simone, & Simone, 2007). Researchers have demonstrated a beneficial effect of lycopene supplementation during radiation therapy to reduce the associated gastrointestinal (GI) side effects in rats (Andic, Garipagaoglu, Yurdakonar, Tuncel, & Kucuk, 2009). Clinical trials evaluating the impact of lycopene supplementation during radiotherapy are limited. The only documentation in the literature of lycopene supplementation during radiotherapy was found in patients with high grade gliomas undergoing radiotherapy and chemotherapy (Puri et al., 2005). Puri et al., (2005) reported a significant increase in serum lycopene levels, no adverse effects and positive, but not statistically significant ($p = 0.1$), outcomes in the lycopene (8 mg supplement) treated group compared to the control group. A summary of some of the studies evaluating the effectiveness of lycopene supplementation during radiation therapy are provided in Table 2.8.

Summary

Despite several clinical trials evaluating the role of lycopene in patients with BPH (Edinger & Koff, 2006; Schwarz, et al., 2008) and prostate cancer (Bowen et al., 2002; Chen et al., 2001; Jatoi et al., 2007; Kim et al., 2003; Kucuk et al., 2001; Rao et al., 1999), data on the efficacy of lycopene supplementation in prostate cancer patients during radiation therapy are lacking. To our knowledge, no studies have examined the effects of lycopene supplementation in

men undergoing radiation therapy for the treatment of prostate cancer. Several researchers have proposed the urgency in evaluating the effectiveness of lycopene supplementation in prostate cancer patients undergoing radiation and androgen oblation therapy (Bowen, 2005; Davis, et al., 2005). Thus, the proposed study evaluated the impact of daily supplementation of three different doses of lycopene on serum lycopene levels, PSA, and select markers of inflammation, and on the onset and severity of specific physical side effects (urinary frequency and urgency, proctitis, and diarrhea) and performance status in patients with localized prostate cancer during radiotherapy.

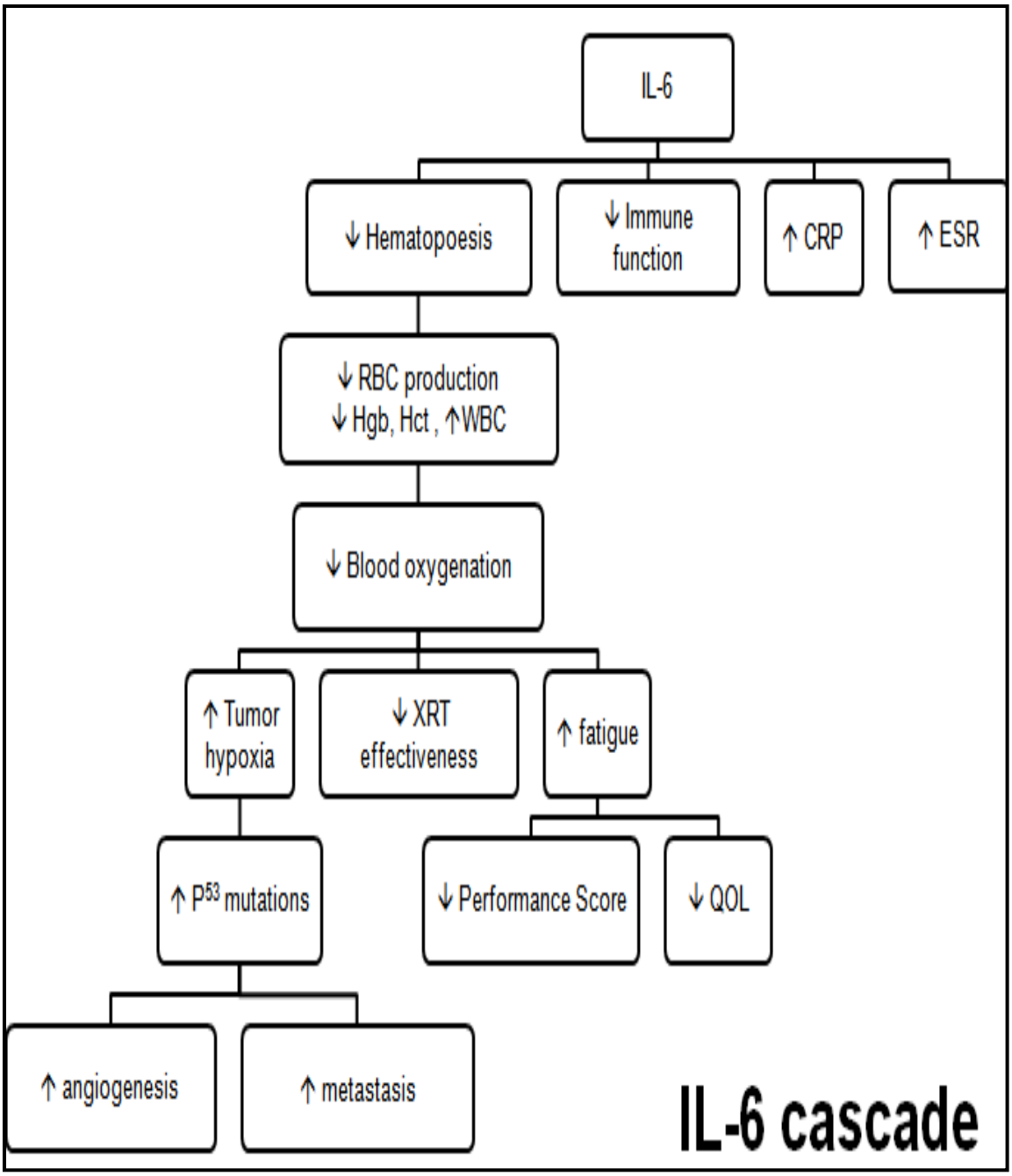

Figure 2.1. Cascade of events reported with increased IL-6 expression. IL-6 = interleukin-6, CRP = c-reactive protein, ESR = erythrocyte sedimentation rate, RBC = red blood cells, Hgb = hemoglobin, Hct = hematocrit, WBC = white blood count, XRT = radiation therapy, QOL = quality of life

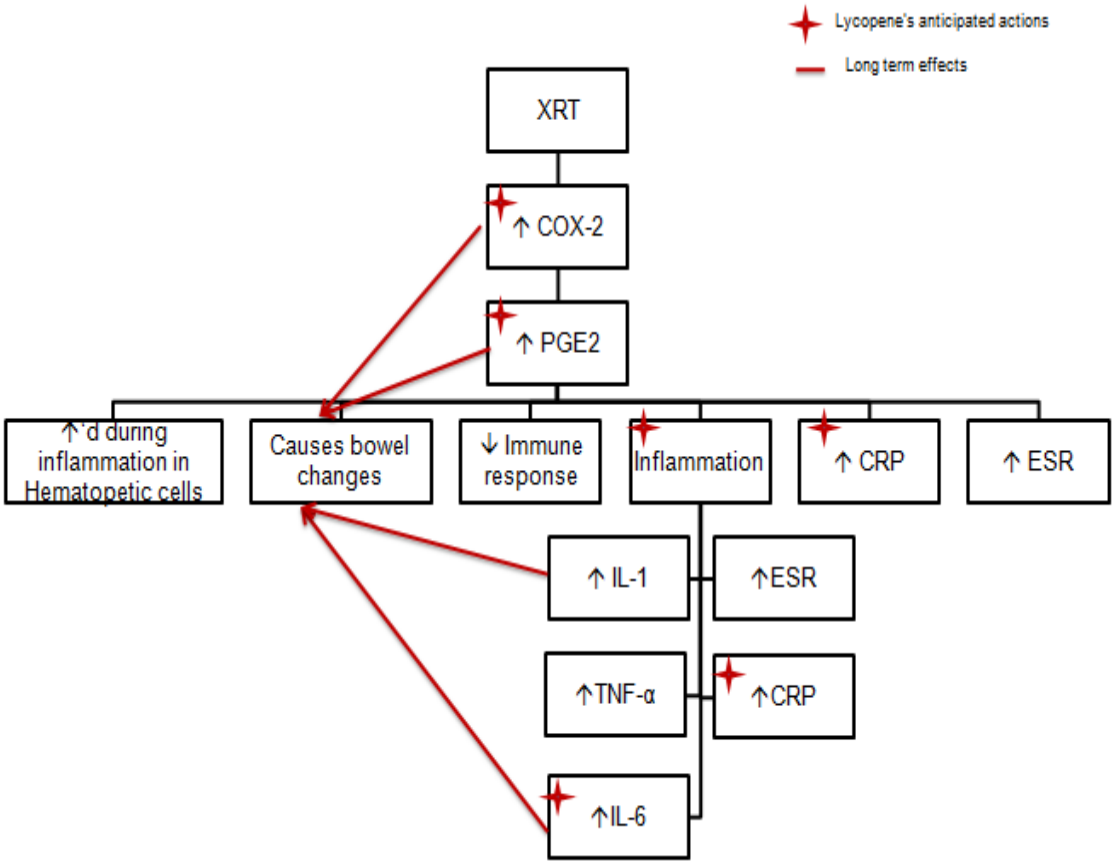

Figure 2.2. Radiation therapy related cascade. XRT = radiation therapy, COX-2 = cyclooxygenase-2, PGE2 = prostaglandin E2, CRP = c-reactive protein, ESR = erythrocyte sedimentation rate, IL-1 = interleukin-1, TNF-α = tumor necrosis factor- α, IL-6 = interleukin-6

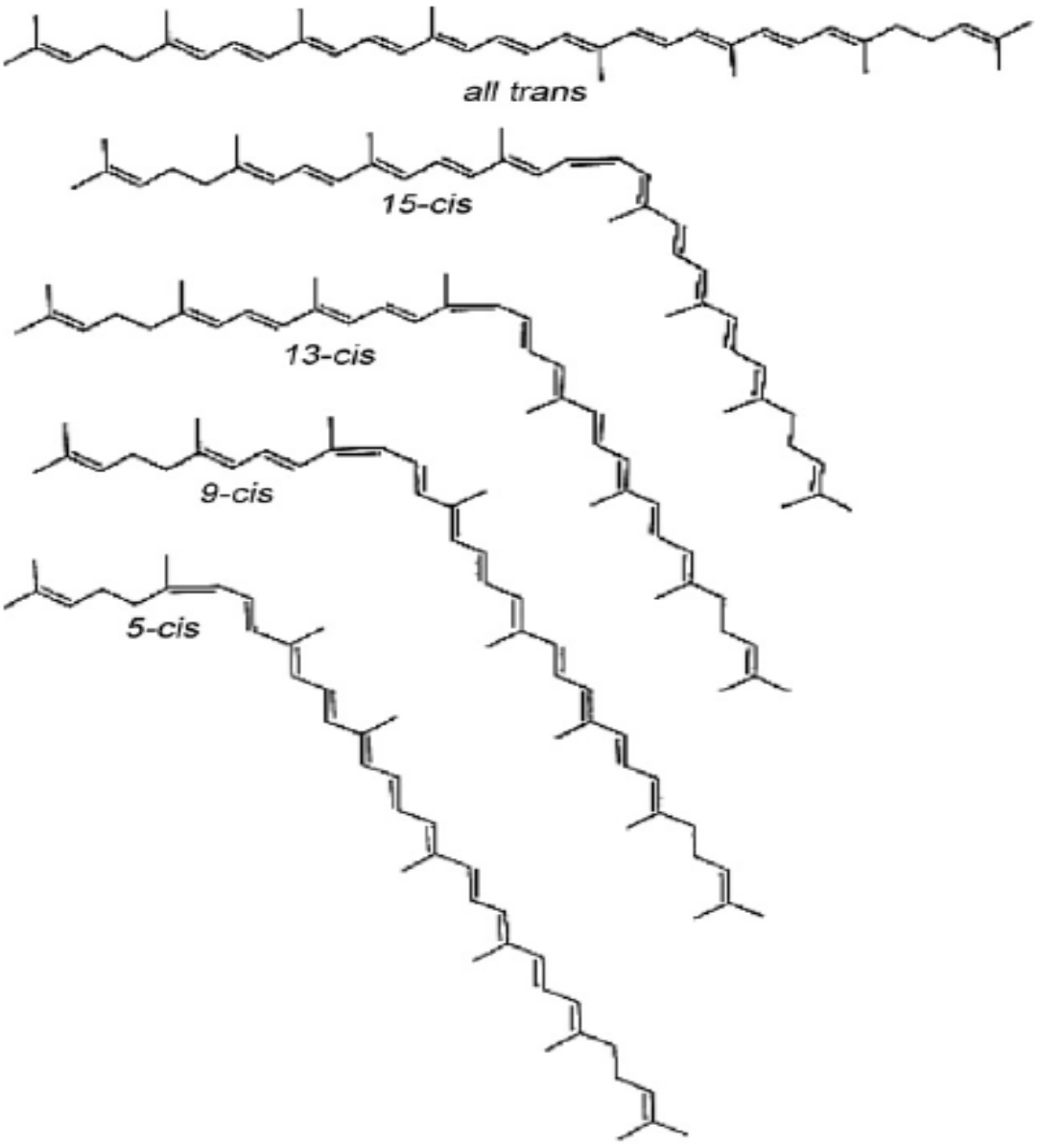

Figure 2.3: All-trans and cis isomers of lycopene (Source – (Rao & Rao, 2007)).

TABLE 2.1. Cell and animal research studies implicating inflammation in prostate tumorigenesis

Study	Animal/ cell line	Tests/Assays	Results
(Quintar et al., 2010)	Rat model	Prostatic tissues immuno-stained for PBP, ACTA2, ErbB1, & ErbB2 receptors, TUNEL, & cell proliferation markers. Dot & Western blots for PBP, ACTA2, ErbB1, ErbB2, & TGFb1	Post infection prostatic epithelium hypertrophied with ↑ PBP, ErbB1 & ErbB2 in a time dependent fashion. @ 72 hrs post infection, the epithelium showed apoptosis & atrophy with ↓ in PBP & ErbB receptors. ↓ in ACTA2 at 72 hr post infection indicating atrophic & proliferative changes promoted by even early stage prostatic inflammation
(Wong, Bray, & Ho, 2009)	LNCaP prostate cancer epithelial cells	Western blots, RNA isolation, cDNA synthesis, & real time quantitative PCR, ELISA, Flow cytometry	LNCaP cells show local proinflam response (NF-kB activation, & ↑ TNFa, IL-1b, & IL-6 expr. in activated macrophage conditioned media). Sig. ↑ VCAM-1 & nuclear estrogen receptor-a indicating potential link between chronic inflam. & its involvement in PC
(Fujita et al., 2002)	LNCaP cells	RNA Extraction and RT-PCR, Quantitative Real-Time PCR, western blot, COX assay, luciferase assay, ELISA	COX-2 mRNA & protein & COX activity in LNCaPCOX-2 cells sig. ↑ compared with parent and control-transfected cells & ↑ both proliferation in vitro & tumor growth rate in vivo. But pro-tumor effect not associated with androgen receptor level/activity. ↑ VEGF seen in LNCaP-COX-2 cells suggesting tumor growth in vivo stimulated by angiogenesis induced by COX-2.
(X.-H. Liu, et al., 2002)	Human PIN cell line	immunoprecipitation, immunoblotting, ELISA, nuclear extract preparation & electrophoretic mobility shift assay	PGE2 demonstrates autocrine upregulation by stimulating soluble IL-6 receptor release, gp130 dimerization, Stat-3 protein phosphorylation, & DNA binding activity, leading to ↑ PIN cell growth. COX-2 inhibitor ↓ cell growth. IL-6 neutralizing antibodies diminished PGE2-stimulated PIN cell growth indicating that ↑ COX-2/PGE2 leads to PC dev. thru activation IL-6 signaling pathway

TABLE 2.2. Human tissue studies implicating inflammation in prostate tumorigenesis

Study	n	Study Design & Population	Tests/Assays	Results
(Ravenna et al., 2009)	20 pairs	human normal and primary prostate tissue	real-time PCR and Western blot analysis in prostate samples	RAGE, P2X7R, COX2, NOS2, PTX3, EGFR, ERa, (but not ERb) up-regulated in tumor samples. Western blot analysis showed nuclear translocation of the NF-kB subunit p65.
(Bouraoui et al., 2008)	47	Tissues from 5 normal, 25 BPH and 17 human PC	Western blot & immune-histochemistry of IL-1, IL-6 & TNF-α, PSA	in BPH, IL-1α, IL-6 and TNF expressed in pts with PSA levels of 0–4 or 4–20 only, but not >20 ng/ml In PC these cytokines only expressed in patients with PSA serum levels > 4 ng/ml.
(Khor et al., 2007)	586	Prostate tissue from men treated with radiation (XRT) and both short (STAD)or long term androgen deprivation (LTAD)	Immunohistochemical staining; any failure, local failure, distant metastasis, biochemical failure, overall mortality, cause specific mortality	intensity of COX-2 staining an indep. predictor of distant mets (p=0·0004); biochem. failure (p=0·008); & any failure (p=0·011). higher the expression of COX-2, greater the chance of failure. COX-2 over-expression most discriminating for those receiving STAD vs LTAD. ↑ COX-2 expr. sig. associated with biochemical failure, distant mets & any failure. LTAD might overcome effects of COX-2 overexpr. COX-2 expr. may be useful in selecting pts needing LTAD.
(Cohen et al., 2006)	60	Prostate cancer specimens from men post radical prostatectomy	immune-histochemistry, slide grading; preoperative PSA, stage, Gleason sum (GS), margin, extraprostatic extension (EPE); seminal vesicle (SV) invasion	At 62-mths follow-up, COX-2 staining predicted progression with 82.4% sensitivity & 81.3% specificity. Sensitivity (86.4%) & specificity (86.7%) improved at ≥100-months follow-up., preop. PSA, EPE, margin, SV invasion & high COX-2 expr. were sig. predictors of biochemical recurrence ($p < 0.05$). In multivariate analysis, preop PSA & COX-2 were indep. prognostic indicators. Pts with PSA > 7 ng/ml & ↑ COX-2 expr. had highest prob. of recurrence.

TABLE 2.2. Human tissue studies implicating inflammation in prostate tumorigenesis, cont'd

Study	n	Study Design & Population	Tests/Assays	Results
(Wang, Bergh, & Damber, 2004)	45	BPH samples.	immunohistochemistry; double staining;	↑ COX-2 expression seen in all prostate luminal epithelial samples; highest proliferation index found in COX-2 +ve stained epithelium.COX-2 expression associated with Bcl-2 immunostaining in atrophic lesions (P<0.0001). T lymphocytes & macrophages predominant inflammatory cells related to the COX-2 expression in prostate epithelium.
(Hughes et al., 2002)	106	PC pts taking part in the Phase III RTOG 86-10 trial	Immunohistochemical staining for COX-2	99% of slides expressed COX-2, samples with GS 7-10 had 81% COX-2 expression, GS 2-6 had only 69%
(Gupta, Srivastava, Ahmad, Bostwick, & Mukhtar, 2000)	12 PC tissue samples 12 control	Diagnosis of PC	semi-quanti. reverse transcription-PCR, immunoblotting, & immunohistochemistry	Mean COX-2 mRNA levels 3.4-x ↑ in PC tissue compared with the paired benign tissue. COX-2 protein over- expressed in 10 of 12 samples;

TABLE 2.3. Clinical trials implicating inflammation in prostate tumorigenesis

Study	n	Study Design & Population	Tests/Assays	Results
(Salinas, et al., 2010)	1001 PC cases, 942 age matched controls	Case control study	Assessment of aspirin and other NSAID use, Genotyping	Significant ↓ (21%) in PC risk seen among current users of aspirin compared with nonusers. Long-term use of aspirin & daily use of low-dose aspirin also associated with ↓ risk. no evidence that the association with aspirin use varied by disease aggressiveness, but there was effect modification. PC risk not related to use of non aspirin NSAIDs or acetaminophen.
(Schenk et al., 2010)	676 cases 683 control	Nested case-control study from placebo arm of the PC Prevention Trial	Baseline serum was analyzed for CRP, TNF-a, sTNF-RI & sTNF-RII, IL-6, & interferon c	↑ CRP associated with ↑ BPH risk (for quartile 4 vs. quartile 1, OR =1.40, 95% CI: 1.04, 1.88; which attenuated after control for BMI (OR = 1.30, 95% CI: 0.95, 1.75). Low sTNFRII & ↑ IL-6 associated with ↑ BPH risk; associations only in men aged <65 years.
(Rebbeck, et al., 2008)	1090	Caucasian PC cases, history of BPH	Genotype analysis	only remaining significant associations involved CYP3A43 P340A genotypes & history of BPH on both Gleason grade (interaction p-value = 0.026) and tumor stage (interaction p-value = 0.017).
(Di Silverio et al., 2003)	3942 case	Retrospective histopathology examination	Histopathology examination	↑% of pts with inflammation associated with BPH. FAA (p = 0.027), PIN (p = 0.036) & incidental PC ↑ sig. with age. Distribution on inflammatory aspects and AAH varied sig. (p = 0.002) based on prostate volume.

TABLE 2.3. Clinical trials implicating inflammation in prostate tumorigenesis, cont'd

Study	n	Study Design & Population	Tests/Assays	Results
(Nickel, Downey, Young, & Boag, 1999)	80	diagnosis of BPH	immunostained for leukocyte common antigen; computerized image-analysis system.	Inflammation present in all pts but only 1.1% of mean tissue surface area involved, 44% prostate specimens showed bacterial growth. no sig. diff. between inflammation pattern, volume or grade of inflammation in those catheterized or not (P=0.15) or culture +ve (pathogenic or not) & culture-negative cases (P=0.06). amount, degree or distribution of inflammation didn't sig. correlate with total PSA or PSA density

Table 2.4. Cell and animal research studies implicating COX-2 upregulation in radiation therapy of the prostate

Study	Animal/ cell line	Tests/Assays	Results
(Keskek, et al., 2006)	Sprague -Dawley rats	abdominal irradiation, Cyclooxygenase-2 Immunostaining, Myeloperoxidase Activity, Malondialdehyde Assay, Histopathology	COX-2 ↑ sig. in vascular endothelial cells on days 4 & 14 post exposure. COX-2 ↑ in fibroblasts immediately post irradiation & remained sig. ↑ during the entire study ($P < 0.001$), there was a peak COX-2 expression on day 14 similar to that observed in endothelial cells. Irradiation significantly ↑ intestinal epithelial damage, MPO activity, & MDA levels compared to the control group in a time-dependent fashion. Treatment with rofecoxib significantly ↓ these ↑ except on day 4
(Li et al., 2003)	PC3 cell line	Glutathione Assay, DCF, ROS assay, PGE2 monoclonal Immunoassay Kit	L-Buthionine sulfoximin ↓ cellular GSH & ↑ cellular ROS in PC-3 cells, whereas lipoic acid & NAC ↑ GSH level & ↓ cellular ROS. Both radiation & H202 similarly up-regulated COX-2 & PGE2 in PC-3 cells. ↑ greater when PC-3 cells pretreated with BSO. Pretreatment with a-lipoic acid or NAC for 24 h, up-regulated both radiation- & H202-induced COX2 & ↓ PGE2 production.
(Steinauer , et al., 2000)	PC3 cell line	Western blot analysis for COX-2 protein expression. PGE_2 measured using Monoclonal Immunoassay Kit. flow cytometry.	dose-dependent ↑ in COX-2 of 37.0%, 79.7%, & 97.5% post irradiation with 5, 10, and 15 Gy, respectively. PGE_2 ↑ in irradiated cells than controls; ↓ PGE_2 levels in cells irradiated in the presence of NS-398. no differences in cell cycle distribution or apoptosis between cells irradiated in the presence or absence of NS-398.

TABLE 2.5. Summary of lycopene research in cell/animal studies

Study	Methods	Lycopene dose	animal/Cell line	Results
(Polívková, et al., 2010)	AMES test, bone marrow micronucleus test	(30 mg and 300 mg per plate) 0, 25 or 50 mg/kg for animal study	TA98, TA100, BALB/c mice	Sig., dose dependent, antimutagenic effects of lycopene noted at various conc. Of AFB1 & IQ in TA98 & TA100. ↑ protective effects seen in AFB1 & IQ compared to MNU. Mice treated for 3 days with 25 or 50 mg/kg of lycopene prior to mutagen admin. show a sig. ↓ micronuclei numbers & tomato puree showed a stronger, dose-depend. antimutagenic effect compared with similar doses of pure lycopene
(Konijeti, et al., 2010)	Serum IGF-I, IGFBP-3 measured by ELISA, serum & prostate lycopene & α-tocopherol & liver tissue deoxy-guanosine & 8-hydroxydeoxyguanosine measured by HPLC	28 mg/kg lycopene from tomato paste (TP) or lycopene beadlets (LB)	TRAMP mice	PC incidence sig. ↓ in LB group compared to controls (60% vs. 95%, P=0.0197) but not between TP & control groups (80% vs. 95%, P=0.34). no diff in prostate wts seen between groups. Both total serum & prostate lycopene levels ↑in LB & TP groups but not control group. ratio of 5-cis-lycopene to trans-lycopene in serum was sig. ↑ in LB group compared to TP group (P=0.0001). sig. ↓ oxidative DNA damage in livers of mice fed LB & TP diets but not control group.
(Kanagaraj, et al., 2007)	IGF-I, IGFBP-3 and IGF-I receptors in lycopene-treated cells, Annexin V & PI binding studies	20, 40 & 60 μM lycopene treated for 24, 48, 72 & 96 hrs	PC-3 cells	lycopene treatment showed a sig. ↓ in cell proliferation. 40 μM lycopene sig. ↑ IGFBP-3. Lycopene induced apoptosis; & DNA fragmentation absent after 24 hrs but not after 48 hrs. sig.↓ in the IGF-IR post cell treatment with lycopene, IGF-I

TABLE 2.5. Summary of lycopene research in cell/animal studies, cont'd

Study	Methods	Lycopene dose	animal/Cell line	Results
(Ivanov, et al., 2007)	Proliferation assay, bromodeoxyuridine incorporation and flow cytometric analysis of cellular DNA content	0-100µM	LNCaP, PC3	LNCaP & PC-3 cells undergo mitotic arrest accumulating in G0/G1 phase - block in G1/S transition due to ↓ cyclin D1 & E, CDK4 & Rb phosphorylation. Corresponding with ↓ IGF-1 receptor expression & activation, ↑ IGFBP-2 expression & ↓AKT activation
(Srinivasan et al., 2007)	TBARS, single cell gel electrophoresis;levels of SOD, catalase, glutathione peroxidase ceruloplasmin, vitamin A, C, E & urinc acid	1.86, 9.31, 18.62 µM	hepatocytes isolated from rat (Sprague-Dawley) liver	pretreatment with lycopene demonstrated a sig. ↓ in TBARS and DNA damage and a sig. ↑ in glutathione, vitamin A, C, E, uric acid and ceruloplasmin. Max protection to hepatocytes noted at 9.31 µM lycopene
(Gunasekera et al., 2007)	Inhibition of cell growth, time course studies, Mitogenic assays for growth factor studies	0, 0.04, 0.4, 5, 10, 20 µM	Dunning R3327AT3 or AT3 cells and DTE	both lycopene & lutein inhibited malignant AT3 cells in a dose and time-dep. manner, but not DTE cells. Lycopene showed more robust response than lutein & no synergistic or additive effect observed with both.
(Gitenay et al., 2007)	Mass Spec, RNA extravtion, real time PCR, western blot	50 mg/kg	Male Wistar rats,PC3AR human cell line	Cx43 expression sig. ↑ post exposure to RTS or YTS for 48 hrs compared to control, LBS effect not sig. diff. similar lycopene levels in cells incubated with RTS & LBS, while YTS contained no lycopene.
(Limpens et al., 2006)	HPLC-prostate and liver to detect lycopene and a,G, D tocopherol	Ly/Vit E-5 or 50 mg/kg; Ly+E - 5 mg/kg	PC-346C	none of the treatments sig. ↓ tumor vol., Lycopene+vit E @5 mg/kg sig. ↓ tumor growth, ↑ median survival by 40%. Lycopene levels ↑ in a dose dep.manner

TABLE 2.5. Summary of lycopene research in cell/animal studies, cont'd

Study	Methods	Lycopene dose	animal/Cell line	Results
(A. Liu, et al., 2006)	subcellular fractionation of LNCaP cells using centrifugation and liquid chromatography-tandem mass spectrometry	1.48 Mmol/L	LNCaP, PC-3, and DU145	After 24 hrs of incubation with 1.48 Mmol/L lycopene, LNCaP cells accumulated most lycopene & only one to express PSA. Levels of PSA ↓ 55% in LNCaP cells after 1.48 Mmol/L lycopene. lycopene not found to be a ligand for ligand-binding domain of the human androgen receptor. majority of lycopene (55%) localized in the nuclear membranes, followed by nuclear matrix (26%), & then microsomes (19%) & none in the cytosol.
(Hwang & Bowen, 2004)	effects of lycopene on cell growth or survival, cell cycle progression, and apoptosis	0, 0.1, 1, and 5 µ*M*	LNCaP	Lycopene at 1 µ*M* inhibited cell growth by 31% compared to placebo post 48-hr incubation. Lycopene at 5 µ*M* ↑ number of cells in G2/M phase of cell cycle from 13% to 28% & ↓S-phase cells from 45% to 29%, no shifts in cell cycle seen in placebo-treated groups. Apoptosis observed at 5 µ*M* lycopene @ late stages during 24 &48 hour treatments.
(Obermuller-Jevic et al., 2003)	Cellular uptake, thymidine assay, flow cytometry, western blot	0.1-5.0µM	PrEC (Human prostate epithelial cells)	Dose dependent inhibition of cell proliferation noted; synchronized cells treated with 0.5µM lycopene showed minimal change, but cells treated with 5.0µM showed a significant accumulation in G0/G1 with no expression of cyclin D1, cyclin E was unaffected.

TABLE 2.5. Summary of lycopene research in cell/animal studies, cont'd

Study	Methods	Lycopene dose	animal/Cell line	Results
(Yaping, Wenli, Weile, & Ying, 2003)	croton oil-induced mouse ear edema model, & glass slide method	0.1, 0.5, 1, 2 g/kg body wt	3-4 week old male mice	Administration of lycopene for four days was associated with ↓ swelling of the treated ear similar to amoxycillin. Lycopene also ↑ coagulation time
(Guttenplan, et al., 2001)	DNA isolation, mutagenesis assay, lycopene assay	0.5, 1 mmol/kg	LacZ male mice	inhibition of spontaneous mutagenesis in prostate and colon observed at 1 mmol/kg of lycopene-rich tomato oleoresin (LTO), benzo[a]pyrene (BaP)-induced mutagenesis inhibited by LTO in prostate.
(Forssberg, Lingen, Ernster, & Lindberg, 1959)	lycopene administered pre 48-12 hrs) & post (7-24 hrs) treatment	0.5-2 mg inj. Intraperitoneally	159 - lycop; 190 - control	lycopene improves survival when given before irradiation & at certain time after. females tolerated ↑ lycopene dose in combination with radiation better than males (males did better at smaller doses); radiation tolerance varies in different stages of menstrual cycle;

TABLE 2.6. Summary of lycopene trials in healthy subjects

study	n	Lyco dose	food vs pill	duration	Participg Populat.	end point	Results
(Talvas et al., 2010)	30	0, 16 mg	Tomato paste, purified lycopene	1 wk supplementation, 2 wk washout period	Health men, 50-70 yrs	Differentiate the effect of tomato matrix from tomato paste or purified lycopene	serum lycopene ↑ after consuming red tomato paste & purified lycopene. No change in lipid profile, antioxidant status, PSA & IGF-I after consumption of tomato paste & lycopene. Sig.↑ in IGFBP-3 & Bax:Bcl-2 ratio & ↓of cyclin-D1, p53, & Nrf-2 in men consuming red tomato paste compared with serum collected after first washout period. Sig. ↑ of IGFBP-3, c-fos, & uPAR in men consuming purified lycopene compared with placebo
(Graydon et al., 2007)	20	15 mg	pill	4 weeks	healthy men 18-60 yrs	Serum IGF-1, IGFBP-3 & lycopene pre & post supplemnt	↑ change in serum lycopene in treatment group compared to control. no diff in median change in IGF-1 or IGFBP-3 between treatment & control groups. Change in lycopene corresponded with the change in IGFBP-3 in treatment group (r=0.78; P=0.008; n=10)
(Goyal, Chopra, Lwaleed, Birch, & Cooper, 2007)	6	22.8 mg	Cream of tomato soup	2 weeks	Healthy men	Blood/ seminal lycopene & antioxidant capacity pre & post supplemt	statistically sig. ↑ in blood & seminal plasma lycopene levels post supplementation, with a strong +ve correlation (r=0.84,P<0.05). no measurable ↑ in total radical scavenging capacity of semen.

TABLE 2.6. Summary of lycopene trials in healthy subjects, cont'd

study	n	Lycopene dose	food vs pill	duration	Participg Populat.	end point	Results
(Riso, Brusamolino, Martinetti, & Porrini, 2006)	20	5.7 mg	Lyc-o-mato drink	26 days	Healthy young men and women	Blood carotenoid, IGF-1 and IGFBP-3 estimation	tomato drink ↑ plasma lycopene, phytoene, phytofluene, & bcarotene ($P < 0.05$). No sig. effect of tomato drink on IGF-1. changes in lycopene pre & post each experimt period inversely & sig. correlated with IGF-1 ($r = –0.33$, $P < 0.05$). No correlation with other carotenoids. sig. ↓ serum IGF-1 (–5.7%) with highest plasma lycopene ($P<0.05$). No change with placebo
(Gustin, et al., 2004)	25	10, 30, 60, 90, 120 mg	30 gm tomato paste + 5 ml olive oil	28 days	healthy men 18-45 yrs	3 parameters of lycopene & isomers after single dose of lycopene; toxicity profile	headache, nausea, diarrhea most common adverse events, toxicity grade 2 or less; max serum lycopene reached in 15-32.6 hrs; half life 28-61.6 hrs; lower doses (10, 30) had the largest ↑ in systemic exposure parameters
(Allen et al., 2003)	36	21 mg, 12 mg, 17 mg	sauce, soup, juice (v8 tomato or veggie)	6 week lycopene free diet-2 wk washout+ 4 week Tx	healthy men and women; 18-65 yr	impact of single daily svgs of 3 tomato prod. on blood & BMC conc. of carotenoid & lycopene isomer	serum & BMC lycopene ↑ with all three (sauce, soup, juice); lycopene plateaued after 2 weeks.

TABLE 2.6. Summary of lycopene trials in healthy subjects, cont'd

study	n	Lycopene dose	food vs pill	duration	Particip Populat.	end point	Results
(Hadley, et al., 2002)	60	35 mg, 23 mg, 25 mg	cond. soup (35 mg), RTS soup (23 mg), V8 veg juice (25 mg)	1 wk lycopene free diet, 15 day lycopene supplementation	healthy >40 yr old men & women	rate & magnitude of plasma lycopene after 1 wk of lycopene free diet & then with 3 diff food sources of lycopene; assess impact on biomarkers of oxidative damage	plasma conc of lycopene ↓ 35% ($p<0.0001$) during washout period, lycopene ↑ 123% cond. soup, 57% RTS soup, 112% V8. No significant change seen in urinary 8-OH-2'-dG & 8-epi-PGF2α, but ex-vivo lipoprotein oxidation lag period (measure of antiox. capacity) ↑ significantly
(A V Rao & Shen, 2002)	12	5, 10, 20 mg	ketchup & lyc-o-mato	2 week washout, then 2 week intervention	healthy men & women, non-smokers, no MVI	serum lycopene, oxidative biomarkers (MDA)	dose dependent ↑ in serum lycopene with both pill and food, levels slightly higher with pill but diff not stat. Sig. MDA ↓ & reduced thiols ↑ with both pill and food all doses.
(Porrini & Riso, 2000)	9	7 mg	25 gm tomato paste + ↓ carotene diet	14 days	healthy women	plasma and lymphocyte carotenoid conc.; lymphocyte resistance to oxidative stress	Inverse relationship between plasma lycopene conc. and lymphocyte lycopene conc.and oxidative DNA damage…small amts of lycopene over short duration can ↑ carotenoid conc and resistance of lymphocytes to oxidative stress.

TABLE 2.6. Summary of lycopene trials in healthy subjects, cont'd

study	n	Lycopene dose	food vs pill	duration	Particip Populat.	end point	Results
(Bohm & Bitsch, 1999)	22	5 mg	tomatoes, tomato juice and pill	2 wks of washout, 6 weeks of supplemt	adult females (20-27 yrs)	serum lycopene, lipid panel, antioxidant capacity of plasma	no change with tomato intake, but TJ (0.22) and oleoresin (0.25) ↑ serum lycopene conc. No affect on the lipid status or antioxidant capacity in any of the 3 groups
(Paetau et al., 1998)	15	70-75 mg	tomato juice, and lycopene oleoresin & beadlets	4 weeks each, 6 week washout period between Tx	healthy men and women 33-61 yr	lycopene plasma response from food and supplements; changes in lycopene oxidation products	lycopene level ↑ with all 3 Tx, no stat significant diff among the three; levels plateaued after 1 wk for supplements and after 2 wks for TJ. TJ ↑ other tomato carotenoids (phytofluene, phytoene). Cyclolycopene (lycopene metabolite) ↑ most with TJ.
(Stahl & Sies, 1992)	6	0.35, 1.25, 2.5µmol/kg	tomato juice - heated and unheated	single dose (absorption, dose dep), once a day x 4 days)	healthy 22-36 yr old (5) male & (1) females	absorption studies, dose dependent response, accumulation	**Absorption:** Peak conc seen within 24-48 hrs post consumption of heated TJ; 1/2 life 2-3 days; **Dose Dep**: lycopene uptake was dose dep, but not very linear; higher absorption with smaller amounts. **Accumulation:** of lycopene & its isomers very linear

TABLE 2.7. Summary of lycopene trials in subjects with prostate involvement

study	N	Lycopene dose (mg)	food vs pill	duration	patient population	end point	results
(Barber et al., 2006)	37	10 mg	lycoplus (with vit C, E etc)	1 year	PC pts; confirm with biopsy, no Tx, watchful waiting	change in PSA velocity with lycopene supplementation	lycopene inhibits DNA synthesis in PEC, results inconclusive!! Large ↑ in PSA doubling time but not stat. sig. Data skewed since some pts eliminated had sig. ↓ in PSA
(Edinger & Koff, 2006)	43	13 mg	50 gm T paste	10 wks	BPH, ↑PSA	PSA	Significant ↓ in PSA
(Mohanty, et al., 2005)	40	0, 4 mg BID	Lyc-o-mato	1 year	HGPIN	PSA, serum lycopene, prostate biopsy, DRE	lycopene can delay or prevent HGPIN from progressing to PC, inverse relationship between lycopene and PSA
(Chang et al., 2005)	118 cases 52 contrl	NA	NA	2 years	Cases- histologically confirmed localized PC Controls- PSA<4ng/ml, -ve DRE	Plasma carotenoid	50% less risk for men with ↑plasma levels of α-carotene, trans-β-carotene, β-cryptoxanthin, lutein & zeaxanthin. No Sig. associations for total lycopene, all-trans lycopene,& cis-lycopene isomer peaks 2, 3,& 5, high levels of cis-lycopene isomer peak inversely associated with risk.
(Jian, Du, Lee, & Binns, 2005)	cases -130, 274 contrl	5 mg	lycoplus (5 mg lycopene + other antox	I year	Cases- PC controls- hospital inpts without cancer	prostate cancer dev	prostate risk ↓ with ↑ing intake of lycopene, α & β-carotene, β-cryptoxanthine, lutein,& zeaxanthine

TABLE 2.7. Summary of lycopene trials in subjects with prostate involvement, cont'd

study	N	Lycopene dose (mg)	food vs pill	duration	patient population	end point	Results
(Ansari & Gupta, 2004)	20	10 mg	pill	3 mths	Metastatic hormone refractory PC	ECOG, Dz response (complete or partial vs stable or prog. dz), bone pain, LUTS	complete response-5%, partial -30%, stable dz -50%) & dz prog.-15%. ECOG Grade 0-25%, Grade I-50% & Grade II-25%. ↓ ECOG from Grade I-0-35% & Grade II-I-15%, ↑ in 15% & unchanged – 35%. LUTS improved in 61% of pts. No supplmt related intol./ toxicity seen.
(H. Kim, et al., 2003)	32-tx; 34-ct	30 mg	3 pasta dishes	3 weeks	prostate cancer, BPH	PSA, lycopene, bax, bcl-2,	no change in bcl-2, but bax ↓ in cancer, ↑ in apoptotic cells in BPH & cancer; PSA ↓
(Bowen et al., 2002)	32	30 mg	3 pasta dishes	3 weeks	localized PC awaiting prostatectomy	PSA, leukocyte oxidative DNA damage	Sig. ↑ in serum & prostate lycopene; leukocyte oxidative DNA & PSA sig. ↓ with lycopene;
(Kucuk et al., 2002)	26	30 mg	lyc-o-mato	3 weeks	PC	PSA, IGF-1, IGFBP-3, prostate tissue	Tx group pts had smaller tumors, ↓ involvement of surgical margins, PSA ↓ 18%; IGF-1 & IGFBP-3 ↓ sig.

TABLE 2.7. Summary of lycopene trials in subjects with prostate involvement, cont'd

study	N	Lycopene dose (mg)	food vs pill	duration	patient population	end point	Results
(van Breemen et al., 2002)	32	30 mg	Tomato sauce based pasta dishes	3 weeks	T1/T2 PC patients waiting prostatectomy	Cis & trans lycopene in serum & prostate tissue	Total lycopene ↑ 2.0-fold in serum & 3.0-fold in prostate tissue post suppl. Mean all-trans-lycopene in prostate tissue ↑ to 22.7% (from 12.4%) of total lycopene post suppl., serum only a 2.8% ↑but statistically sig.
(Chen et al., 2001)	32	30 mg	3 pasta dishes	3 weeks	PC pts waiting prostatectomy	PSA, leukocyte oxidative DNA damage	Sig.↑ in serum & prostate lycopene; leukocyte oxidative DNA & PSA sig. ↓ with lycopene
(Kucuk, et al., 2001)	26	30 mg	lyc-o-mato	3 weeks	localized PC patients waiting prostatectomy	PSA, connexin43, bax, bcl-2	serum lycopene ↓ post intervention. Lycopene suppl. may ↓ PC growth; bcl-2, bax same between groups, IGF-1 ↓ in both groups

TABLE 2.7. Summary of lycopene trials in subjects with prostate involvement, cont'd

study	N	Lycopene dose (mg)	food vs pill	duration	patient population	end point	Results
(Lu et al., 2001)	65 cases 132 control	NA	NA	4 years	Cases- pathologically confirmed PC Control – healthy cancer free men	Plasma tocopherols, retinol & carotenoids, NCI FFQ	Sig. inverse associations with PC observed with plasma lycopene [OR, 0.17; 95% CI, 0.04–0.78; *P* for trend, 0.0052] and zeaxanthin (OR,0.22; 95% CI, 0.06–0.83; *P* for trend, 0.0028) Borderline associations found for lutein (OR, 0.30; 95% CI, 0.09 –1.03; *P* for trend, 0.0064) & b-cryptoxanthin (OR, 0.31; 95% CI, 0.08 –1.24; *P* for trend, 0.0666).
(A Venket Rao, Fleshner, & Agarwal, 1999)	12 cases 12 age matchedcontrols	NA	NA	NA	Cases - Histologically conformed & untreated PC Controls- untreated muscle invasive bladder cancer, but no BPH or PC	Serum & tissue lycopene, lutein, cryptoxanthin, β-carotene, lipid and protein oxidation	Sig ↓ serum & tissue lycopene but not other carotenoids noted in cases; while no diff in serum lipid peroxidation noted, serum protein thiol sig ↓ in cases. Role of lycopene in preventing oxidative damage and ↓ risk of PC should be evaluated further.

TABLE 2.8. Cell and animal research studies investigating lycopene and radiation

Study	animal/ Cell line	Test/Assays	Lycopene dose	Results
(Saada, Rezk, & Eltahawy, 2010)	Male albino rats	6 Gy γ-radiation (XRT); Ileum tissues for xanthine oxidase; thiobarbituric acid reactive substances (TBARS); xanthine dehydrogenase; Superoxide dismutase (SOD), catalase (CAT), serotonin (5-HT), dopamine (DA), glutathione (GSH); norepinephrine (NE), epinephrine (EPI); monoamine-oxidase (MAO)	5 mg/kg body weight	Irradiated animals showed sloughing villi, ulcers, & ruptured goblet cells, shrinkage of submucosa layers & fibroblasts. Histopathological changes associated with sig. ↑ in TBARS & change in xanthine oxidoreductase system (XOR). sig. ↓ in reduced GSH, SOD & CAT seen. XRT also induced a sig. ↓ in level of: 5-HT, DA, NE, & EPI associated with an ↑ in MAO activity. Lycopene pretreatment sig. ↑ the oxidant/antioxidant status, associated with sig. regeneration of small intestine, & improved monoamines levels. Thus, lycopene may protect small intestine against radiation-induced damage.
(Andic, et al., 2009)	Wistar albino rats	8 Gy abdominal and pelvic radiation; Study endpoints: weight loss, diarrhea, duration of diarrhea, survival, & plasma level of TBARS.	5 mg/kg body wt/day	Rats receiving RT only had sig. ↑ wt. loss rate compared to the lycopene + RT group (P = 0.001). Plasma TBARS levels after RT were also sig. ↑ in the RT only group compared to lycopene + RT group (P = 0.001). Lycopene supplementation sig. ↓ wt. loss & prevented oxidative stress in rats treated with abdomino-pelvic radiation.

TABLE 2.8. Cell and animal research studies investigating lycopene and radiation, cont'd

Study	animal/ Cell line	Test/Assays	Lycopene dose	Results
(Srinivasan, Devipriya, Kalpana, & Menon, 2009)	Cultured human lymphocytes	γ-radiation at 1, 2 and 4 Gy. The cellular changes were estimated by using TBARS, hydroperoxides (HP), the antioxidants SOD, CAT, GPx & GSH. The DNA damage was analyzed by cytokinesis blocked micronucleus assay (CBMN), dicentric aberration (DC) and translocation frequency.	1, 5 & 10μg/ml	Diff. doses of γ-radiation led to sig. ↑ in # of DC, micronuclei (MN), translocation frequency, TBARS & HP level, but levels of GSH & antiox. enzymes sig. ↓ compared with control. Max. damage to lymphocytes @ 4Gy. Lycopene pretreatment (1, 5 &10μg/ml) sig. ↓ frequency of MN, DC & translocation when compared with control. level of TBARS, HP were also ↓ & activities of SOD, CAT & GPx were sig. ↑ along with GSH levels when compared with control. 5μg/ml lycopene more effective than other two doses-offering protection to normal lymphocytes against γ-radiation-induced cellular damage.
(Srinivasan, et al., 2007)	cultured rat hepatocytes	Cellular changes estimated using TBARS, SOD, CAT, glutathione peroxidase (GPx), GSH, ceruloplasmin, vitamins A, E, C and uric acid. DNA damage analysed by single cell gel electrophoresis (comet assay).	1.86, 9.31 & 18.62 μM	↑ severity of DNA damage observed with ↑ in γ-radiation dose (1, 2 & 4 Gy) in cultured rat hepatocytes. TBARS ↑ sig. while levels of GSH, vitamins C, E and A, ceruloplasmin, uric acid & antiox.enzymes sig.↓in γ-irradiated groups. Max. damage to hepatocytes observed at 4 Gy irradiation. Pretreatment with lycopene (1.86, 9.31& 18.62 μM) showed a sig. ↓ in levels of TBARS & DNA damage. Antiox.enzymes ↑ sig. along with the levels of GSH, vitamins A, E, C, uric acid & ceruloplasmin. Max. protection of hepatocytes observed at 9.31 μM lycopene pretreatment.

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CHAPTER III

DIETARY AND SERUM LYCOPENE LEVELS IN PROSTATE CANCER PATIENTS UNDERGOING IMRT

Submitting to: Cancer Causes & Control

Abstract

Objectives: To determine the effect of radiotherapy and supplementing three different amounts of tomato juice on serum lycopene levels in men with prostate cancer.

Methods: Dietary lycopene intake was calculated using the National Cancer Institute (NCI) Diet History Questionnaire. Gastrointestinal (GI) tolerance of tomato juice was evaluated using the NCI Cancer Therapy Evaluation Program: Common Toxicity Criteria v 2.0. Serum lycopene levels were determined using liquid chromatography-mass spectrometry. Both serum and dietary lycopene were used as covariates in data analysis.

Results: Daily tomato juice supplementation (4, 8 or 12 oz) was tolerated without any adverse GI effects. Serum lycopene decreased in control group participants, while increasing from 0.33±0.11 µg/mL to 0.41± 0.12 µg/ in the intervention group. No correlation between serum and dietary lycopene was observed. Control group participants lost weight, while participants in the intervention group did not.

Major conclusions: Tomato juice, a food source of lycopene (a potent antioxidant) is well tolerated and does increase serum lycopene levels in men with prostate cancer undergoing radiotherapy. Weight change should be monitored and evaluated in prostate cancer patients during radiotherapy. Larger clinical trials are needed to validate tomato juice use as a way to increase serum/dietary lycopene intake during radiotherapy in men with prostate cancer.

Introduction

Dietary phytochemicals (such as lycopene) may halt carcinogenesis by suppressing the initiating transforming and inflammatory processes (Dorai & Aggarwal, 2004). Epidemiological studies (Gann et al., 1999; Giovannucci, 1999, 2005; Vogt et al., 2002) have documented an inverse relationship between intake of lycopene rich foods and the development of prostate cancer. Researchers have also evaluated the impact of lycopene in patients with benign prostate hypertrophy (Edinger & Koff, 2006; Schwarz et al., 2008), and prostate cancer patients prior to initiating treatment (Bowen et al., 2002; Chen et al., 2001; Jatoi et al., 2007; Kim et al., 2003; Kucuk et al., 2001; Rao, Fleshner, & Agarwal, 1999) and after failure of treatment (Clark et al., 2006). Data on the effectiveness of lycopene supplementation during radiation therapy are lacking and researchers have proposed an urgency in evaluating the effectiveness of lycopene supplementation in prostate cancer patients undergoing radiation and androgen oblation therapies (Clinton, 2005; Davis et al., 2005), in order to formulate evidence-based recommendations for this population.

Serum antioxidant levels have been used to evaluate nutritional status and oxidative stress, since many of these antioxidants are essential and are utilized in physiological defense mechanisms (Polidori, Stahl, Eichler, Niestroj, & Sies, 2001). Oxidative stress generated secondary to chemotherapy and radiotherapy may further decrease tissue antioxidant levels, thereby increasing oxidative stress (Simone, Simone, Simone, & Simone, 2007), and may worsen cancer

treatment related side effects and existing subclinical or clinical nutrient deficiencies (Kucuk, 2002). A relatively new trend in cancer research is utilizing chemopreventive agents either by themselves or as adjuncts to halt disease progression, prevent secondary cancers or reduce treatment toxicities (Kucuk, 2002). In a case-controlled study, researchers demonstrated that prostate cancer patients have significantly lower (44%; $p < 0.004$) serum lycopene levels compared to matched controls (Rao, et al., 1999). Clinical trials evaluating the impact of lycopene supplementation during radiotherapy are limited, and since carotenoids have been reported to be preferentially utilized in oxidative states (Polidori, et al., 2001), we were interested in determining the level of serum lycopene in prostate cancer patients undergoing radiation therapy, and further evaluating the impact of tomato juice supplementation on serum lycopene levels. Consequently, we conducted this randomized controlled trial to evaluate the impact of three different volumes of tomato juice on serum lycopene levels during radiation therapy in men with localized prostate cancer. We also obtained diet history information to evaluate routine dietary lycopene intake among participants of this study. This study was part of a Phase I clinical trial evaluating the impact of three different volumes of tomato juice on various inflammatory markers and selected side effects of radiation therapy.

Materials and Methods

This randomized controlled trial conducted in men newly diagnosed with localized prostate cancer consisted of four study arms (control group participants

consumed their normal diets, and three intervention groups who received 4, 8 or 12 ounces (oz) of tomato juice in addition to their routine dietary intake). Participants were instructed to refrain from making any changes to their diet or consuming any nutritional (vitamin, mineral or other nutraceutical) supplements for the duration of the study. All participants received image guided intensity modulated radiation therapy (IMRT) to the prostate gland (treatment volume included prostate alone or prostate and seminal vesicles). In order to minimize time spent at the cancer center and avoid unnecessary follow-up visits, all assessments, blood draws and tomato juice administration (intervention groups only) were scheduled to coincide with each patient's physician, procedure planning or radiation therapy appointment times.

Seventeen men with newly diagnosed localized prostate cancer, receiving ≥ 72 Gray (Gy) of external radiation to the prostate alone or prostate and seminal vesicles completed this pilot study. Sample recruitment was consecutive and nonprobabilistic, since we included all patients meeting the eligibility criteria who were referred to the Hayworth Cancer Center at High Point Regional Health System (HPRHS) between April 2009 and October 2010. Participant screening was conducted in two stages: a preliminary screening was conducted by the radiation oncologist (BF) at the time of initial consult based on treatment area, type and dose. Patients meeting preliminary screening criteria signed a Health Insurance Portability and Accountability Act (HIPAA) form granting access to their medical information for detailed medical record review. In addition, patient

interviews were also conducted (MD) to thoroughly screen potential participants based on the study eligibility criteria. The Institutional Review Boards at the University of North Carolina Greensboro (UNCG) and HPRHS approved the study protocol. All participants provided written informed consent prior to enrolling in the study.

The *eligibility criteria* included – histologically confirmed localized prostate adenocarcinoma with no lymph node involvement or metastasis; normal immune, liver and renal function; and Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1. *Exclusion criteria* included: post prostatectomy, chemotherapy or other prior treatment for prostate cancer. Patients who received hormone therapy or participated in other treatment based clinical trials concurrently while receiving radiation therapy were also not eligible to participate in this study. Patients who were allergic to tomato products or red dye; had pre-existing uncontrolled gastro-esophageal reflux disease and other malabsorptive disorders, or hyperkalemia; or routinely consumed (and unwilling to stop these supplements during treatment) fiber, saw palmetto, lycopene, omega-3 fatty acids/fish oil, EPA/DHA, vitamin C, E, A; β-carotene, flaxseeds and flaxseed oil supplements were also ineligible to participate in this study. Any potential participants consuming these specified supplements at the time of the initial interview were asked to discontinue these supplements until the end of treatment. This allowed for a wash-out period of several weeks before radiation treatment commenced. Verbal confirmation was obtained on the first day of

treatment to insure that the participants had discontinued use of supplements as previously instructed.

Names of each group (control, 4, 8, or 12 oz) and the participant number for that group were written on a piece of folded paper. The patient or accompanying family member then picked one of the folded pieces of paper and handed it to the investigator (MD) who transcribed the group assignment, writing it on the participant screening form and informing the participant and family members.

Assigned volume of tomato juice (4, 8 or 12 oz) was initiated two days prior to participants receiving the first dose of radiation therapy and continued daily until the last day of treatment. Participants in the intervention groups were provided with the measured amount of their assigned volume of tomato juice for consumption over the weekend and on holidays. Verbal confirmation of tomato juice consumption was obtained at the subsequent treatment day. Participants were instructed to consume tomato juice with either a meal or a snack with at least five grams of fat to facilitate lycopene absorption. During treatment days, participants were asked to consume their assigned volume of tomato juice and crackers (providing at least five grams of fat) after their radiation treatment, prior to leaving the cancer center, which was observed by the researcher (MD). Caloric contribution of the tomato juice and crackers was 121 kcal (4 oz), 146 kcal (8 oz) and 171 kcal (12 oz) daily (Table 3.3). Participants were asked to maintain their normal diets during the study. We used the National Cancer

Institute (NCI) Diet History Questionnaire (DHQ) ("Diet History Questionnaire,") to obtain participant's diet history and routine dietary lycopene intake once during the study period. The DHQ was given to the participants prior to starting radiation therapy and all participants except one returned the completed DHQ within two weeks of starting treatment. Participants were weighed by the nursing staff on the first day of treatment (baseline) and then once a week during treatment on a stationary platform scale in the radiation therapy department at HPRHS.

In order to evaluate (gastrointestinal) tolerance of tomato juice supplementation, the NCI Cancer Therapy Evaluation Program: Common Toxicity Criteria (CTC) v 2.0 ("Cancer therapy evaluation program. Common Toxicity Criteria, v2.0," 1999) was used. The CTC evaluates adverse events on a scale of 0-5 (TABLE 3.1) and any participant exhibiting toxicity grade ≥3 would have been withdrawn from the study by the researchers.

Standardized planning and filming protocol was followed, and participants were treated on a Linear Accelerator 2100 iX (Varian Medical Systems, CA). A diagnostic image obtained prior to each treatment was approved by the treating Radiation Oncologist. The prescribed radiation dose ranged between 72.50-79.20 Gy and treatment days varied between 29-44 days. Consequently, daily fractions ranged from 1.8-2.5 Gy.

Participants provided blood samples at baseline, midpoint (end of three weeks of treatment) and on the last day of treatment. Whole blood was collected in aluminum foil wrapped vacutainer tubes, and transported on ice to the

laboratory at UNCG. In order to minimize loss of lycopene, tubes were wrapped in aluminum foil immediately after collection and blood samples were processed under a yellow light. After centrifuging (3000 rpm x 20 min at 4^0C) whole blood, the separated serum was divided into aliquot tubes labeled with patient ID, time point of blood draw (baseline, midpoint or endpoint) and stored at -80^0C until analyzed. Serum and tomato juice samples were shipped under dry ice to Dr. Wei Jia's laboratory at the Kannapolis Research Campus, NC, for lycopene analysis using liquid chromatography-mass spectrometry (LC-MS).

Stock solution for lycopene standard was prepared by dissolving ~1 mg of the standard in 1 mL of chloroform followed by storage at -80 °C. Fresh calibration solutions were prepared each day from the stock solution. Calibration curves were obtained using freshly prepared lycopene standard solutions in the range of 0.005-10 µg/mL in acetonitrile/methyl tert-butyl ether (MTBE) (1:1, v/v). Aliquots of 10 *µ*L of each standard solution were injected onto the column for LC analysis, and calibration curves were constructed by linear regression analysis of the area versus the concentration of lycopene.

Lycopene from tomato juice was extracted with an extraction mixture (hexane, methanol, acetone, 2:1:1, v/v/v, containing 2.5% butylated hydroxytoluen (BHT)). Tomato juice (200 µL) was extracted with the extraction mixture (600 µL). Samples were vortexed for one min and then centrifuged for 5 min at 13,000 rpm at 4^0C. Aliquots of 10 µL of the supernatant were diluted to 1 mL with the mobile phase for the HPLC injection. To prepare serum for lycopene

analysis, 300 µL serum was mixed with same amount of ethanol. The mixture was extracted twice with 600 µL of hexane containing 100mg/L BHT. The hexane extracts were collected after being centrifuged at 13,000 rpm for 5 min (4^0C), combined, and evaporated to dryness under vacuum. The extract was reconstituted in 100 µL of acetonitrile/MTBE (1:1, v/v). An aliquot of 10 µL was injected onto the LC for lycopene measurement. An Agilent HPLC 1200 system equipped with a binary solvent delivery manager and a sample manager (Agilent Corporation, Santa Clara, CA, USA) is used with chromatographic separations performed on a 4.6 × 150 mm 5 µm Agilent ZORBAX Eclipse XDB-C18 chromatography column. The flow rate was 1 mL/min. Elution solvent A was acetonitrile and solvent B was MTBE. The LC elution conditions are optimized with isocratic acetonitrile/MTBE (45:55, v/v). The column is maintained at 20^0C. The detection wavelength was set at 472 nm. A 10 µL aliquot reference standards and 10 µL samples were injected onto the column, respectively.

On the last day of treatment, the researcher (MD) asked each participant about his reasons for participating in the study, whether he was glad to have participated, and if he would recommend any changes to the study protocol.

Descriptive statistics were used to analyze key participant and cancer related characteristics. Between group differences were detected using the non-parametric Wilcoxon Rank Sum analysis. We also conducted Spearman's correlation to determine any associations between dietary and serum lycopene levels and select lifestyle characteristics, and stepwise regression to detect the

strength of this relationship. Repeated measures were used to evaluate within group change over time. One-sided level of significance was established at $p \leq 0.05$. Between-group comparisons with p between 0.051-0.10 were identified as demonstrating a trend towards significance.

Results

The flow diagram describes participant screening, final enrollment, attrition and reasons for participant exclusion (Figure 3.1). Seventeen men between the ages of 61 and 77 years completed this pilot study. Seventy-one percent of participants had clinical stage T1c prostate adenocarcinoma, followed by 18% with stage T2a and 6% each with stage T2b and T2c (data not shown). The largest proportion of our participants was non-Hispanic Whites (71%), followed by African-Americans (24%) and 6% of Peruvian descent. Despite a higher prevalence of prostate cancer reported among African-Americans ("Cancer Facts and Figures 2010," 2010), only 24% of our participants were African-American. This may be due to poor/limited access to health care and/or poor likelihood of localized prostate cancer diagnosis in African-Americans ("Cancer Facts and Figures 2010," 2010; Jemal, Siegel, Xu, & Ward, 2010). Height was statistically different between the control group and 12 oz group participants ($p = 0.026$); however, no differences were detected among groups for weight or body mass index (BMI). A majority (82%) of study participants in our study were married, and 71% reported less than or equivalent to a high school education (Table 3.2).

The most frequently (88%) observed medical diagnoses among study participants were hypercholesterolemia and hypertension. Thirty-five percent of participants had a diagnosis of diabetes but did not report following a “strict” diet for diabetes management/control. Only about 6% of participants reported still smoking compared with 65% of participants who reported consuming alcohol. One participant in the 4 oz treatment group reported changing his diet and activity significantly in the months prior to starting radiation therapy, but this was unrelated to his cancer diagnosis (data not shown). Overall, 71% of participants reported consuming one or more over-the-counter herbal/nutritional supplements at the time of enrollment. A multivitamin was the most commonly consumed (47%) supplement followed by fish oil/omega-3 fatty acid (29%). Other nutritional supplements consumed included vitamin D (18%), fiber (12%), vitamin C (12%), and 6% reported consuming either vitamin E, flax seeds, saw palmetto or coenzyme Q10 (Table 3.2). Twelve percent of the participants also reported using “eye” vitamin drops. Participants discontinued use of all supplements for the duration of the study. No statistically significant differences were detected between groups for supplement use. We did, however, find a significant positive correlation between supplement use and diagnosis of hypertension ($r = 0.556$, $p = 0.009$, $n = 17$) and observed a significant trend between supplement use and education level ($r = 0.337$, $p = 0.093$, $n = 17$).

Participants in all three treatment groups tolerated the tomato juice well with no reported gastrointestinal side effects (nausea, vomiting, or heartburn)

(data not shown). Participants in the intervention groups received 121 kcal (4 oz), 146 kcal (8 oz) and 171 kcal (12 oz) daily from the tomato juice and crackers (Table 3.3). When asked, some participants reported changes in their daily meal pattern or quantity of food intake in the meal closest to the time they received their tomato juice supplement at the cancer center. Not surprisingly, lycopene from tomato juice was statistically different between groups (Table 3.3). Macro and select micro nutrient analysis obtained from participant-reported DHQs are reported in Table 3.4. While a large variation was noted in energy intakes reported by participants among the four groups, caloric intake between only the control group and 8 oz group was statistically significant ($p = 0.0476$). Calculated dietary lycopene intake between the control group and 12 oz group ($p = 0.0357$) and 4 and 12 oz groups ($p = 0.0286$) were statistically significant. Intervention group participants received an estimated additional 8 mg, 18 mg, or 28 mg daily dose of lycopene from their assigned volume (4, 8 or 12 oz per day) of tomato juice. Total dietary lycopene (diet+supplemetal) intake was also statistically significantly different between groups (Table 3.4). We found a significant positive correlation ($r = 0.517$; $p = 0.017$; $n = 17$) with education level and reported dietary lycopene intake and a significant trend between hypertension and reported dietary lycopene intake ($r = 0.335$, $p = 0.094$, $n = 17$), indicating that participants with higher education and a diagnosis of hypertension reported higher intake of dietary lycopene.

No significant differences in body weight were detected among participants in any group at the beginning of the study. While very little variation in body weight was observed among the 4 oz and the 12 oz intervention group participants from the beginning to the end of the study, participants in the control group lost nine pounds while participants in the 8 oz group gained about eight pounds (Table 3.5).

Mean serum lycopene level for all study participants at baseline was 0.30 µg/mL and ranged between 0 to 1.04 µg/mL. When baseline levels were evaluated by study group, participants in the 8 oz group had the highest baseline lycopene level (0.51±0.17 µg/mL). Two participants in both 4 and 12 oz groups had no detectable serum lycopene at baseline. We detected a significant decrease ($p = 0.009$) in serum lycopene levels among control group participants over time, but not any of the intervention groups. However, overall serum lycopene levels increased with daily tomato juice supplementation. Intervention group mean serum lycopene increased from 0.33±0.11 µg/mL at baseline to 0.41± 0.12 µg/mL at endpoint. Participants in the 4 oz group demonstrated a progressive, yet non-significant decrease, while 8 oz group participants demonstrated a significant increase ($p = 0.056$ comparing percent change from baseline to midpoint and $p = 0.095$ comparing percent change from baseline to endpoint) in serum lycopene throughout treatment. All participants in the 12 oz group had measureable levels at midpoint and endpoint versus only one at baseline. We detected significant between-group differences in serum lycopene

levels at various time points (Table 3.4). Total lycopene intake per kilogram of body weight varied considerably: 0.07 mg/Kg (control group), 0.14 mg/Kg (4 oz), 0.27 mg/kg (8oz) and 0.53 mg/kg (12 oz group). These values were statistically significantly different ($p < 0.01$) between groups.

A significant positive correlation was detected between serum lycopene, weight ($r = 0.525$; $p = 0.015$; $n = 17$) and BMI ($r = 0.541$; $p = 0.012$; $n = 17$), and a negative correlation between serum lycopene and prior nutritional supplement use ($r = -0.464$; $p = 0.030$; $n = 17$). While not statistically significant, we also observed a negative trend between serum lycopene, smoking ($r = -0.359$; $p = 0.078$; $n = 17$) and diagnosis of hypertension ($r = -0.356$; $p = 0.080$; $n = 17$). However, in the final stepwise regression model, BMI (but not weight) and smoking were the two variables that explained 56% of the variance [$F(2, 14) = 8.833$, $p = 0.003$] in serum lycopene level.

The majority of participants (65%) reported taking part in the study because they “liked the idea of tomato juice helping reduce side effects of treatment.” Forty seven percent of the participants also reported participating in the hopes that the results of the study would “help other people.” Ninety-four percent of participants reported being glad that they participated in our clinical trial. Only one participant was dissatisfied, primarily because he was randomized into the control group and he wanted to participate in one of the intervention groups. While several participants remarked on the length of the DHQ while

completing the questionnaire, only one participant suggested shortening the DHQ during the exit interviews.

Discussion

We evaluated food based lycopene (tomato juice) supplementation in this randomized control trial in men undergoing radiation therapy for localized prostate cancer. The primary goal of this study was to evaluate serum lycopene levels during prostate radiation therapy and the impact of three different volumes of tomato juice administered daily on serum lycopene levels during radiation therapy. Secondary goals were to determine reported dietary lycopene intake in participants enrolled in our study and their reasons for participating in this clinical trial. We were able to demonstrate an increase in serum lycopene levels with daily tomato juice supplementation in men with prostate cancer undergoing radiation therapy. Serum lycopene levels ranging from 0.43 µmol/L in BPH (Schwarz, et al., 2008) to 0.64 µmol/L in prostate cancer patients (Bowen, et al., 2002) have been reported, similar to levels (0.55 µmol/L or 0.30 µg/mL) that we obtained at baseline. While Mayne et al (Mayne et al., 1999) have reported an association between serum and dietary lycopene intake, we did not observe this association. Inaccurate reporting of dietary lycopene, inconsistent digestion and absorption have been postulated as some of the reason why a poor correlation between dietary intake and serum levels of lycopene may be observed (Hadley, Miller, Schwartz, & Clinton, 2002) . We also did not detect an association between alcohol intake, ethnicity, marital status or age as reported by Porrini and

Riso (Porrini & Riso, 2005). While an optimum time (before or after radiation exposure) for initiating an antioxidant supplement has not been identified (S. L. Brown et al., 2010), we choose to initiate tomato juice supplement two days before radiation therapy started. Researchers have reported that lycopene reaches maximum concentration in the serum 15-48 hours after consumption (Gustin et al., 2004; Stahl & Sies, 1992), and our goal was to insure the presence of lycopene in the serum of participants at the commencement of radiation therapy, to evaluate the impact of radiation induced oxidative stress on serum lycopene levels with daily tomato juice intake during radiation treatment.

Dietary lycopene intake was estimated once using the DHQ. Mean reported dietary lycopene intake among participants was 7.24±1.48 mg/day (range 1.56-23.59 mg/day). Matulka et al (Matulka, Hood, & Griffiths, 2004) reported mean daily intake of lycopene to be about 8.2 mg/day, and lycopene intake ranging between 0.32-15.03 mg/d (Yong et al., 1994) has also been reported. We observed great variability among our participants in the reported consumption of lycopene. We found significant differences in reported dietary lycopene intake between the 12 oz group and 4 oz and control groups. Since most participants completed the DHQ within the first few weeks of treatment, we do not believe that the tomato juice supplement accounts for any of the differences in calculated dietary lycopene consumption. We found a positive correlation between education and dietary lycopene intake, perhaps indicating that participants with higher education included more variety (higher fruits and

vegetables) in their diet. While we did not find a correlation between education and dietary supplement use, a positive trend was observed, indicating that these men may be trying to lead a more “healthier” lifestyle, as reported by Wiygul et al who also reported similar supplement use in men diagnosed with prostate cancer (Wiygul et al., 2005).

We observed some weight loss in control group participants, while intervention group participants either maintained or gained weight (Table 3.5). Participants in the intervention groups received additional 121 kcal (4 oz), 146 kcal (8 oz) and 171 kcal (12 oz) daily (Table 3.4), which may account for perhaps 1.47 pounds weight gain in the 4 oz group, 1.61 pounds in the 8 oz group and 1.89 pounds in the 12 oz group if they maintained their usual dietary intake. Since we did not routinely monitor dietary intake beyond verbally ascertaining several times during the study that participants were maintaining their usual diet intake, we are unable to quantify whether any change in weight was related to the calories provided by the tomato juice and crackers or to other factors (such as fluid shifts/changes). In future studies, an isocaloric beverage with same amount of crackers should be provided to the control group participants to insure no discrepancies in caloric and volumetric intake. Similar trends in weight change have been reported in animal studies. Andic et al (Andic, Garipagaoglu, Yurdakonar, Tuncel, & Kucuk, 2009) reported weight loss in rats receiving radiation therapy without supplemental lycopene, and speculated that this may be a result of radiation induced anorexia and nausea. While control group

participants did not report any changes in appetite, other factors such as fatigue or biochemical changes (cytokine expression) may have contributed to their weight loss and should be monitored in these patients (Plata-Salamán, 1996).

Participants in our study tolerated the tomato juice supplemented daily during radiation therapy, as evidenced by no reported GI side effects (heartburn, nausea, or vomiting). Based on the responses received during the exit interview, our participants were enthusiastic about participating in a food-based trial, in order to reduce side effects of treatment. Reasons for participation were similar to those reported by Jatoi et al (Jatoi, et al., 2007), however, our participants did not report any adverse effects with daily tomato juice supplementation. Our participants had received no prior treatment for prostate cancer, and their disease was also localized, unlike the participant demographics reported by Jatoi et al (Jatoi, et al., 2007). Reasons for differences in tomato juice tolerance remain unclear.

This study has several strengths**.** To our knowledge, this is the first clinical trial to evaluate serum lycopene levels, tolerance of food-based lycopene supplementation and its impact on serum lycopene levels in men with localized prostate cancer undergoing radiation therapy. Lycopene intake was estimated using the DHQ. The DHQ was selected for estimating lycopene rich foods since it has been tested with older adults (Subar et al., 2001) and because it would require less work for the participants as compared to other available methods such as maintaining a food diary. It calculates average intake over a 12 month

period so that day-to-day or seasonal variations in dietary intake are included in the estimated amounts. Since we used a whole food approach instead of an isolated nutrient, there is a potential for greater beneficial effects due to the synergistic effect of all nutrients present (Norman et al., 2003) in tomato juice. We chose tomato juice as a vehicle for lycopene delivery because it is a processed tomato product, convenient to administer and consume. Additionally, this is a safe and effective way to increase consumption of vegetable intake among a population of men who typically have poor reported intake of fruits and vegetables.

This study had several limitations as well. These include a small sample size, which may have prevented us from detecting statistical significance and limits generalizability of these results. Due to variations in scheduling radiation treatments, blood was also not drawn from participants after an overnight fast. Serum lycopene levels may be influenced by any lycopene intake in a recent meal, however, researchers have demonstrated that the serum concentration of carotenoids does not change significantly for up to four hours after a meal (E. D. Brown, Rose, Craft, Seidel, & Smith, 1989; Mejia & Arroyave, 1983; Mejia, Pineda, Noriega, Benitez, & Falla, 1984). Additionally, we only measured total lycopene content in the serum. Cis and trans isomers of lycopene should also be evaluated to determine clinical correlates.

To conclude, tomato juice supplementation to increase dietary lycopene intake as a means to improve serum lycopene is feasible and easily accepted

among this sample of prostate cancer patients undergoing radiation therapy. Since lower serum lycopene levels have been reported in prostate cancer patients (Rao, et al., 1999) and antioxidants may decrease due to oxidative stress generated by radiation therapy, processed tomato juice supplementation can be used to increase serum lycopene levels in men with prostate cancer undergoing radiation therapy. While we did not observe a dose-dependent response to tomato juice supplementation, we did observe a variable response to increased intake of tomato juice. Although we did not detect an association between dietary and serum lycopene, this lack of association may perhaps be explained by tissue lycopene uptake which we did not measure. These biomarker results need to be validated in larger clinical trials.

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Table 3.1. Supplement (tomato juice) toxicity evaluation ("Cancer therapy evaluation program. Common Toxicity Criteria, v2.0," 1999)

Criteria	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Weight loss	None (<5%)	5-<10% of baseline, intervention not indicated	10-<20% of baseline; nutrition support indicated	> 20% of baseline; TF or TPN indicated	---
Nausea	None	Able to eat reasonable intake	Intake significantly decreased but can eat	No significant intake	-----
Vomiting	None	1 episode in 24 hours over pretreatment	2-5 episodes in 24 hours; IV fluids indicated < 24 hrs	≥ 6 episodes in 24 hours, IV fluids or TPN indicated > 24 hrs	Life threatening consequences
Heartburn	None	Mild	Moderate	Severe	-

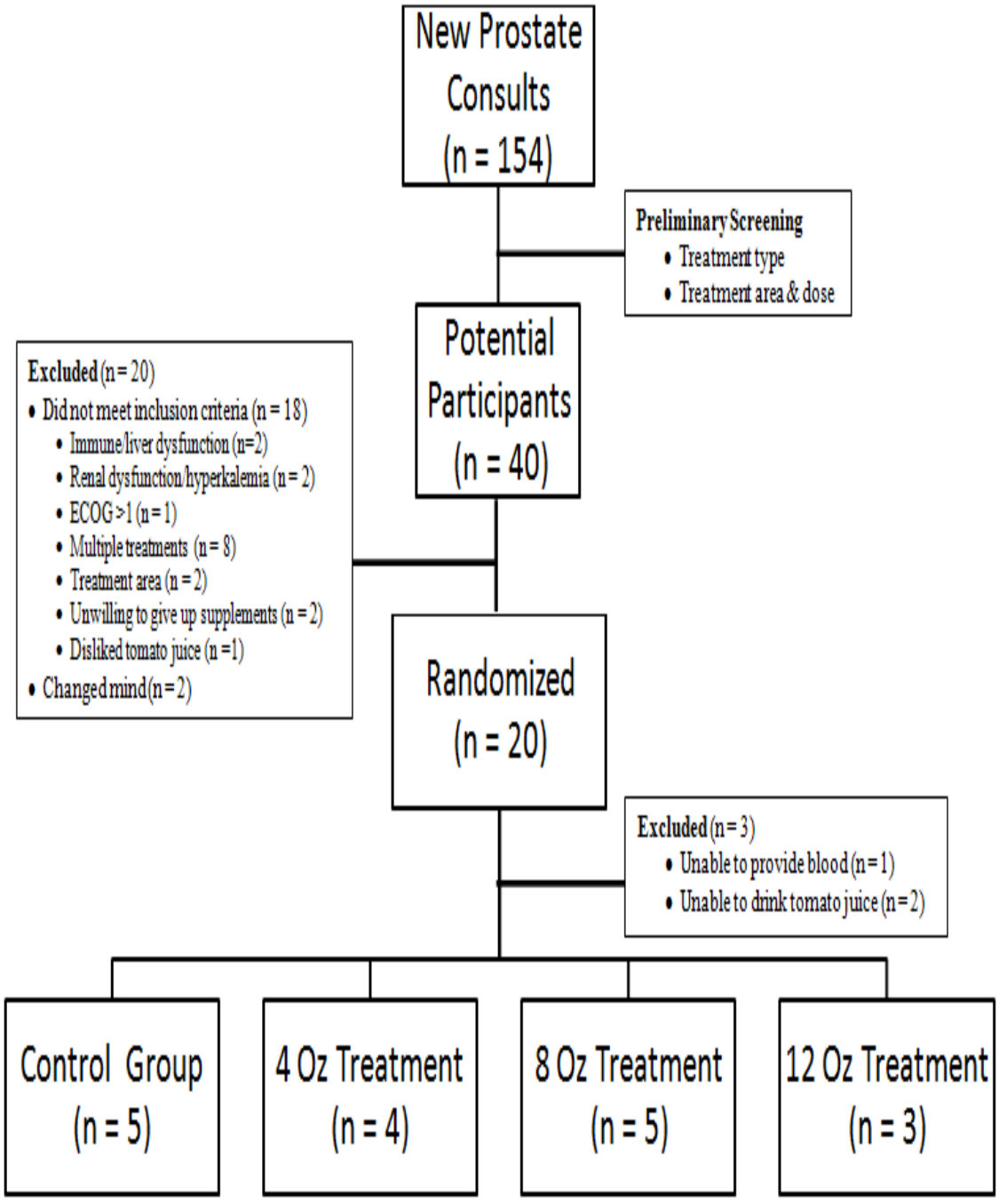

Figure 3.1: Flow diagram representing recruitment and randomization of study participants

Table 3.2. Participant characteristics. Data are reported as mean ± standard error of the mean or number (%).

	Control (n = 5)	4 Oz (n = 4)	8 Oz (n = 5)	12 Oz (n = 3)
Age	69.40±1.91	66.25±1.89	68.20±2.22	72.33±3.28
Height*	66.40± 0.87*	70.50±1.44	70.60±1.36	71.17±0.93*
Weight	167±13.11	193±14.61	221±30.74	168±28.41
BMI (kg/m^2)	27±1.97	27±1.30	30±3.22	23±4.10
Ethnicity				
African American	2 (40)	1 (25)	1 (20)	0
Caucasian	2 (40)	3 (75)	4 (80)	3 (100)
Other	1 (20)	0	0	0
Education				
< High School	1 (20)	2 (50)	2 (40)	0
High School	3 (60)	1 (25)	1 (20)	2 (67)
> High School	1 (20)	1 (25)	2 (40)	1 (33)
Marital Status				
Married	4 (80)	4 (100)	4 (80)	2 (67)
Divorced	1 (20)	0	1 (20)	1 (33)
Diabetes				
Yes	2 (40)	2 (50)	1 (20)	1 (33)
No	3 (60)	2 (50)	4 (80)	2 (67)
Hypertension				
Yes	5 (100)	4 (100)	4 (80)	2 (67)
No	0	0	1 (20)	1 (33)
Hypercholesterolemia				
Yes	4 (80)	4 (100)	4 (80)	3 (100)
No	1 (20)	0	1 (20)	0
Routine use of supplements				
Yes	4 (80)	3 (75)	4 (80)	1 (33)
No	1 (20)	1 (25)	1 (20)	2 (67)
Supplements Evaluated				
Fiber†	0	0	2 (40)	0
Multivitamin†	3 (60)	1 (25)	4 (80)	1 (33)
Saw Palmetto†	0	0	1 (20)	0
Vitamin C†	0	1 (25)	1 (20)	0
Vitamin D†	2 (40)	0	1 (20)	0
Vitamin E†	0	0	1 (20)	0
Omega-3 Fatty acids/Fish oil†	1 (20)	1 (25)	3 (60)	0
Flax seeds OR flaxseed oil†	0	1 (25)	0	0
Coenzyme Q10†	0	1 (25)	0	0
Eye Vitamin†	0	0	2 (40)	0

† Data reported indicate affirmative responses from participants using these supplements. *p < 0.05 (one sided)

Table 3.3: Nutritional contribution from crackers and tomato juice provided to participants daily during IMRT

	4 Oz (n = 4)	**8 Oz (n = 5)**	**12 Oz (n = 3)**
6 crackers			
Calories (kcal)	96	96	96
Fat (gm)	5.4	5.4	5.4
Sodium (mg)	162	162	162
Potassium (mg)	12	12	12
Tomato Juice			
Calories (kcal)	25	50	75
Sodium (mg)	340	680	1020
Potassium (mg)	215	430	645
Lycopene (mg)	7.85[ab]	17.52[ac]	28.09[bc]

ac; $p = 0.011$ (between group differences)

b; $p < 0.001$ (between group differences)

Table 3.4: Reported nutritional information obtained using the Diet History Questionnaire ("Diet History Questionnaire,"), measured and percent change in serum lycopene levels. Data are reported as mean ± standard error of the mean.

Nutrients	**Control (n = 5)**	**4 Oz (n = 4)**	**8 Oz (n = 5)**	**12 Oz (n = 3)**
Energy (Kcal)	1634±276.8[a]	2202±496.3	3015±668.2[a]	2296±969.4
Protein (gm)	56±7.3	89±25.0	84±21.01	72±31.2
Total Fat (gm)	63±10.9	81±18.8	88±16.0	86±45.7
Saturated fat (gm)	17±3.42	25±6.75	27±4.82	23±10.1
Monounsaturated fat (gm)	24±4.02	32±6.84	38±8.20	34±18.5
Polyunsaturated fat (gm)	17±3.36	18±3.75	17±2.90	23±14.6
Cholesterol (mg)	187±39.95	278±110.50	306±63.48	160±56.4
Dietary Fiber	19±8.16	21±2.89	20±3.08	23±8.9
Dietary Lycopene (mg)	4.77±1.31[b]	4.00±1.23[c]	9.35±3.88	12.19±3.58[bc]
Total (diet+TJ) dietary lycopene (mg)	4.77[de]	11.85[fg]	26.87[dfh]	40.27[egh]
Measured Serum Lycopene (µg/mL)				
Baseline	0.209±0.07	0.295±0.21	0.514±0.17[i]	0.082±0.08[i]
Midpoint	0.137±0.05[j]	0.272±0.08[k]	0.534±0.09[jkl]	0.069±0.01[l]
Endpoint	0.167±0.07[m]	0.257±0.07	0.622±0.25[m]	0.253±0.20
Percent Change Serum lycopene				
Midpoint-Baseline	-135.21	-572.15	8.83	-30.74
Midpoint-Endpoint	-21.87	-3.34	-53.33	3.15
Endpoint-Baseline	-151.29	-97.79	-47.15	-15.21

$p<0.05$ (one sided)

Between group differences

[abchil]$p< 0.05$ [deg]$p\leq 0.001$ [fm]$p< 0.03$ [j]$p< 0.005$ [k]$p< 0.01$

Table 3.5: Measured weekly weight of participants undergoing IMRT. Data are reported as mean ± standard error of the mean.

	Control (n = 5)	**4 Oz (n = 4)**	**8 Oz (n = 5)**	**12 Oz (n = 3)**
Starting Weight (lbs)	167±13.11	193±14.61	221±30.74	168±28.42
Week 1 (lbs)	167±12.94	195±14.01	220±29.75	168±26.82
Week 2 (lbs)	167±13.09	194±13.81	221±30.42	168±26.39
Week 3 (lbs)	168±13.52	194±14.08	221±30.10	169±27.02
Week 4 (lbs)	166±13.00	195±14.60	223±30.46	168±26.91
Week 5 (lbs)	167±13.37	195±13.91	222±30.48	168±27.06
Week 6 (lbs)	158±17.33	195±13.67	224±38.66	168±27.15
Week 7 (lbs)	158±17.19	195±13.74	229±38.20	168±26.24
Weight Change (lbs)	-9 lbs	+2 lbs	+8 lbs	None

One sided level of significance ($p<0.05$). No significant between group differences detected.

Lbs = pounds

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CHAPTER IV

EFFECT OFTOMATO JUICE SUPPLEMENTATION ON INFLAMMATORY RESPONSE IN MEN UNDERGOING IMRT FOR PROSTATE CANCER

Submitting to: Cancer Letters

Abstract

Purpose: Evaluate selected inflammatory mediator levels in serum and the impact of different volumes of tomato juice on these, in prostate cancer patients undergoing radiotherapy.

Principle results: All participants exhibited inflammation at baseline. Increased c-reactive protein (CRP) and interleukin-6 (IL-6) was observed in control group, while decreases in serum CRP, IL-6 and prostaglandin E2 (PGE2) levels were observed in intervention groups. Serum tumor necrosis factor-α was not detected in these participants at any time point.

Major Conclusions: Systemic inflammation was observed pretreatment and tomato juice supplementation appears to decrease serum CRP, IL-6 and PGE2 levels in localized prostate cancer patients undergoing radiotherapy.

Introduction

Inflammation is now one of the acknowledged causes of carcinogenesis (Baniyash, 2006). However, the link between prostate cancer and inflammation is still considered “suggestive” (De Marzo, Marchi, Epstein, & Nelson, 1999; De Marzo et al., 2007; Palapattu et al., 2005; Schottenfeld & Beebe-Dimmer, 2006; Sciarra et al., 2008; Wagenlehner et al., 2007) since a direct causal relationship has not yet been demonstrated (Haverkamp, Charbonneau, & Ratliff, 2008). Biopsies obtained from patients with benign prostate hypertrophy and prostate cancer (Lehrer et al., 2005) do provide evidence of the presence of chronic inflammation in prostate carcinogenesis. Several pro-inflammatory mediators such as cytokines, chemokines, inflammatory enzymes, etc., have been implicated in fostering chronic inflammation (Aggarwal, Shishodia, Sandur, Pandey, & Sethi, 2006). Increased levels of c-reactive protein (CRP), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2) and other inflammatory markers have been reported in various stages of prostate carcinogenesis (McArdle, McMillan, Sattar, Wallace, & Underwood, 2004; Pfitzenmaier et al., 2003). Researchers have recently also reported the up-regulation of several inflammatory markers in men with prostate cancer undergoing radiation therapy (Christensen et al., 2009; Johnke et al., 2009).

CRP, an acute phase protein (Ford, Liu, Mannino, Giles, & Smith, 2003; Helzlsouer, Erlinger, & Platz, 2006; Lehrer, et al., 2005), is a sensitive, yet non-specific marker of inflammation and is reported to be elevated in cancer patients

(Walsh, Mahmoud, & Barna, 2003). Radiation therapy has also been proposed as an independent cause of CRP elevation (Cengiz, Akbulut, Atahan, & Grigsby, 2001). CRP levels are inversely associated with circulating levels of lycopene along with other carotenoids, retinoids and antioxidants (Ford, et al., 2003; McMillan et al., 2002).

Tumor necrosis factor- α (TNF-α) is a key player in the body's inflammatory response, and can promote carcinogenesis by facilitating the growth of initiated cancer cells and by involving surrounding inflammatory cells (Balkwill, 2006). TNF-α is recognized not only for its role in carcinogenesis, but also for its ability to induce other inflammatory mediators such as NF-kB (Balkwill, 2006), and ultimately the acute phase response along with increasing prostaglandin, leukotriene and collegenase synthesis (Huang, Ghai, & Ho, 2004). TNF-α may induce a cascade of secondary mediators such as IL-1 that may be responsible for some of the physiological effects observed in various inflammatory conditions, independent of serum TNF-α levels (Kevin J. Tracey & Cerami, 1994).

IL-6 is a pleiotropic cytokine (Song & Kellum, 2005) that is involved in several key cellular functions such as proliferation, differentiation, angiogenesis and apoptosis (Culig, Steiner, Bartsch, & Hobisch, 2005), and has also been implicated in the development and progression of several tumors including the prostate (Culig, et al., 2005; Stark et al., 2009). Researchers have reported higher IL-6 levels in patients with prostate cancer, and have linked IL-6 along

with TNF-α and IL-1 with cancer progression (Bouraoui et al., 2008). While TNF-α and IL-1 stimulate the release of IL-6, it remains in the plasma longer than TNF-α and IL-1 and, consequently, is considered a key indicator of the activation of pro-inflammatory cytokines (Song & Kellum, 2005). Researchers have also recently demonstrated that IL-6 is upregulated in the plasma of men with prostate cancer undergoing radiation therapy (Christensen, et al., 2009; Johnke, et al., 2009).

Prostaglandin E2 (PGE2) is a key player in the immune suppression associated with inflammation (Ben-Baruch, 2006). It is synthesized by COX-2 and together they have demonstrated great potential as targets for cancer therapy (Ben-Baruch, 2006). COX-2 and PGE2 are over-expressed in both prostate intraepithelial neoplasia and prostate cancer (Kirschenbaum, Liu, Yao, & Levine, 2001). Radiation therapy may also induce inflammation and up-regulate PGE2 (Dorai & Aggarwal, 2004; Milas & Hanson, 1995; Steinauer et al., 2000) and other inflammatory markers. COX-2 can be induced by other pro-inflammatory cytokines such as TNF-α and IL-1 (Keskek et al., 2006). Sustained levels of COX-2 may play a key role in radiation-induced intestinal side effects/toxicities, by amplifying toxicities and by increasing their duration and severity (Keskek, et al., 2006). Upregulation of PGE2 has been implicated in radiation induced inflammatory changes in the bowel (Cole, Slater, Sokal, & Hawkey, 1993). Enhanced patient response to radiation therapy has been

proposed with the inhibition of COX-2 levels in prostate cancer patients (Khor et al., 2007).

Radiotherapy targets normal tissues and organs along with the cancer tissues by generating free radicals (Prasad, Cole, Kumar, & Prasad, 2002), inducing oxidative stress through generation of reactive oxygen species (ROS) and subsequent imbalance between pro-oxidants and antioxidants in the cell (Srinivasan et al., 2007). Tissue antioxidant levels can become depleted as a result of oxidative stress generated secondary to chemotherapy and radiotherapy, thereby increasing the level of oxidative stress (Simone, Simone, Simone, & Simone, 2007). Radiation therapy has been reported to induce cytokine expression (Friedman, 2002) along with triggering the acute phase response (Cengiz, et al., 2001; Koc, Taysi, Sezen, & Bakan, 2003). Researchers hypothesize that expression of radiation induced cytokines may be tissue specific (Stone, Coleman, Anscher, & McBride, 2003) and that these may serve as useful indicators of toxicity to the cells and tissues during radiation therapy for prostate cancer (Christensen, et al., 2009). Antioxidants can modulate cytokine levels in tissues, consequently reducing the inflammatory response (Okunieff et al., 2008). Several mechanisms have been proposed to explain the anti-inflammatory actions of tomato products/lycopene. These include antioxidative action, inhibition of IL-6, induction of phase II enzymes (Wertz, 2009), and modulation of the cyclo-oxygenase pathway (De Stefano et al., 2007; Sengupta, Ghosh, Das, Bhattacharjee, & Bhattacharya, 2006). We conducted this randomized controlled

trial to evaluate the levels of select serum markers of inflammation (CRP, TNF-α, IL-6 and PGE2) in men with localized prostate cancer undergoing radiation therapy. We were also interested in determining the impact of different volumes of tomato juice, dietary and serum lycopene on these select serum markers of inflammation during radiation therapy in this patient population.

Materials and Methods

Seventeen of 20 eligible patients with localized prostate cancer recruited between April 2009 and October 2010 completed the study. Participants were recruited consecutively at High Point Regional Health System's (HPRHS) Hayworth Cancer Center in High Point, North Carolina. Preliminary screening based on treatment area, type and dose was performed by the Radiation Oncologist (BF) at the time of initial consult. Participants meeting these criteria were referred to the primary investigator (MD) for detailed medical record review and patient interviews. Health Insurance Portability and Accountability Act and informed consent forms were obtained from participants. Institutional Review Board approvals for the study protocol were obtained at the University of North Carolina at Greensboro (UNCG) and HPRHS.

Patients were eligible for this study if they had a new diagnosis of localized prostate cancer and were scheduled to receive ≥ 72 Gray (Gy) external image guided intensity modulated radiation therapy (IMRT) to the prostate alone or prostate and seminal vesicles included in the treatment volume. Patients were ineligible to participate in this study if they had received prior treatment for

prostate cancer or were scheduled to receive other treatments concurrently with IMRT; or if they had abnormal liver, renal or immune function; hyperkalemia; uncontrolled gastro-esophageal reflux disease and other malabsorptive disorders; allergy to tomato products or red dye; ECOG score > 1; continued use of fiber supplements and other nutraceuticals with antioxidant properties (multivitamin, omega-3 fatty acids/fish oil, EPA/DHA, lycopene, vitamin C, E, A; B-carotene, flaxseeds and flaxseed oil) or action on the prostate (saw palmetto), and unwillingness to discontinue these for the study duration. Participant assessments, blood draws and tomato juice administration were scheduled during each patient's planning procedure and radiation therapy appointment time. Potential participants picked one out of four folded pieces of paper with different group name printed on each. The investigator (MD) recorded the group and individual participant ID on the participant screening form and informed the participant and family members of group assignment.

We used the National Cancer Institute (NCI) Diet History Questionnaire (DHQ) to obtain participant diet information and estimate dietary lycopene intake. The DHQ is a 124 item questionnaire that has been tested for reliability and validity. It is easy to use, takes about an hour to complete and includes questions on food portion sizes as well as dietary supplements ("Diet History Questionnaire,").

Tomato juice supplementation commenced two days before the first dose of radiation therapy and continued daily till the last day of treatment. During

treatment days, participants consumed tomato juice and crackers (providing at least five grams of fat, to facilitate lycopene absorption) prior to leaving the cancer center, in the presence of the researcher (MD). Tomato juice was provided to participants for consumption over weekends and holidays with the instruction to consume it with food providing at least five grams of fat. Suggestions for snack and meals containing at least five grams of fat were discussed with participants. Verbal verification of tomato juice consumption was obtained from participants at their subsequent treatment day.

Blood samples were collected at baseline, at the end of three weeks of treatment (midpoint) and on the last day of treatment (endpoint). In order to protect serum lycopene from light, serum tubes for whole blood collection were wrapped in aluminum foil. Due to the length of transport time (20 min) from collection site to processing site at UNCG, collected blood was transported on ice. Blood samples were processed (centrifuged at 3000 rpm x 20 min at 4^0C) under a yellow light to further minimize loss of lycopene. Separated serum, divided into aliquot tubes, was stored at -80^0C until analyzed. Each aliquot tube was labeled with time point of blood draw (baseline, midpoint or endpoint) and patient ID. Standard Enzyme-Linked Immuno Sorbent Assay (ELISA) kits were used to test for serum PGE2, CRP, TNF- α, and IL-6 levels at the UNCG laboratory using the manufacturer's (R&D) instructions. We also used control samples for each kit to establish quantitative controls. Optical density was measured at 450nM using the Synergy^{TM} HT (Bio-Tek Instrument, Inc) multi-

detection microplate reader. The minimum detectable limits for PGE2, CRP, TNF-α, and lL-6 were 30.9 pg/mL, 0.010 ng/mL, 1.6 pg/mL and 0.70 pg/mL respectively. Since the CRP levels were not normally distributed, we log transformed the serum levels. We chose not to use PSA as an endpoint in this study, even though it has been used as an endpoint in several lycopene clinical trials (Ansari & Gupta, 2004; Barber et al., 2006; Edinger & Koff, 2006; Vaishampayan et al., 2007). Since radiation therapy results in a decline in PSA levels post treatment, we would not be able to identify if the observed effect was due to radiation therapy or lycopene.

Serum samples for lycopene analysis were packed in dry ice and transported to Dr. Wei Jia's laboratory at the Kannapolis Research Campus, NC. Samples were analyzed for lycopene using high performance liquid chromatography and mass spectrometry (LC-MS).

All study participants received image guided IMRT, on a Linear Accelerator 2100 iX (Varian Medical Systems, CA) following same standardized planning and filming protocol. For treatment, participants were placed supine on the treatment table with their head resting on a square sponge and legs placed in a "W" shaped cushion. Patients were instructed to keep hands away from the treatment area by placing their hands on their chest. Daily treatment fractions were delivered utilizing specified photons with a prescribed field treatment plan. Each treatment day a diagnostic image was obtained by radiation therapy staff

and approved by the treating radiation oncologist to verify proper positioning of the isocenters.

Key participant and cancer related characteristics were analyzed using descriptive statistics. Inflammatory mediator data are reported as mean±standard error of the mean. Between-group differences were examined using the Wilcoxon rank sum test, and within-group differences were detected with repeated measures. Spearman's rho correlation was used to evaluate an association between the inflammatory mediators, serum lycopene and select lifestyle (weight, age) and cancer related characteristics (PSA, gleason sum (GS), treatment dose, daily fractions). One-sided level of significance was established at $p \leq 0.05$, with significant trends observed between 0.051-0.10.

Results

Forty seven percent of participants had a GS score 7, and 71% participants had tumor stage T1cN0M0. The proportion of participants who received 2 Gy, 1.8 Gy and 2.5 Gy fractions daily were 53%, 29% and 18% respectively. Treatment dose ranged between 72.50-79.20 Gy (Table I).

Serum lycopene levels during radiation therapy

Overall serum lycopene at baseline was 0.30 µg/mL, and 0.33±0.11 µg/mL in the intervention group at baseline. We did observe a small non-significant increase in serum lycopene levels with daily tomato juice supplementation, in the intervention group towards the end of the study (0.41±0.12 µg/mL). A progressive statistically significant decrease ($p = 0.009$) in serum lycopene was observed in

control group participants (Table II). Participants with GS 6 had lower serum lycopene levels at baseline (0.21±0.11 µg/mL) compared to participants with GS 7 (0.39±0.11 µg/mL).

CRP was detected in the serum of all participants at baseline. Participants in the 12 oz group had the lowest CRP, while participants in the 8 oz group had the highest CRP levels at baseline (Table II). Additionally, participants with GS 6 had higher CRP levels at baseline compared with participants with GS 7 (8.24±0.59 vs 8.10±0.14). No statistically significant within group differences were detected. While an increase in CRP was observed in control group participants throughout treatment, CRP levels decreased in the intervention groups. A statistically significant difference ($p = 0.018$) at midpoint was observed between control group and 12 oz group participants (Table II). While we did not detect a statistical significance, we did observe a trend towards significance ($p = 0.071$) in the baseline CRP levels between 8 and 12 oz groups, and midpoint levels ($p = 0.057$) between 4 and 12 oz groups. A statistically significant positive correlation was detected between serum IL-6 and CRP levels at baseline ($r = 0.417$, $p = 0.048$, $n = 17$) and endpoints ($r = 0.566$, $p = 0.009$, $n = 17$).

No TNF-α levels were detected in the sera of study participants. IL-6 was detected in the serum in all but two participants (n=1 each control group and 8 oz groups) at baseline. Overall baseline IL-6 among study participants was 2.15 pg/mL. Unlike CRP, participants with GS 7 had higher IL-6 levels at baseline

(2.46±0.76 vs 1.87±0.54 pg/mL). Highest IL-6 level at baseline was observed in the 4 oz group. Interestingly, a progressive increase in IL-6 levels was observed in control group participants, while a progressive decrease was observed in the 4 oz group means. While IL-6 levels in both 8 and 12 oz participants at end point were lower than baseline (Table II), the within group differences for 12 oz group only were statistically significant when comparing percent change at baseline and endpoint with midpoint ($p = 0.014$). While we did not detect a statistical significance, we did observe a trend towards significance ($p = 0.0952$) in the midpoint IL-6 levels between 4 and 8 oz groups.

Control group participants had the highest level of PGE2 at all time points. No PGE2 was detected in the serum of two participants (n=1 each, 4 and 8 oz groups) at any time point. Participants with GS 6 had higher PGE2 levels at baseline compared with participants with GS 7 (514.56±120.26 vs 440.08±217.68 pg/mL). While PGE2 levels in the 4 oz group were lower at endpoint compared to baseline (Table II), both 8 and 12 oz participants demonstrated a progressive decrease, but within group differences for only the 12 oz group were statistically significant ($p = 0.001$) when comparing percent change at baseline with midpoint and endpoint ($p = 0.003$). We did observe a trend towards significance in the baseline PGE2 levels between 4 oz and control groups ($p = 0.0952$) and between 4 and 12 oz groups ($p = 0.0857$). We detected no correlation between inflammatory markers, cancer characteristics (PSA, GS,

radiation dose or daily fractions) and dietary or serum lycopene (data not reported).

Discussion

This study evaluated the levels of selected inflammatory mediators during radiation therapy of the prostate and the impact of lycopene supplementation via tomato juice on these mediators of inflammation. Overall, control group participants demonstrated a progressive but non-significant increase in CRP and IL-6 levels and a slight non-significant decrease in PGE2 level throughout treatment. High CRP levels in patients undergoing radiation therapy have been reported by other researchers (Cengiz, et al., 2001; Koc, et al., 2003). Since CRP is virtually undetectable in healthy adults the midpoint elevations observed in the 4 oz group participants may also represent an increase in the acute phase response. A considerable decline in the endpoint CRP levels was observed in the 8 oz group (percent change -2976% between baseline and endpoint), and a much lower magnitude of response was observed in the 4 (change -64%) and 12 oz (change -70%) group CRP levels between baseline and endpoint. Daily lycopene consumed as tomato juice may explain these results. An inverse relationship has been previously been reported between CRP and serum lycopene by Ford et al (Ford, et al., 2003). Participants with lower GS had higher CRP levels. The clinical implications of this remain unclear at the present time.

Serum IL-6 levels mirrored CRP levels in all groups. A progressive, non-significant increase in IL-6 levels was observed in control group and a non-

significant decrease observed in the intervention group participants. Higher IL-6 levels observed in participants with higher GS have also been reported by Alcover et al. (Alcover et al., 2010). Other researchers have also reported elevated IL-6 levels in prostate cancer patients undergoing IMRT (Christensen, et al., 2009; Johnke, et al., 2009). However, Johnke et al (Johnke, et al., 2009) reported normalization of IL-6 levels to pre-treatment levels after about two weeks of IMRT, results that we did not observed in this study. IL-6 levels increased progressively until the end in our control group participants. While we did observe an elevation at midpoint (end of 3 weeks of treatment) in the 8 and 12 oz groups, endpoint levels were below baseline and we observed a progressive decline in 4 oz group participants, as well. This may indicate a physiological role of tomato juice supplementation, even though we did not observe a dose dependent response.

We observed high PGE2 levels at baseline prior to participants receiving radiation therapy, confirming role of inflammation in carcinogenesis (Kirschenbaum, et al., 2001). Baseline PGE2 levels were highest among control and 12 oz groups, almost four times higher than participants in the 4 oz group. The non-significant decrease observed in intervention group participants overtime, likely indicates a beneficial effect of tomato juice to these participants. Lack of PGE2 levels observed in two patients may be due to gene polymorphisms (Zhang, Dhakal, Lang, & Kadlubar, 2010).

While we did not detect any TNF-α in the serum of our participants, researchers have reported higher TNF-α levels in prostate cancer patients undergoing IMRT (Christensen, et al., 2009). However, some researchers have also reported that detecting serum TNF-α may be problematic, perhaps as a result of a short half-life or binding to its competitive inhibitor soluble receptor (Bossola et al., 2000). TNF-α levels may also be suppressed by non steroidal anti-inflammatory agents, production of TGF-β, IL-10 and PGE2 (K. J. Tracey & Cerami, 1993), and binding with receptors (Kevin J. Tracey & Cerami, 1994). Akimoto et al reported 85% of prostate cancer patients in their study had TNF-α levels below the detectable limit (Akimoto, Okumura, & Fuse, 1998). However, TNF-α may induce a cascade of secondary mediators such as IL-1, that may be responsible for some of the physiological effects observed in various inflammatory conditions, independent of the serum TNF-α levels (Kevin J. Tracey & Cerami, 1994). Researchers have also reported lower TNF-α levels in patients with localized disease compared with metastatic prostate cancer (Michalaki, Syrigos, Charles, & Waxman, 2004). Since we did not observe any TNF-α in the serum of our participants, we cannot conclusively state that TNF-α was not induced. Future studies may need to test TNF receptors, IL-1 and other downstream secondary inflammatory mediators.

Increase in CRP at mid point may be reflective of acute radiation toxicity among those participants (data not reported). A progressive decline in CRP and PGE2 and a statistically significant within group differences when comparing

baseline and endpoint with midpoint ($p = 0.014$) for the 12 oz group may perhaps be a result of higher lycopene intake. This relationship needs to be evaluated further in prostate cancer patients undergoing radiation therapy. We did not detect a correlation between the different volumes of tomato juice intake and the levels of specific inflammatory mediators in our study. As we did not exclude participants for using anti-inflammatory medication, future studies should control for this variable as has also been recommended by other researchers (Grainger et al., 2008).

While recent publications have demonstrated higher serum levels of different cytokines during radiation therapy, we believe this is the first study evaluating the impact of food-based lycopene supplementation on select inflammatory mediators (CRP, TNF-α, lL-6 and PGE2) during radiotherapy in prostate cancer patients. Impact of dietary lycopene supplementation on the level of inflammatory mediators during radiotherapy also needs to be evaluated in men with prostate cancer receiving hormone therapy along with more aggressive stages of cancer.

This study has several limitations. In order to minimize disruption of daily activities among the study participants, blood draws were scheduled around the time of their prescheduled radiation treatment appointment. Consequently, patients were not in a fasted state for the blood draws. The impact of non-fasted state on these test parameters remains unknown. A small sample size of this study limits generalization of these results and may have prevented us from

detecting statistical significance. Due to the emerging research interest in cytokine expression during radiation therapy in prostate cancer patients, specific reference values relating to the magnitude of increase that can be expected in this population are lacking.

In conclusion, as expected, systemic inflammation was observed in men with localized prostate cancer prior to treatment. Despite the small sample size and mixed inflammatory mediator levels observed among the treatment group, our findings suggest that 8-12 oz tomato juice may be helpful in blunting the inflammatory response during radiation therapy in this population. Larger clinical trials are needed to validate these results and to ascertain if food based lycopene supplementation is an acceptable adjunct during radiation therapy.

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Table 4.1. Participant cancer related characteristics. Data are reported as mean ± standard error of the mean.

	Control (n = 5)	**4 Oz (n = 4)**	**8 Oz (n = 5)**	**12 Oz (n = 3)**
PSA	7.33±1.83	5.97±0.88	7.09±0.94	7.79±1.27
0-4.99 ng/mL	3 (60)	2 (50)	0	0
5-9.99 ng/mL	1 (20)	2 (50)	4 (80)	3 (100)
10-14.99 ng/mL	1 (20)	0	1 (20)	0
Gleason Sum*	6.40±0.25	6.75±0.25	6.40±0.25	6.33±0.33
6	3 (60)	1 (25)	3 (60)	2 (67)
7	2 (40)	3 (75)	2 (40)	1 (33)
Tumor Stage				
$T1_cN_0M_0$	4 (80)	3 (75)	3 (60)	2 (67)
$T2_aN_0M_0$	1 (20)	1 (25)	0	1 (33)
$T2_bN_0M_0$	0	0	1 (20)	0
$T2_cN_0M_0$	0	0	1 (20)	0
Treatment Dose (Gy)	74.60±0.86 (72.50-76.00)	78.40±0.80 (76.00-79.20)	76.58±1.25 (72.50-79.20)	77.33±0.67 (76.00-78.00)
Treatment Days	34±2.21	43±1.50	39±2.75	39±0.33

Level of significance ($p<0.05$). No statistical significance detected.

Table 4.2. Serum lycopene and inflammatory markers at three time points in all participants. Data are reported as mean ± standard error of the mean

	Baseline	Midpoint	Endpoint
Lycopene (µg/mL)			
Control*	0.209±0.07	0.137±0.05[b]	0.167±0.07[e]
4 oz	0.295±0.21	0.272±0.08[c]	0.257±0.07
8 oz	0.514±0.17[a]	0.534±0.09[bcd]	0.622±0.25[e]
12 oz	0.082±0.08[a]	0.069±0.01[d]	0.253±0.20
Log CRP			
Control	7.65±0.49	8.57±0.51[f]	8.34±1.04
4 oz	7.96±0.25	8.07±0.14	7.53±0.36
8 oz	9.22±0.78	8.92±0.96	8.02±0.53
12 oz	7.60±0.32	7.37±0.22[f]	7.18±0.10
IL-6 (pg/mL)			
Control	1.433±0.517	1.911±0.406	2.655±1.383
4 oz	3.442±1.263	1.844±0.891	1.580±0.586
8 oz	2.289±0.909	5.888±3.181	1.914±0.519
12 oz	1.374±0.619	2.094±0.398	1.073±0.171
PGE2 (pg/mL)			
Control	707.47±307.80	684.81±428.24	664.58±428.64
4 oz	188.03±78.42	277.21±185.76	170.40±98.38
8 oz	351.13±176.75	183.79±162.06	98.31±40.99
12 oz	702.16±221.48	510.71±101.29	244.56±69.61

Statistical significance: $p < 0.05$ one-sided

[*] within group difference p = 0.009

[a] p <0.05 (between group differences)

[b] p <0.005 (between group differences)

[c] p <0.01 (between group differences)

[def] p <0.03 (between group differences)

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CHAPTER V

IMPACT OF TOMATO JUICE SUPPLEMENTATION ON ACUTE TREATMENT TOXICITY AND PERFORMANCE STATUS DURING IMRT FOR PROSTATE CANCER

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Abstract

Purpose: to evaluate the impact of three different volumes of tomato juice on several frequently reported acute side effects (diarrhea, proctitis and urinary urgency and frequency) of radiation therapy and performance status in men with localized prostate cancer.

Materials and Methods: The National Cancer Institute's Cancer Therapy Evaluation Program: Common Toxicity Criteria v 2.0 was used to evaluate tolerance of tomato juice; and Common Terminology Criteria for Adverse Events was used to evaluate severity of side effects related to radiation therapy. Performance status was assessed using the Eastern Cooperative Oncology Group scale.

Results: Men with localized prostate cancer (tumor grade $T_{1c\text{-}2c}N_0M_0$) scheduled to receive external radiation to the prostate alone or prostate and seminal vesicles were recruited between April 2009 and October 2010. Seventy one percent of participant tumors were staged $T_{1c}N_0M_0$. Daily radiation fractions varied between 1.8-2.5 Gray. Control group participants reported higher (worse) performance status scores than participants in the intervention groups. A strong positive correlation was detected between performance status, reported alcohol intake and diagnosis of hypertension at baseline. No differences in urinary frequency and urgency were detected between control and intervention groups.

However, tomato juice supplementation (especially 12 oz tomato juice) appeared to have a protective effect on gastrointestinal acute toxicity (diarrhea and proctitis) for the first three weeks of treatment.

Discussion: Daily tomato juice supplementation during intensity modulated radiation therapy in men with localized prostate cancer resulted in a gastrointestinal protective effect for the first three weeks of treatment and in performance status though out treatment. These results need to be validated in larger clinical trials using 12 ounce tomato juice or equivalent tomato products.

Introduction

In 2010, 217,730 new cases of prostate cancer were projected in the US and more than 90% of these cases were expected to be loco-regional cases with 100% five-year survival (Jemal, Siegel, Xu, & Ward, 2010). This is significant considering primary treatment strategies in addition to active surveillance include surgery and radiation therapy,("Cancer Facts and Figures 2010," 2010) and reducing side effects of therapy will enhance quality of life (Andic, Garipagaoglu, Yurdakonar, Tuncel, & Kucuk, 2009). Side effects of radiation therapy may be either acute or chronic. Acute side effects of radiation therapy are those observed during or within three months of radiation, while symptoms occurring after three months are classified as chronic(Hauer-Jensen, Wang, Boerma, Fu, & Denham, 2007) or late effects (Stone, Coleman, Anscher, & McBride, 2003). Despite improved biochemical control, intensity modulated radiation therapy (IMRT) may still cause toxicity (Christensen et al., 2009). While the timeliness, severity and type of expression is dependent on the site of the irradiated tissue and cytokine cascade activation (Bentzen, 2006; Stone, et al., 2003), rate of tissue renewal, presence of microvascular (diabetes, hypertension) auto-immune or collagen vascular diseases, and radiation dose (Andreyev, 2007; Okunieff, Chen, Maguire, & Huser, 2008) may also impact treatment tolerance. Pre-existing medical conditions such as hypertension (HTN), coronary artery disease (CAD) and diabetes (DM) may actually increase the potential for higher toxicity

upon exposure to radiation therapy (Chon & Loeffler, 2002; Houterman, Janssen-Heijnen, Hendrikx, Berg, & Coebergh, 2006; Okunieff, Chen, et al., 2008).

The most common acute toxicity symptoms of pelvic radiation are gastrointestinal (GI) and genitourinary (GU) symptoms. De Meerleer et al reported that the incidence of acute GI toxicity was observed in about 37% of patients (Grade 1: 44% and Grade 2: 29%), while 38% patients reported mild to moderately severe GU toxicity (Grade 1: 47%, Grade 2: 36% and Grade 3: 7%) (De Meerleer et al., 2004). Other researchers (Fonteyne, Villeirs, Lumen, & De Meerleer, 2009; Lips et al., 2008) have reported similar incidence of acute toxicity in prostate cancer patients undergoing radiation therapy. Symptoms of early radiation toxicity may be subtle and transient, but they can impact quality of life and functional status of patients considerably during treatment (Hauer-Jensen, et al., 2007), even in patients with milder symptoms (Christiansen et al., 2007). While acute toxicities may be transient and of short duration, it is imperative to treat or minimize these, since acute toxicities have been reported to be one of the significant predictors of long term “chronic” toxicities (Denham et al., 1999; Hovdenak et al., 2003; O'Brien, 2001; Schultheiss et al., 1997).

Researchers have proposed that various cytokines may be predictive, prognostic or diagnostic markers of radiation toxicity (Okunieff, Chen, et al., 2008). The release of a “cytokine storm” has been suggested to occur immediately after tissue irradiation, and that the intensity of these cytokines may be a predictor of toxicities in patients (Okunieff, Chen, et al., 2008). Higher levels

of serum interleukin-6 (IL-6) has been reported in prostate cancer patients undergoing IMRT (Christensen, et al., 2009), and increased levels of IL-6 along with tumor necrosis factor-α (TNF-α) have been reported in men with prostate cancer who develop radiation-induced proctitis (Christiansen, et al., 2007). Other researchers have reported elevation of IL-6 and other cytokines in patients with radiation induced pathologies (Indaram, Visvalingam, Locke, & Bank, 2000; McBride, 1995). Prostaglandins have also been implicated in radiation induced enteritis, but the results are not conclusive (Lifshitz, Savage, Taylor, Tewfik, & Van Orden, 1982).

Antioxidants can modulate cytokine levels in tissues, consequently reducing the inflammatory response (Okunieff et al., 2008). The potential for using radioprotective compounds to limit radiation–induced toxicity by protecting normal tissues and enhancing the therapeutic benefits has been explored by researchers and clinicians (Grdina, Murley, & Kataoka, 2002; Weiss & Landauer, 2003). Synthetic antioxidants such as amifostine have been successfully used in head and neck, ovarian, and non-small cell lung cancer patients to reduce treatment related toxicities (Grdina, et al., 2002). This radioprotective effect is not limited to synthetic antioxidants (Weiss & Landauer, 2003). Several phytochemicals (genistein, caffeine etc.) Have been identified that have both antioxidant as well as radioprotective effects in vivo (Weiss & Landauer, 2003). We limited our focus to lycopene which is a potent antioxidant and anti-inflammatory agent (Huang, Ghai, & Ho, 2004; Rafi, Yadav, & Reyes, 2007).

Researchers (Saada, Rezk, & Eltahawy, 2010; Srinivasan, Devipriya, Kalpana, & Menon, 2009; Srinivasan et al., 2007) have demonstrated that lycopene can protect cells against γ-radiation induced cellular damage and decrease acute GI side effects (diarrhea) observed during pelvic radiation therapy (Andic, et al., 2009). We undertook this study to evaluate the impact of a food based source of lycopene (tomato juice) on the frequently reported acute GI (diarrhea and proctitis) and GU (urinary urgency and frequency) side effects and performance status in men undergoing radiation therapy for localized prostate cancer.

Materials and Methods

Participants were selected consecutively and non-probabilistically at High Point Regional Health System's (HPRHS) Hayworth Cancer Center in High Point, NC between April 2009 and October 2010. Of the 154 new prostate cancer referrals to the cancer center, 40 participants met the preliminary screening criteria based on treatment area, type and dose. Eighteen participants did not meet the other eligibility criteria and two participants expressed no interest in participating in the study. Of the twenty participants who were randomized into four treatment arms (control, 4, 8 or 12 oz tomato juice), two dropped out due to inability to consume the assigned volume of tomato juice (n = 1 each from 8 and 12 oz groups) and one participant (4 oz group) was withdrawn by the investigators due to inability to obtain access for blood draws. The study protocol was approved by the Institutional Review Boards at the University of North Carolina at Greensboro (UNCG) and HPRHS. All participants signed a Health

Insurance Portability and Accountability Act (HIPAA) and informed consent forms prior to study enrollment.

The *eligibility criteria* included: newly diagnosed localized prostate cancer patients scheduled to receive ≥72 Gray (Gy) IMRT, treatment volume included prostate alone or prostate and seminal vesicles; no concurrent hormones, chemotherapy or other treatments for cancer; Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1. Patients were ineligible to participate in this study if they had pre-existing uncontrolled gastro-esophageal reflux disease and other malabsorptive disorders, renal or liver disease; compromised immune system; hyperkalemia; allergy to tomato products or red dye; or routinely used and unwilling to stop these supplements- fiber, saw palmetto, lycopene, omega-3 fatty acids/fish oil, EPA/DHA, vitamin C, E, A; β-carotene, flaxseeds and flaxseed oil supplements. In order to minimize time spent at the cancer center and to avoid additional follow-up visits, assessments, blood draws and tomato juice administration were scheduled around each patient's appointment times (planning, radiation therapy, etc.).

A Linear Accelerator 2100 ix (Varian Medical Systems, CA) was used to provide image guided IMRT to all participants in the study. Standardized planning and treatment protocols were followed for radiation therapy delivery under close supervision of the treating radiation oncologist. Researchers (MD) assessed performance status weekly using the ECOG performance scale. The NCI Cancer Therapy Evaluation Program: Common Toxicity Criteria (CTC) v 2.0 was used to

assess any GI adverse effects (nausea, vomiting, heartburn) from tomato juice supplementation, and the NCI Common Terminology Criteria for Adverse Events (CTCAE) was used to evaluate severity of side effects (urinary frequency and urgency, diarrhea and proctitis) related to radiation therapy.

Participants in the three intervention groups commenced tomato juice consumption two days before treatment and continued drinking it daily until the last day of treatment. During week days, tomato juice and crackers containing five grams of fat (to aid in the absorption of lycopene) were provided at the cancer center. The researcher (MD) observed participants drinking the juice and eating the crackers each treatment day. Participants were provided containers of their assigned volume of tomato juice for weekends and holidays with the instructions to consume tomato juice with either a meal or a snack containing at least five grams of fat. A few examples of snacks and meals containing five grams of fat were discussed with participants. Verbal confirmation of tomato juice consumption was obtained on the next radiation therapy day.

Data are reported as group means ± standard error of the mean. We utilized the Wilcoxon rank sum test to detect between-group differences for performance status and side-effects of treatment, and repeated measures were used to detect within-group differences at different time points. Chi-square was used to detect differences in proportions. We also conducted Spearman's rho correlation to evaluate the association of performance status scores and treatment side effects with select lifestyle characteristics. Differences were

considered statistically significant at one-sided $p \leq 0.05$, and a trend towards significance was observed with p between 0.051-0.10.

Results

Participants in our study were between 61 and 77 years of age, with no statistically significant differences in age distribution among participants in the four groups. Majority (71%) of our participants were Caucasian, 82% of participants were married, and 71% had at least a high school education. The TNM staging ranged from $t_{1c}n_0m_0$ to $t_{2c}n_0m_0$, with 71% of participant tumors in the $t_{1c}n_0m_0$ stage. Treatment radiation dose ranged from 7250-7920 cgy and treatment days varied between 29 (18%), 38 (41%), 39 (12%) and 44 (29%). Overall 88% participants had a diagnosis of HTN and hyperlipidema, 35% were diagnosed with CAD and DM. Ninety-four percent of participants reported either never smoking or quitting some time ago and 65% of reported currently consuming alcohol. Seventy-one percent of participants reported using one or more over-the-counter herbal/nutritional supplements prior to entering the study but agreed to stop taking them for the duration of the study (Table 5.1). No statistically significant between-group differences were detected for any of these variables.

Control group participants had higher ECOG scores with increasing cumulative doses of radiation. A consistent peak was observed at weeks 3-5 followed by a slight decline at week 6, as a result of one participant in this group completing therapy. No change in performance status was reported by

participants in the 12 oz group, while a slightly increased group mean was noted in the 8 oz group beginning week 6. However, no statistically significant between-group or within-group differences were detected (Table 5.2).

Utilizing the CTCAE measurement criteria, urinary frequency and urgency were evaluated using participant reported baseline or pretreatment levels. Statistically significant differences ($p = 0.039$) in urinary frequency were detected at week 5 between 4 and 8 oz groups. A significant trend was observed at week 5 between control group and 8 oz group participants ($p = 0.059$) and between 8 and 12 oz groups ($p = 0.089$). Urinary urgency was statistically significant at weeks 2 ($p = 0.008$) and 5 ($p = 0.048$) between control and 4 oz groups, and at weeks 5 ($p = 0.004$) and 7 ($p = 0.029$) between control and 8 oz groups. A significant trend was observed at week 2 between control group and 8 oz group participants ($p = 0.083$) (Table 5.3). No within-group differences were detected in control, 4 or 12 oz groups, but a trend towards significance was observed, in the 8 oz group for urinary frequency ($p = 0.099$) and urinary urgency ($p = 0.069$).

Statistically significant differences were observed in the reported incidence of diarrhea at weeks 6 and 7 ($p = 0.029$) between control and 8 oz groups. Participants in the 12 oz group reported a very minimal increase in stool frequency. While no within-group differences were observed in control and 12 oz groups for diarrhea, we observed a trend towards significance ($p= 0.065$) in 4 oz group and statistical significance ($p = 0.000$) in the 8 oz group. No incidence of proctitis was reported among participants in the 12 oz group. Despite reported

proctitis in the other three groups, no statistically significant differences were detected between or within-groups. Overall it appears that tomato juice supplementation exhibited a protective effect on reported diarrhea incidence during the first three weeks of treatment (Table 5.4).

The relationship between certain lifestyle factors (supplement usage, diagnosis of HTN, CAD, DM), tomato juice supplementation and symptoms was investigated using the Spearman's correlation. There was a positive correlation between ECOG score and alcohol intake (beginning week 3) and hypertension (baseline to week 7) and ECOG scores (Table 5.5). Prior dietary supplement use showed a statistically significant association with ECOG score at week 1 ($r = 0.555$, $p = 0.010$, $n = 17$) and 2 ($r = 0.433$, $p = 0.041$, $n = 17$), and a significant trend at baseline and weeks 3-5 (Data not shown). No significant correlation was observed between ECOG score and DM.

Discussion

The primary objective of this paper was to examine the impact of three different doses of tomato juice on the performance status and selected radiation therapy related side effects in men with localized prostate cancer compared with participants in the control group. We did not measure fatigue in our participants, but nursing staff observed higher fatigue in control group participants, which may be reflected in the higher ECOG scores observed in control group participants. While not statistically significant, these results are clinically relevant and have implications beyond our study population, if these results can be replicated in

larger trials. If consumption of tomato products can alleviate radiation therapy induced fatigue in patients, there is considerable potential for improved quality of life during treatment with daily consumption of at least 8-12 oz of tomato juice.

Overall no significant differences in urinary frequency and urgency were observed between control and intervention group participants. While some between-group differences were detected for both urinary frequency and urgency, these may not be good outcome measures. Recommendations to increase fluid intake during treatment, personal tolerance and individual level of discomfort would greatly impact the subjective assessment of these variables.

Tomato juice supplementation appears to have a GI protective effect for the first three weeks of treatment. While the incidence of diarrhea throughout radiation therapy appears to be lower in the control group, one participant reported taking Imodium® to manage his diarrhea, which resulted in a lower group mean for diarrhea (Table 5.4), but a higher weekly participant score (Grade 2: increased stool frequency, bleeding mucus discharge or rectal discomfort requiring medication; anal fissure) and group mean for proctitis (Table 5.4). Despite consistency in treatment planning and delivery, significant variability in the incidence and severity of side effects is not uncommon (Bentzen, 2006). Radiation therapy may induce new microscopic colitis or exacerbate preexisting GI conditions causing diarrhea observed during therapy. Such effects may account for the increase in the incidence of diarrhea observed in the 4 and 8 oz tomato juice groups. Participants in the 12 oz group demonstrated the best GI

protective response. No proctitis was observed in this group and the incidence of diarrhea was minimal. Only one participant in the 12 oz group reported higher stool frequency (change from every other day to daily bowel movement). Researchers have reported decreased severity of diarrhea and acute GI toxicity in rats that were supplemented with lycopene and underwent pelvic radiation (Andic, et al., 2009). Our small sample size may have precluded us from detecting statistical significance. We did detect a significant positive association between performance status (ECOG score) and the diagnosis of hypertension or consumption of alcohol and but not serum or total dietary lycopene, DM or CAD. Chon and Loeffler have also reported lower radiation tolerance in patients with a diagnosis of diabetes or hypertension (Chon & Loeffler, 2002).

To our knowledge, this is the very first study evaluating the tolerance of lycopene supplementation (from processed tomato juice) during radiation therapy in prostate cancer patients. We assessed the tolerance of three doses of tomato juice during radiotherapy and evaluated their impact on performance status as well as both GI and GU symptoms during radiotherapy. While we did not observe any GU protective effect, we did observe a GI protective effect in the intervention groups. Tomato juice was well tolerated by the participants of this study, with no reported incidence of nausea, vomiting or heartburn. This study also expands the scope of lycopene research from preventive to an effective adjunct during radiation therapy.

This study has several limitations. We used validated clinical assessment tools to monitor participant side effects of treatment and performance status. However, we did not find the criteria to be sensitive enough for the purposes of our study. For instance, when evaluating the incidence of diarrhea, participants with increased daily stool frequency of 1-3 stools were all classified on the CTCAE scale as grade 1 toxicity. Some of our participants reported no more than an additional stool per day over baseline, while other participants reported an increase of 2-3 stools per day. However, by using the CTCAE criteria, both groups of participants were classified as grade 1. More objective criteria to evaluate treatment toxicities need to be investigated for future clinical trials. Additionally, the small sample size of this study limits generalization of these results and also may have prevented us from detecting consistent statistically significant differences between groups.

Conclusions

Tomato juice supplementation had no effect on acute GU side effects of radiation treatment, but appeared to offer a GI protective effect in men with prostate cancer receiving at least 8-12 oz of tomato juice daily during radiation therapy. Larger clinical trials are needed to validate these results.

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Table 5.1. Frequency of lifestyle characteristics of study participants.

		Control (n = 5)	4 Oz (n = 4)	8 Oz (n = 5)	12 Oz (n = 3)
Diabetes					
	Yes	2 (40)	2 (50)	1 (20)	1 (33)
	No	3 (60)	2 (50)	4 (80)	2 (67)
Hypertension					
	Yes	5 (100)	4 (100)	4 (80)	2 (67)
	No	0	0	1 (20)	1 (33)
Cardiovascular Disease					
	Yes	2 (40)	3 (75)	1 (20)	0
	No	3 (60)	1 (25)	4 (80)	3 (100)
Hypercholesterolemia					
	Yes	4 (80)	4 (100)	4 (80)	3 (100)
	No	1 (20)	0	1 (20)	0
Smoker					
	Yes	0	1 (25)	0	0
	No	5 (100)	3 (75)	5 (100)	3 (100)
Alcohol Consumption					
	Yes	3 (60)	1 (25)	5 (100)	2 (67)
	No	2 (40)	3 (75)	0	1 (33)
Routine use of supplements					
	Yes	4 (80)	3 (75)	4 (80)	1 (33)
	No	1 (20)	1 (25)	1 (20)	2 (67)

$p<0.05$ (one-sided). No statistical significance detected.

Table 5.2. Reported weekly Eastern Cooperative Oncology Group performance status ("Eastern Cooperative Oncology Group Performance Status," 1982). Data are reported as mean ± standard error of the mean.

	Control (n = 5)	**4 Oz (n = 4)**	**8 Oz (n = 5)**	**12 Oz (n = 3)**
Week 1	0.20±0.20	0.25±0.25	0.20±0.20	0.33±0.33
Week 2	0.40±0.25	0.25±0.25	0.20±0.20	0.33±0.33
Week 3	0.60±0.25	0.25±0.25	0.20±0.20	0.33±0.33
Week 4	0.60±0.25	0.25±0.25	0.20±0.20	0.33±0.33
Week 5	0.60±0.25	0.25±0.25	0.20±0.20	0.33±0.33
Week 6	0.33±0.33	0.25±0.25	0.25±0.25	0.33±0.33
Week 7	0.67±0.67	0.25±0.25	0.25±0.25	0.33±0.33

$p<0.05$ (one-sided). No statistical significance detected.

Table 5.3. Reported weekly incidence of acute genitourinary side effects. Data are reported as mean ± standard error of the mean.

Urinary frequency	**Control (n = 5)**	**4 Oz (n = 4)**	**8 Oz (n = 5)**	**12 Oz (n = 3)**
Week 1	0.60±0.60	0.25±0.25	0.40±0.25	0.00±0.00
Week 2	0.40±0.25	0.75±0.25	0.60±0.25	0.00±0.00
Week 3	0.40±0.25	0.75±0.25	0.40±0.25	0.33±0.33
Week 4	0.40±0.25	0.50±0.29	0.60±0.25	0.33±0.33
Week 5	0.40±0.25	0.25±0.25[a]	1.20±0.20[a]	0.33±0.33
Week 6	0.33±0.33	0.50±0.29	1.00±0.41	0.00±0.00
Week 7	0.00±0.00	0.75±0.25	1.00±0.41	0.67±0.33
Urinary Urgency				
Week 1	0.00±0.00	0.50±0.29	0.40±0.25	0.33±0.33
Week 2	0.00±0.00[b]	1.00±0.00[b]	0.60±0.25	0.33±0.33
Week 3	0.40±0.25	0.50±0.29	0.80±0.20	0.67±0.33
Week 4	0.40±0.25	0.75±0.25	0.80±0.20	0.33±0.33
Week 5	0.00±0.00[cd]	0.75±0.25[d]	1.00±0.00[c]	0.33±0.33
Week 6	0.00±0.00	0.75±0.25	0.75±0.25	0.67±0.33
Week 7	0.00±0.00[e]	0.75±0.25	1.00±0.00[e]	0.33±0.33

Level of significance ($p \leq 0.05$).

[bc]$p \leq 0.01$ [ade]$p \leq 0.05$

Table 5.4. Reported weekly incidence of acute gastrointestinal side effects. Data are reported as mean ± standard error of the mean.

Diarrhea	**Control (n = 5)**	**4 Oz (n = 4)**	**8 Oz (n = 5)**	**12 Oz (n = 3)**
Week 1	0.20±0.20	0.00±0.00	0.20±0.20	0.00±0.00
Week 2	0.60±0.25	0.00±0.00	0.40±0.25	0.00±0.00
Week 3	0.20±0.20	0.50±0.29	0.60±0.25	0.00±0.00
Week 4	0.40±0.25	0.75±0.25	0.80±0.20	0.33±0.33
Week 5	0.20±0.20	0.50±0.29	0.80±0.20	0.33±0.33
Week 6	0.00±0.00[a]	0.75±0.25	1.25±0.00[a]	0.33±0.33
Week 7	0.00±0.00[a]	0.75±0.25	1.00±0.00[a]	0.33±0.33
Proctitis				
Week 1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Week 2	0.00±0.00	0.00±0.00	0.20±0.20	0.00±0.00
Week 3	0.00±0.00	0.00±0.00	0.40±0.25	0.00±0.00
Week 4	0.00±0.00	0.00±0.00	0.20±0.20	0.00±0.00
Week 5	0.60±0.40	0.50±0.50	0.20±0.20	0.00±0.00
Week 6	0.67±0.67	0.50±0.50	0.50±0.50	0.00±0.00
Week 7	0.67±0.67	0.50±0.50	0.50±0.50	0.00±0.00
Week 8	1.00±1.00	0.00±0.00	0.67±0.67	0.00±0.00

p≤0.05, one-sided

[a]$p \leq 0.05$

Table 5.5: Spearman's rho correlations for ECOG score ("Eastern Cooperative Oncology Group Performance Status," 1982)

	ECOG Score							
	Baseline N=17	Wk1 N=17	Wk2 N=17	Wk3 N=17	Wk4 N=17	Wk5 N=17	Wk6 N=14	Wk7 N=14
Alcohol	0.304	0.171	0.334	.485*	.485*	.485*	.519*	.536*
Hypertension	.789**	.658**	.566**	.494*	.494*	.494*	.645**	.575*

Correlation is significant at
**p≤0.01 level (1-tailed)
* p≤0.05 level (1-tailed)

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CHAPTER VI

EPILOGUE

Nutrition care of the oncology patient has always been a passion of mine. This interest was sparked while I was doing my dietetic internship at the All India Institute of Medical Sciences in New Delhi, India. Patients were housed in cubicle like compartments in a large hall with glass partitions, sequestered from the outside world including friends and family. The diagnosis of cancer was a certain death sentence in the 1990's, and this, along with social isolation and ravages of the disease and treatment, led to poor nutritional intake and progressive weight loss. It boggles my mind to this day why the health care providers were surprised when they observed weight loss in these patients. While working as a dietitian in the US, the majority of the consults received for Medical Nutrition Therapy (MNT) were for "weight loss. Poor PO intake." However, I have observed more intensive monitoring of cancer patients receiving chemotherapy due to obvious impediments to intake such as nausea, vomiting and diarrhea. Radiation therapy patients, more specifically patients with head and neck cancer, were referred for MNT only after extensive weight loss. We are now more aware of the effects of treatment on normal tissue, cytokine milieu created as a result of treatment, and a host of other factors that contribute to decreased intake and the common outcomes of weight loss and overall treatment tolerance. Proactive nutrition care in cancer patients can help improve treatment tolerance and quality

of life. Researchers are now beginning to explore nutrients as radiosensitizers to enhance the effect of radiation therapy on tumors while protecting normal tissue and reducing potential side effects of treatment.

My dissertation examined the effect of tomato juice, a rich source of lycopene, in prostate cancer patients undergoing radiation therapy. The overall goals of this project were to evaluate whether dietary lycopene increases serum lycopene levels, delays onset of some of the most common side effects of radiation therapy and consequently improves performance status during therapy. So why lycopene?

My interest in lycopene was piqued while researching the link between carotenoids and lung cancer for NTR 673:Research Methodology, in Spring 2007. Lycopene, a potent antioxidant, has been a source of fascination to researchers for several decades. Researchers as early as the1950s have been utilizing lycopene to mitigate the effects of radiation (Forssberg, Lingen, Ernster, & Lindberg, 1959). Several epidemiological studies (Gann et al., 1999; Giovannucci, 1999, 2005; Vogt et al., 2002) have documented an inverse relationship between intake of lycopene rich foods and the development of prostate cancer. Using cell and animal studies as well as retrospective and prospective trials in humans, researchers have evaluated the impact of lycopene in patients with benign prostate hypertrophy (Edinger & Koff, 2006; Schwarz et al., 2008), high grade prostatic intraepithelial neoplasia (Mohanty, Saxena, Singh, Goyal, & Arora, 2005) and prostate cancer patients prior to initiating

treatment (Bowen et al., 2002; Chen et al., 2001; Jatoi et al., 2007; Kim et al., 2003; Kucuk et al., 2001; Rao, Fleshner, & Agarwal, 1999) or after treatment failure (Clark et al., 2006). However, there is paucity of research investigating the impact of lycopene/tomato products supplementation in prostate cancer patients undergoing treatment. Researchers have demonstrated several mechanisms of action for lycopene's effectiveness. Some of the mechanisms of action that are relevant to this project include inhibition of IL-6 expression and modulation of the cyclooxygenase pathway. Therefore, this research project was developed from research gaps identified in the areas of vulnerable populations, exposures, mechanisms and biomarkers (Davis et al., 2005).

Due to the exploratory nature of this research, we encountered several challenges. The primary challenge was lack of sufficient funding to approach multiple cancer centers for patient recruitment, compensating a professional laboratory for blood sample assessment, and providing compensation to participants. We also had to limit the number of inflammatory mediators that we could test due to limited funding. However, this project could not have been conducted without the funding support we did receive from the University of North Carolina at Greensboro's Faculty Grant.

Initially, when participant recruitment commenced, we had some challenges with potential participant appointment notifications. Since the lycopene study was outside the routine job responsibilities of the cancer center staff, I had to work with them to insure I was notified about patient appointment

times and any subsequent changes in their appointment schedule, in order not to miss meeting with the participants. After making a few repeat trips to the cancer center, and missing a few screening appointments, we devised a system of notification that overcame this obstacle.

We did not achieve our intended target of recruiting 40 total participants. This may have prevented us from observing statistical significance where statistical differences did exist. The possibility of reduced side effects from a food product consumed during radiation therapy did appear to be an incentive for at least some of the participants who volunteered for our study, as evidenced by participant enthusiasm and enrollment. It was a fascinating, educational experience working with the participants of this study. When asked during the exit interviews, 65% of participants reported enrolling in the study to reduce their side effects of treatment, and 47% of participants also reported participating for purely altruistic reasons. These men were extremely motivated and inquisitive about the impact of nutrition and nutritional supplements on prostate health and were further intrigued by the possibility of something as simple as a tomato making them “feel better” along with the possibility of “chemicals” in the blood that could predict their radiation response! Several participants requested additional information on risk vs benefits of supplements, and lowering sodium intake in their diet. Since the information requested would not have influenced the study results, information was provided to the participants when requested.

Due to the number of blood variables being tested, we had to insure that blood collection and processing procedures could be standardized. All collected blood samples were treated the same, thus, we did not add indomethacin to the blood samples to halt synthesis of prostaglandins as stipulated in the PGE_2 ELISA kit. Omitting this step may result in higher PGE_2 expression. Also considering the labile nature of the cytokines, blood processing methodology needs to be carefully considered, specifically the temperature at which blood samples should be processed. Since lycopene is affected by heat and light, we covered the collection tubes containing participant blood immediately with aluminum foil and placed them on ice post collection for transportation to the UNCG laboratory.

Based on the results of this study, participant comments and researcher observations, tomato juice was well tolerated by men over 60 years of age, diagnosed with prostate cancer, undergoing radiation therapy. Only two participants withdrew from the study due to inability to consume the assigned volume of tomato juice. We recognize that tomato juice may not be acceptable to all men as a vehicle for the delivery of dietary lycopene. Consequently, other tomato products providing similar amounts of lycopene may need to be substituted.

Participants in our study did demonstrate high systemic inflammation, which was not unexpected. We did not observe a significant correlation between inflammatory markers and side effects of treatment. The most noteworthy

difference that we did observe was performance status in the intervention groups. Control group participants reported higher fatigue than the intervention group participants and this was reflected in the reported performance status responses from week to week. Another challenge observed during the study was the measurement of urinary frequency and urgency. Considering the age of the participants and frequent complaints of difficulty urinating with this prostate pathology, and also since all participants in the study were instructed by the Radiation Oncologist to increase their fluid intake, it was difficult to truly assess if the urinary frequency and urgency complaints by participants were radiation therapy related. Consequently this may not be an appropriate endpoint for lycopene intervention trials. We observed weight loss in the control group and a protective/delayed gastrointestinal response with lycopene supplementation, confirming results from an animal study (Andic, Garipagaoglu, Yurdakonar, Tuncel, & Kucuk, 2009). Overall, the results of this study may help expand the role of lycopene research from preventive to therapeutic.

Strengths and Limitations

Strengths

1. To our knowledge, this is the first study evaluating serum lycopene levels and the impact of food-based lycopene supplementation during radiotherapy in prostate cancer patients.
2. Lycopene intake was assessed using a validated food frequency questionnaire, the National Cancer Institute's (NCI) diet history

questionnaire (DHQ) which has been validated with older adults. Additionally, since the DHQ calculates average intake over a 12 month period, day-to-day or seasonal variations in intake are not reflected.

3. The DHQ was also selected for estimating lycopene rich foods over other available methods (food diary) mainly because it would require less work for the patients as compared to maintaining a food diary during their treatment regimen.
4. Tolerance of food-based lycopene supplementation during radiotherapy was assessed using the NCI Cancer Therapy Evaluation Program: Common Toxicity Criteria (CTC) v 2.0.
5. Impact of lycopene supplementation on both performance status and select side effects during radiotherapy was assessed.
6. Impact of total lycopene on key inflammatory markers was evaluated.
7. This study expands the scope of lycopene research from preventive to an effective adjunct during radiation therapy in patients with localized prostate cancer.

Limitations

1. In order to minimize disruption of daily activities among the study participants, blood draws were scheduled around the time of their prescheduled radiation treatment appointment. Consequently, patients were not in a fasted state for the blood draws. Several researchers have demonstrated that the serum concentration of carotenoids do not change

significantly for up to four hours after a meal (Brown, Rose, Craft, Seidel, & Smith, 1989; Mejia & Arroyave, 1983; Mejia, Pineda, Noriega, Benitez, & Falla, 1984). However, the impact of non-fasted state on the other test parameters remains unknown.

2. We were unable to use food records to monitor intake of lycopene rich foods during study duration.
3. While the DHQ require less work for the patients as compared to maintaining a food diary, it relies heavily on patient memory in its reporting.
4. Efficacy of lycopene supplementation was only evaluated in men with localized prostate cancer during radiotherapy. It also needs to be evaluated in prostate cancer patients receiving other treatments such as hormone therapy.
5. We only measured total serum lycopene levels in our participants. Role of specific lycopene isomers (cis vs trans) should also be evaluated in future trials.
6. Small sample size of this study limits generalization of these results.

Future Work

Based on the results of this research study, a phase II trial utilizing 8-12 oz of tomato juice or equivalent quantity of tomato product should be conducted. Participants in the 12 oz group demonstrated the least side effects of treatment and a progressive decline in CRP and PGE2 expression between the three time

points. Despite a small increase in IL-6 expression at midpoint, the endpoint results were lower than the baseline results in the 12 oz group. While participants in the 4 oz group demonstrated a progressive decline in IL-6 between the three time points, some participants did demonstrate other side effects of treatment. Lower fatigue was observed among all intervention group participants but not in the control group. This was an unexpected finding. While we did not measure fatigue scores, we speculate that it impacted the performance status scores observed. Since we excluded patients who concurrently received hormone therapy or were treated with brachytherapy, or received radiation to the whole pelvic bed, the impact of lycopene supplementation in these groups also needs to be investigated. Additionally, no information exists on the impact of this supplementation in reducing long term complications of radiation therapy and in survivors. A retrospective review of long-term side effects in our study participants should also be conducted to identify the impact of lycopene on these side effects.

Overall, patients who volunteered to participate in our study appeared to have a desire for more “natural” alternatives to manage side effects of radiation therapy. This research also provides valuable data on the impact of tomato juice as a source of the carotenoid lycopene on select markers of inflammation. Links exploring the relationship between cytokine expression, radiation side-effects and lycopene need to be explored further in larger clinical trials. We hope that the

results of this study will allow us to secure funding in the future to conduct trials with a larger sample of patients to validate these results.

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APPENDIX A. RECTUITMENT FLYER

Effect of Tomato Juice during Radiation Therapy in men with Prostate Cancer

Are you a man, who

- has recently been diagnosed with prostate cancer,
- has not undergone hormone therapy, surgery or other treatment for prostate cancer,
- is about to start radiation treatment,
  AND
- is interested in participating in a clinical trial?

If you responded YES to each of the above statements, then call us!

The purpose of this research is to investigate the effect of lycopene (a nutrient found in tomato juice) on radiation therapy related side effects in men with prostate cancer undergoing radiation treatment.

To be eligible to participate in this study you must

- Have a new diagnosis of localized prostate cancer
- Have not yet initiated treatment;
- Have normal immune, liver and kidney function;
- Be willing to sign informed consents
- Be willing to provide small blood samples (2 tablespoons three different times)

You will **not be eligible to participate in this study** if you

- Have metastasized prostate cancer or have previously received treatment for prostate cancer;
- Are allergic to tomato products
- Are unwilling to provide blood samples
- Have certain medical conditions - High blood potassium levels, uncontrolled gastro-esophageal reflux disease (GERD) , malabsorption disorders, liver or kidney problems
- Routinely **use the following supplements** –MVI, fiber, saw palmetto, lycopene, Omega-3 Fatty acids/Fish oil, Vitamin C, E, A and B-carotene, flaxseeds or flaxseed oil supplements;
- Are unable/unwilling to sign the informed consent

For additional information, please contact
Dr. Bart Frizzell at 878 - 6036
or
Mri Datta at 307 - 9020

APPENDIX B. HEALTH INFORMATION PORTABILITY AND ACCOUNTABILITY ACT (HIPAA) FORM – ENGLISH

HIGH POINT REGIONAL HEALTH SYSTEM
INSTITUTIONAL REVIEW BOARD

HIPAA – AUTHORIZATION FOR RESEARCH

For the Use and/or Disclosure of
Protected Health Information

You may qualify for participation in a research study. This "HIPAA Authorization for Research" form gives you information about how health information collected about you for a research study would be used by researchers and disclosed to others involved in the research study. Your signature on this form permits High Point Regional to give your information to the research team.

Study Title: Food-based lycopene supplementation in prostate cancer patients undergoing radiotherapy

Principal Investigator: __Martha Taylor, PhD_____

I authorize the use and/or disclosure of my protected health information as described below:

1. The following entity/person(s) is *authorized to use or disclose* my information:

   - **High Point Regional Health System**
     601 North Elm Street
     High Point, NC 27262
     (336) 878-6000

2. The following entity/person(s) is *authorized to receive* my information:

   - The Researchers (members, agents or successors of the research team, such as the Principal Investigator, Co-Investigators and members of their research staff)
   - The sponsor of the research study, and its agents and contractors
   - Representatives of government organizations, review boards, and other persons who are required to watch over the safety and effectiveness of medical products and therapies and the conduct of research

3. *Description of the information* that may be used or disclosed:

   - Health information in my medical records that is relevant to the study

     (Taking part in this research study may involve collecting and disclosing health information that you consider confidential or private that directly identifies you.

Information in your medical record may include results of tests, procedures, interventions, interactions, questionnaires or surveys. All information may be used and possibly disclosed and re-disclosed to monitor your health status, to measure the effects of drugs/devices/procedures /interventions as stated in this study, to determine research results and outcomes, and possibly develop new drugs/devices/tests/procedures and commercial products.)

4. My information will be used or disclosed for the following *purposes*:

    - By the Researchers, among themselves and with other participating researchers to conduct the Research;
    - By the research Sponsor, as will be described in the Informed Consent Form;
    - By the representatives of government organizations, review boards, and other persons to watch over the safety and effectiveness of medical products and therapies and the conduct of research.

    (Your health information may be used by, disclosed to and re-disclosed for: research, quality assurance, or regulatory purposes, by: members, agents or successors of the research team, such as the Principal Investigator, Co-Investigators and members of their research staff ("Researchers"); other researchers and their staff involved with this study at other medical centers, institutions, hospitals, central data centers, the U.S. Food and Drug Administration, the Department of Health and Human Services, the Federal Office of Human Research Protection, High Point Regional Health System and/or the sponsor of this research study.)

5. Study data that does not directly identify you may be published in medical journals or shared with others as part of scientific discussions.

6. You have the right to see and copy your personal health information related to the research study for as long as this information is held by the Investigator or a research institution. However, to ensure the scientific integrity of the study, you will not be able to review some of the study information until after the study has been complete.

7. This authorization will expire when the last component of this research study is completed. (Some research studies include more than one component, each of which may span a different time period; therefore, this authorization extends to the time when the last component is completed.)

8. I understand that I may revoke this authorization at any time. My revocation must be in writing and contain my signature. I am aware that my revocation is not effective to the extent that the entity/person(s) I have authorized to use or disclose my information have acted in reliance upon this authorization. To revoke my authorization, I understand that I need to contact the IRB Office at the address and phone number listed above in item #1 above and complete the appropriate paperwork.

9. I have been advised, and I understand, that this authorization is voluntary. I do not have to sign this authorization and my refusal to sign will not affect my abilities to obtain treatment from High Point Regional Health System, nor will it affect my eligibility for benefits. **However, I understand that I will be required to sign this authorization form prior to receiving research-related treatment.**

10. I understand that if my information is disclosed to someone who is not required to comply with the federal privacy protection regulations, then such information may be re-disclosed and would no longer be protected.

11. I certify that I have received a copy of this authorization.

_____ _____

Signature of Patient Date

_____

Print Name

In the event this form is completed by someone other than the patient, please indicate below:

_____ _____

Signature Date

_____ _____

Print Name Relationship to Patient

03-03; revised: 12-18-03; revised 01-09-06

APPENDIX C. HEALTH INFORMATION PORTABILITY AND ACCOUNTABILITY ACT (HIPAA) FORM – SPANISH

High Point Regional Health System
Comité de Ética de Investigación Clínica

Autorización para Investigación-HIPAA
Para el Uso y/o Divulgación de
Información Médica Protegida

Usted puede calificar para participar en un estudio de investigación. Este formulario "Autorización para Investigación de HIPPA" le da información acerca de cómo su información medica colectada para el estudio de investigación será usada por los investigadores y será divulgada a otros envueltos en el estudio de investigación. Su firma en este formulario permite a High Point Regional dar su información al equipo de investigación.

Título del Estudio:

Investigador Principal: ____Martha Taylor, PhD_____

Yo autorizo el uso y/o divulgación de mi información médica protegida como se describe abajo:

1. La siguiente entidad/persona(s) son autorizadas para usar o divulgar mi información:

   - High Point Regional Health System
     601 North Elm Street
     High Point, NC 27262
     (336) 878-6000

2. La siguiente entidad/persona(s) esta autorizada para recibir mi información:

   - Los investigadores (miembros, agentes o sucesores del equipo de investigación, tales como el Investigador Principal, Co-Investigadores y miembros del personal de investigación)
   - El patrocinador del estudio de investigación y sus agentes y contratistas
   - Representantes de organizaciones gubernamentales, comités de ética, y otras personas que les es requerido vigilar la seguridad y la efectividad de los productos médicos y terapias y el conducto de la investigación.

3. Descripción de la información que puede ser usada y divulgada:

   - Información médica en mis expedientes médicos que sea pertinente al estudio

     (El tomar parte en este estudio puede envolver el colectar y divulgar información médica que usted considera confidencial o privada que directamente lo identifica. Información médica en su expediente médico puede incluir los resultados de exámenes, procedimientos, intervenciones, interacciones, cuestionarios o encuestas. Toda la

información puede ser usada y posiblemente divulgada y revelada para monitorear su estado de salud para medir los efectos de los medicamentos/aparatos/procedimientos/intervenciones como se ha expuesto en este estudio, para determinar los resultados y consecuencias y posiblemente desarrollar nuevos medicamentos/aparatos/exámenes/procedimientos y productos comerciales.)

4. Mi información será usada o divulgada con los siguientes *propósitos:*

   - Por los Investigadores, entre ellos y con otros investigadores participantes para conducir la Investigación.
   - Por el investigador Patrocinador, como será descrito en el Consentimiento Informado;
   - Por los representantes de organizaciones gubernamentales, comités de ética y otras personas que vigilan la seguridad y la efectividad de los productos médicos y terapias y el conducto de la investigación.

   (Su información puedes ser usada, divulgada y revelada para: investigación, control de calidad o propósitos reguladores, por: miembros, agentes o sucesores de el equipo de investigación, tal como el Investigador Principal, Co-Investigadores y miembros del personal de investigación ("Investigadores"); otros investigadores y su personal envueltos con este estudio en sus centros médicos, instituciones, hospitales, centro de datos central, Administración de Alimentos y Fármacos de EE. UU., el Departamento de Salud y Servicios Humanos, La Oficina Federal para la Protección de los Seres Humanos en la Investigación, High Point Regional Health System y/o el patrocinador de este estudio de investigación.

5. Información del estudio que no lo identifique directamente puede se publicada en diarios médicos o compartida con otros en discusiones científicas.

6. Usted tiene el derecho de ver y copiar su información personal médica relacionada con la investigación mientras que el Investigador o la institución de investigación tenga esta información. Sin embargo, para asegurar la integridad científica de la investigación usted no podrá repasar alguna de la información de la investigación hasta después de que se haya completado la investigación.

7. Esta autorización se vence cuando el ultimó componente de esta investigación se complete. (Algunos estudios de investigación incluyen más de un componente, lo cual cada uno tiene un lapso de un período de tiempo diferente; por consiguiente, esta autorización se extiende al tiempo cuando el ultimó componente es completado.)

8. Yo entiendo que puedo revocar esta autorización en cualquier momento. Mi revocación debe ser por escrito y debe contener mi firma. Estoy al tanto de que mi revocación no es efectiva a la entidad/persona(s) que he autorizado y han actuado según esta autorización para usar y divulgar mi información. Para revocar mi autorización, yo

entiendo que necesito contactar la oficina del IRB a la dirección y número de teléfono indicado arriba en el artículo #1 arriba y completar el papeleo apropiado.

9. Se me ha aconsejado y entiendo que esta autorización es voluntaria. No tengo que firmar esta autorización y mi rechazo a firmar no afectará mis habilidades a obtener tratamiento de High Point Regional Health System, ni tampoco afectará mi elegibilidad para beneficios. **Sin embargo, yo entiendo que se requiere que firme esta autorización antes de recibir tratamiento relacionado a la investigación.**

10. Yo entiendo que si mi información es divulgada a alguien que no se le requiera cumplir con las regulaciones federales de privacidad, después la información puede ser revelada y ya no seria protegida.

11. Yo certifico que he recibido una copia de esta autorización.

_____ _____
Firma del paciente Fecha

_____
Escriba su nombre en letra de molde

En el caso de que este formulario sea completado por alguien que no es el paciente, por favor indíquelo abajo:

_____ _____
Firma Fecha

_____ _____
Escriba su nombre en letra de molde Relación al paciente

APPENDIX D. CONSENT FORM – ENGLISH

HIGH POINT REGIONAL HEALTH SYSTEM
INSTITUTIONAL REVIEW BOARD
INFORMED CONSENT

What is a research study?

You are invited to be in a research study. Research studies are designed to gain scientific knowledge that may help other people in the future. You may or may not receive any benefit from being part of the study. There may also be risks associated with being part of research studies. You are being asked to take part in this study because you have been diagnosed with prostate cancer and are about to begin radiation therapy for it. Your participation in this study is voluntary. Please take your time to make your decision and ask your study doctor or the study staff to explain any words or information that you do not understand. You may also discuss the study with your friends and family.

Who is sponsoring the study?

The researchers do not hold a direct financial interest in the sponsor or the product being studied.

Why is the study being done?

This study is being done to test if lycopene, a natural substance found in tomatoes, tomato products and some other foods helps reduce radiation therapy related side effects in men with prostate cancer, receiving external beam radiation therapy.

In this study, the effect of three different amounts of tomato juice is being evaluated. In this study, you will receive 4 ounces, 8 ounces or 12 ounces of tomato juice.

How many people will take part in the study?

Forty men with prostate cancer about to initiate radiation therapy for treatment will be invited to participate in this study.

What is involved in the study?

If you agree to participate, we will need to check your blood work to make sure your kidneys and liver are functioning adequately and check your bloods lycopene level and other immune function related labs. This will require a blood sample (about 2 tablespoons of blood) to be drawn at the beginning of the study. These tests will be repeated at the end of three weeks of treatment and on the last day you receive your radiation treatment.

We will ask you to drink your assigned amount of tomato juice daily while you are receiving your radiation treatment, starting at least 2 days before you begin your treatment. On Friday of each week (during treatment), you will be provided with two additional doses of the beverage to take home with you. One dose of the beverage should be consumed on Saturday and the other on Sunday.

You will be asked to complete the National Cancer Institute's Diet History Questionnaire. This will help us estimate your usual intake of lycopene rich foods. There is no right or wrong answer, and you do not need to change your eating habits just because you are participating in this study. Please answer the questions honestly about how frequently you have been consuming specific foods.

At the end of each week, you receive treatment we will also ask you to report your level of fatigue and ability to carry out your daily activities and any symptoms you may be experiencing.

How long will I be in the study?

You will be in the study for the entire time you are receiving radiation therapy.

The researcher may decide to take you off the study if: 1) continued participation is not in your best medical interest, 2) health conditions occur which would make your participation possibly dangerous, or 3) new information becomes available.

While we encourage you to continue till the end of the study, you may of course, withdraw from the study at any time. This will not comprise or affect the quality of your care that you may receive from this institution. If you decide to stop participating at any time, we encourage you to talk with the investigator or study staff first to learn about any potential health or safety consequences.

What are the risks of the study?

Being in this study may or may not involve any risk to you. You should discuss the risk of being in this study with the study staff.

Blood draw: You may experience discomfort, bruising, and/or bleeding where the needle is inserted. There may be bruising at the puncture site when blood is drawn and occasionally some redness and swelling. Occasionally some people become dizzy, lightheaded or feel faint. Infection may occur on rare occasions.

Risks and side effects related to the study treatments:

Study supplement: Lycopene is a naturally substance found in tomatoes, tomato products and some other foods. Processed tomato juice, which is the chosen food to provide lycopene in this study, may potentially cause some of the following side effects:

- heartburn
- abdominal distention
- flatulence (gas)(Jatoi *et al.*, 2007)
- nausea
- vomiting
- diarrhea

There may be other side effects that we cannot predict or are unexpected. If you have any unusual symptoms, report them immediately to your doctor and the study staff. You should also tell the research staff about all medications, vitamins and supplements you take and any medical conditions you have. This may help avoid side effects, interactions and other risks.

Will I benefit from taking part in the study?

You may or may not receive any benefit from taking part in this research study. We hope the information learned from this study will benefit other patients in the future. The benefit of participating in this study may be a decrease in radiation therapy related side effects.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

A record of your progress will be kept in a confidential file at the hospital or doctor's office where you receive treatment. Organizations that may inspect and/or copy your research and medical records (blood samples etc.) for quality assurance, research, and data analysis include groups such as:

Comprehensive cancer center at Wake Forest University (CCCWFU)

National Cancer Institute (NCI) and its representative

Food and Drug Administration (FDA)

Office of Human Research protections (OHRP)

Department of Health and Human Services (DHHS)

Institutional Review Board (IRB) at High Point Regional Health System

Possible other federal or state government agencies

If your record is used out given out for governmental purposes, it will be done under conditions that will protect your privacy to the fullest extent possible consistent with the laws relating to public disclosure of information and law-enforcement responsibilities of the agency. These agencies may review the research to see that it is being done safely and correctly.

You authorize the use of clinical information contained in your records, but any publication that includes such information or data shall not reveal your name, show

your picture or contain any other personally identifying information, except as otherwise required by law.

What are the costs of taking part in this study?

There are no costs to you for taking part in this study. All the study costs, including any study medications and procedures directly related to the study, will be paid for by the study. Costs or your regular medical care/treatment, which are not related to this study, will be your own responsibility.

You will not be paid to participate in this study.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, Bart Frizzell, M.D. if you feel that you have been injured because if taking part in this study. You can tell the doctor in person or call him at 336-878-6036.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. This study will not pay for medical treatment. Although no funds or monies have been set aside to compensate you in the event of injury or illness related to the study treatment or procedures, you do not waive any of your legal rights for compensation by signing this form.

You or your insurance company will be charged for continuing medical care and/or hospitalization.

What are my rights if I take part in this study?

Taking part in this study is voluntary. You may choose to take part, not to take part or may leave the study at any time. No matter what decision you make, there will be no penalty for you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care or result in any penalty or loss of benefits to which you are entitled.

Even after you agree to take part in this study, you may withdraw at anytime. Before you withdraw, you should talk to one of the researchers. This will allow them to inform you of any medical problems that could result from stopping your treatment. Your decision to stop taking part in this study will not affect your medical treatment or your relationship with those treating you or with this institution. If you withdraw from the study, you will still be offered all available care that meets your medical condition. You are free to seek care from a doctor of your choice at any time. The investigators also have the right to stop your participation in the study at any time. This could happen if you lose too much weight or have side effects from either the radiation therapy or the beverage that prevents you from continuing in the study.

We will tell you about new information that may affect your health, welfare or willingness to stay in the study. You may be asked to sign another consent form in response to new information.

Who can answer my questions about the study?

For questions about the study or a research-related injury, contact your doctor, Bart Frizzell, M.D. at 336-878-6036.

You can contact the study coordinator, Mri Datta at 336-307-9020 if you have further questions.

For questions about your rights as a research participant, contact the High Point Regional health System Institutional Review Board (which is a group of people at the hospital where you receive treatment, who review the research to protect your rights) at 336-878-6207.

Participant Contract

I have been offered the opportunity to ask questions about this study and all questions have been answered to my satisfaction. The contents of this form have been explained to

me and I understand them. I agree to allow the research personnel specified above the access to my medical records.

It may be necessary for my doctor to contact me at a future date regarding new information about the treatment I received; therefore, I agree to notify my doctor of any change of address and/or telephone number.

My signature below means that I have voluntarily agreed to participate in this research study. I will be given a copy of all 6 pages of this consent. I have read it or it has been read to me, I may also request a copy of the study (complete study plan)

_____ _____ _____

(Participant Name) (Participant Signature) (Date)

I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in the research study and have answered any questions that have been raised.

_____ _____ _____

Name of person obtaining consent) (Signature) (Date)

I, _____, withdraw my consent to participate in this study and refuse to have clinical data collected from my medical record (s).

Participant name _____ study ID#_____

(please print name clearly)

Participant signature _____ Date_____

Witness signature _____ Date_____

APPENDIX E. CONSENT FORM – SPANISH

HIGH POINT REGIONAL HEALTH SYSTEM
COMITÉ DE ÉTICA DE INVESTIGACIÓN CLÍNICA

CONSENTIMIENTO INFORMADO

¿Qué es un estudio de investigación?

Usted es invitado a participar en un estudio de investigación. Los estudios de investigación son diseñados para obtener conocimiento científico que pueda ayudar a otras personas en el futuro. Quizá reciba o no beneficios por ser parte del estudio. También pueden existir riesgos asociados por ser parte de los estudios de investigación. Se le ha pedido ser parte de este estudio porque usted ha sido diagnosticado con cáncer de la próstata y está a punto de recibir radio terapia para ella. Su participación en este estudio es voluntaria. Por favor de tomarse el tiempo para tomar su decisión y pregúntele a su doctor o a el personal del estudio que le expliquen cualquier palabra o información que no entienda. También puede hablar con su familia y amistades acerca del estudio.

¿Quién esta patrocinando el estudio?

Los investigadores no tienen ningún interés financiero al patrocinar el producto que se está estudiando.

¿Por qué se está haciendo este estudio?

Este estudio se esta haciendo para probar si el licopeno, una sustancia natural que se halla en tomates, productos de tomates y otros alimentos, puede ayudar a reducir los efectos secundarios relacionados con la radioterapia en hombres con cáncer de la próstata que reciben radioterapia.

En este estudio, se está evaluando el efecto de tres diferentes cantidades de jugo de tomate. En este estudio usted recibirá 4 onzas, 8 onzas y 12 onzas de jugo de tomate.

¿Cuántas personas participarán en el estudio?

Cuarenta hombres con cáncer de la próstata que están por iniciar la radioterapia serán invitados a participar en este estudio.

¿Qué envuelve el estudio?

Si usted está de acuerdo en participar, necesitamos revisar sus análisis de sangre para asegurarnos de que sus riñones e hígado estén funcionando adecuadamente y analizaremos en su sangre su nivel de licopeno y otras funciones inmunes. Esto requiere que tomemos una muestra de su sangre (como 2 cucharadas de sangre) al principio del estudio. Estos exámenes se repetirán al final de tres semanas de tratamiento y al último día que reciba la radioterapia.

Le pediremos que tome a diario su bebida de jugo de tomate con la cantidad asignada mientras esté recibiendo su radioterapia, comenzando por lo menos 2 días antes de empezar su tratamiento. El viernes de cada semana (durante el tratamiento) se le proveerá con dos dosis adicionales de la bebida para que la lleve a casa. Debe consumir una dosis de la bebida el sábado y la otra el domingo.

Se le pedirá que llene el Cuestionario de Historial Alimenticio del Instituto Nacional de Cáncer. Esto nos ayudará a estimar su consumo de alimentos ricos en licopeno. No hay ninguna contestación correcta o incorrecta y no tiene que cambiar sus hábitos de alimentación solo porque va a participar en este estudio. Por favor de contestar todas las preguntas lo más honesto posible, de que tan frecuente ha estado consumiendo alimentos específicos.

Al final de cada semana que reciba tratamiento también le pediremos que reporte su nivel de fatiga y habilidad de llevar a cabo sus actividades diarias y cualquier síntoma que experimente.

¿Cuánto tiempo participaré en el estudio?

Usted participará en el estudio todo el tiempo que reciba la radioterapia.

El investigador puede decidir removerlo del estudio si: 1) su participación no es lo más conveniente para su salud 2) ocurren condiciones de salud que harían su participación posiblemente peligrosa o 3) sale disponible información nueva.

Aunque le animamos a que continué hasta el final, usted puede retirarse del estudio en cualquier momento. Esto no comprometerá o afectará la calidad de cuidado que recibe de esta institución. Si decide no participar en cualquier momento le animamos a que hable con el investigador o personal del estudio primero para ver si hay alguna consecuencia potencial en su salud o seguridad.

¿Cuáles son los riesgos del estudio?

El participar en este estudio puede o no envolver riesgos a usted. Hable con el personal del estudio acerca de los riesgos por participar en el.

Extracción de sangre: Puede experimentar incomodidad, moretón y/o sangrado donde introducen la aguja. Pueden obtener un moretón en el sitio de la punción cuando extraigan la sangre y ocasionalmente enrojecimiento e hinchazón. Ocasionalmente algunas personas se marean o sienten que van a desmayar. En raras ocasiones puede producirse una infección.

Riesgos y efectos secundarios relacionados a los tratamientos del estudio:

Suplemento del estudio: Licopeno es una sustancia natural que se encuentra en tomates, productos de tomates y otros alimentos. El jugo de tomate procesado, el alimento escogido para proveer licopeno en este estudio, puede potencialmente causar los siguientes efectos secundarios:

- acidez/agruras
- distensión abdominal
- flatulencia (gas)
- náusea
- vomito
- diarrea

Puede haber otros efectos secundarios que no podemos predecir o que son inesperados. Si tiene algún síntoma inusual repórtelos de inmediato a su doctor o al el personal del estudio. También déjele saber a el personal de investigación sobre todo medicamento, vitaminas o suplementos que toma y cualquier condición de salud que tenga. Esto puede ayudar a evitar efectos secundarios, interacciones y otros riesgos.

¿Me beneficiaré por participar en el estudio?

Puede que si o que no reciba ningún beneficio al participar en el estudio de investigación. Esperamos que la información que obtengamos del estudio le podrá beneficiar a otros pacientes en el futuro. El beneficio de participar en este estudio puede ser una disminución en los efectos secundarios relacionados con la radioterapia.

¿Se mantendrá privada mi información médica?

Haremos lo mejor para asegurarnos de que su información personal en su expediente médico se mantenga privada. Sin embargo, no le podemos garantizar privacidad total. Su información personal puede ser divulgada si es requerido por la ley. Si la información de este estudio es publicada o presentada en juntas científicas, no se usará su nombre o alguna otra información personal.

El expediente de su progreso se mantendrá en un archivo confidencial en el hospital o la oficina del doctor donde usted recibe el tratamiento. Las organizaciones que quizá inspeccionen y/o copien su estudio o expediente médico (muestras de sangre, etc.) para garantía de calidad, investigación y análisis de datos incluyen los grupos como:

Centro exhaustivo de cáncer en La Universidad Wake Forest (CCCWFU)
Instituto Nacional de Cáncer (NCI) y sus representantes
Administración de Alimentos y Fármacos (FDA)
Oficina para la Protección de los Seres Humanos en la Investigación (OHRP)
Departamento de Salud y Servicios Humanos (DHHS)
Comité de Ética de Investigación Clínica en High Point Regional Health System
Posiblemente otras agencias gubernamentales federales o estatales

Si su expediente se libera con propósitos gubernamentales, será bajo condiciones que proteja su privacidad hasta donde las leyes relacionadas a la divulgación pública de información y las responsabilidades de la agencia a ejecutar la ley lo permitan. Estas agencias pueden examinar la investigación para ver si se esta haciendo de una manera segura y correcta.

Usted autoriza el uso de información clínica que contiene su expediente, pero cualquier publicación que incluya tal información o datos no deben revelar su nombre, mostrar su fotografía o contener cualquier otra información que lo identifique, a menos que se requiera por ley.

¿Cuáles con los costos por participar en este estudio?

No hay ningún costo a usted por participar en este estudio. Todos los costos del estudio, incluyendo cualquier medicamento o procedimientos directamente relacionados con el estudio, serán pagados por el estudio. Los costos de su tratamiento/cuidado médico regular, que no están relacionados con el este estudio, serán su responsabilidad.

No se le pagará por participar en este estudio.

¿Qué pasa si me lastimo por participar en este estudio?

Es importante que le diga a su doctor, Bart Frizzell, , M.D. si siente que ha sido lastimado al participar en este estudio. Puede decírselo al doctor en persona o llamarlo al 336-878-6036.

Usted recibirá tratamiento médico si es lastimado como resultado de participar en este estudio. Se le cobrará a usted y/o plan de salud por este tratamiento. Este estudio no pagará por tratamiento médico. Aunque no se ha reservado fondos o dinero para compensarlo en evento de ser lastimado o por enfermedad relacionada con el tratamiento o procedimientos del estudio, al firmar este formulario usted no esta renunciando sus derechos legales por compensación.

Se le cobrará a usted o su compañía de seguro por cuidado médico continuo y/o por ser hospitalizado.

¿Cuáles son mis derechos si participo en este estudio?

El participar en este estudio es voluntario. Puede optar por participar, no participar o por dejar el estudio en cualquier momento. No importa cual sea su decisión, no habrá ninguna penalización y no perderá ninguno de sus beneficios regulares. El que deje el estudio no afectará su cuidado médico o resultará en ninguna penalización o pérdida de beneficios a los cuales tenga derecho.

Aun si concuerda en participar en este estudio, usted puede retirarse en cualquier momento. Antes de retirarse debe hablar con los investigadores. Esto les permitirá informarle de cualquier problema que pueda surgir como resultado por dejar el tratamiento. Su decisión de dejar de participar en el estudio no afectará su tratamiento médico o su relación con quienes lo atienden o con esta institución. Si se retira del estudio, aún se le ofrecerá todo cuidado disponible que reúna los requisitos a su condición médica. Tiene el derecho de buscar cuidado de cualquier doctor en cualquier momento. Los investigadores también tienen el derecho de detener su participación en este estudio en cualquier momento. Esto ocurre si baja demasiado de peso o tiene efectos secundarios, ya sea de la radioterapia o la bebida, que prevenga a que continúe en el estudio.

Le informaremos acerca de nueva información que pueda afectar su salud, bienestar o disposición de permanecer en el estudio. Puede que se le pida firmar otro consentimiento en reacción a la nueva información.

¿Quién puede contestar mis preguntas acerca del estudio?

Para preguntas acerca del estudio o herida/lesión relacionado con el estudio, contacte a su doctor, Bart Frizzell, M.D. al 336878-6036.

Puede contactar a la coordinadora del estudio, Mri Datta al 336-307-9020 si tiene más preguntas.

Para preguntas sobre sus derechos como participante de la investigación, contacte a el Comité de Ética de Investigación Clínica de High Point Regional Health System (lo cual es un grupo de personas en el hospital donde recibe tratamiento, quienes revisan el proyecto de investigación para proteger sus derechos: al 336-878-6207.

Contrato del Participante

Se me ha ofrecido la oportunidad de hacer preguntas acerca del estudio y todas las preguntas han sido contestadas a mi satisfacción. Se me ha explicado el contenido de este formulario y lo entiendo. Estoy de acuerdo en permitir el personal de la investigación especificado arriba a tener acceso a mi expediente médico.

Quizá sea necesario que mi doctor me contacte en el futuro sobre nueva información acerca del tratamiento que recibí; por lo tanto yo concuerdo en notificar a mi doctor con cualquier cambio de dirección y/o número de teléfono.

Mi firma abajo significa que yo voluntariamente concuerdo en participar en esta investigación. Se me dará una copia de las 5 páginas de este consentimiento. Yo lo he leído o se me ha leído, también puedo pedir una copia del estudio (plan completo del estudio)

_____	_____	_____
(Nombre del Participante)	(Firma del Participante)	(Fecha)

Yo certifico que he explicado al individuo mencionado arriba la naturaleza y el propósito, los beneficios potenciales y posibles riesgos asociados con la participación en la investigación y he contestado cualquier pregunta que ha surgido.

_____ _____ _____

(Nombre de la persona obteniendo el consentimiento) (Firma) (Fecha)

Yo, _____, retiro mi consentimiento a participar en este estudio y me rehúso a que colecten datos de mi expediente(s) médico.

Nombre del participante _____ # de identificación de estudio

(por favor escriba claramente su nombre en letra de molde)

Firma del participante _____Fecha _____

Firma del testigo _____ Fecha _____

APPENDIX F. SCREENING FORM

Participant Information/Screening

Name:_____ Age:_____

Ethnicity: ❑ African American ❑ Caucasian ❑ Other_____

Height:_____ reported wt:_____ how long ago:_____

Weight change: ❑ loss (#/time)_____ ❑ gain (#/time)_____

Marital Status: ❑ married ❑widowed ❑ divorced ❑ never married

Highest level of Education: ❑ < high school ❑ high school ❑ > high school

Other significant information:_____

Group Assignment:		**Participant ID:**

No. Of treatments prescribed: _____ Gy, over _____ days

Labs	**Results**	
	Date:	**Date:**
PSA		
Na		
K		
Gluc		
BUN		
Cr.		
Ca		
albumin		
SGOT		
SGPT		
LDH		
Alk. Phos		

Medical History	
❑ HTN	❑
❑ DM	❑
❑ CAD	❑
❑ Hyper chol	❑

Meds & Supplements: Prescription & OTC

Medications	**Supplements**

	Yes	No
ECOG performance score 0 or 1		
Are you allergic to tomatoes or red food coloring?		
Are you allergic to any other foods? If yes, please specify foods allergic to__		
Have you ever been diagnosed with		
Problems with your immune function		
liver disease		
kidney disease		
malabsorption disorders/ Irritable bowel/ Celiac disease		
Uncontrolled gastro-esophageal reflux disease		
Are you currently taking any fiber supplements/pills: Metamucil, Citrucel, Fibercon, Benefiber		
Do you exercise? If yes, how often?_____		
Do you smoke? If yes how many packs a day_____		
Do you drink alcohol? If yes, please specify how many drinks		
Daily ___ drinks a day of _____ Monthly ___drinks per month of __ Weekly____ drinks a week of _____ Socially ___ drink_____		
Since being diagnosed with cancer, have you made any changes in (specify)		
Activity		
Diet		
Lifestyle		
Are you currently taking any of the following supplements		
Multivitamin supplement with lycopene		
Saw Palmetto		
Lycopene		
Vitamin A		
Vitamin C		
Vitamin E		
Beta-carotene		
Omega-3 Fatty acids		
Fish oil		
Flax seeds		
Flaxseed oil		
EPA/DHA		
_____Other Supplements (please specify)		
Are you willing to stop taking these supplements for the duration of the study?		
Would you be willing to sign an informed consent form?		
Notes:		

APPENDIX G. INSTRUCTIONS FOR STUDY PARTICIPANTS – ENGLISH

Instructions for tomato juice and prostate cancer study participants

1. Please **continue with your normal eating habits and meal patterns**. Do not make any changes in your diet just because you are participating in this study.
2. During your participation in the study, each Friday you will be provided with two beverage containers. Please consume the beverage in one container on Saturday and the other on Sunday.
3. Consume beverage within 30 minutes of eating a meal to aid in the digestion of the beverage.
4. **Do not** consume any of the following supplements for the duration of your treatment/study participation as these may change how your body handles the treatment you are receiving.

a. Multivitamin	e. Vitamin A	i. Fish Oil
b. Saw Palmetto	f. Vitamin C	j. EPA/DHA
c. Lycopene	g. Vitamin E	k. Flaxseeds
d. Beta-Carotene	h. Omega-3	l. Flaxseed oil

5. Before taking any other nutritional supplement or vitamins, please check with your radiation doctor and the study coordinator. Some of these supplements may decrease the benefits of the radiation treatment you are receiving.
6. You will be asked to complete a **food frequency questionnaire** at the beginning of the study. This should take you about an hour to complete. Please answer all questions as accurately as you can and return ***the questionnaire to the study coordinator, one week before your last treatment***. There is no right or wrong answer.
7. Please let your radiation doctor and study coordinator know if there are any changes in your bowel movements, bladder problems or you experience other problems such as nausea, vomiting, or heartburn.

You can reach the study coordinator Mri Datta at 307-9020

APPENDIX H. INSTRUCTIONS FOR STUDY PARTICIPANTS – SPANISH

Instrucciones para los participantes en el estudio de cáncer de la próstata y jugo de tomate

1. Por favor **continúe con sus hábitos y patrones normales de alimentación.** No haga ningún cambio en su dieta solo porque esta participando en este estudio.

2. Durante su participación en el estudio, cada viernes se le proveerá con dos envases de bebidas. Por favor de consumir la bebida de un envase el sábado y la otra el domingo.

3. Consuma la bebida dentro de 30 minutos de haber comido para ayudar con la digestión de la bebida.

4. **No** consuma ninguno de los siguientes suplementos por la duración de su participación en el tratamiento/estudio, ya que estos pueden cambiar la manera como su cuerpo maneja el tratamiento que esta recibiendo.

a. Multivitamínico	e. Vitamina A	i. Aceite de pescado
b. Palma enana americana	f. Vitamina C	j. EPA/DHA
c. Licopeno	g. Vitamina E	k. Linaza
d. Beta Caroteno	h. Omega-3	l. Aceite de linaza

5. Antes de tomar algún otro suplemento o vitaminas, por favor revise con su doctor de radiación y el coordinador del estudio. Algunos de estos suplementos pueden disminuir los beneficios del tratamiento de radiación que está recibiendo.

6. Se le pedirá completar un **cuestionario de frecuencia de alimentos** al principio del estudio. Le tomará una hora completarlo. Por favor de contestar todas las preguntas lo más preciso posible y **entréguelo al coordinador del estudio, una semana antes de su último tratamiento.** No hay ninguna contestación correcta o incorrecta.

7. Por favor déjele saber a su doctor de radiación y al coordinador del estudio si hay algún cambio en sus defecaciones, problemas de la vejiga o si experimenta otros problemas como náusea, vomito o ardor de estómago.

Se puede comunicar con la coordinadora del estudio Mri Datta al 307-9020

APPENDIX I. NATIONAL CANCER INSTITUTE DIET HISTORY QUESTIONNAIRE

NATIONAL INSTITUTES OF HEALTH

Diet History Questionnaire

GENERAL INSTRUCTIONS

- **Answer each question as best you can. Estimate if you are not sure. A guess is better than leaving a blank.**
- **Use only a black ball-point pen. Do not use a pencil or felt-tip pen. Do not fold, staple, or tear the pages.**
- **Put an X in the box next to your answer.**
- **If you make any changes, cross out the incorrect answer and put an X in the box next to the correct answer. Also draw a circle around the correct answer.**
- **If you mark NEVER, NO, or DON'T KNOW for a question, please follow any arrows or instructions that direct you to the next question.**

BEFORE TURNING THE PAGE, PLEASE COMPLETE THE FOLLOWING QUESTIONS.

Today's date:

MONTH	DAY		YEAR
☐ Jan	|___|___|		☐ 2007
☐ Fe b	☐0	☐0	☐ 2008
☐ Mar	☐1	☐1	☐ 2009
☐ Ap r	☐2	☐2	☐ 2010
☐ May	☐3	☐3	☐ 2011
☐ J un		☐4	
☐ Ju l		☐5	
☐ Au g		☐6	
☐ Sep		☐7	
☐ Oct		☐8	
☐ No v		☐9	
☐ Dec			

In what month were you born?

- ☐ Jan
- ☐ Fe b
- ☐ Mar
- ☐ Ap r
- ☐ May
- ☐ J un
- ☐ Ju l
- ☐ Au g
- ☐ Sep
- ☐ Oct
- ☐ No v
- ☐ Dec

In what year were you born?

19 |___|___|

☐0	☐0
☐1	☐1
☐2	☐2
☐3	☐3
☐4	☐4
☐5	☐5
☐6	☐6
☐7	☐7
☐8	☐8
☐9	☐9

Are you male or female?

- ☐ Male
- ☐ Female

BAR CODE LABEL OR SUBJECT ID HERE

|___|___|___|___|___|___|___|___|

1. Over the past 12 months, how often did you drink **tomato juice** or **vegetable juice**?

☐ NEVER (GO TO QUESTION 2)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

1a. Each time you drank **tomato juice** or **vegetable juice**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1¼ cups (6 to 10 ounces)
☐ More than 1¼ cups (10 ounces)

2. Over the past 12 months, how often did you drink **orange juice** or **grapefruit juice?**

☐ NEVER (GO TO QUESTION 3)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

2a. Each time you drank **orange juice** or **grapefruit juice**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1¼ cups (6 to 10 ounces)
☐ More than 1¼ cups (10 ounces)

3. Over the past 12 months, how often did you drink **other 100% fruit juice** or **100% fruit juice mixtures** (such as apple, grape, pineapple, or others)?

☐ NEVER (GO TO QUESTION 4)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

3a. Each time you drank **other fruit juice** or **fruit juice mixtures**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1½ cups (6 to 12 ounces)
☐ More than 1½ cups (12 ounces)

Question 4 appears in the next column

Over the past 12 months…

4. How often did you drink other **fruit drinks** (such as cranberry cocktail, Hi-C, lemonade, or Kool-Aid, diet or regular)?

☐ NEVER (GO TO QUESTION 5)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

4a. Each time you drank **fruit drinks**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 2 cups (8 to 16 ounces)
☐ More than 2 cups (16 ounces)

4b. How often were your fruit drinks **diet** or **sugar-free drinks**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

5. How often did you drink **milk as a beverage** (NOT in coffee, NOT in cereal)? (Please include chocolate milk and hot chocolate.)

☐ NEVER (GO TO QUESTION 6)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

5a. Each time you drank **milk as a beverage**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 1½ cups (8 to 12 ounces)
☐ More than 1½ cups (12 ounces)

5b. What kind of **milk** did you usually drink?

☐ Whole milk
☐ 2% fat milk
☐ 1 % fat milk
☐ Skim, nonfat, or ½% fat milk
☐ Soy milk
☐ Rice milk
☐ Other

Question 6 appears on the next page

Over the <u>past 12 months</u>…

6. How often did you drink **meal replacement, energy, or high-protein beverages** such as Instant Breakfast, Ensure, Slimfast, Sustacal or others?

☐ NEVER (GO TO QUESTION 7)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

6a. Each time you drank **meal replacement beverages**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 1½ cups (8 to 12 ounces)
☐ More than 1½ cups (12 ounces)

7. Over the past 12 months, did you drink **soft drinks, soda,** or **pop**?

☐ NO (GO TO QUESTION 8)

☐ YES

7a. How often did you drink **soft drinks, soda,** or **pop IN THE SUMMER**?

☐ NEVER

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

7b. How often did you drink **soft drinks, soda,** or **pop DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

7c. Each time you drank **soft drinks, soda,** or **pop,** how much did you usually drink?

☐ Less than 12 ounces or less than 1 can or bottle
☐ 12 to 16 ounces or 1 can or bottle
☐ More than 16 ounces or more than 1 can or bottle

7d. How often were these soft drinks, soda, or pop **diet** or **sugar-free**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

7e. How often were these soft drinks, soda, or pop **caffeine-free**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

8. Over the <u>past 12 months</u>, did you drink **beer**?

☐ NO (GO TO QUESTION 9)

☐ YES

8a. How often did you drink **beer IN THE SUMMER**?

☐ NEVER

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

8b. How often did you drink **beer DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

8c. Each time you drank **beer**, how much did you usually drink?

☐ Less than a 12-ounce can or bottle
☐ 1 to 3 12-ounce cans or bottles
☐ More than 3 12-ounce cans or bottles

Question 8 appears in the next column

Question 9 appears on the next page

Over the <u>past 12 months</u>…

9. How often did you drink **wine** or **wine coolers**?

☐ NEVER (GO TO QUESTION 10)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

9a. Each time you drank **wine** or **wine coolers,** how much did you usually drink?

☐ Less than 5 ounces or less than 1 glass
☐ 5 to 12 ounces or 1 to 2 glasses
☐ More than 12 ounces or more than 2 glasses

10. How often did you drink **liquor** or **mixed drinks**?

☐ NEVER (GO TO QUESTION 11)

☐ 1 time per month or less
☐ 2–3 times per month
☐ 1–2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

10a. Each time you drank **liquor** or **mixed drinks,** how much did you usually drink?

☐ Less than 1 shot of liquor
☐ 1 to 3 shots of liquor
☐ More than 3 shots of liquor

11. Over the <u>past 12 months</u>, did you eat **oatmeal, grits,** or **other cooked cereal?**

☐ NO (GO TO QUESTION 12)

☐ YES

11a. How often did you eat **oatmeal, grits,** or **other cooked cereal IN THE WINTER**?

☐ NEVER

☐ 1–6 times per winter
☐ 7–11 times per winter
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

11b. How often did you eat **oatmeal, grits,** or **other cooked cereal DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

11c. Each time you ate **oatmeal, grits,** or **other cooked cereal,** how much did you usually eat?

☐ Less than ¾ cup
☐ ¾ to 1¼ cups
☐ More than 1¼ cups

12. How often did you eat **cold cereal**?

☐ NEVER (GO TO QUESTION 13)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

12a. Each time you ate **cold cereal**, how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 2½ cups
☐ More than 2½ cups

12b. How often was the cold cereal you ate **Total, Product 19,** or **Right Start**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

12c. How often was the cold cereal you ate **All Bran, Fiber One, 100% Bran,** or **Bran Buds?**

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 12 appears in the next column

Question 13 appears on the next page

Over the <u>past 12 months</u>…

12d. How often was the cold cereal you ate **some other bran** or **fiber cereal** (such as Cheerios, Shredded Wheat, Raisin Bran, Bran Flakes, Grape-Nuts, Granola, Wheaties, or Healthy Choice)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

12e. How often was the cold cereal you ate any **other type of cold cereal** (such as Corn Flakes, Rice Krispies, Frosted Flakes, Special K, Froot Loops, Cap'n Crunch, or others)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

12f. Was **milk** added to your cold cereal?

- ☐ NO (GO TO QUESTION 13)
- ☐ YES

12g. What kind of **milk** was usually added?

- ☐ Whole milk
- ☐ 2% fat milk
- ☐ 1% fat milk
- ☐ Skim, nonfat, or ½% fat milk
- ☐ Soy milk
- ☐ Rice milk
- ☐ Other

12h. Each time **milk was added to your cold cereal**, how much was usually added?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

13. How often did you eat **applesauce**?

- ☐ NEVER (GO TO QUESTION 14)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 14 appears in the next column

13a. Each time you ate **applesauce**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

14. How often did you eat **apples**?

- ☐ NEVER (GO TO QUESTION 15)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

14a. Each time you ate **apples**, how many did you usually eat?

- ☐ Less than 1 apple
- ☐ 1 apple
- ☐ More than 1 apple

15. How often did you eat **pears** (fresh, canned, or frozen)?

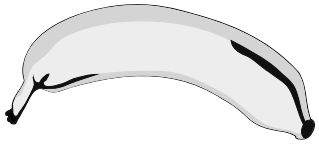
- ☐ NEVER (GO TO QUESTION 16)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

15a. Each time you ate **pears**, how many did you usually eat?

- ☐ Less than 1 pear
- ☐ 1 pear
- ☐ More than 1 pear

16. How often did you eat **bananas?**

- ☐ NEVER (GO TO QUESTION 17)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day



Question 17 appears on the next page

Over the <u>past 12 months</u>…

16a. Each time you ate **bananas**, how many did you usually eat?

- ☐ Less than 1 banana
- ☐ 1 banana
- ☐ More than 1 banana

17. How often did you eat **dried fruit**, such as prunes or raisins (not including dried apricots)?

- ☐ NEVER (GO TO QUESTION 18)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

17a. Each time you ate **dried fruit**, how much did you usually eat (not including dried apricots)?

- ☐ Less than 2 tablespoons
- ☐ 2 to 5 tablespoons
- ☐ More than 5 tablespoons

18. Over the <u>past 12 months</u>, did you eat **peaches, nectarines,** or **plums**?

- ☐ NO (GO TO QUESTION 19)
- ☐ YES

18a. How often did you eat **fresh peaches**, **nectarines**, or **plums WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

18b. How often did you eat **peaches, nectarines,** or **plums** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

18c. Each time you ate **peaches, nectarines,** or **plums,** how much did you usually eat?

- ☐ Less than 1 fruit or less than ½ cup
- ☐ 1 to 2 fruits or ½ to ¾ cup
- ☐ More than 2 fruits or more than ¾ cup

19. How often did you eat **grapes**?

- ☐ NEVER (GO TO QUESTION 20)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

19a. Each time you ate **grapes**, how much did you usually eat?

- ☐ Less than ½ cup or less than 10 grapes
- ☐ ½ to 1 cup or 10 to 30 grapes
- ☐ More than 1 cup or more than 30 grapes

20. Over the <u>past 12 months</u>, did you eat **cantaloupe**?

- ☐ NO (GO TO QUESTION 21)
- ☐ YES

20a. How often did you eat **fresh cantaloupe WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

20b. How often did you eat **fresh** or **frozen cantaloupe DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 19 appears in the next column

Question 21 appears on the next page

Over the <u>past 12 months</u>…

20c. Each time you ate **cantaloupe**, how much did you usually eat?

- ☐ Less than ¼ melon or less than ½ cup
- ☐ ¼ melon or ½ to 1 cup
- ☐ More than ¼ melon or more than 1 cup

21. Over the <u>past 12 months</u>, did you eat **melon, other than cantaloupe** (such as watermelon or honeydew)?

- ☐ NO (GO TO QUESTION 22)
- ☐ YES

21a. How often did you eat **fresh melon, other than cantaloupe** (such as watermelon or honeydew) **WHEN IN SEASON**?

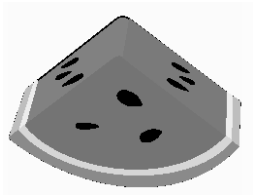
- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

21b. How often did you eat **fresh** or **frozen melon, other than cantaloupe** (such as watermelon or honeydew) **DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

21c. Each time you ate **melon other than cantaloupe**, how much did you usually eat?

- ☐ Less than ½ cup or 1 small wedge
- ☐ ½ to 2 cups or 1 medium wedge
- ☐ More than 2 cups or 1 large wedge



Question 22 appears in the next column

22. Over the <u>past 12 months</u>, did you eat **strawberries**?

- ☐ NO (GO TO QUESTION 23)
- ☐ YES

22a. How often did you eat **fresh strawberries WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

22b. How often did you eat **fresh** or **frozen strawberries DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

22c. Each time you ate **strawberries**, how much did you usually eat?

- ☐ Less than ¼ cup or less than 3 berries
- ☐ ¼ to ¾ cup or 3 to 8 berries
- ☐ More than ¾ cup or more than 8 berries

23. Over the <u>past 12 months</u>, did you eat **oranges, tangerines,** or **tangelos**?

- ☐ NO (GO TO QUESTION 24)
- ☐ YES

23a. How often did you eat **fresh oranges, tangerines,** or **tangelos WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 24 appears on the next page

Over the <u>past 12 months</u>…

23b. How often did you eat **oranges, tangerines,** or **tangelos** (fresh or canned) **DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

23c. Each time you ate **oranges, tangerines,** or **tangelos,** how many did you usually eat?

- ☐ Less than 1 fruit
- ☐ 1 fruit
- ☐ More than 1 fruit

24. Over the <u>past 12 months</u>, did you eat **grapefruit**?

- ☐ NO (GO TO QUESTION 25)
- ☐ YES

24a. How often did you eat **fresh grapefruit WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

24b. How often did you eat **grapefruit** (fresh or canned) **DURING THE REST OF THE YEAR?**

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

24c. Each time you ate **grapefruit**, how much did you usually eat?

- ☐ Less than ½ grapefruit
- ☐ ½ grapefruit
- ☐ More than ½ grapefruit

25. How often did you eat **other kinds of fruit**?

- ☐ NEVER (GO TO QUESTION 26)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

25a. Each time you ate **other kinds of fruit**, how much did you usually eat?

- ☐ Less than ¼ cup
- ☐ ¼ to ¾ cup
- ☐ More than ¾ cup

26. How often did you eat **COOKED greens** (such as spinach, turnip, collard, mustard, chard, or kale)?

- ☐ NEVER (GO TO QUESTION 27)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

26a. Each time you ate **COOKED greens**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

27. How often did you eat **RAW greens** (such as spinach, turnip, collard, mustard, chard, or kale)? (*We will ask about lettuce later.)*

- ☐ NEVER (GO TO QUESTION 28)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

27a. Each time you ate **RAW greens**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

Question 25 appears in the next column

Question 28 appears on the next page

Over the <u>past 12 months</u>…

28. How often did you eat **coleslaw**?

☐ NEVER (GO TO QUESTION 29)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

28a. Each time you ate **coleslaw**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ¾ cup
☐ More than ¾ cup

29. How often did you eat **sauerkraut** or **cabbage** (other than coleslaw)?

☐ NEVER (GO TO QUESTION 30)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

29a. Each time you ate **sauerkraut** or **cabbage**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1 cup
☐ More than 1 cup

30. How often did you eat **carrots** (fresh, canned, or frozen)?

☐ NEVER (GO TO QUESTION 31)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

30a. Each time you ate **carrots**, how much did you usually eat?

☐ Less than ¼ cup or less than 2 baby carrots
☐ ¼ to ½ cup or 2 to 5 baby carrots
☐ More than ½ cup or more than 5 baby carrots

Question 31 appears in the next column

31. How often did you eat **string beans** or **green beans** (fresh, canned, or frozen)?

☐ NEVER (GO TO QUESTION 32)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

31a. Each time you ate **string beans** or **green beans**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

32. How often did you eat **peas** (fresh, canned, or frozen)?

☐ NEVER (GO TO QUESTION 33)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

32a. Each time you ate **peas**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ¾ cup
☐ More than ¾ cup

33. Over the <u>past 12 months</u>, did you eat **corn**?

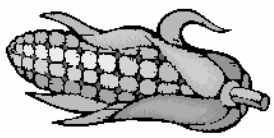
☐ NO (GO TO QUESTION 34)

☐ YES

33a. How often did you eat **fresh corn WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season
☐ 7–11 times per season
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day



Question 34 appears on the next page

Over the <u>past 12 months</u>…

33b. How often did you eat **corn** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

33c. Each time you ate **corn**, how much did you usually eat?

☐ Less than 1 ear or less than ½ cup
☐ 1 ear or ½ to 1 cup
☐ More than 1 ear or more than 1 cup

34. Over the <u>past 12 months</u>, how often did you eat **broccoli** (fresh or frozen)?

☐ NEVER (GO TO QUESTION 35)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

34a. Each time you ate **broccoli**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1 cup
☐ More than 1 cup

35. How often did you eat **cauliflower** or **Brussels sprouts** (fresh or frozen)?

☐ NEVER (GO TO QUESTION 36)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

35a. Each time you ate **cauliflower** or **Brussels sprouts**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ½ cup
☐ More than ½ cup

36. How often did you eat mixed **vegetables**?

☐ NEVER (GO TO QUESTION 37)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

36a. Each time you ate **mixed vegetables**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

37. How often did you eat **onions**?

☐ NEVER (GO TO QUESTION 38)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

37a. Each time you ate **onions**, how much did you usually eat?

☐ Less than 1 slice or less than 1 tablespoon
☐ 1 slice or 1 to 4 tablespoons
☐ More than 1 slice or more than 4 tablespoons

38. Now think about all the **cooked vegetables** you ate in the <u>past 12 months</u> and how they were prepared. How often were your vegetables **COOKED WITH** some sort of **fat**, including oil spray? *(Please do not include potatoes.)*

☐ NEVER (GO TO QUESTION 39)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

Question 36 appears in the next column

Question 39 appears on the next page

Over the <u>past 12 months</u>…

38a. Which fats were usually added to your vegetables **DURING COOKING**? *(Please do not include potatoes. **Mark all that apply.**)*

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Olive oil
- ☐ Corn oil
- ☐ Canola or rapeseed oil
- ☐ Oil spray, such as Pam or others
- ☐ Other kinds of oils
- ☐ None of the above

39. Now, thinking again about all the **cooked vegetables** you ate in the <u>past 12 months</u>, how often was some sort of fat, sauce, or dressing added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes.)*

- ☐ NEVER (GO TO QUESTION 40)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 times per day
- ☐ 3 or more times per day

39a. Which fats, sauces, or dressings were usually added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes. **Mark all that apply.**)*

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Salad dressing
- ☐ Cheese sauce
- ☐ White sauce
- ☐ Other

39b. If margarine, butter, lard, fatback, or bacon fat was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- ☐ Did not usually add these
- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

39c. If salad dressing, cheese sauce, or white sauce was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- ☐ Did not usually add these
- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

Question 40 appears in the next column

40. Over the <u>past 12 months</u>, how often did you eat **sweet peppers** (green, red, or yellow)?

- ☐ NEVER (GO TO QUESTION 41)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

40a. Each time you ate **sweet peppers**, how much did you usually eat?

- ☐ Less than ⅛ pepper
- ☐ ⅛ to ¼ pepper
- ☐ More than ¼ pepper

41. Over the <u>past 12 months</u>, did you eat **fresh tomatoes** (including those in salads)?

- ☐ NO (GO TO QUESTION 42)
- ☐ YES

41a. How often did you eat **fresh tomatoes** (including those in salads) **WHEN IN SEASON**?

- ☐ NEVER
- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

41b. How often did you eat **fresh tomatoes** (including those in salads) **DURING THE REST OF THE YEAR**?

- ☐ NEVER
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

41c. Each time you ate **fresh tomatoes**, how much did you usually eat?

- ☐ Less than ¼ tomato
- ☐ ¼ to ½ tomato
- ☐ More than ½ tomato

Question 42 appears on the next page

Over the <u>past 12 months</u>…

42. How often did you eat **lettuce salads** (with or without other vegetables)?

☐ NEVER (GO TO QUESTION 43)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

42a. Each time you ate **lettuce salads**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1¼ cups
☐ More than 1¼ cups

43. How often did you eat **salad dressing** (including low-fat) on salads?

☐ NEVER (GO TO QUESTION 44)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

43a. Each time you ate **salad dressing** on salads, how much did you usually eat?

☐ Less than 2 tablespoons
☐ 2 to 4 tablespoons
☐ More than 4 tablespoons

44. How often did you eat **sweet potatoes** or **yams**?

☐ NEVER (GO TO QUESTION 45)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

44a. Each time you ate **sweet potatoes** or **yams**, how much did you usually eat?

☐ 1 small potato or less than ¼ cup
☐ 1 medium potato or ¼ to ¾ cup
☐ 1 large potato or more than ¾ cup

45. How often did you eat **French fries, home fries, hash browned potatoes**, or **tater tots**?

☐ NEVER (GO TO QUESTION 46)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

45a. Each time you ate **French fries**, **home fries**, **hash browned potatoes**, or **tater tots** how much did you usually eat?

☐ Less than 10 fries or less than ½ cup
☐ 10 to 25 fries or ½ to 1 cup
☐ More than 25 fries or more than 1 cup

46. How often did you eat **potato salad**?

☐ NEVER (GO TO QUESTION 47)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

46a. Each time you ate **potato salad**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

47. How often did you eat **baked, boiled,** or **mashed potatoes**?

☐ NEVER (GO TO QUESTION 48)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

47a. Each time you ate **baked, boiled,** or **mashed potatoes**, how much did you usually eat?

☐ 1 small potato or less than ½ cup
☐ 1 medium potato or ½ to 1 cup
☐ 1 large potato or more than 1 cup

Question 45 appears in the next column

Question 48 appears on the next page

Over the <u>past 12 months</u>…

47b. How often was **sour cream** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- ☐ Almost never or never (GO TO QUESTION 47d)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

47c. Each time **sour cream** was added to your potatoes, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

47d. How often was **margarine** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

47e. How often was **butter** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

47f. Each time **margarine** or **butter** was added to your potatoes, how much was usually added?

- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

47g. How often was **cheese** or **cheese sauce** added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- ☐ Almost never or never (GO TO QUESTION 48)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

47h. Each time **cheese** or **cheese sauce** was added to your potatoes, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

48. How often did you eat **salsa**?

- ☐ NEVER (GO TO QUESTION 49)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

48a. Each time you ate **salsa**, how much did you usually eat?

- ☐ Less than 1 tablespoon
- ☐ 1 to 5 tablespoons
- ☐ More than 5 tablespoons

49. How often did you eat **catsup**?

- ☐ NEVER (GO TO QUESTION 50)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

49a. Each time you ate **catsup**, how much did you usually eat?

- ☐ Less than 1 teaspoon
- ☐ 1 to 6 teaspoons
- ☐ More than 6 teaspoons

50. How often did you eat **stuffing, dressing,** or **dumplings**?

- ☐ NEVER (GO TO QUESTION 51)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

50a. Each time you ate **stuffing, dressing,** or **dumplings**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

Question 48 appears in the next column

Question 51 appears on the next page

Over the <u>past 12 months</u>…

51. How often did you eat **chili**?

☐ NEVER (GO TO QUESTION 52)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

51a. Each time you ate **chili**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1¾ cups
☐ More than 1¾ cups

52. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas)?

☐ NEVER (GO TO QUESTION 53)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

52a. Each time you ate **Mexican foods**, how much did you usually eat?

☐ Less than 1 taco, burrito, etc.
☐ 1 to 2 tacos, burritos, etc.
☐ More than 2 tacos, burritos, etc.

53. How often did you eat **cooked dried beans** (such as baked beans, pintos, kidney, blackeyed peas, lima, lentils, soybeans, or refried beans)? *(Please don't include bean soups or chili.)*

☐ NEVER (GO TO QUESTION 54)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

53a. Each time you ate **beans**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

53b. How often were the beans you ate **refried beans, beans prepared with any type of fat**, or **with meat added**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

54. How often did you eat **other kinds of vegetables**?

☐ NEVER (GO TO QUESTION 55)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

54a. Each time you ate **other kinds of vegetables**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ½ cup
☐ More than ½ cup

55. How often did you eat **rice** or **other cooked grains** (such as bulgur, cracked wheat, or millet)?

☐ NEVER (GO TO QUESTION 56)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

55a. Each time you ate **rice** or **other cooked grains**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1½ cups
☐ More than 1½ cups

55b. How often was **butter, margarine**, or **oil** added to your rice **IN COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 54 appears in the next column

Question 56 appears on the next page

Over the <u>past 12 months</u>…

56. How often did you eat **pancakes, waffles,** or **French toast**?

☐ NEVER (GO TO QUESTION 57)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

56a. Each time you ate **pancakes, waffles,** or **French toast**, how much did you usually eat?

☐ Less than 1 medium piece
☐ 1 to 3 medium pieces
☐ More than 3 medium pieces

56b. How often was **margarine** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

56c. How often was **butter** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

56d. Each time **margarine** or **butter** was added to your pancakes, waffles, or French toast, how much was usually added?

☐ Never added
☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

56e. How often was **syrup** added to your pancakes, waffles, or French toast?

☐ Almost never or never (GO TO QUESTION 57)
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 57 appears in the next column

56f. Each time **syrup** was added to your pancakes, waffles, or French toast, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 4 tablespoons
☐ More than 4 tablespoons

57. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini**? *(Please do not include spaghetti or other pasta.)*

☐ NEVER (GO TO QUESTION 58)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

57a. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini**, how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 2 cups
☐ More than 2 cups

58. How often did you eat **macaroni and cheese**?

☐ NEVER (GO TO QUESTION 59)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

58a. Each time you ate **macaroni and cheese**, how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 1½ cups
☐ More than 1½ cups

59. How often did you eat **pasta salad** or **macaroni salad**?

☐ NEVER (GO TO QUESTION 60)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

Question 60 appears on the next page

Over the <u>past 12 months</u>…

59a. Each time you ate **pasta salad** or **macaroni salad**, how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

60. Other than the pastas listed in Questions 57, 58, and 59, how often did you eat **pasta, spaghetti**, or **other noodles**?

- ☐ NEVER (GO TO QUESTION 61)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

60a. Each time you ate **pasta, spaghetti**, **or other noodles**, how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 3 cups
- ☐ More than 3 cups

60b. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITH meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

60c. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITHOUT meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

60d. How often did you eat your pasta, spaghetti, or other noodles with **margarine, butter, oil,** or **cream sauce**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 61 appears in the next column

61. How often did you eat **bagels** or **English muffins**?

- ☐ NEVER (GO TO INTRODUCTION TO QUESTION 62)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

61a. Each time you ate **bagels** or **English muffins**, how many did you usually eat?

- ☐ Less than 1 bagel or English muffin
- ☐ 1 bagel or English muffin
- ☐ More than 1 bagel or English muffin

61b. How often was **margarine** (including low-fat) added to your bagels or English muffins?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

61c. How often was **butter** (including low-fat) added to your bagels or English muffins?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

61d. Each time **margarine** or **butter** was added to your bagels or English muffins, how much was usually added?

- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 2 teaspoons
- ☐ More than 2 teaspoons

61e. How often was **cream cheese** (including low-fat) spread on your bagels or English muffins?

- ☐ Almost never or never (GO TO INTRODUCTION TO QUESTION 62)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Introduction to Question 62 appears on the next page

Over the <u>past 12 months</u>…

61f. Each time **cream cheese** was added to your bagels or English muffins, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 2 tablespoons
- ☐ More than 2 tablespoons

The next questions ask about your intake of breads other than bagels or English muffins. First, we will ask about bread you ate as part of sandwiches only. Then we will ask about all other bread you ate.

62. How often did you eat **breads** or **rolls AS PART OF SANDWICHES** (including burger and hot dog rolls)?

- ☐ NEVER (GO TO QUESTION 63)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

62a. Each time you ate **breads** or **rolls AS PART OF SANDWICHES**, how many did you usually eat?

- ☐ 1 slice or ½ roll
- ☐ 2 slices or 1 roll
- ☐ More than 2 slices or more than 1 roll

62b. How often were the breads or rolls that you used for your sandwiches **white bread** (including burger and hot dog rolls)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

62c. How often was **mayonnaise** or **mayonnaise-type dressing** (including low-fat) added to your sandwich bread or rolls?

- ☐ Almost never or never (GO TO QUESTION 62e)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 62e appears in the next column

Question 63 appears in the next column

62d. Each time **mayonnaise** or **mayonnaise-type dressing** was added to your sandwich breads or rolls, how much was usually added?

- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

62e. How often was **margarine** (including low-fat) added to your sandwich bread or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

62f. How often was **butter** (including low-fat) added to your sandwich bread or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

62g. Each time **margarine** or **butter** was added to your sandwich breads or rolls, how much was usually added?

- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 2 teaspoons
- ☐ More than 2 teaspoons

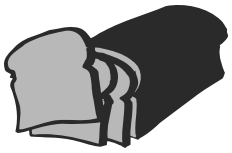
63. How often did you eat **breads** or **dinner rolls, NOT AS PART OF SANDWICHES**?

- ☐ NEVER (GO TO QUESTION 64)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

63a. Each time you ate **breads** or **dinner rolls, NOT AS PART OF SANDWICHES**, how much did you usually eat?

- ☐ 1 slice or 1 dinner roll
- ☐ 2 slices or 2 dinner rolls
- ☐ More than 2 slices or 2 dinner rolls



Question 64 appears on the next page

Over the <u>past 12 months</u>…

63b. How often were the breads or rolls you ate **white bread**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63c. How often was **margarine** (including low-fat) added to your breads or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63d. How often was **butter** (including low-fat) added to your breads or rolls?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63e. Each time **margarine** or **butter** was added to your breads or rolls, how much was usually added?

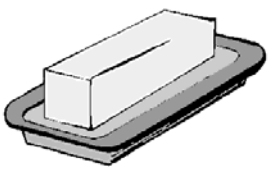
- ☐ Never added
- ☐ Less than 1 teaspoon
- ☐ 1 to 2 teaspoons
- ☐ More than 2 teaspoons

63f. How often was **cream cheese** (including low-fat) added to your breads or rolls?

- ☐ Almost never or never (GO TO QUESTION 64)
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

63g. Each time **cream cheese** was added to your breads or rolls, how much was usually added?

- ☐ Less than 1 tablespoon
- ☐ 1 to 2 tablespoons
- ☐ More than 2 tablespoons



Question 64 appears in the next column

64. How often did you eat **jam, jelly,** or **honey** on bagels, muffins, bread, rolls, or crackers?

- ☐ NEVER (GO TO QUESTION 65)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

64a. Each time you ate **jam, jelly,** or **honey**, how much did you usually eat?

- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

65. How often did you eat **peanut butter** or **other nut butter**?

- ☐ NEVER (GO TO QUESTION 66)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

65a. Each time you ate **peanut butter** or **other nut butter**, how much did you usually eat?

- ☐ Less than 1 tablespoon
- ☐ 1 to 2 tablespoons
- ☐ More than 2 tablespoons

66. How often did you eat **roast beef** or **steak IN SANDWICHES**?

- ☐ NEVER (GO TO QUESTION 67)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

66a. Each time you ate **roast beef** or **steak IN SANDWICHES**, how much did you usually eat?

- ☐ Less than 1 slice or less than 2 ounces
- ☐ 1 to 2 slices or 2 to 4 ounces
- ☐ More than 2 slices or more than 4 ounces

Question 67 appears on the next page

Over the <u>past 12 months</u>…

67. How often did you eat **turkey** or **chicken COLD CUTS** (such as loaf, luncheon meat, turkey ham, turkey salami, or turkey pastrami)? (*We will ask about other turkey or chicken later.)*

☐ NEVER (GO TO QUESTION 68)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

67a. Each time you ate **turkey or chicken COLD CUTS**, how much did you usually eat?

☐ Less than 1 slice
☐ 1 to 3 slices
☐ More than 3 slices

68. How often did you eat **luncheon** or **deli-style ham**? *(We will ask about other ham later.)*

☐ NEVER (GO TO QUESTION 69)

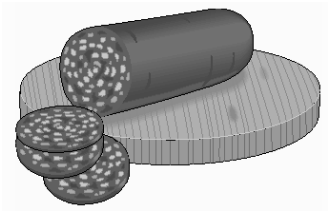
☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

68a. Each time you ate **luncheon** or **deli-style ham**, how much did you usually eat?

☐ Less than 1 slice
☐ 1 to 3 slices
☐ More than 3 slices

68b. How often was the luncheon or deli-style ham you ate **light**, **low-fat**, or **fat-free**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always



Question 69 appears in the next column

69. How often did you eat **other cold cuts** or **luncheon meats** (such as bologna, salami, corned beef, pastrami, or others, including low-fat)? *(Please do not include ham, turkey, or chicken cold cuts.)*

☐ NEVER (GO TO QUESTION 70)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

69a. Each time you ate **other cold cuts** or **luncheon meats**, how much did you usually eat?

☐ Less than 1 slice
☐ 1 to 3 slices
☐ More than 3 slices

69b. How often were the other cold cuts or luncheon meats you ate **light, low-fat**, or **fat-free cold cuts** or **luncheon meats**? *(Please do not include ham, turkey, or chicken cold cuts.)*

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

70. How often did you eat **canned tuna** (including in salads, sandwiches, or casseroles)?

☐ NEVER (GO TO QUESTION 71)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

70a. Each time you ate **canned tuna**, how much did you usually eat?

☐ Less than ¼ cup or less than 2 ounces
☐ ¼ to ½ cup or 2 to 3 ounces
☐ More than ½ cup or more than 3 ounces

70b. How often was the canned tuna you ate **water-packed tuna**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 71 appears on the next page

Over the <u>past 12 months</u>…

70c. How often was the canned tuna you ate **prepared with mayonnaise** or **other dressing** (including low-fat)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

71. How often did you eat **GROUND chicken** or **turkey**? *(We will ask about other chicken and turkey later.)*

- ☐ NEVER (GO TO QUESTION 72)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

71a. Each time you ate **GROUND chicken** or **turkey**, how much did you usually eat?

- ☐ Less than 2 ounces or less than ½ cup
- ☐ 2 to 4 ounces or ½ to 1 cup
- ☐ More than 4 ounces or more than 1 cup

72. How often did you eat **beef hamburgers** or **cheeseburgers**?

- ☐ NEVER (GO TO QUESTION 73)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

72a. Each time you ate **beef hamburgers** or **cheeseburgers**, how much did you usually eat?

- ☐ Less than 1 patty or less than 2 ounces
- ☐ 1 patty or 2 to 4 ounces
- ☐ More than 1 patty or more than 4 ounces

72b. How often were the beef hamburgers or cheeseburgers you ate made with **lean ground beef**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 73 appears in the next column

73. How often did you eat **ground beef in mixtures** (such as meatballs, casseroles, chili, or meatloaf)?

- ☐ NEVER (GO TO QUESTION 74)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

73a. Each time you ate **ground beef in mixtures**, how much did you usually eat?

- ☐ Less than 3 ounces or less than ½ cup
- ☐ 3 to 8 ounces or ½ to 1 cup
- ☐ More than 8 ounces or more than 1 cup

74. How often did you eat **hot dogs** or **frankfurters**? *(Please do not include sausages or vegetarian hot dogs.)*

- ☐ NEVER (GO TO QUESTION 75)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

74a. Each time you ate **hot dogs** or **frankfurters**, how many did you usually eat?

- ☐ Less than 1 hot dog
- ☐ 1 to 2 hot dogs
- ☐ More than 2 hot dogs

74b. How often were the hot dogs or frankfurters you ate **light** or **low-fat hot dogs**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 75 appears on the next page

Over the <u>past 12 months</u>…

75. How often did you eat beef mixtures such as **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables**?

☐ NEVER (GO TO QUESTION 76)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

75a. Each time you ate **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables,** how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 2 cups
☐ More than 2 cups

76. How often did you eat **roast beef** or **pot roast**? *(Please do not include roast beef or pot roast in sandwiches.)*

☐ NEVER (GO TO QUESTION 77)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

76a. Each time you ate **roast beef** or **pot roast** (including in mixtures), how much did you usually eat?

☐ Less than 2 ounces
☐ 2 to 5 ounces
☐ More than 5 ounces

77. How often did you eat **steak** (beef)? (*Do not include steak in sandwiches*)

☐ NEVER (GO TO QUESTION 78)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

77a. Each time you ate **steak** (beef), how much did you usually eat?

☐ Less than 3 ounces
☐ 3 to 7 ounces
☐ More than 7 ounces

77b. How often was the steak you ate **lean steak**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

78. How often did you eat **pork** or **beef spareribs**?

☐ NEVER (GO TO QUESTION 79)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

78a. Each time you ate **pork** or **beef spareribs**, how much did you usually eat?

☐ Less than 4 ribs
☐ 4 to 12 ribs
☐ More than 12 ribs

79. How often did you eat **roast turkey, turkey cutlets,** or **turkey nuggets** (including in sandwiches)?

☐ NEVER (GO TO QUESTION 80)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

79a. Each time you ate **roast turkey, turkey cutlets,** or **turkey nuggets**, how much did you usually eat? *(Please note: 4 to 8 turkey nuggets = 3 ounces.)*

☐ Less than 2 ounces
☐ 2 to 4 ounces
☐ More than 4 ounces

80. How often did you eat **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures**?

☐ NEVER (GO TO QUESTION 81)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

Question 78 appears in the next column

Question 81 appears on the next page

Over the <u>past 12 months</u>…

80a. Each time you ate **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures,** how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1½ cups
- ☐ More than 1½ cups

81. How often did you eat **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets)? *(Please do not include chicken in mixtures.)*

- ☐ NEVER (GO TO QUESTION 82)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

81a. Each time you ate **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets), how much did you usually eat?

- ☐ Less than 2 drumsticks or wings, less than 1 breast or thigh, or less than 4 nuggets
- ☐ 2 drumsticks or wings, 1 breast or thigh, or 4 to 8 nuggets
- ☐ More than 2 drumsticks or wings, more than 1 breast or thigh, or more than 8 nuggets

81b. How often was the chicken you ate **fried chicken** (including deep fried) or **chicken nuggets**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

81c. How often was the chicken you ate **WHITE meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

81d. How often did you eat chicken **WITH skin**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 82 appears in the next column

82. How often did you eat **baked ham** or **ham steak**?

- ☐ NEVER (GO TO QUESTION 83)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

82a. Each time you ate **baked ham** or **ham steak**, how much did you usually eat?

- ☐ Less than 1 ounce
- ☐ 1 to 3 ounces
- ☐ More than 3 ounces

83. How often did you eat **pork** (including chops, roasts, and in mixed dishes)? *(Please do not include ham, ham steak, or sausage.)*

- ☐ NEVER (GO TO QUESTION 84)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

83a. Each time you ate **pork**, how much did you usually eat?

- ☐ Less than 2 ounces or less than 1 chop
- ☐ 2 to 5 ounces or 1 chop
- ☐ More than 5 ounces or more than 1 chop

84. How often did you eat **gravy** on meat, chicken, potatoes, rice, etc.?

- ☐ NEVER (GO TO QUESTION 85)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

84a. Each time you ate **gravy** on meat, chicken, potatoes, rice, etc., how much did you usually eat?

- ☐ Less than ⅛ cup
- ☐ ⅛ to ½ cup
- ☐ More than ½ cup

Question 85 appears on the next page

Over the <u>past 12 months</u>…

85. How often did you eat **liver** (all kinds) or **liverwurst**?

☐ NEVER (GO TO QUESTION 86)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

85a. Each time you ate **liver** or **liverwurst**, how much did you usually eat?

☐ Less than 1 ounce
☐ 1 to 4 ounces
☐ More than 4 ounces

86. How often did you eat **bacon** (including low-fat)?

☐ NEVER (GO TO QUESTION 87)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

86a. Each time you ate **bacon**, how much did you usually eat?

☐ Fewer than 2 slices
☐ 2 to 3 slices
☐ More than 3 slices

86b. How often was the bacon you ate **light, low-fat,** or **lean bacon**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

87. How often did you eat **sausage** (including low-fat)?

☐ NEVER (GO TO QUESTION 88)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

87a. Each time you ate **sausage**, how much did you usually eat?

☐ Less than 1 patty or 2 links
☐ 1 to 3 patties or 2 to 5 links
☐ More than 3 patties or 5 links

87b. How often was the sausage you ate **light, low-fat**, or **lean sausage**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

88. How often did you eat **fish sticks** or **fried fish** (including fried seafood or shellfish)?

☐ NEVER (GO TO QUESTION 89)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

88a. Each time you ate **fish sticks** or **fried fish**, how much did you usually eat?

☐ Less than 2 ounces or less than 1 fillet
☐ 2 to 7 ounces or 1 fillet
☐ More than 7 ounces or more than 1 fillet

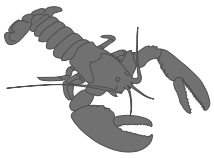
89. How often did you eat **fish** or **seafood that was NOT FRIED** (including shellfish)?

☐ NEVER (GO TO INTRODUCTION TO QUESTION 90)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

89a. Each time you ate eat **fish** or **seafood that was NOT FRIED**, how much did you usually eat?

☐ Less than 2 ounces or less than 1 fillet
☐ 2 to 5 ounces or 1 fillet
☐ More than 5 ounces or more than 1 fillet



Question 88 appears in the next column

Introduction to Question 90 appears on the next page

Over the <u>past 12 months</u>…

Now think about all the meat, poultry, and fish you ate in the <u>past 12 months</u> and how they were prepared.

90. How often was **oil, butter, margarine**, **or other fat** used to **FRY, SAUTE, BASTE, OR MARINATE** any meat, poultry, or fish you ate? *(Please do not include deep frying.)*

- ☐ NEVER (GO TO QUESTION 91)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

90a. Which of the following **fats** were regularly used to prepare your meat, poultry, or fish? ***(Mark all that apply.)***

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Olive oil
- ☐ Corn oil
- ☐ Canola or rapeseed oil
- ☐ Oil spray, such as Pam or others
- ☐ Other kinds of oils
- ☐ None of the above

91. How often did you eat **tofu, soy burgers,** or **soy meat-substitutes**?

- ☐ NEVER (GO TO QUESTION 92)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

91a. Each time you ate **tofu, soy burgers,** or **soy meat-substitutes**, how much did you usually eat?

- ☐ Less than ¼ cup or less than 2 ounces
- ☐ ¼ to ½ cup or 2 to 4 ounces
- ☐ More than ½ cup or more than 4 ounces

Question 92 appears in the next column

92. Over the <u>past 12 months</u>, did you eat **soups**?

- ☐ NO (GO TO QUESTION 93)
- ☐ YES

92a. How often did you eat **soup DURING THE WINTER**?

- ☐ NEVER

- ☐ 1–6 times per winter
- ☐ 7–11 times per winter
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

92b. How often did you eat **soup DURING THE REST OF THE YEAR**?

- ☐ NEVER

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

92c. Each time you ate **soup**, how much did you usually eat?

- ☐ Less than 1 cup
- ☐ 1 to 2 cups
- ☐ More than 2 cups

92d. How often were the soups you ate **bean soups**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

92e. How often were the soups you ate **cream soups** (including chowders)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 93 appears on the next page

Over the <u>past 12 months</u>…

92f. How often were the soups you ate **tomato** or **vegetable soups**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

92g. How often were the soups you ate **broth soups** (including chicken) **with** or **without noodles** or **rice**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

93. How often did you eat **pizza**?

- ☐ NEVER (GO TO QUESTION 94)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

93a. Each time you ate **pizza**, how much did you usually eat?

- ☐ Less than 1 slice or less than 1 mini pizza
- ☐ 1 to 3 slices or 1 mini pizza
- ☐ More than 3 slices or more than 1 mini pizza

93b. How often did you eat pizza with **pepperoni, sausage**, or **other meat**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

94. How often did you eat **crackers**?

- ☐ NEVER (GO TO QUESTION 95)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 95 appears in the next column

94a. Each time you ate **crackers**, how many did you usually eat?

- ☐ Fewer than 4 crackers
- ☐ 4 to 10 crackers
- ☐ More than 10 crackers

95. How often did you eat **corn bread** or **corn muffins**?

- ☐ NEVER (GO TO QUESTION 96)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

95a. Each time you ate **corn bread** or **corn muffins,** how much did you usually eat?

- ☐ Less than 1 piece or muffin
- ☐ 1 to 2 pieces or muffins
- ☐ More than 2 pieces or muffins

96. How often did you eat **biscuits**?

- ☐ NEVER (GO TO QUESTION 97)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

96a. Each time you ate **biscuits**, how many did you usually eat?

- ☐ Fewer than 1 biscuit
- ☐ 1 to 2 biscuits
- ☐ More than 2 biscuits

97. How often did you eat **potato chips, tortilla chips,** or **corn chips** (including low-fat, fat-free, or low-salt)?

- ☐ NEVER (GO TO QUESTION 98)
- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 98 appears on the next page

Over the <u>past 12 months</u>…

97a. Each time you ate **potato chips, tortilla chips,** or **corn chips**, how much did you usually eat?

- ☐ Fewer than 10 chips or less than 1 cup
- ☐ 10 to 25 chips or 1 to 2 cups
- ☐ More than 25 chips or more than 2 cups

97b. How often were the chips you ate **Wow chips** or other **chips made with fat substitute** (Olean or Olestra)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

97c. How often were the chips you ate other **low-fat** or **fat-free chips**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

98. How often did you eat **popcorn** (including low-fat)?

☐ NEVER (GO TO QUESTION 99)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

98a. Each time you ate **popcorn**, how much did you usually eat?

- ☐ Less than 2 cups, popped
- ☐ 2 to 5 cups, popped
- ☐ More than 5 cups, popped

99. How often did you eat **pretzels**?

☐ NEVER (GO TO QUESTION 100)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 100 appears in the next column

99a. Each time you ate **pretzels**, how many did you usually eat?

- ☐ Fewer than 5 average twists
- ☐ 5 to 20 average twists
- ☐ More than 20 average twists

100. How often did you eat **peanuts, walnuts, seeds,** or **other nuts**?

☐ NEVER (GO TO QUESTION 101)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

100a. Each time you ate **peanuts, walnuts, seeds,** or **other nuts**, how much did you usually eat?

- ☐ Less than ¼ cup
- ☐ ¼ to ½ cup
- ☐ More than ½ cup

101. How often did you eat **energy, high-protein**, or **breakfast bars** such as **Power Bars, Balance, Clif,** or **others**?

☐ NEVER (GO TO QUESTION 102)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

101a. Each time you ate **energy, high-protein**, or **breakfast bars**, how much did you usually eat?

- ☐ Less than 1 bar
- ☐ 1 bar
- ☐ More than 1 bar

102. How often did you eat **yogurt** (NOT including frozen yogurt)?

☐ NEVER (GO TO QUESTION 103)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 103 appears on the next page

Over the <u>past 12 months</u>…

102a. Each time you ate **yogur**t, how much did you usually eat?

- ☐ Less than ½ cup or less than 1 container
- ☐ ½ to 1 cup or 1 container
- ☐ More than 1 cup or more than 1 container

103. How often did you eat **cottage cheese** (including low-fat)?

- ☐ NEVER (GO TO QUESTION 104)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

103a. Each time you ate **cottage cheese**, how much did you usually eat?

- ☐ Less than ¼ cup
- ☐ ¼ to 1 cup
- ☐ More than 1 cup

104. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches or subs)?

- ☐ NEVER (GO TO QUESTION 105)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

104a. Each time you ate **cheese**, how much did you usually eat?

- ☐ Less than ½ ounce or less than 1 slice
- ☐ ½ to 1½ ounces or 1 slice
- ☐ More than 1½ ounces or more than 1 slice

104b. How often was the cheese you ate **light** or **low-fat cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

104c. How often was the cheese you ate **fat-free cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

105. How often did you eat **frozen yogurt, sorbet,** or **ices** (including low-fat or fat-free)?

- ☐ NEVER (GO TO QUESTION 106)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

105a. Each time you ate **frozen yogurt, sorbet,** or **ices**, how much did you usually eat?

- ☐ Less than ½ cup or less than 1 scoop
- ☐ ½ to 1 cup or 1 to 2 scoops
- ☐ More than 1 cup or more than 2 scoops

106. How often did you eat **ice cream, ice cream bars,** or **sherbet** (including low-fat or fat-free)?

- ☐ NEVER (GO TO QUESTION 107)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

106a. Each time you ate **ice cream, ice cream bars,** or **sherbet**, how much did you usually eat?

- ☐ Less than ½ cup or less than 1 scoop
- ☐ ½ to 1½ cups or 1 to 2 scoops
- ☐ More than 1½ cups or more than 2 scoops

106b. How often was the ice cream you ate **light, low-fat,** or **fat-free ice cream** or **sherbet**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 105 appears in the next column

Question 107 appears on the next page

Over the <u>past 12 months</u>…

107. How often did you eat **cake** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 108)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

107a. Each time you ate **cake**, how much did you usually eat?

☐ Less than 1 medium piece
☐ 1 medium piece
☐ More than 1 medium piece

107b. How often was the cake you ate **light, low-fat,** or **fat-free cake**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

108. How often did you eat **cookies** or **brownies** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 109)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

108a. Each time you ate **cookies** or **brownies**, how much did you usually eat?

☐ Less than 2 cookies or 1 small brownie
☐ 2 to 4 cookies or 1 medium brownie
☐ More than 4 cookies or 1 large brownie

108b. How often were the cookies or brownies you ate **light, low-fat,** or **fat-free cookies** or **brownies**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 109 appears in the next column

109. How often did you eat **doughnuts, sweet rolls, Danish,** or **pop-tarts**?

☐ NEVER (GO TO QUESTION 110)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

109a. Each time you ate **doughnuts, sweet rolls, Danish,** or **pop-tarts**, how much did you usually eat?

☐ Less than 1 piece
☐ 1 to 2 pieces
☐ More than 2 pieces

110. How often did you eat **sweet muffins** or **dessert breads** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 111)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

110a. Each time you ate **sweet muffins or dessert breads**, how much did you usually eat?

☐ Less than 1 medium piece
☐ 1 medium piece
☐ More than 1 medium piece

110b. How often were the sweet muffins or dessert breads you ate **light, low-fat,** or **fat-free sweet muffins** or **dessert breads**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

111. How often did you eat **fruit crisp, cobbler,** or **strudel**?

☐ NEVER (GO TO QUESTION 112)

☐ 1–6 times per year	☐ 2 times per week
☐ 7–11 times per year	☐ 3–4 times per week
☐ 1 time per month	☐ 5–6 times per week
☐ 2–3 times per month	☐ 1 time per day
☐ 1 time per week	☐ 2 or more times per day

Question 112 appears on the next page

Over the <u>past 12 months</u>…

111a. Each time you ate **fruit crisp, cobbler,** or **strudel,** how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

112. How often did you eat **pie**?

- ☐ NEVER (GO TO QUESTION 113)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

112a. Each time you ate **pie**, how much did you usually eat?

- ☐ Less than ⅛ of a pie
- ☐ About ⅛ of a pie
- ☐ More than ⅛ of a pie

The next four questions ask about the kinds of pie you ate. Please read all four questions before answering.

112b. How often were the pies you ate **fruit pie** (such as apple, blueberry, others)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

112c. How often were the pies you ate **cream, pudding, custard,** or **meringue pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

112d. How often were the pies you ate **pumpkin** or **sweet potato pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 113 appears in the next column

112e. How often were the pies you ate **pecan pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

113. How often did you eat **chocolate candy**?

- ☐ NEVER (GO TO QUESTION 114)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

113a. Each time you ate **chocolate candy**, how much did you usually eat?

- ☐ Less than 1 average bar or less than 1 ounce
- ☐ 1 average bar or 1 to 2 ounces
- ☐ More than 1 average bar or more than 2 ounces

114. How often did you eat **other candy**?

- ☐ NEVER (GO TO QUESTION 115)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

114a. Each time you ate **other candy**, how much did you usually eat?

- ☐ Fewer than 2 pieces
- ☐ 2 to 9 pieces
- ☐ More than 9 pieces

115. How often did you eat **eggs, egg whites,** or **egg substitutes** (NOT counting eggs in baked goods and desserts)? *(Please include eggs in salads, quiche, and soufflés.)*

- ☐ NEVER (GO TO QUESTION 116)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

Question 116 appears on the next page

Over the <u>past 12 months</u>…

115a. Each time you ate **eggs**, how many did you usually eat?

- ☐ 1 egg
- ☐ 2 eggs
- ☐ 3 or more eggs

115b. How often were the eggs you ate **egg substitutes**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115c. How often were the eggs you ate **egg whites only**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115d. How often were the eggs you ate **regular whole eggs**?

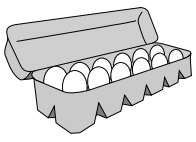
- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115e. How often were the eggs you ate **cooked in oil, butter,** or **margarine**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

115f. How often were the eggs you ate part of **egg salad**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always



Question 116 appears in the next column

116. How many cups of **coffee**, caffeinated or decaffeinated, did you drink?

- ☐ NEVER (GO TO QUESTION 117)
- ☐ Less than 1 cup per month
- ☐ 1–3 cups per month
- ☐ 1 cup per week
- ☐ 2–4 cups per week
- ☐ 5–6 cups per week
- ☐ 1 cup per day
- ☐ 2–3 cups per day
- ☐ 4–5 cups per day
- ☐ 6 or more cups per day

116a. How often was the coffee you drank **decaffeinated**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

117. How many glasses of **ICED tea,** caffeinated or decaffeinated, did you drink?

- ☐ NEVER (GO TO QUESTION 118)
- ☐ Less than 1 cup per month
- ☐ 1–3 cups per month
- ☐ 1 cup per week
- ☐ 2–4 cups per week
- ☐ 5–6 cups per week
- ☐ 1 cup per day
- ☐ 2–3 cups per day
- ☐ 4–5 cups per day
- ☐ 6 or more cups per day

117a. How often was the iced tea you drank **decaffeinated** or **herbal tea**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

118. How many cups of **HOT tea**, caffeinated or decaffeinated, did you drink?

- ☐ NEVER (GO TO QUESTION 119)
- ☐ Less than 1 cup per month
- ☐ 1–3 cups per month
- ☐ 1 cup per week
- ☐ 2–4 cups per week
- ☐ 5–6 cups per week
- ☐ 1 cup per day
- ☐ 2–3 cups per day
- ☐ 4–5 cups per day
- ☐ 6 or more cups per day

118a. How often was the hot tea you drank **decaffeinated** or **herbal tea**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

Question 119 appears on the next page

Over the <u>past 12 months</u>…

119. How often did you add **sugar** or **honey** to your coffee or tea?

☐ NEVER (GO TO QUESTION 120)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

119a. Each time **sugar** or **honey** was added to your coffee or tea, how much was usually added?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

120. How often did you add **artificial sweetener** to your coffee or tea?

☐ NEVER (GO TO QUESTION 121)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

120a. What kind of **artificial sweetener** did you usually use?

☐ Equal or aspartame
☐ Sweet N Low or saccharin

121. How often was **non-dairy creamer** added to your coffee or tea?

☐ NEVER (GO TO QUESTION 122)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

121a. Each time **non-dairy creamer** was added to your coffee or tea, how much was usually used?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

Question 122 appears in the next column

121b. What kind of **non-dairy creamer** did you usually use?

☐ Regular powdered
☐ Low-fat or fat-free powdered
☐ Regular liquid
☐ Low-fat or fat-free liquid

122. How often was **cream** or **half and half** added to your coffee or tea?

☐ NEVER (GO TO QUESTION 123)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

122a. Each time **cream** or **half and half** was added to your coffee or tea, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 2 tablespoons
☐ More than 2 tablespoons

123. How often was **milk** added to your coffee or tea?

☐ NEVER (GO TO QUESTION 124)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

123a. Each time **milk** was added to your coffee or tea, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 3 tablespoons
☐ More than 3 tablespoons

123b. What kind of **milk** was usually added to your coffee or tea?

☐ Whole milk
☐ 2% milk
☐ 1% milk
☐ Skim, nonfat, or ½% milk
☐ Evaporated or condensed (canned) milk
☐ Soy milk
☐ Rice milk
☐ Other

Question 124 appears on the next page

Over the <u>past 12 months</u>…

124. How often was **sugar** or **honey** added to foods you ate? (Please do not include sugar in coffee, tea, other beverages, or baked goods.)

☐ NEVER (GO TO INTRODUCTION TO QUESTION 125)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

124a. Each time **sugar** or **honey** was added to foods you ate, how much was usually added?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

The following questions are about the kinds of margarine, mayonnaise, sour cream, cream cheese, and salad dressing that you eat. If possible, please check the labels of these foods to help you answer.

125. Over the <u>past 12 months</u>, did you eat **margarine**?

☐ NO (GO TO QUESTION 126)

☐ YES

125a. How often was the margarine you ate **regular-fat margarine** (stick or tub)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

125b. How often was the margarine you ate **light** or **low-fat margarine** (stick or tub)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 126 appears in the next column

125c. How often was the margarine you ate **fat-free margarine**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

126. Over the past 12 months, did you eat **butter**?

☐ NO (GO TO QUESTION 127)

☐ YES

126a. How often was the butter you ate **light** or **low-fat butter**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

127. Over the <u>past 12 months</u>, did you eat **mayonnaise** or **mayonnaise-type dressing**?

☐ NO (GO TO QUESTION 128)

☐ YES

127a. How often was the mayonnaise you ate **regular-fat mayonnaise**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

127b. How often was the mayonnaise you ate **light** or **low-fat mayonnaise**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 128 appears on the next page

Over the <u>past 12 months</u>…

127c. How often was the mayonnaise you ate **fat-free mayonnaise**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

128. Over the <u>past 12 months</u>, did you eat **sour cream**?

- ☐ NO (GO TO QUESTION 129)
- ☐ YES

128a. How often was the sour cream you ate **regular-fat sour cream**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

128b. How often was the sour cream you ate **light, low-fat,** or **fat-free sour cream**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

129. Over the <u>past 12 months</u>, did you eat **cream cheese**?

- ☐ NO (GO TO QUESTION 130)
- ☐ YES

129a. How often was the cream cheese you ate **regular-fat cream cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

129b. How often was the cream cheese you ate **light, low-fat,** or **fat-free cream cheese**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

130. Over the <u>past 12 months</u>, did you eat **salad dressing**?

- ☐ NO (GO TO INTRODUCTION TO QUESTION 131)
- ☐ YES

130a. How often was the salad dressing you ate **regular-fat salad dressing** (including oil and vinegar dressing)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

130b. How often was the salad dressing you ate **light** or **low-fat salad dressing**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

130c. How often was the salad dressing you ate **fat-free salad dressing**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

The following two questions ask you to summarize your usual intake of vegetables and fruits. Please do not include salads, potatoes, or juices.

131. Over the <u>past 12 months</u>, how many servings of **vegetables** (not including salad or potatoes) did you eat per week or per day?

- ☐ Less than 1 per week
- ☐ 1–2 per week
- ☐ 3–4 per week
- ☐ 5–6 per week
- ☐ 1 per day
- ☐ 2 per day
- ☐ 3 per day
- ☐ 4 per day
- ☐ 5 or more per day

Question 130 appears in the next column

Over the past 12 months…

132. Over the past 12 months, how many servings of **fruit** (not including juices) did you eat per week or per day?

- ☐ Less than 1 per week
- ☐ 1–2 per week
- ☐ 3–4 per week
- ☐ 5–6 per week
- ☐ 1 per day
- ☐ 2 per day
- ☐ 3 per day
- ☐ 4 per day
- ☐ 5 or more per day

133. Over the past month, which of the following foods did you eat **AT LEAST THREE TIMES?** ***(Mark all that apply.)***

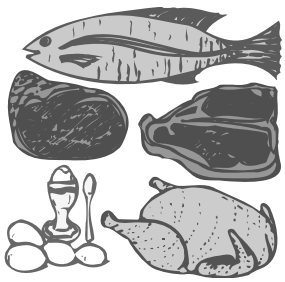
- ☐ Avocado, guacamole
- ☐ Cheesecake
- ☐ Chocolate, fudge, or butterscotch toppings or syrups
- ☐ Chow mein noodles
- ☐ Croissants
- ☐ Dried apricots
- ☐ Egg rolls
- ☐ Granola bars
- ☐ Hot peppers
- ☐ Jello, gelatin
- ☐ Milkshakes or ice-cream sodas
- ☐ Olives
- ☐ Oysters
- ☐ Pickles or pickled vegetables or fruit
- ☐ Plantains
- ☐ Pork neckbones, hock, head, feet
- ☐ Pudding or custard
- ☐ Veal, venison, lamb
- ☐ Whipped cream, regular
- ☐ Whipped cream, substitute
- ☐ NONE

134. For **ALL** of the past 12 months, have you followed any type of **vegetarian diet**?

- ☐ NO (GO TO INTRODUCTION TO QUESTION 135)
- ☐ YES

134a. Which of the following foods did you **TOTALLY EXCLUDE** from your diet? ***(Mark all that apply.)***

- ☐ Meat (beef, pork, lamb, etc.)
- ☐ Poultry (chicken, turkey, duck)
- ☐ Fish and seafood
- ☐ Eggs
- ☐ Dairy products (milk, cheese, etc.)



Introduction to Question 135 appears in the next column

The next questions are about your use of fiber supplements or vitamin pills.

135. Over the past 12 months, did you take any of the following types of **fiber or fiber supplements** on a regular basis (more than once per week for at least 6 of the last 12 months)? ***(Mark all that apply.)***

- ☐ NO, didn't take any fiber supplements on a regular basis (GO TO QUESTION 136)
- ☐ YES, psyllium products (such as Metamucil, Fiberall, Serutan, Perdiem, Correctol)
- ☐ YES, methylcellulose/cellulose products (such as Citrucel, Unifiber)
- ☐ YES, Fibercon
- ☐ YES, Bran (such as wheat bran, oat bran, or bran wafers)

136. Over the past 12 months, did you take any **multivitamins**, such as One-a-Day-, Theragran-, or Centrum-type multivitamins (as pills, liquids, or packets)?

- ☐ NO (GO TO INTRODUCTION TO QUESTION 138)
- ☐ YES

137. How often did you take **One-a-day-, Theragran-, or Centrum-type** multivitamins?

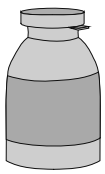
- ☐ Less than 1 day per month
- ☐ 1–3 days per month
- ☐ 1–3 days per week
- ☐ 4–6 days per week
- ☐ Every day

137a. Does your **multivitamin** usually contain **minerals** (such as iron, zinc, etc.)?

- ☐ NO
- ☐ YES
- ☐ Don't know

137b. For how many years have you taken **multivitamins**?

- ☐ Less than 1 year
- ☐ 1–4 years
- ☐ 5–9 years
- ☐ 10 or more years



Introduction to Question 138 appears on the next page

Over the <u>past 12 months</u>…

137c. Over the <u>past 12 months</u>, did you take any vitamins, minerals, or other herbal supplements other than your multivitamin?

☐ NO

Thank you *<u>very much</u>* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- **Did not skip any pages and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

☐ YES (GO TO INTRODUCTION TO QUESTION 138)

These last questions are about the vitamins, minerals, or herbal supplements you took that are <u>NOT</u> part of a One-a-day-, Theragran-, or Centrum-type of multivitamin.

Please include vitamins taken as part of an antioxidant supplement.

138. How often did you take **Beta-carotene** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 139)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

138a. When you took **Beta-carotene**, about how much did you take in one day?

☐ Less than 10,000 IU
☐ 10,000–14,999 IU
☐ 15,000–19,999 IU
☐ 20,000–24,999 IU
☐ 25,000 IU or more
☐ Don't know

138b. For how many years have you taken **Beta-carotene**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

Question 139 appears in the next column

139. How often did you take **Vitamin A** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 140)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

139a. When you took **Vitamin A**, about how much did you take in one day?

☐ Less than 8,000 IU
☐ 8,000–9,999 IU
☐ 10,000–14,999 IU
☐ 15,000–24,999 IU
☐ 25,000 IU or more
☐ Don't know

139b. For how many years have you taken **Vitamin A**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

140. How often did you take **Vitamin C** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 141)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

140a. When you took **Vitamin C**, about how much did you take in one day?

☐ Less than 500 mg
☐ 500–999 mg
☐ 1,000–1,499 mg
☐ 1,500–1,999 mg
☐ 2,000 mg or more
☐ Don't know

140b. For how many years have you taken **Vitamin C**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

Question 141 appears on the next page

Over the <u>past 12 months</u>…

141. How often did you take **Vitamin E** (**NOT** as part of a multivitamin in Question 137)?

- ☐ NEVER (GO TO QUESTION 142)
- ☐ Less than 1 day per month
- ☐ 1–3 days per month
- ☐ 1–3 days per week
- ☐ 4–6 days per week
- ☐ Every day

141a. When you took **Vitamin E**, about how much did you take in one day?

- ☐ Less than 400 IU
- ☐ 400–799 IU
- ☐ 800–999 IU
- ☐ 1,000 IU or more
- ☐ Don't know

141b. For how many years have you taken **Vitamin E**?

- ☐ Less than 1 year
- ☐ 1–4 years
- ☐ 5–9 years
- ☐ 10 or more years

142. How often did you take **Calcium** or **Calcium-containing antacids** (**NOT** as part of a multivitamin in Question 137)?

- ☐ NEVER (GO TO QUESTION 143)
- ☐ Less than 1 day per month
- ☐ 1–3 days per month
- ☐ 1–3 days per week
- ☐ 4–6 days per week
- ☐ Every day

142a. When you took **Calcium** or **Calcium-containing antacids**, about how much elemental calcium did you take in one day? *(If possible, please check the label for elemental calcium.)*

- ☐ Less than 500 mg
- ☐ 500–599 mg
- ☐ 600–999 mg
- ☐ 1,000 mg or more
- ☐ Don't know

142b. For how many years have you taken **Calcium** or **Calcium-containing antacids**?

- ☐ Less than 1 year
- ☐ 1–4 years
- ☐ 5–9 years
- ☐ 10 or more years

The last two questions ask you about other supplements you took <u>more than once per week</u>.

143. Please mark any of the following **single supplements** you took <u>more than once per week</u> (**NOT** as part of a multivitamin in Question 137):

- ☐ B-6
- ☐ B-complex
- ☐ Brewer's yeast
- ☐ Cod liver oil
- ☐ Coenzyme Q
- ☐ Fish oil (Omega-3 fatty acids)
- ☐ Folic acid/folate
- ☐ Glucosamine
- ☐ Hydroxytryptophan (HTP)
- ☐ Iron
- ☐ Niacin
- ☐ Selenium
- ☐ Zinc

144. Please mark any of the following **herbal** or **botanical supplements** you took <u>more than once per week</u>.

- ☐ Aloe Vera
- ☐ Astragalus
- ☐ Bilberry
- ☐ Cascara sagrada
- ☐ Cat's claw
- ☐ Cayenne
- ☐ Cranberry
- ☐ Dong Kuai (Tangkwei)
- ☐ Echinacea
- ☐ Evening primrose oil
- ☐ Feverfew
- ☐ Garlic
- ☐ Ginger
- ☐ Ginkgo biloba
- ☐ Ginseng (American or Asian)
- ☐ Goldenseal
- ☐ Grapeseed extract
- ☐ Kava, kava
- ☐ Milk thistle
- ☐ Saw palmetto
- ☐ Siberian ginseng
- ☐ St. John's wort
- ☐ Valerian
- ☐ Other

Thank you *<u>very much</u>*** **for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:**

- **Did not skip any pages and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

Question 143 appears in the next column

APPENDIX J. WEEKLY SYMPTOM, TOXICITY AND ECOG ASSESSMENT FORM

Enrollment ID: **NCI Symptom and Toxicity Evaluation** **Date:**

Criteria	Pt response	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Weight loss		None (<5%)	5-<10% of baseline, intervention not indicated	10-<20% of baseline; nutrition support indicated	> 20% of baseline; TF or TPN indicated	---
Nausea		None	Able to eat reasonable intake	Intake significantly decreased but can eat	No significant intake	-----
Vomiting		None	1 episode in 24 hours over pretreatment	2-5 episodes in 24 hours; IV fluids indicated < 24 hrs	≥ 6 episodes in 24 hours, IV fluids or TPN indicated > 24 hrs	Life threatening consequences
Heartburn		None	Mild	Moderate	Severe	-
Urinary frequency/ urgency		None	Increase in frequency or nocturia upto 2 x normal	Increase in frequency or nocturia more than 2 x normal but less frequent than every hr	frequency of urination hourly or with more urgency, requiring catheter	----
Diarrhea (patients without colostomy)		None	Increase of < 4 stools/day over pre-treatment	Increase of 4 – 6 stools/day or nocturnal stools	Increase of ≥ 7 stools/day or incontinence; or need for parenteral support for dehydration	Physiologic consequences requiring intensive care;or hemodynamic collapse
Proctitis		None	increased stool frequency, occasional blood streaked stools or rectal discomfort (including hemorrhoids not requiring meds	increased stool frequency, bleeding mucus discharge or rectal discomfort requiring medication; anal fissure	increased stool frequency/diarrhea requiring parenteral support; rectal bleeding requiring transfusions or persistent mucus discharge, necessitating pads	perforation, bleeding or necrosis or other life threatening complication requiring surgical intervention (eg colostomy)

Grade	Patient response	ECOG Criteria
0		Fully active, able to carry on all pre-disease performance without restriction
1		Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2		Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3		Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4		Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair

APPENDIX K. EXIT INTERVIEW FORM

Tomato Juice study participant evaluation

Please tell us why you chose to participate in this study **(please select all that apply)**.

- ❑ Liked the idea of tomato juice helping with radiation therapy side effects
- ❑ Wanted to participate in a research study
- ❑ Wanted to help student research
- ❑ Other (please specify)_____
  _____
  _____

Are you glad you participated in this study? **(Please select only one response)**.

- ❑ Yes
- ❑ Maybe, not sure
- ❑ No (please elaborate why not_____)

Based on your experience participating in this study, what changes (if any) would you recommend the researchers make to improve participation in this study for other men with prostate cancer undergoing radiation therapy?

- ❑ No changes
- ❑ Recommend the following changes
  a. _____
  b. _____
  c. _____

Thank you for your valuable participation in this research study.

APPENDIX L. SUPPLEMENTAL FIGURES

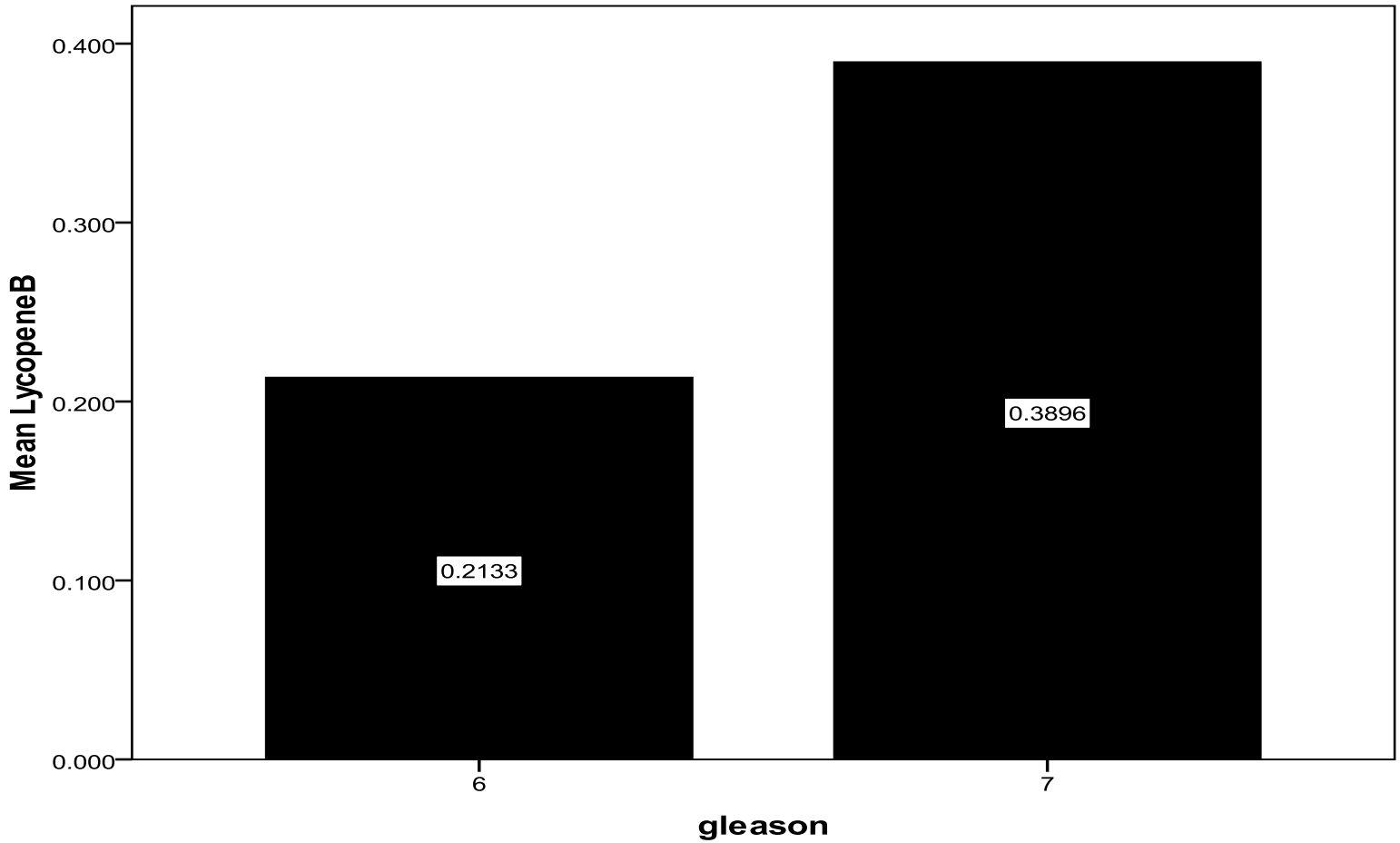


Figure 1. Serum lycopene levels at baseline among study [participants based on Gleason sum.

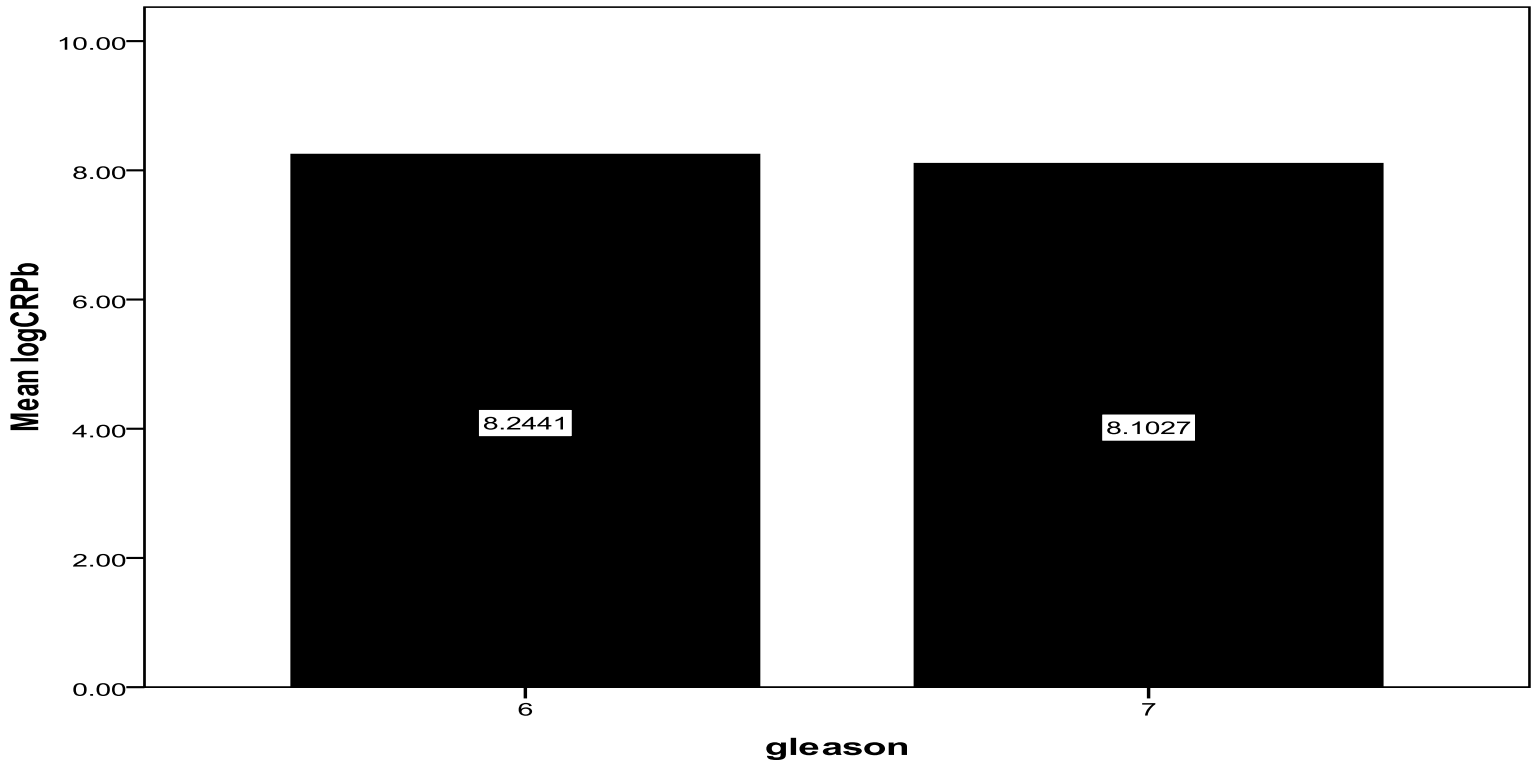


Figure 2. Serum log c-reactive protein levels at baseline based on Gleason Sum

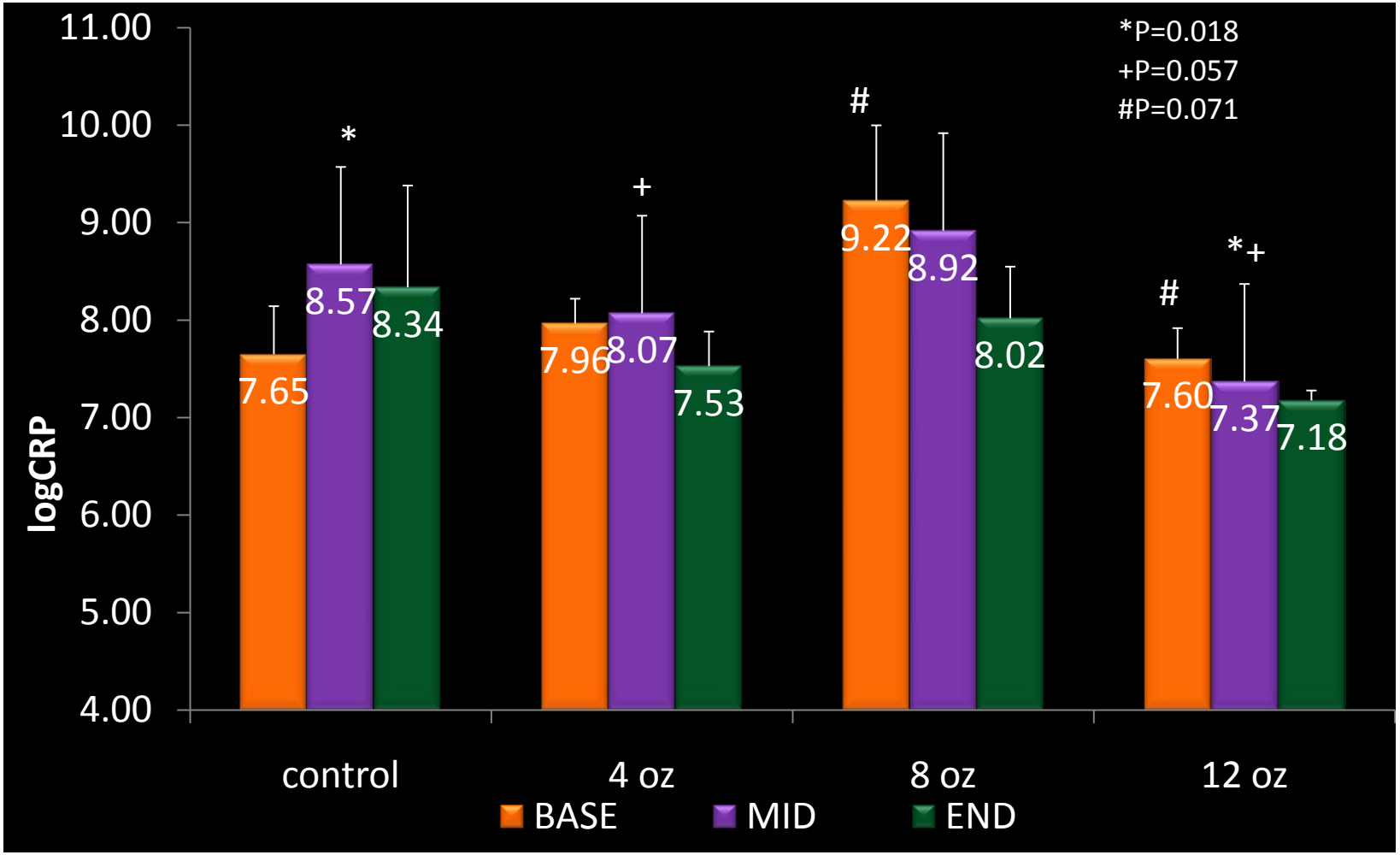


Figure 3. Serum log c-reactive protein levels among study groups at three time points.

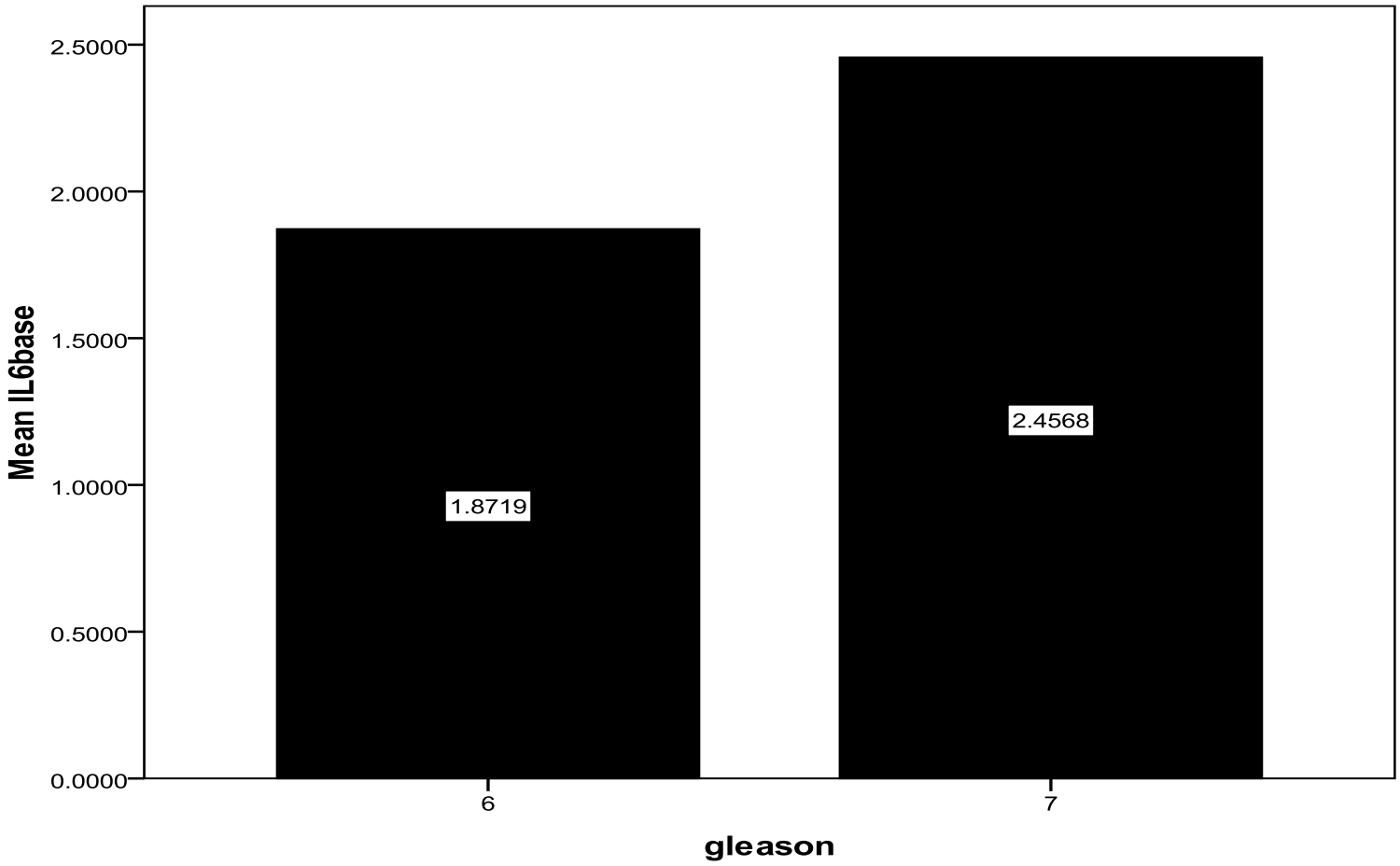


Figure 4. Serum interleukin-6 levels at baseline based on Gleason Sum

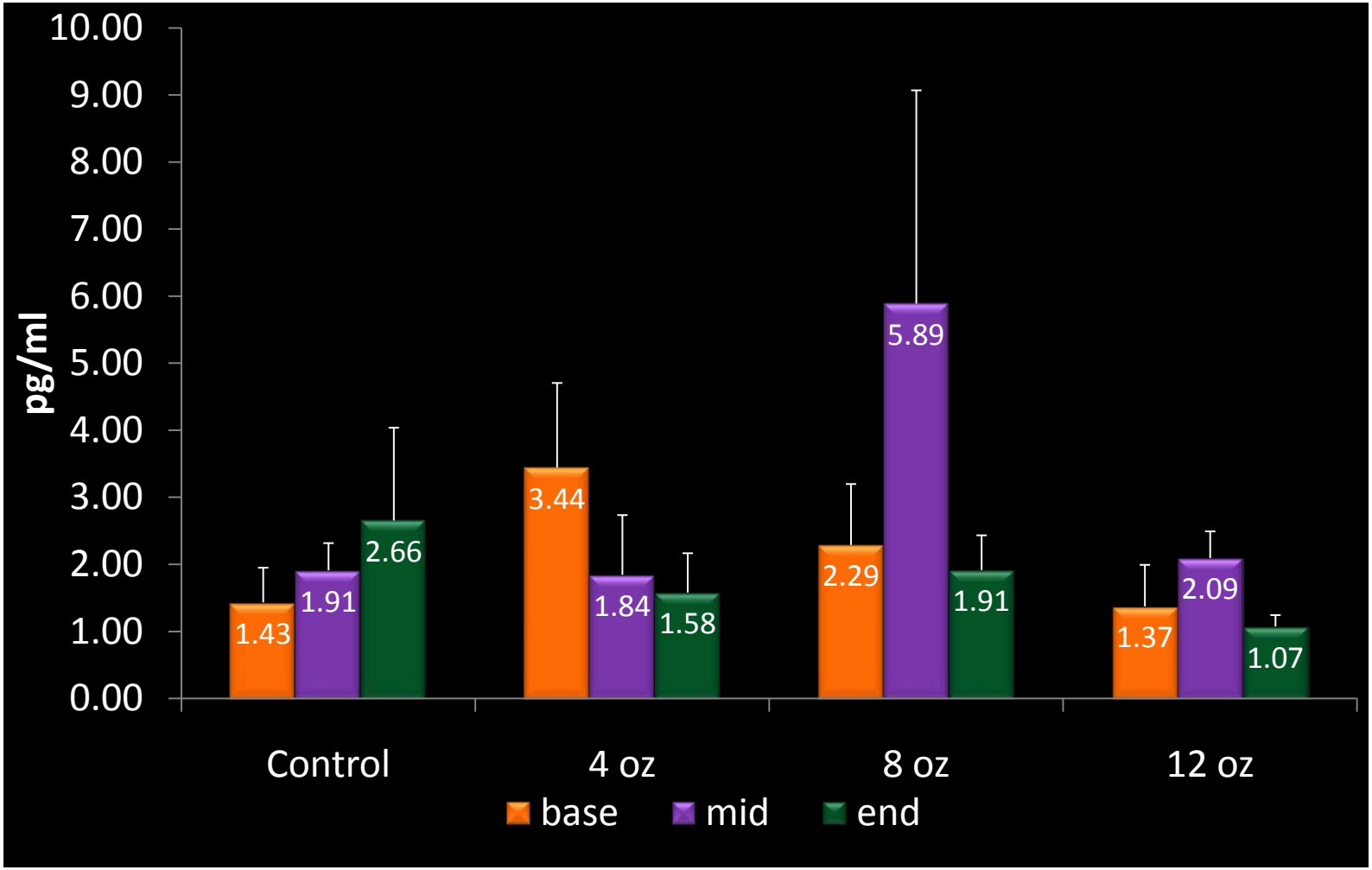


Figure 5. Serum Interleukin-6 levels among study groups at three time points.

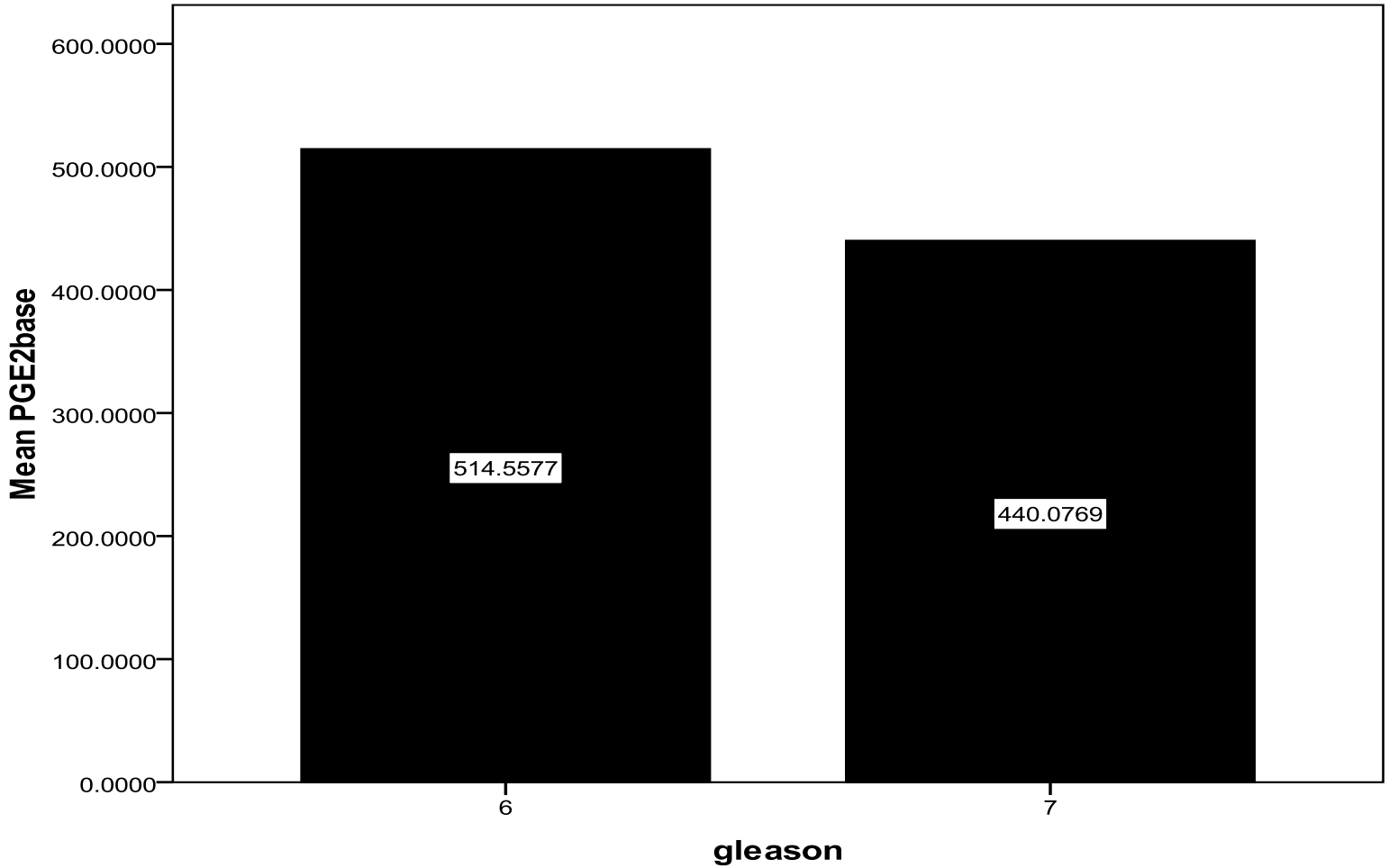


Figure 6. Serum prostaglandin E2 levels at baseline based on Gleason Sum

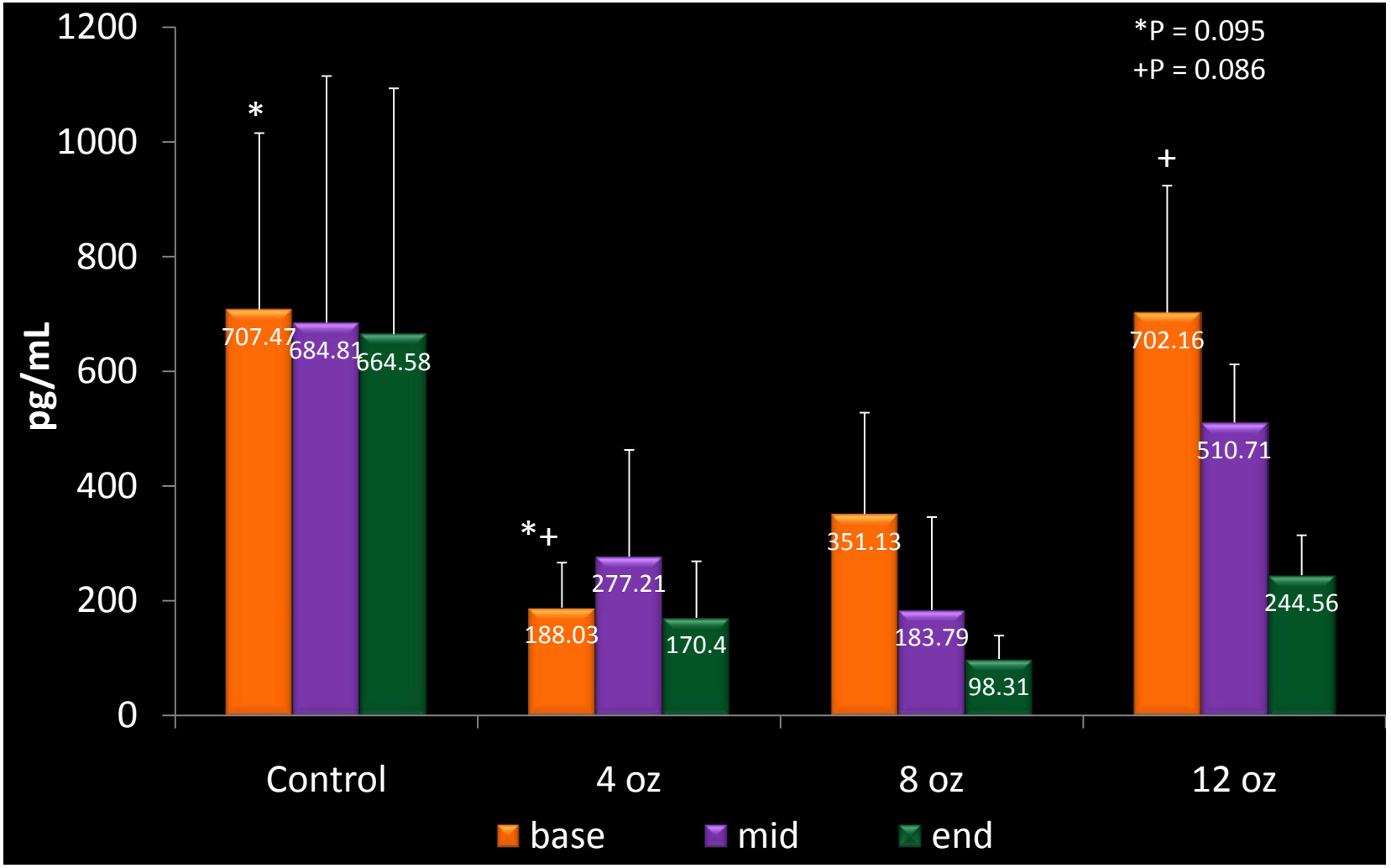


Figure 7. Serum prostaglandin E2 levels among study groups at three time points.

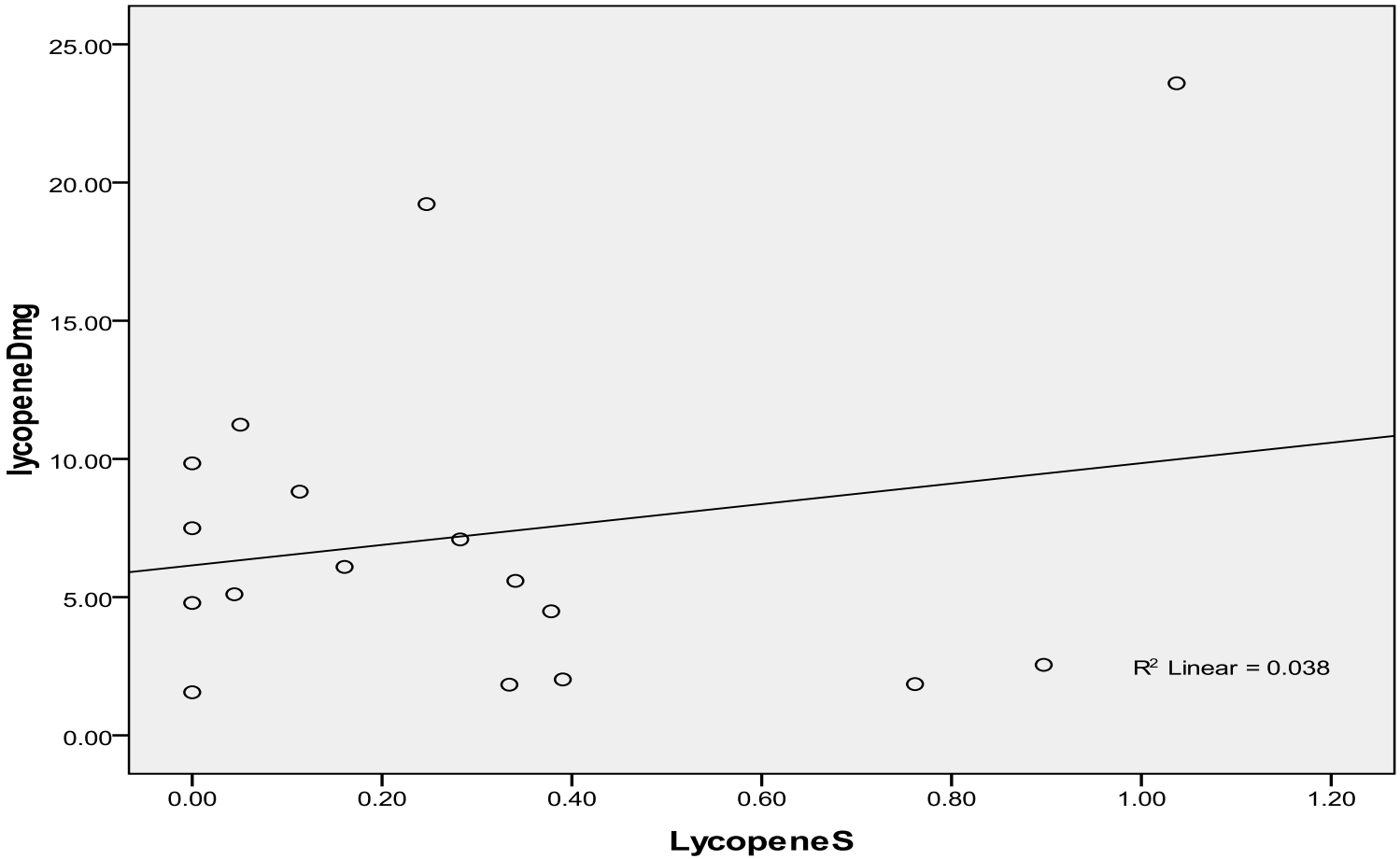


Figure 8. Scatter plot of serum lycopene plotted against total lycopene intake

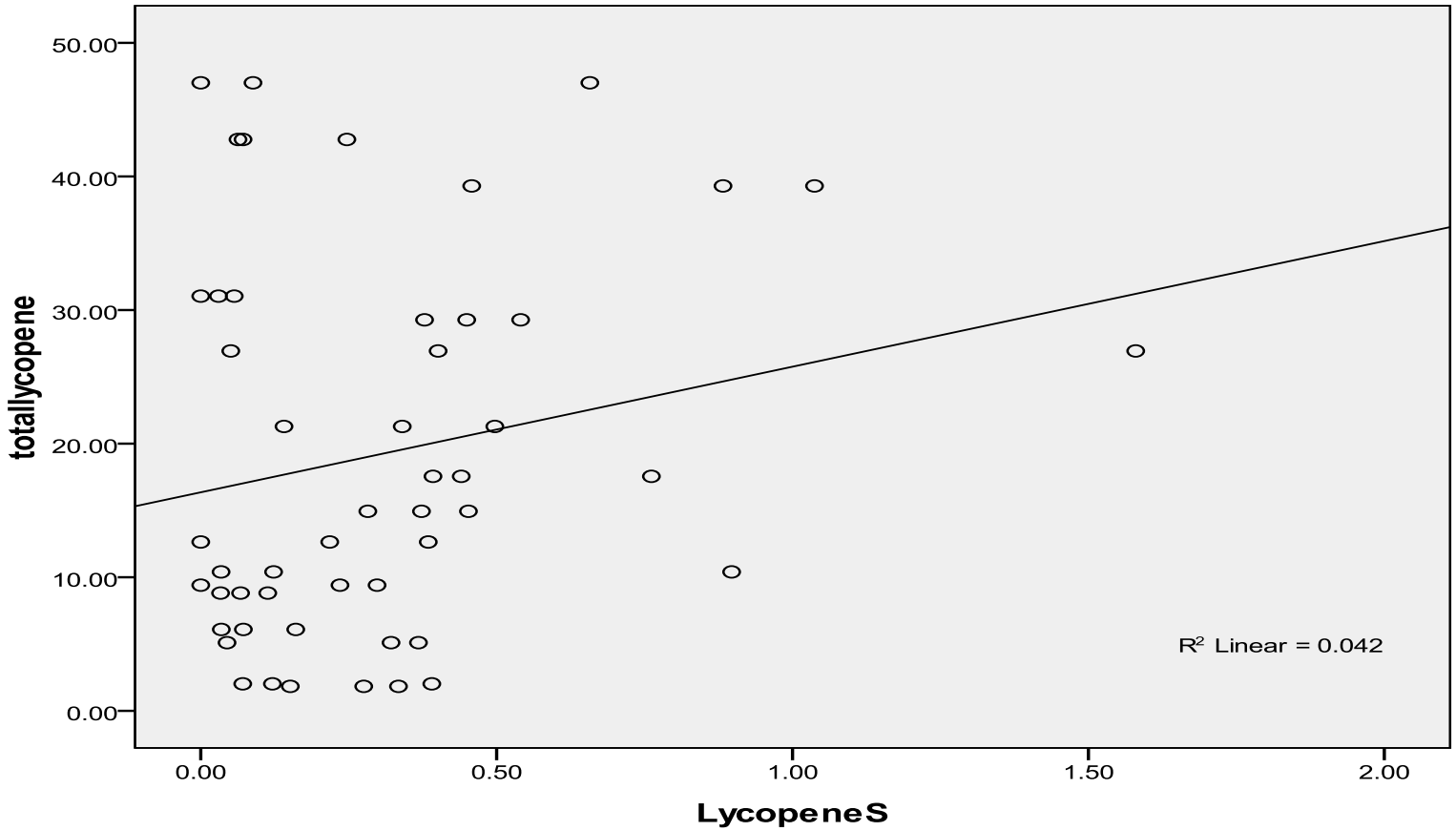


Figure 9. Scatter plot of serum lycopene plotted against total lycopene intake

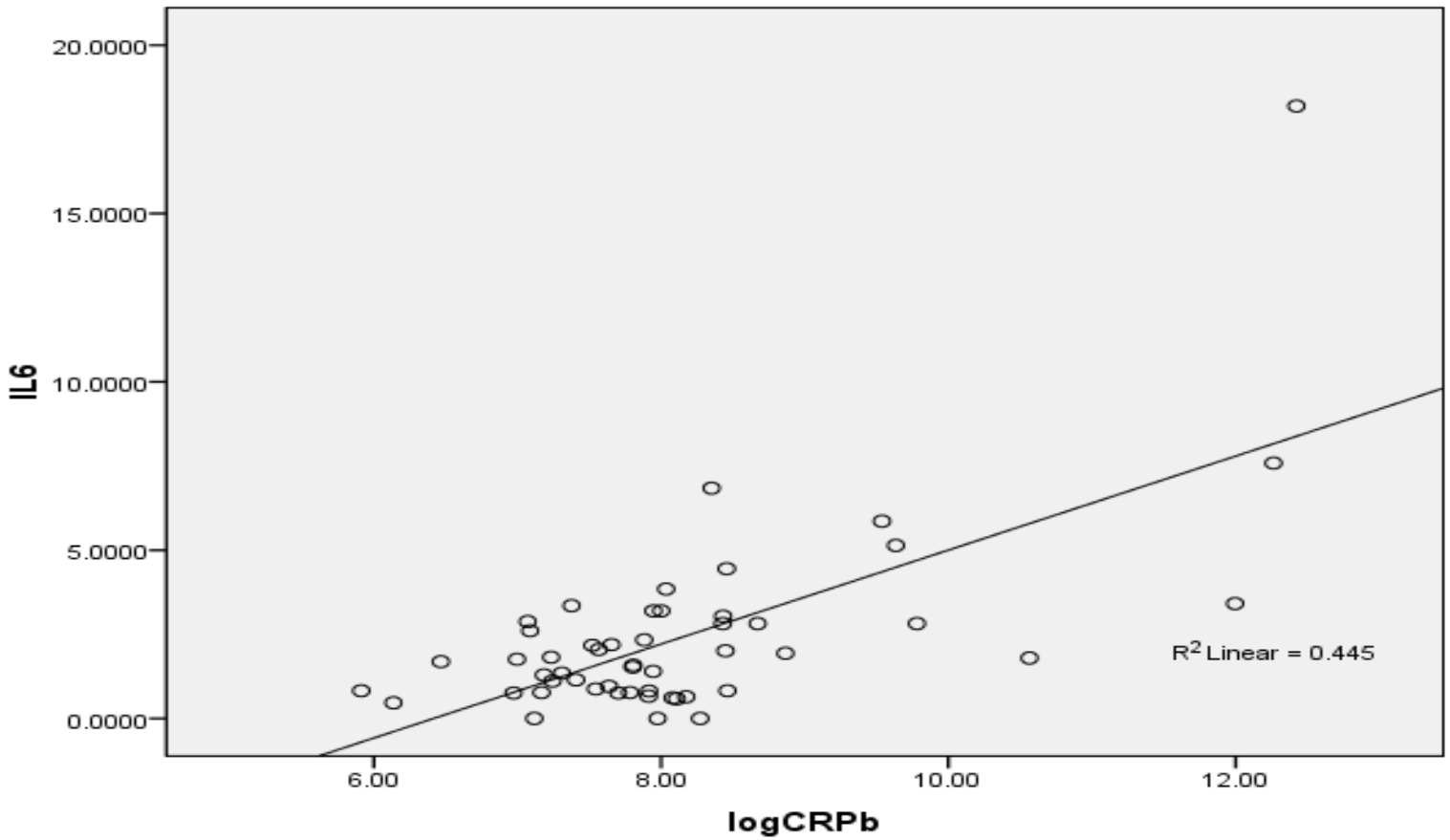


Figure 10. Scatter plot of serum CRP log transformed plotted against serum interleukin-6

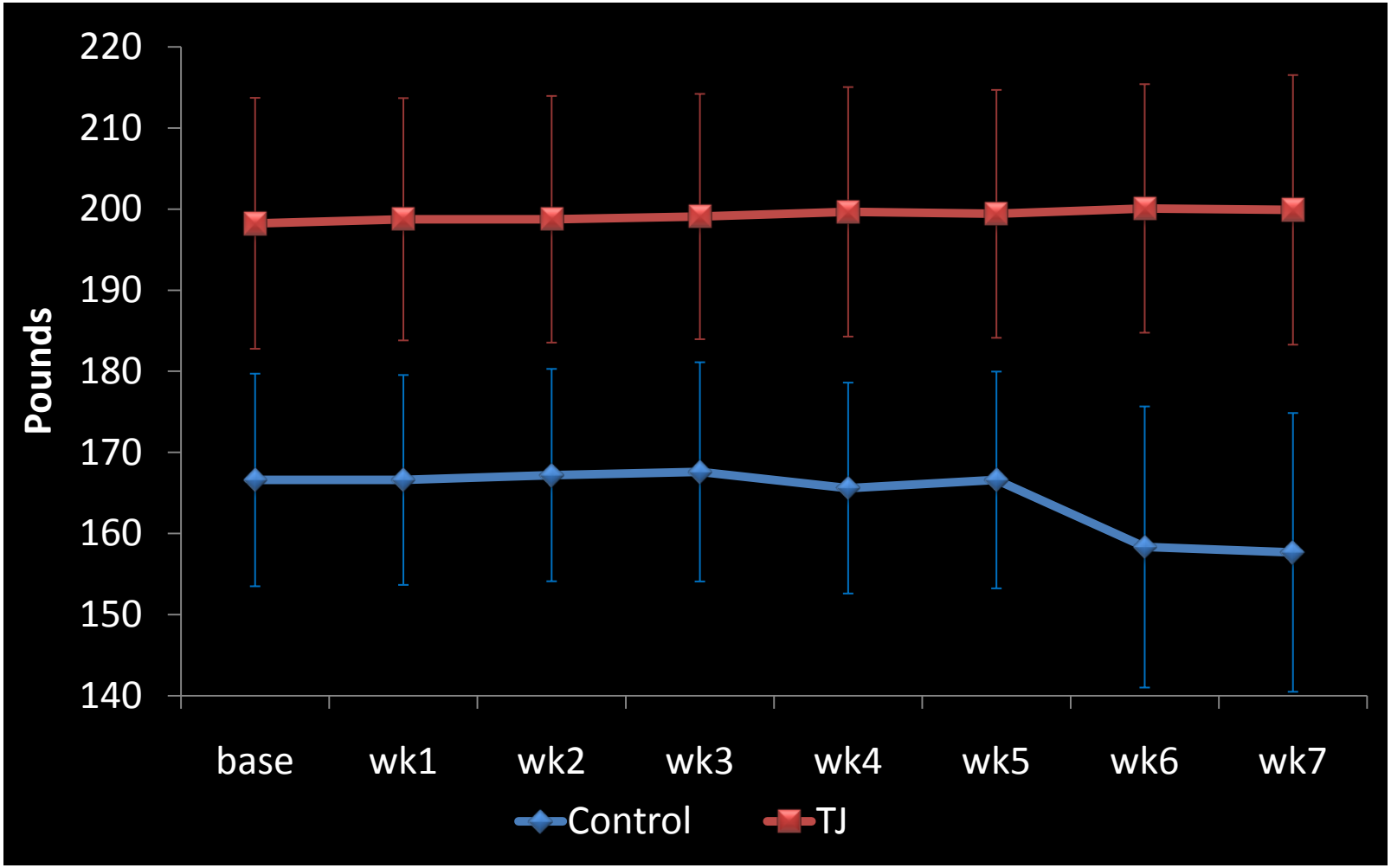


Figure 11. Weight change during treatment in control and all treatment groups

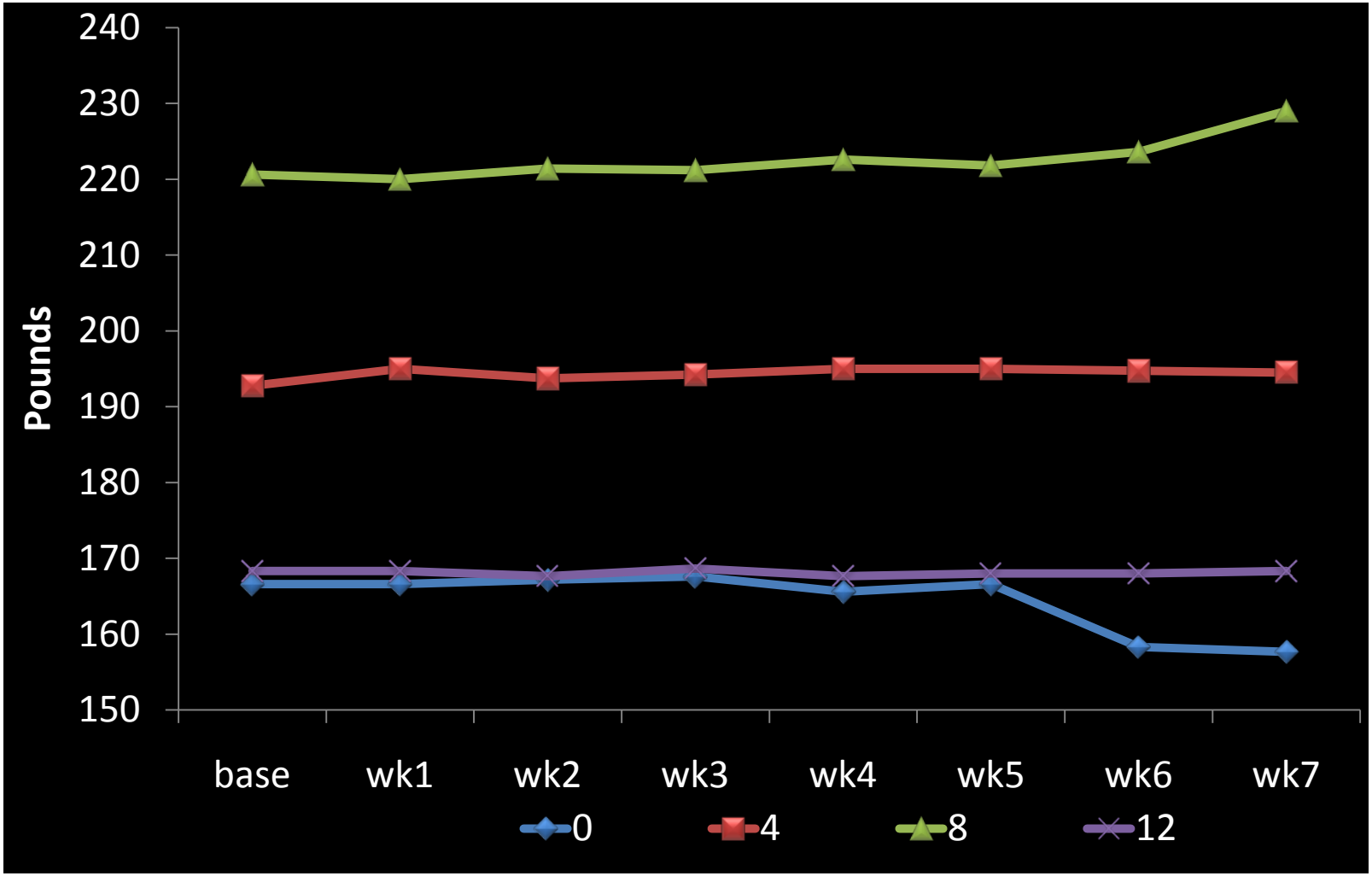


Figure 12. Weight change during treatment in all groups

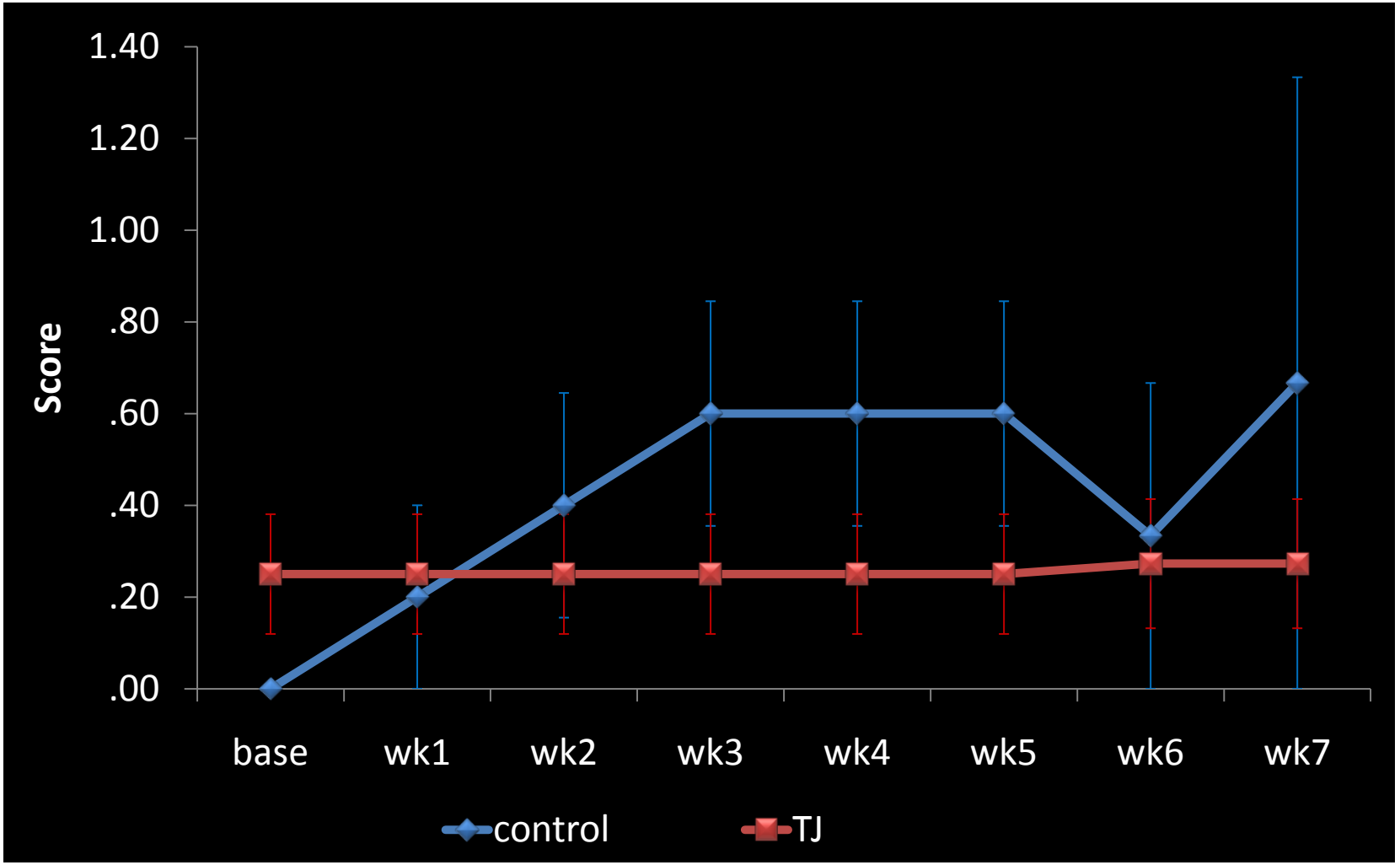


Figure 13. ECOG score incidence during treatment in all groups

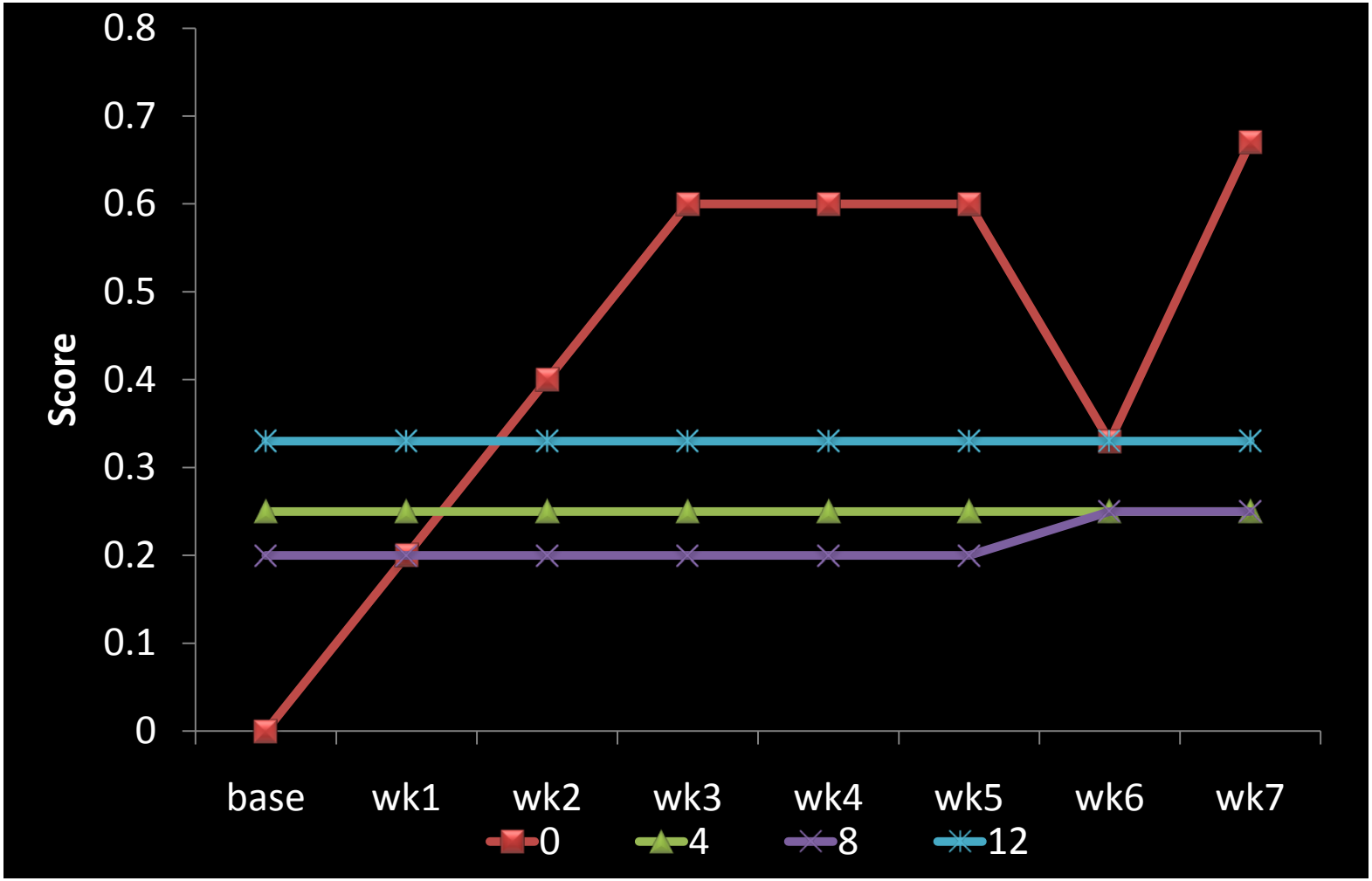


Figure 14. ECOG scores during treatment in all groups

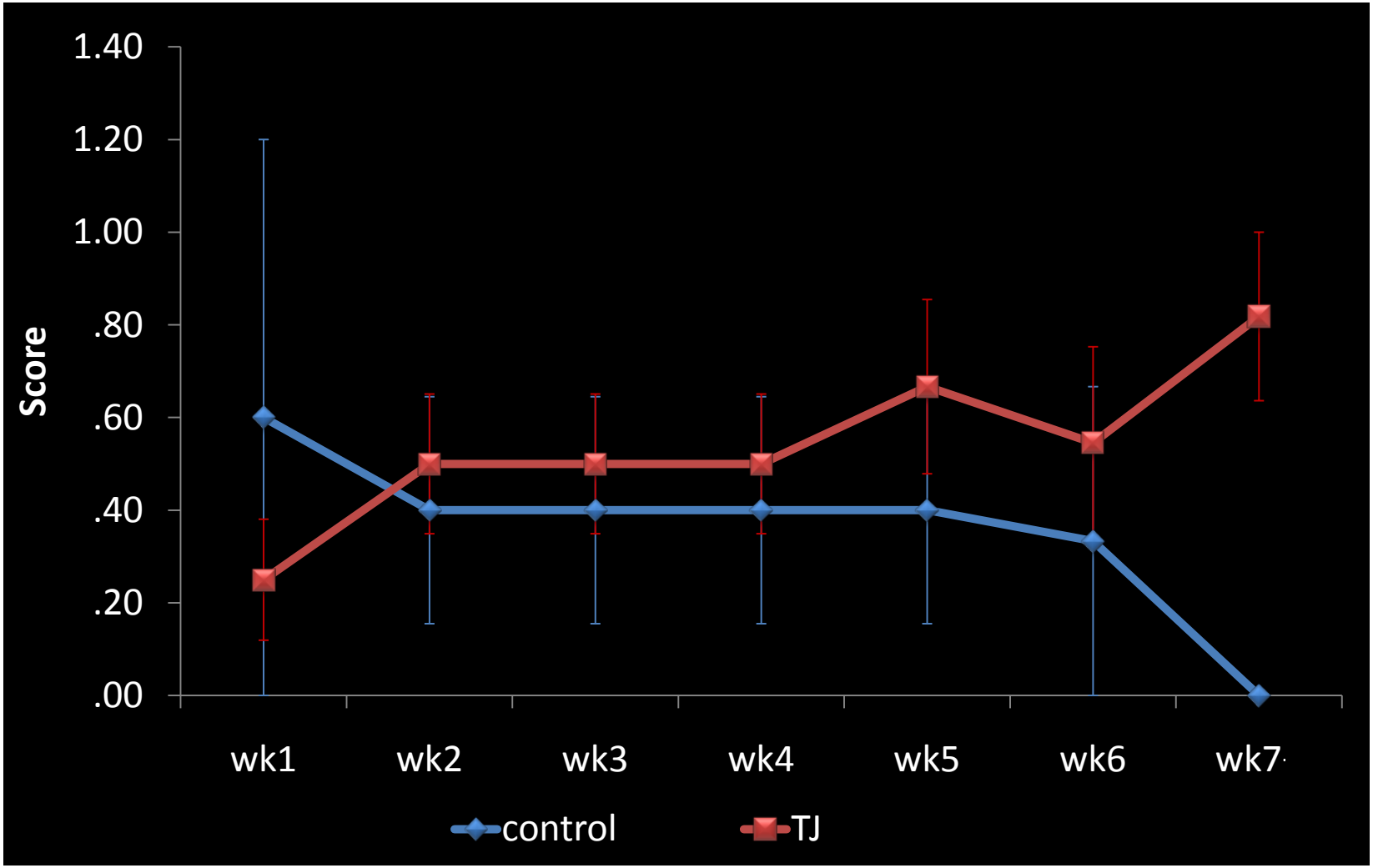


Figure 15. Urinary frequency during treatment in control and all treatment groups

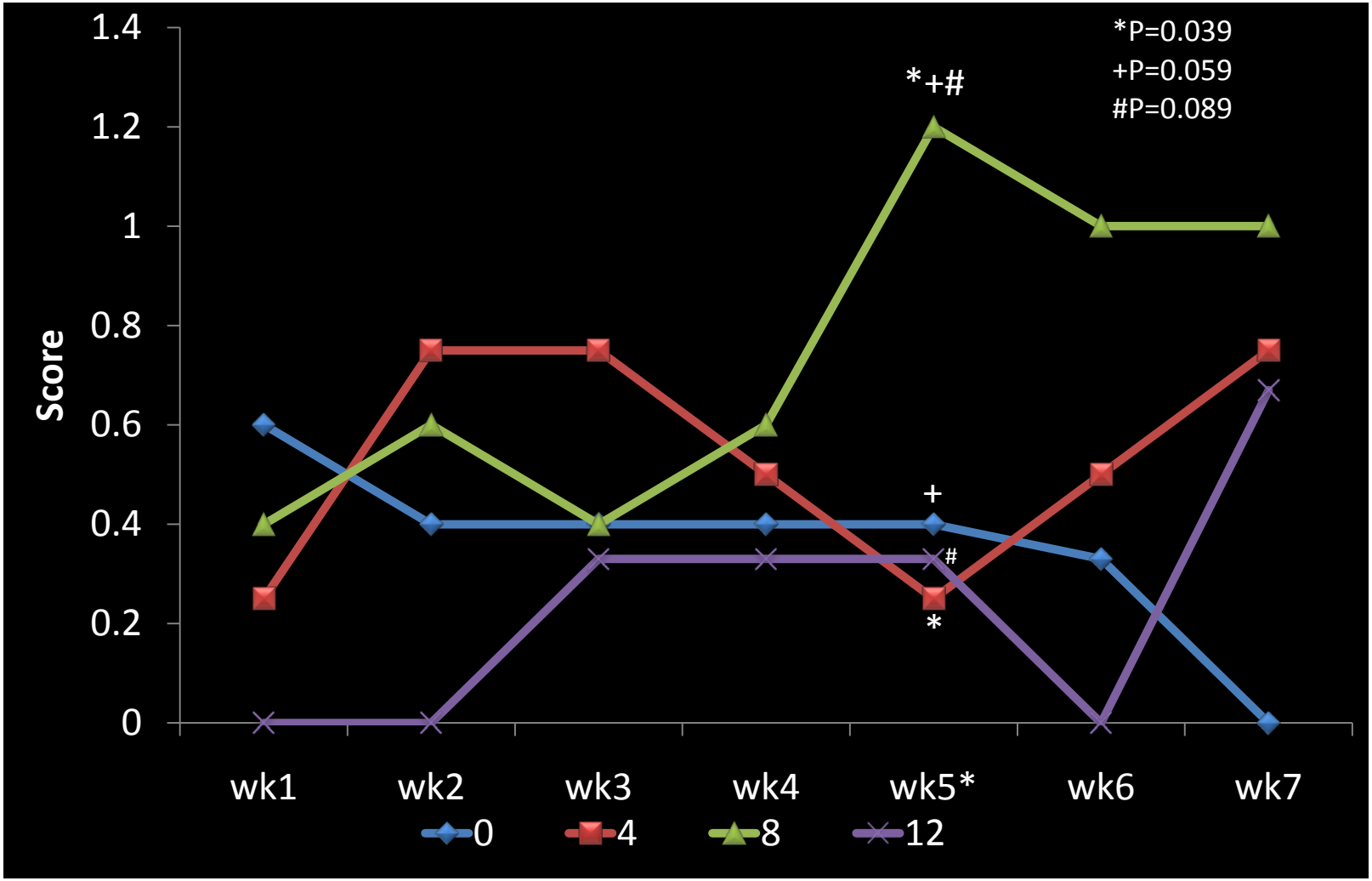


Figure 16. Urinary frequency incidence during treatment in all groups

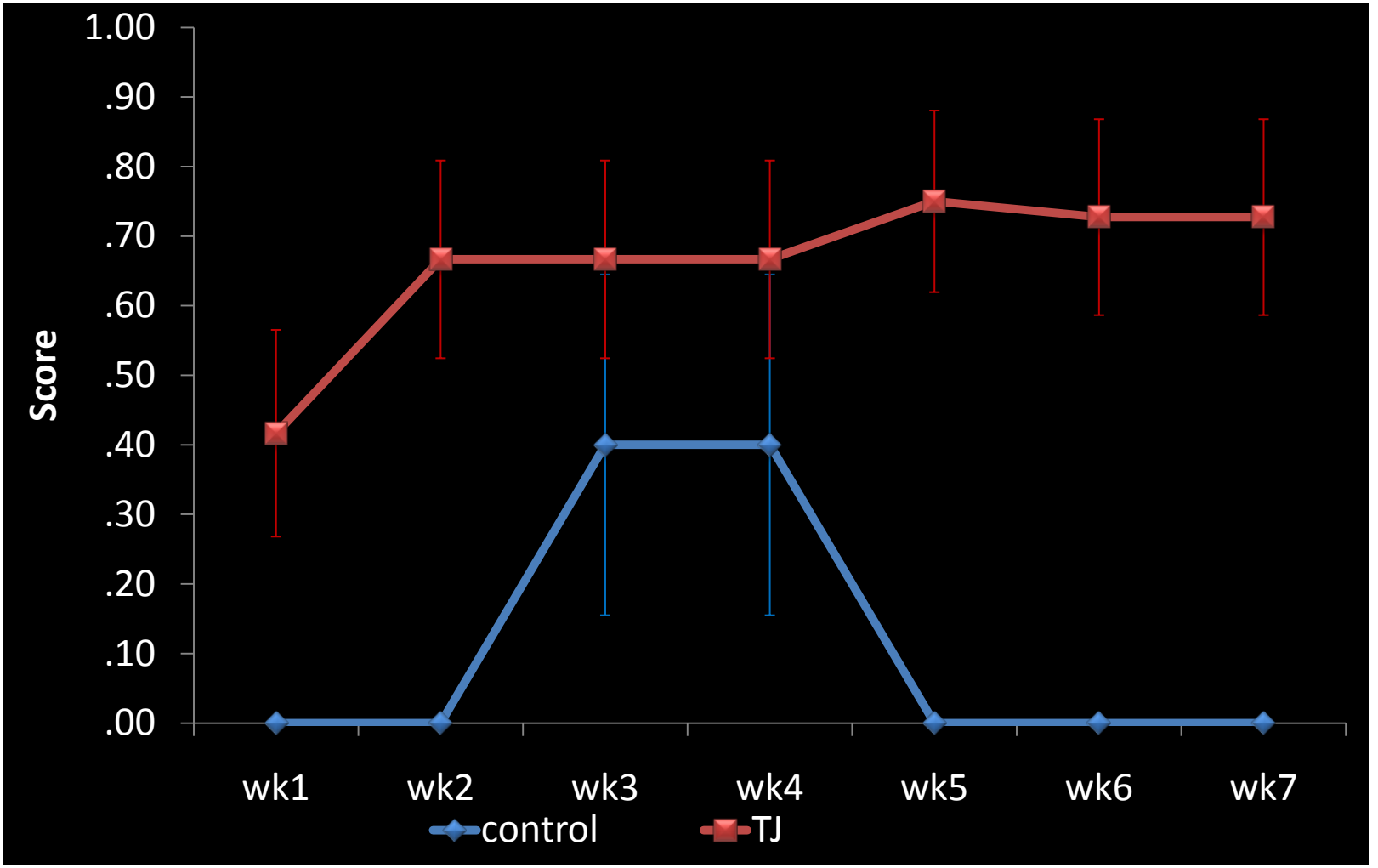


Figure 17. Urinary urgency during treatment in control and all treatment groups

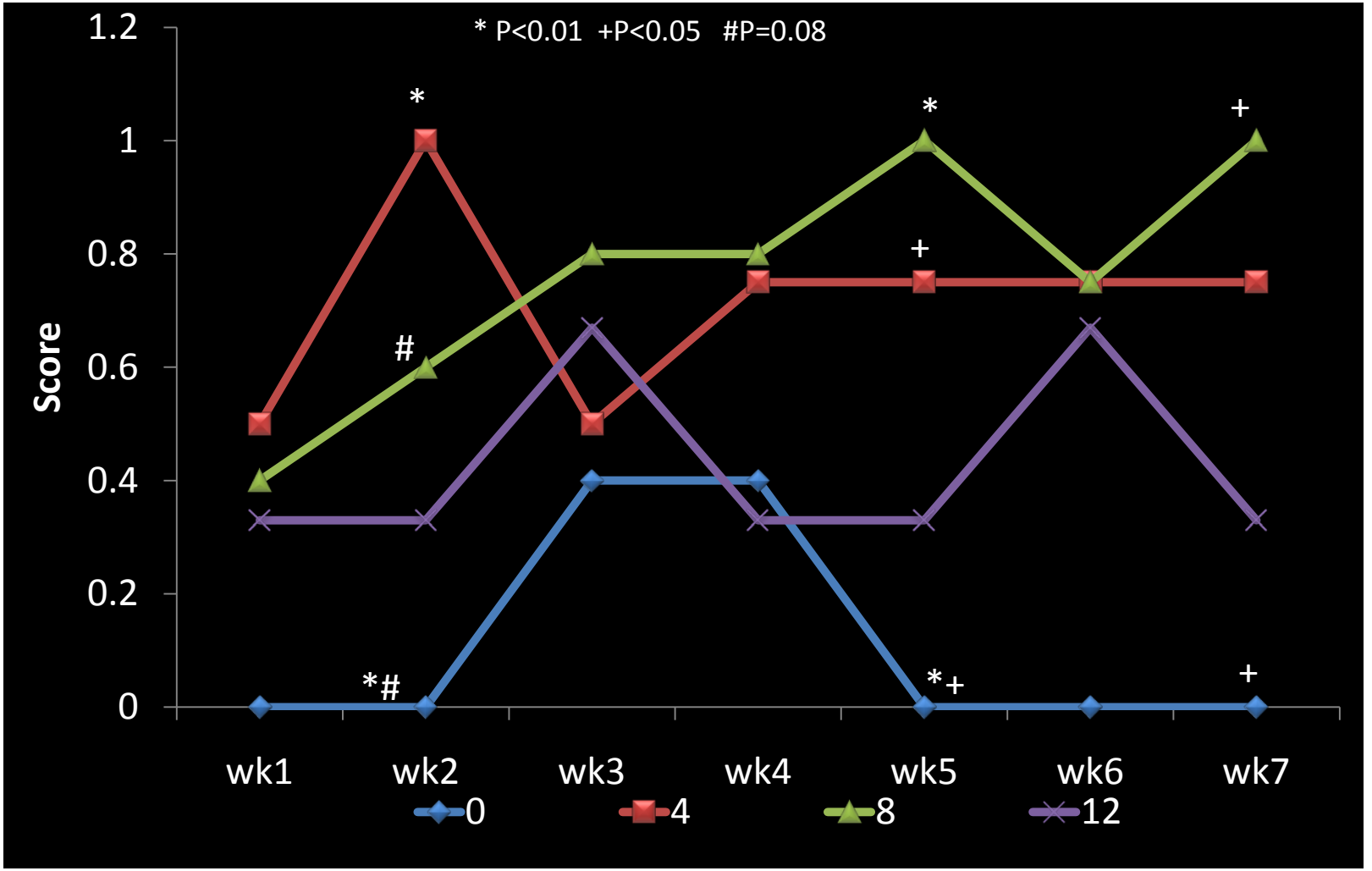


Figure 18. Urinary urgency incidence during treatment in all groups

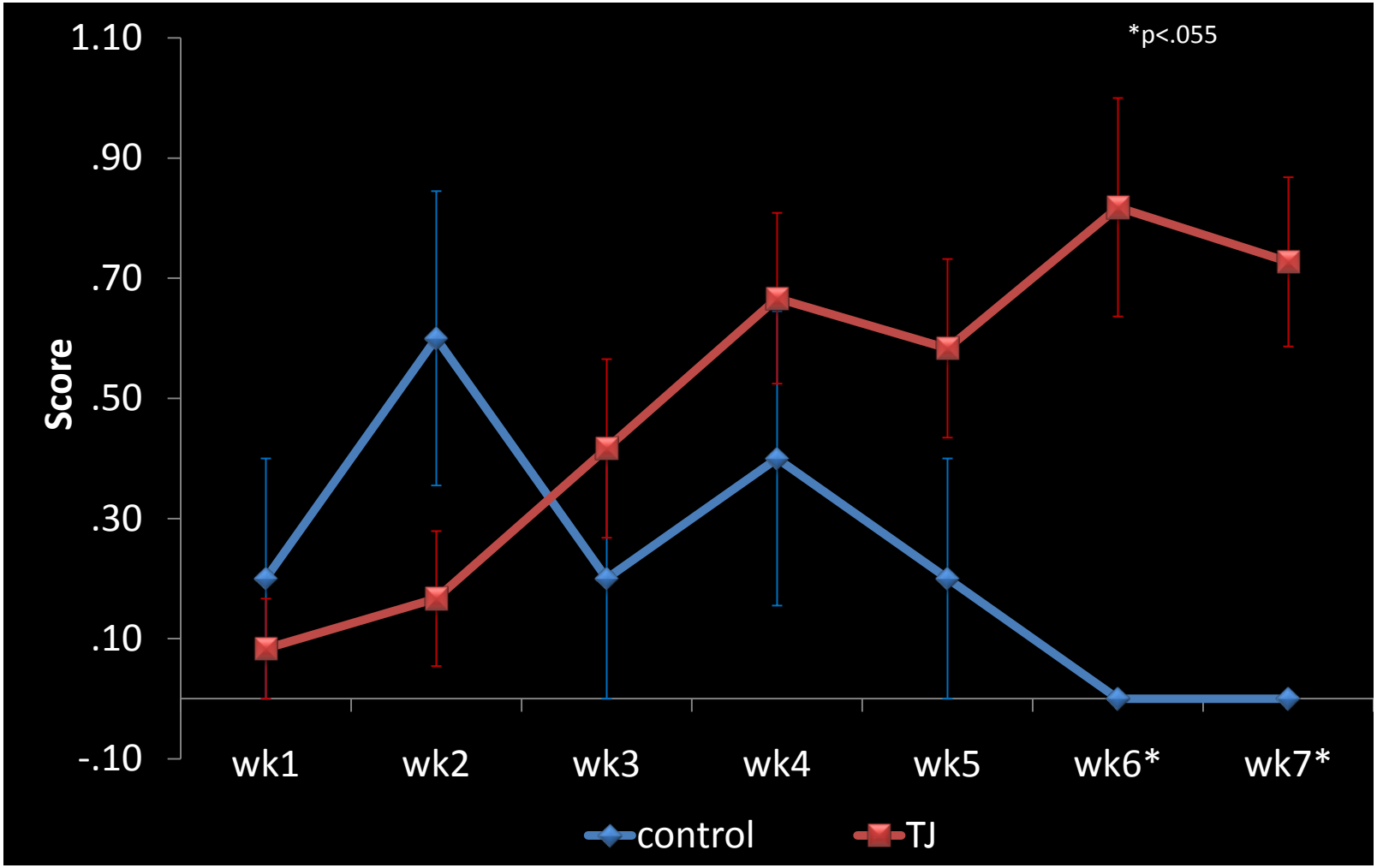


Figure 19. Diarrhea incidence during treatment in control and all treatment groups

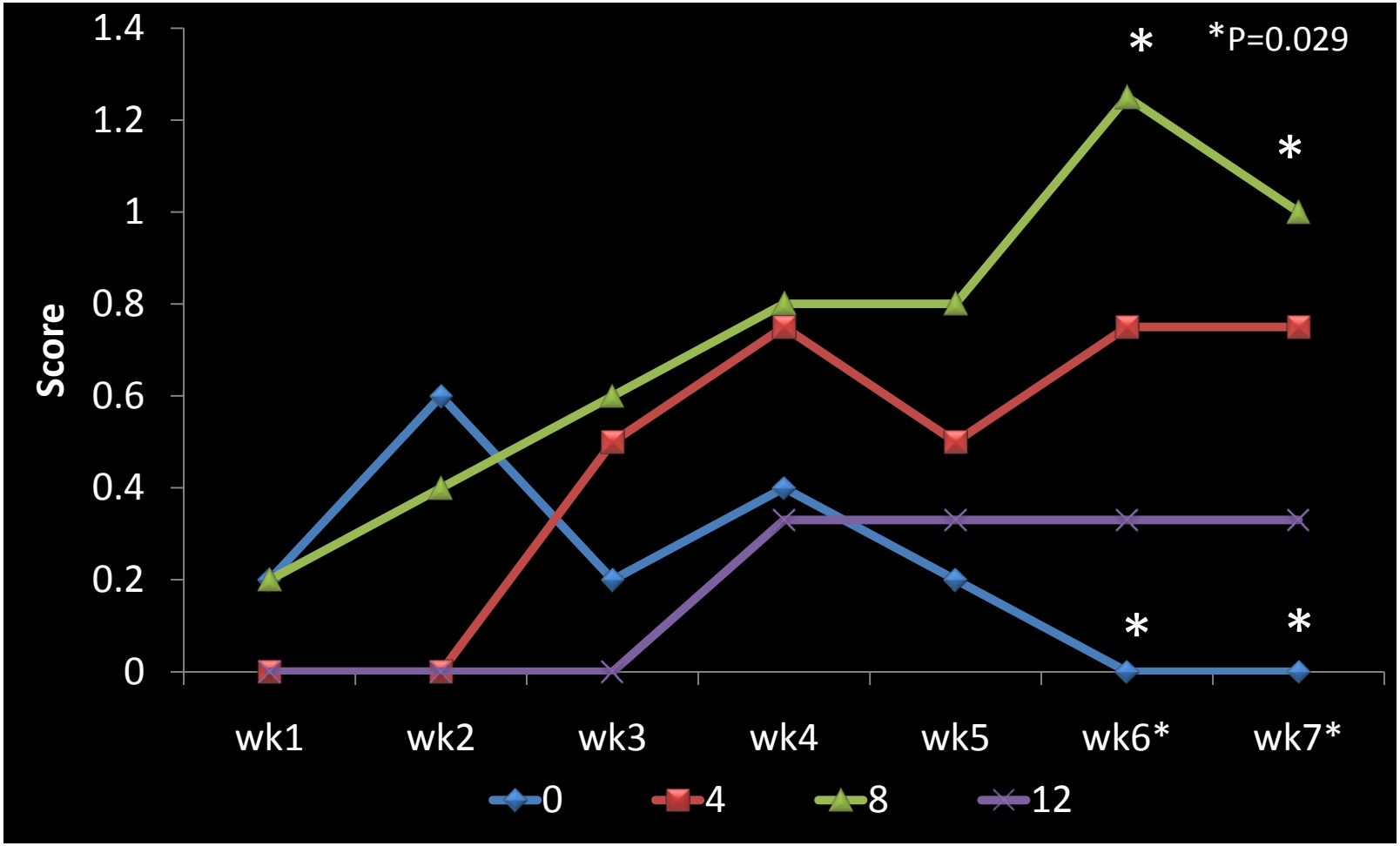


Figure 20. Diarrhea incidence during treatment in all groups

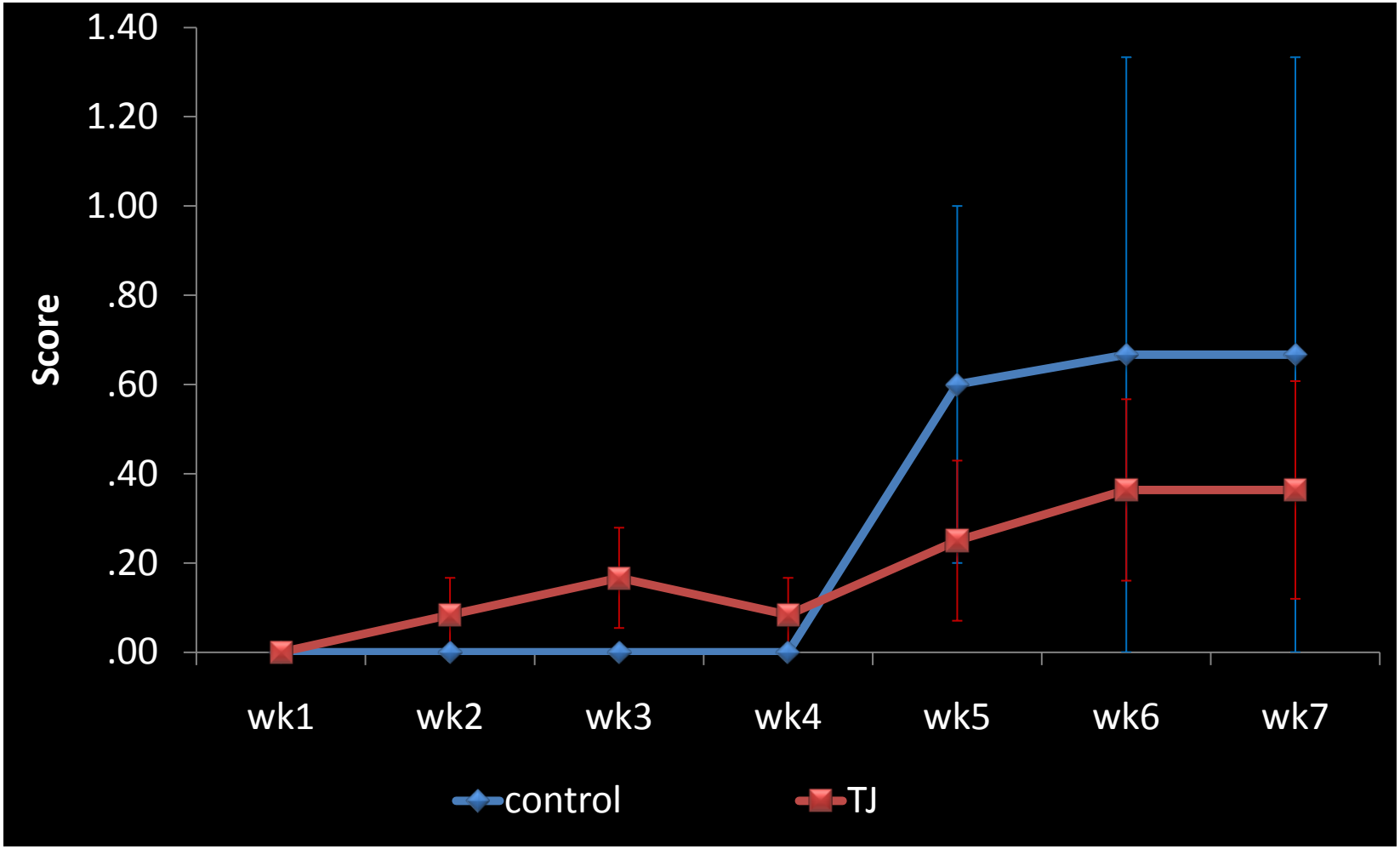


Figure 21. Proctitis incidence during treatment in control and all treatment groups

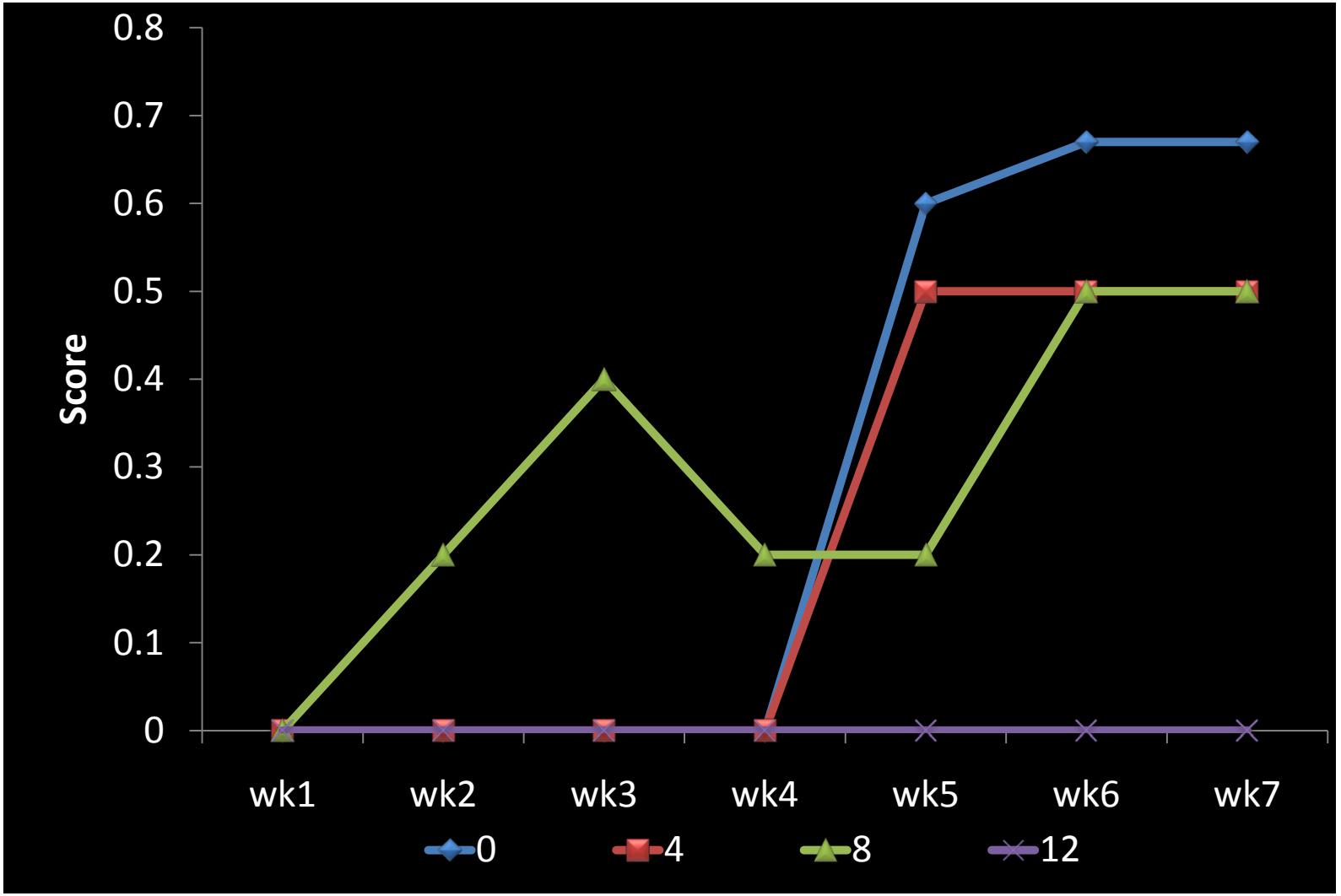


Figure 22. Proctitis incidence during treatment in all groups