THE ROLE OF LYCOPENE ON HUMAN PROSTATE CANCER
AND BONE CELLS IN-VITRO

by

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A thesis submitted in conformity with the requirements
for the degree of M. Sc.
Graduate Department of Nutritional Sciences
University of Toronto

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Master of Science, 2001
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ABSTRACT

Lycopene is found naturally in fruits and vegetables with tomatoes and tomato products being the main dietary source. Epidemiological studies have shown that serum and tissue lycopene levels are inversely related to chronic disease risk. The objective of this study was two-fold; to measure the effects of a water-dispersible lycopene on 1) the proliferation of LNCaP human prostate cancer cells and 2) on the proliferation and differentiation of human osteoblastic SaOS-2 cells. For the LNCaP cells, lycopene, in a dose-dependent manner, inhibited the proliferation of LNCaP cells after 24, 48, 72 and 96hrs. For the SaOS-2 cells, lycopene stimulated the proliferation of cells when incubated for 96 and 144hrs. Dexamethasone (Dex) enhanced alkaline phosphatase activity (ALPase) in a time-course manner, indicating differentiation of the cells. Based on these observations, it is suggested that lycopene may have implication for a significant role in the prevention of human prostate cancer and osteoporosis.
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Chapter One

INTRODUCTION
1. INTRODUCTION

Chronic diseases such as cancer, cardiovascular diseases, diabetes and osteoporosis are gaining particular attention as they are becoming more and more prevalent in the Western world (3). Although genetic factors and age are important in determining their risk, diet is also considered as a major risk factor associated with these diseases. The role of reactive oxygen species (ROS) and oxidative damage to biomolecules is one of the main foci of recent research related to chronic diseases (5, 6, 63, 64, 130, 157, 187). More specifically, oxidative stress has been widely postulated to be involved in the causation and progression of several chronic diseases (6, 64, 130, 157). Moreover, there is compelling evidence to suggest that antioxidants act as protective agents to inactivate ROS and provide protection from oxidative damage, which can prevent chronic diseases.

A dietary approach to the prevention of cancer, which has included assessment and evaluation of the anticancer effect of various components of micronutrients in the diet, has been prevalent for many years. Numerous epidemiological studies have demonstrated that consumption of fruits and vegetables reduces the risk of several human cancers (170, 190). Fruits and vegetables are important sources of dietary antioxidants such as polyphenols, vitamin C, vitamin E and carotenoids. They have been extensively studied and implicated as important cancer-preventive strategic molecules (5, 6, 63, 64, 130, 157, 187).

More recently, one specific antioxidant carotenoid, which has been gaining much interest because of its role in the prevention of cancer, is lycopene. Lycopene is a natural pigment present in some plants and microorganisms. Although present in many fruits and vegetables, the main sources in the western diet are from tomatoes and tomato products. Over the past ten years, there have been many studies showing evidence for the role of lycopene in chronic diseases. In a
recent meta-analysis of 72 studies, 57 studies have reported inverse association between tomato intake or circulating lycopene levels and the risk of several types of cancers (57). Thirty-five of the inverse associations observed were statistically significant and none have reported any adverse effects of high tomato intake or high lycopene levels. The majority of these studies have been epidemiological and have suggested that lycopene decreases the risk of breast, bladder, pancreas, lung and prostate cancer (28, 83, 114, 123, 148, 151, 159). Tissue culture, animal and human (experimental) studies are now beginning to be undertaken to investigate the role of lycopene in the prevention of cancer and the underlying mechanisms of action. The overall objective of this research was to study the role of lycopene on the proliferation of human prostate cancer cells, as well as the proliferation and differentiation of bone cells in a tissue culture model.
1.1 HYPOTHESIS AND OBJECTIVES

1.1.1 Rationale

Lycopene, one of the most prevalent carotenoid in the Western diet and the most abundant in human serum, has attracted much attention in recent years because a number of reports have associated high intakes of this carotenoid with reduced risk of certain chronic diseases. However, most of the evidence has come from epidemiological and animal studies and very few in-vitro studies have been reported. In addition, chronic diseases such as prostate cancer and osteoporosis are becoming increasingly prevalent in the world, and in order to understand the role of lycopene in these diseases not only in vivo and clinical studies, but tissue culture studies are also need to be undertaken.

1.1.2 Overall Hypothesis

Lycopene, acting as an antioxidant, will influence the growth and activity of human prostate cancer and osteoblast cells.

1.1.3 Overall Objective

To investigate the role of lycopene on the proliferation of human prostate LNCaP cancer cells and human osteoblast-like SaOS-2 cells in-vitro and to measure the alkaline phosphatase (ALPase) activity in SaOS-2 cells, as a biochemical indicator for cell differentiation.
1.1.4 Specific Objectives

To establish cell cultures of LNCaP and SaOS-2 cells for studying the following effects of lycopene:

1. The dose-dependent effect of lycopene on the proliferation of human prostate cancer LNCaP cells using a wide range of lycopene concentrations.

2. a) The dose-dependent effect of lycopene on human osteoblast-like SaOS-2 cell proliferation and,
   b) The effect of lycopene on alkaline phosphatase activity in SaOS-2 cells at 2 different stages of maturation.
Chapter Two

LITERATURE REVIEW
2. LITERATURE REVIEW

2.1. EPIDEMIOLOGY OF PROSTATE CANCER

Prostate cancer is the most common human malignancy and the second leading cause of cancer deaths among men in Western nations (28). Descriptive epidemiological evidence suggests that exposure to the environment such as diet, play an important causative role in the progression of prostate cancer (13, 52). Several autopsy studies have confirmed that small foci of prostate cancer exist in all races and geographic regions in 42-80% of males in their 8th decade (38). In North America, with a high incidence and death rate from prostatic carcinoma (CAP), these foci of cancer appear to be more voluminous, higher grade and multifocal compared to the low rates of CAP from countries such as China and Japan. These observations have led to the concept of a late stage environmental promoter that converts latent prostatic cancer into clinical CAP. The conversion of latent to clinical prostate cancer appears to take place at a rapid rate with the latent disease beginning in the 4th decade of life whereas clinical disease manifests in the 6th or 7th (38). Strategies to lower the incidence of CAP have therefore focused on delaying the progression of the latent stages of prostate cancer.

2.2. EPIDEMIOLOGY OF OSTEOPOROSIS

Osteoporosis is a silent disease defined as a condition characterized by reduced bone mass and disruption of bone micro-architecture. This results in increased bone fragility and increased fracture risk (55) thereby making osteoporosis an increasing public health problem. Some of the risk factors for osteoporosis include estrogen deficiency in postmenopausal women, inadequate level of testosterone in men, thin, small-boned Caucasian or Asian, age 50 or older,
smoking, alcohol, inactivity and diet (55, 134). Osteoporosis-related fractures, which cause pain and debilitation, occur in 1 of 2 postmenopausal women and 1 out of 4 men and have been shown to increase dramatically with age (55, 134). Although a number of drugs have been approved for the treatment and prevention of osteoporosis, these drugs have only been shown to put back only 1-8% of bone during 3 to 5 year clinical trials. In general, these drugs are accompanied by some undesirable side effects. Therefore, diet, offers an attractive alternative strategy for the prevention and perhaps reversal of osteoporosis. Nutrients important in the prevention of osteoporotic fractures act by increasing bone strength, through increasing bone mineral density or increasing the strength of the bone matrix in other ways (188). Dietary habit throughout life will affect bone strength, especially during the period for accretion of peak bone mass. In particular, evidence has been shown that calcium and vitamin D intakes are essential factors in the prevention and treatment of osteoporosis (36).

2.3. OXIDATIVE STRESS AND CHRONIC DISEASES

There is convincing evidence to suggest that Reactive Oxygen Species (ROS) generated both endogenously and also in response to external factors such as diet and lifestyle may be a significant factor in the etiology of several degenerative diseases including cancer, cardiovascular diseases, diabetes and osteoporosis (5, 6, 63, 64, 130, 157, 187). ROS are generated endogenously as byproducts of normal metabolic processes and cause oxidative damage to important biomolecules such as lipids, proteins and DNA, which if unrepaired, accumulates and leads to physiological attrition and an increased risk of several chronic diseases.

Oxidation of low density lipoproteins (LDL) is an important factor in atherosclerotic plaque formation leading to coronary heart disease (187). Intracellular protein oxidation results
in functional changes modulating cellular metabolism (5). Oxidative modification of DNA bases leads to mutation and altered gene function resulting in an increased risk of cancer (6, 93). Oxidative damage and mutations in mitochondrial DNA lead to mitochondrial dysfunctions resulting in a variety of pathological disorders (179). ROS have been known to induce the expression of a wide variety of transcription factors, such as NFkB, AP1 and oncogenes, such as c-fos and c-jun (62, 168, 182, 183). Furthermore, ROS also induce conformational changes in p53 protein, which mimics the mutant phenotype. Therefore, by inducing these alterations, ROS influence cell cycle mechanisms and ultimately lead to the development of cancer and other chronic diseases (62, 168, 182, 183).

Several biomarkers of oxidized lipids, protein and DNA have been found to be increased in the tissues and body fluids of patients with cancer and cardiovascular diseases, and during aging (5, 6, 63, 64, 130, 157, 187). Lipid peroxidation products have been found to increase in a variety of oxidative stress conditions (187). Premenopausal women with high mammographic tissue densities, a strong risk factor for breast cancer, were found to excrete more malondialdehyde, a lipid peroxidation product (16,17). Increased oxidation of proteins and DNA was also shown to be involved in the aging process (3, 156). Cancer tissues also show an increased amount of DNA adducts of oxidative damage (93, 110, 180).

2.4. PROSTATE CANCER

2.4.1 OXIDATIVE STRESS

Prostate cancer continues to be the most frequently diagnosed cancer in men in North America (40). Cellular exposure to chronic oxidative stress may be one possible etiologic factor
in the development of many cancers, including prostate cancer. Oxidative stress generates a wider variety of oxygen radicals that can attack DNA directly and result in the accumulation of potentially promutagenic oxidized DNA bases such as 8-hydroxydeoxyguanosine (8-Oxo-dG) (39). In addition, chronic oxidant stress may also result in lipid peroxidation and the subsequent generation of a range of reactive products that can damage DNA. Oxidative damage in aging tissues, including the prostate, is evidence by intracellular accumulation of peroxidized membrane lipoproteins, lipofuscin, and by the accumulation of and the oxidized DNA bases in both the nuclear and mitochondrial genome (39). Several epidemiological and laboratory observations illustrate the importance of oxidative damage in prostatic carcinogenesis. These observations are mainly derived from the association between dietary fat consumption (49) and prostate cancer coupled with recent data about oxidative biomarkers and glutathione-s-transferase activity in prostate tissue. A study by Fleshner et al.(51), was conducted to determine whether or not supplemental Vitamin E, a potent intracellular antioxidant, had an effect on the high-fat promoted growth of transplanted LNCaP cells in the athymic mouse. The results indicated that oxidative stress was an important mechanism by which dietary fat induced tumor growth and in turn, supplemental vitamin E as a preventative agent, was beneficial in inhibiting high-fat diet induced growth of human prostate cancer cells. Moreover, bio-oxidative properties of androgen (142), as well as putative beneficial properties of antioxidants also add credence to this hypothesis. Diets high in fat consumption may be associated with chronically high levels of androgens thus in part providing a biologically plausible reason for the association between fat and prostate cancer (64). Therefore, the prostate may be particularly vulnerable to oxidative stress because androgen activity may alter the prooxidant-antioxidant balance of prostate cells. Ripple et al. (142) demonstrated that physiologic levels of androgens increase mitochondrial
activity and oxidative stress in androgen-responsive human LNCaP prostate cancer cell lines. Oxidative stress also can be exacerbated by prostatitis. Acute and chronic inflammatory cells generate superoxide, hydrogen peroxide, and other ROS (10). Additionally, inflammatory stimuli such as cytokines and growth factors can induce the cyclooxygenase (COX) isoform, COX-2. COX-2 effectively oxygenates a wide array of fatty acid substrates, including arachidonic acid (132), producing prostanoid hydroperoxides that may themselves become a source of ROS (143, 165).

2.4.2. BIOLOGY OF HUMAN PROSTATE CANCER CELLS

Human prostate cancer cell lines are particularly difficult to establish. Most available human prostate cancer cell models either grow only in vivo as xenografts or do not exhibit features commonly seen in human prostate cancer, specifically prostate-specific antigen (PSA) expression (47). This deficiency is especially important since expression of PSA is not only a hallmark of the prostatic phenotype but has also become an invaluable marker of prostate cancer progression (23, 59, 150). Hence, well-established human prostate carcinoma cell lines are necessary in order to study the biologic behaviour of tumor growth and cell proliferation. Several cell lines, PC-3 and DU-145, which are of undifferentiated phenotype have been studied in previous literature. These two aggressive cell lines do not exhibit androgen sensitivity or the ability to secrete prostate specific proteins and thus, may be most commonly used for the most advanced forms of prostate cancer. On the other hand, the LNCaP cells, a less aggressive cell line than the undifferentiated lines, can be readily propagated in the laboratory by routine cell culture methods (70). They are a unique cell line derived from a supraclavicular lymph node metastasis of human prostate carcinoma, and they exhibit increased proliferation in response to
androgens in vitro (71). In concert with its androgen sensitivity, LNCaP cells synthesize and secrete prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), markers of differentiated prostate epithelial cell function (66). They also have a mutation in the androgen-binding domain of the androgen receptor and respond not only to androgens but also to antiandrogens, estrogens and progestins. Yet, despite this response to a broad range of hormones, LNCaP cells have been used widely as a model for prostate cancer research, both because of the lack of alternative models and because mutations in the androgen receptor gene are not uncommon in metastatic prostate cancer and some of these mutations increase the sensitivity of the cells to hormones, including androgens (112).

2. 5. OSTEOPOROSIS

2. 5. 1. OXIDATIVE STRESS

As the average age of the population rises, the incidence of chronic age-related diseases including osteoporosis also increases. There are several reports suggesting that oxidative stress and certain nutritional deficiencies may contribute to the aging process and to many age-related diseases (5, 176). Previous studies reported in the literature have shown that oxidative stress may influence bone cells, which in turn, may have an effect on osteoporosis. The cytotoxicity and free radical production induced by vanadium compounds were investigated in osteoblast and osteosarcoma cell lines in culture (31). It was shown that vanadate induced cell toxicity in osteoblast-like cells, resulting in induced cell death. In addition, a clinical study by Varanasi et al. (176) showed that a patient with severe osteoporosis had a DNA deletion in almost 50% of his mitochondria compared to another patient with less severe osteoporosis, which was later confirmed as the presence of systemic oxidative stress. These findings suggest a possible
relation between oxidative stress and accelerated bone loss. Similarly, oxidative stress has also been studied in relation to osteoclasts and it was demonstrated that ROS produced by osteoclasts stimulated bone resorption. However, the mechanisms of action of oxidative stress in the pathogenesis of osteoporosis, at present, are not fully understood and therefore require further studies.

2.5.2. BIOLOGY OF BONE CELLS

Bone turnover or remodeling is a tightly regulated coupling between the resorption of old bone by osteoclasts and the formation of new bone by osteoblasts. This process continues throughout life and is fundamental to normal bone physiology (109, 134). Osteoblasts and osteoclasts are the two major bone cell types that are responsible for the regulation of bone remodeling. Any disturbances in such coupling can lead to bone diseases (109, 134), with osteoporosis being the most prevalent. Although estrogen deficiency is now known to be an important cause of osteoporosis, other factors that cause the imbalance in the coupling between resorption and formation have also been identified, with oxidative stress being a potential candidate (176). This imbalance can either be due to decreased ability of osteoblasts to form bone and/or the increased ability of osteoclasts to resorb bone (109, 134). One of the factors that can mediate the coupling between osteoblasts and osteoclasts that has recently been discovered, is the osteoclast differentiation factor (ODF). ODF is synthesized by osteoblasts, which in turn stimulates osteoclasts to resorb bone by acting via its receptor RANK-OPG, which is on the surface of osteoclasts. Osteoprotegerin (OPG), a soluble receptor for ODF that is also synthesized by osteoblasts can bind ODF and thus inhibit osteoclast activity (69, 105). ODF and OPG, as coupling factors, are now gaining prominence for having important roles in the
pathogenesis of osteoporosis. Because it is not yet known how oxidative stress influences ODF and/or OPG, investigation of future studies need to be undertaken.

2.6. LYCOPENE

2.6.1 BIOCHEMISTRY AND PHYSIOLOGY

In general, carotenoids are made up of two tetraterpenes units joined by tail-to-tail bond. Most carotenoids have one or two ring structures (5 or 6 membered) formed by the cyclization of the end groups. In addition to carbon and hydrogen, they may also contain oxygen atoms. The color and other physical properties of carotenoids are defined by their unique chemical structures. Lycopene is a carotenoid, an acyclic isomer of β-carotene, and does not possess any vitamin A activity (139, 162). It is a 40 carbon atom, open straight chain hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds arranged in a linear array (Figure 2-1). Lycopene is one of the most potent antioxidants (41, 103, 108, 189) and its singlet oxygen quenching ability is twice that of β-carotene and 10 times that of α-tocopherol (41). As a polyene, it undergoes cis-trans isomerization induced by light, thermal energy or chemical reactions (113,193). Lycopene from natural plant sources exists predominantly in its all-trans configuration, the most thermodynamically stable form. In human plasma, lycopene is an isomeric mixture containing 50% of the total lycopene as cis isomers. All trans, 5-cis, 9-cis, 13-cis and 15-cis are most commonly identified isomeric forms of lycopene (27). The biological significance of these isomers of lycopene is unclear.
Empirical Formula: \( \text{CH}_{40}\text{H}_{56} \)
Molecular Weight: 536.85

**Figure 2-1. Chemical Structure of Lycopene**
2. 6. 2. DIETARY SOURCES OF LYCOPENE

Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals. Some red fruits and vegetables, including tomatoes, watermelons, pink grapefruit and pink guavas are rich sources of lycopene. However, the major source of lycopene in the North American diet is from tomatoes and processed tomato products, which accounts for more than 85% (Figure 2-2). Lycopene content in tomatoes depends on variety and ripeness. Processed tomato products, such as juice, ketchup, paste, sauce and soup, are all very rich sources of lycopene (139).

2.6.3. UPTAKE, METABOLISM AND TISSUE DISTRIBUTION OF LYCOPENE

Many factors influence absorption and hence, the bioavailability of dietary lycopene. The release of lycopene from the food matrix due to processing, presence of dietary lipids, heat induced isomerization from all trans to cis conformation enhance the bioavailability (160). Ingestion of cooked tomato juice in oil medium increased serum lycopene levels by three fold, whereas consumption of an equivalent amount of unprocessed juice did not influence its absorption (160). The presence of other carotenoids such as β-carotene has also been shown to increase lycopene absorption (80).

Estimation of daily intake of lycopene has been difficult to determine due to differences in intake estimates between food consumption databases. Daily intake values have ranged from 0.7 mg/day in Finland to 16.15mg/day in the U.S. Furthermore, in a more recent study (137), the average daily dietary lycopene intake levels were assessed by administering a food frequency questionnaire and was estimated to be 26 mg/day with processed tomato products accounting for
Figure 2-2. Lycopene Content of Common Fruits and Vegetables
50% of the total intake. In general, a direct relationship between dietary intake of lycopene and serum and tissue levels has been suggested. However, experimental data in support of this relationship is lacking.

Serum lycopene levels are affected by several biological and lifestyle factors. Fasting serum lycopene levels were found to be higher and more reproducible than post-prandial levels indicating an induced metabolic stress effect (138). Although blood lycopene levels do not differ significantly between men and women, levels in women were found to be affected by the phases of menstrual cycle with a peak during mid-lutea phase (53). Lifestyle factors such as smoking has also been shown to affect blood lycopene levels (19, 127, 146). A recent study demonstrated that there were no significant differences in serum lycopene levels between smokers and non-smokers. However, the serum lycopene levels fell by 40% with a 40% increase in lipid peroxidation in smokers immediately following smoking three cigarettes (138). Similarly, in-vitro exposure of fresh plasma to cigarette smoke depleted lycopene and several other lipophilic antioxidants (65). Alcohol consumption has also been found to decrease serum lycopene levels (19).

Lycopene is the most predominant carotenoid in human plasma and has a half-life of about 2-3 days (162). Owing to their lipophilic nature, lycopene and other carotenoids are found to concentrate in the LDL and VLDL fractions of the serum (162). Lycopene is known to accumulate in animal and human tissues, with the testes, adrenal gland, liver and prostate showing higher levels compared to other organs (28, 83, 114, 123, 148, 151, 159). The exact biochemical mechanism for the high concentration in these tissues is not clear. One hypothesis is that these tissues have a large number of lipoprotein receptors and lycopene is mainly transported through lipoprotein (83). Tissue specific carotenoid distribution suggests that certain
carotenoids may exert unique biological effects in some tissues but not in others. Adipose tissue has been suggested as a good marker for the assessment of body lycopene status (83, 114, 123, 148, 151, 159). Lycopene and other carotenoids have been recently identified in several body fluids. Thirty-four carotenoids, including 14 geometric isomers, and 2 lycopene oxidation products were recently reported in human milk (86). Lycopene and β-carotene were also found in human seminal plasma and their levels were lower in immuno-infertile men compared to normal individuals (121).

2.7. BIOCHEMICAL EFFECTS OF LYCOPENE

2.7.1 OXIDATIVE EFFECTS

Endogenous or exogenously generated ROS have been implicated in the pathogenesis of various human diseases (5, 6, 63, 64, 130, 157, 187). However, it has been demonstrated that consumption of carotenoid rich food is often associated with several health benefits. Most of the important health benefits are hypothesized to occur through their ability to protect against oxidative damage (103, 107). In-vitro studies have indicated that lycopene is an effective antioxidant and free radical scavenger (41, 103, 107). In particular, because of its high number of conjugated dienes, lycopene is known to be the most potent singlet oxygen quencher among natural carotenoids (41). In addition, lycopene was reported to inactivate hydrogen peroxide and nitrogen dioxide (14, 94). Mortensen et al. (108), using pulse radiolysis technique, demonstrated carotenoids’ ability to scavenge nitrogen dioxide (NO$_2^*$) and sulphonyl (RSO$_2^*$) radicals. In recent studies, lycopene was found to be at least twice as active as B-carotene in protecting lymphocytes against NO$_2^*$ radical induced membrane damage and cell death (14, 167). Although the in-vitro antioxidant properties of lycopene have been studied extensively, the in-
vivo antioxidant effects and interaction with host and other dietary antioxidant defenses are only beginning to be investigated now. Lycopene is extremely hydrophobic and is most commonly located within cell membranes and other lipoprotein components. Therefore, it is expected that interactions of lycopene and ROS are to be more profound in a lipophilic environment. Lycopene was shown to protect human LDL against photosensitized oxidative damage (119). Skin lycopene was utilized preferentially over β-carotene during ultraviolet light exposure in humans, suggesting a preferential role of lycopene over β-carotene in mitigating oxidative damage in tissues (141).

2.7.2 NON-OXIDATIVE

Although the antioxidant properties of lycopene have been the main focus of research, recent studies have shown other non-oxidative related biological effects of lycopene. Gap junctional communication between cells was shown to be influenced by carotenoids such as B-carotene, canthaxanthin and lycopene, suggesting a potential basis for their protective effects towards cancer development (139). Another recent study reported differential dose related effects of β-carotene and lycopene on gap junctional communication in rat liver. The ability of carotenoids to influence gap junctional communication (between adjacent cells) and their singlet oxygen quenching abilities or antioxidant properties are thought to be independent of each other (158). Lycopene has also been shown to act as a moderate hypocholestremic agent mediated through its inhibitory effect on 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) reductase, the rate limiting enzyme in cholesterol synthesis (54). The liver drug metabolizing enzyme cytochrome P-450 2E1 activity was also shown to be modulated by lycopene (9).
2.8. STUDIES OF LYCOPENE IN CELL CULTURE IN-VITRO

Given the possible etiologic role of oxidative injury in the carcinogenic process, several agents have been suggested as defenses against cellular oxidant injury. These include those agents with antioxidant properties such as lycopene, as well as those that specifically target enzymes known to be involved in the production of ROS. Supporting evidence for the biological role of lycopene comes from tissue culture studies using various cell lines, animal models to study anti-tumorogenic properties of lycopene and epidemiological studies done with control and at risk populations (32, 57, 59, 101, 104).

Tissue culture studies have provided the first evidence for molecular and biochemical effects of lycopene in normal and malignant cell lines (11, 14, 32, 89, 101, 172). To date, the work has mainly concentrated on the antitumorogenic activity of lycopene. Countryman et al. (32) showed that lycopene caused approximately 40% inhibition of cell growth in human leukemia cell lines. Moreover, when lycopene was added with retinoic acid, which has been known to cause cellular differentiation, it potentiated the effects of lycopene (11). Lycopene has also been shown to inhibit endometrial (Ishikawa), mammary (MCF-7) and lung (NCI-H226) human cancer cell proliferation and suppressed insulin-like growth factor-I-stimulated growth (89). In a C3H/10T1/2 mouse embryo fibroblast cell line, lycopene inhibited the methylcholanthrene induced malignant transformation (12). In mouse embryo fibroblast cells, lycopene was also shown to enhance the expression of connexin-43, a gene encoding major gap junction protein, and thereby upregulated gap junction communication and acted as an anti-carcinogen (194, 195). Improved survival and suppression of lipid peroxidation in carbon tetrachloride (CCL₄)-exposed rat hepatocytes was observed after lycopene treatment (87). The retinoids and α-tocopherol showed none to very little effects in the same system. It was
hypothesized that lycopene might have arrested $G_0/G_1$ cell cycle phase by suppressing carcinogen-induced phosphorylation of regulatory proteins such as p53 or Rb antioncogene by non-oxidative mechanisms (101). In an in-vitro study using Raji cells (Epstein-Barr virus genome carrying lymphoblastoid cells), lycopene inhibited 12-O-tetradecanoylphorbol-13-acetate induced Epstein-Barr virus activation almost as much as $\beta$-carotene (172) and in the J774A/1 macrophage cell line, lycopene was shown to act as a hypocholesterolemic agent by inhibiting the HMG-CoA reductase pathway (54).

2.9. ANTIOXIDANTS AND HUMAN PROSTATE CANCER CELLS IN-VITRO

To date, very few studies have been performed on the effects of antioxidants on human prostate cancer cells in-vitro. However, one study conducted in 1999 (186), showed that cells treated with beta-carotene significantly slowed the in-vitro growth rates in three different human prostate cancer cell lines: PC-3, DU-145 and LNCaP when incubated for 72hrs. Pastori et al. (126) investigated the effect of lycopene alone or in association with $\alpha$-tocopherol on the growth of two different human prostate carcinoma cell lines: DU-145 and PC-3. They found that lycopene alone was not a potent inhibitor of prostate carcinoma cell proliferation. However, the simultaneous addition of lycopene together with $\alpha$-tocopherol, at physiological concentrations, resulted in a strong inhibitory effect of prostate carcinoma cell proliferation, which reached values close to 90%. In a more recent study conducted in 1999, Xu et al. (191) examined the uptake and possible effect of lycopene on human prostate cancer LNCaP cells in tissue culture. They were able to show that lycopene solubilized in micelles was stable for at least 96hrs under standard cell conditions and lycopene was taken up by LNCaP cells and reached a plateau at approximately 12h. They also observed that neither the micelles themselves nor lycopene
solubilized in micelles in the cell culture media produced cytotoxicity or inhibition of cell proliferation in LNCaP human prostate cells. Accordingly, tissue culture studies involving prostate cancer cells and various antioxidants are still limited and need to be further investigated.

2.10. ANTIOXIDANTS AND OSTEOBLASTS IN-VITRO

Previous literature has indicated that antioxidants may play a role in chronic diseases such as osteoporosis. Evidence from epidemiological studies revealed that specific antioxidants including vitamin C, E and beta-carotene may reduce the risk of osteoporosis (101, 106, 154). While there exists one epidemiological study that positively correlates bone mineral density with the dietary carotenoid, beta-carotene (154), there have been almost no in-vitro studies examining the role of lycopene on osteoblasts.

A number of studies have demonstrated the role of other antioxidants and their effect on bone cells, particularly those in mouse osteoblast cells. Very few studies have investigated human osteoblast cells. One study by Hitomi et al. (68), investigated the effect of L-ascorbic acid 2-phosphate in a human osteoblast cell line, HuO-3N1. They showed that the activity of alkaline phosphatase (ALPase) was significantly enhanced when it was cultured in the presence of L-ascorbic acid 2-phosphate. The level of ALPase activity increased approximately 3-fold without any effect on either the morphology or growth rate. In another study, Park et al. (122), showed that retinol and β-carotene inhibited the proliferation and DNA synthesis in mouse osteoblastic MC3T3-E1 cells in a dose-dependent manner. Retinol induced differentiation of the MC3T3-E1 cells by increasing alkaline phosphatase activity dose dependently in a range from 1 to 100nM. Beta-carotene increased alkaline phosphatase activity in a dose-related manner in a range from 0.1 to 5μM. Alpha-carotene, canthaxanthin and lycopene also inhibited cell
proliferation at 1uM and increased alkaline phosphatase activity, but less potently so than β-carotene. Thus, although a number of studies on the effect of antioxidants on osteoblasts have been reported, there has not been any report involving the more potent antioxidant, lycopene. More importantly, our studies are the first to show the effects of lycopene on human osteoblast cells in-vitro.
Chapter Three

EFFECT OF LYCOPENE ON THE PROLIFERATION OF HUMAN PROSTATE LNCaP CANCER CELLS IN CULTURE
Introduction

Prostate cancer is a commonly diagnosed cancer (40) and the second most common cause of cancer deaths among North American men (124). Although genetic factors and age are important determinants of risk, there is increasing evidence in epidemiological, experimental and metabolic studies, suggesting that environmental exposures including diet, play an important role in the progression of prostate cancer. More recently, nutritional intervention studies have focused on possible agents with the potential to prevent prostate cancer. The role of reactive oxygen species (ROS) and oxidative damage to biomolecules is one of the main foci of recent research related to cancer and chronic diseases. Oxidative stress has been widely postulated to be involved in the causation and progression of several chronic diseases including prostate cancer (4, 5, 62, 63, 130, 187).

Dietary antioxidants, which inactivate ROS and provide protection from oxidative damage (4, 5, 62, 63, 130, 187), are being considered as important preventive strategic molecules for cancer and other chronic diseases. These antioxidants possess the ability to quench singlet oxygen free radicals, and thus protect against cellular oxidative damage (35). A number of epidemiologic, laboratory and clinical investigations suggest that antioxidants may be beneficial in preventing the progression of prostate cancer. Vitamin E, the major intracellular antioxidant in cell membranes inhibits lipid peroxidation and has been demonstrated to have a wide range of anti-cancer properties (35, 45, 177), including protection against carcinogenesis and inhibition of tumor progression. The growth of a wide range of in-vitro and in-vivo cell lines from a variety of human primary cancers have been shown to be inhibited by vitamin E (45, 150, 177). Similarly, selenium, an antioxidant trace mineral essential for the activity of glutathione
Peroxidase has also been shown to prevent the incidence of prostate cancer (67, 74, 174, 175, 192).

Carotenoids, a class of yellow to deep-red pigments, often found in many fruits and vegetables also have antioxidant properties and play a role in the prevention of chronic diseases such as cancer (143). More specifically, lycopene, a predominant carotenoid naturally present in tomatoes and other fruits, is a potent antioxidant and the most significant free radical scavenger (41). These antioxidant properties of lycopene have raised interest in the tomato as a food with potential anticancer properties (162). Lycopene has been shown to concentrate in prostate tissues (40). Among the various carotenoids present in the prostate gland, lycopene was observed to have highest levels (83, 159). Epidemiological data have indicated that lycopene may be beneficial in preventing prostate cancer (27). One large comprehensive dietary study showed that intakes of β-carotene, α-carotene, lutein and β-cryptoxanthin were not significantly associated with a reduction in prostate cancer risk, but high lycopene intake resulted in a statistically significant risk reduction of 21% (72). Combined intake of tomatoes and tomato-based products, accounting for 82% estimated lycopene intake, was associated with a 35% reduced risk of total prostate cancer. For the more advanced or aggressive prostate cancers, which are more likely to cause death, the apparent protective effects were even stronger (58). This unique association with more advanced disease suggests that lycopene supplementation may be beneficial in preventing the progression of prostate cancer. Although the effect of lycopene on the growth of several cancer cell lines have been studied (89), very little evidence is reported with prostate cancer cells of human origin.

The objective of the present study was to measure the effect of lycopene on the growth and proliferation on human prostate cancer LNCaP cells.
Materials and Methods

Cell Culture

The human prostate cancer cell line, LNCaP, was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were maintained in a 75cm² flask in RPMI-1640 medium containing 2 mM glutamine, 10 ug/ml streptomycin, and supplemented with 10% fetal bovine serum and antibiotics and subcultured weekly. For experimental use, the cells were plated into 12-well culture dishes with a density of 5 x 10^5 cells/ml. Forty-eight hrs after subculture, the unattached cells were washed off and fresh medium with different concentrations of lycopene was added and the cells were further cultured for 24, 48, 72, 96 hrs under standard cell culture conditions. Respective vehicle control of similar dilution for each concentration of lycopene was also evaluated. All cell cultures were maintained in a CO2 incubator at 37°C with a 5% CO2 atmosphere.

Lycopene Sample Preparation

A new water-dispersible lycopene (LycoRed Natural Products Industries Ltd., Israel) in an appropriate vehicle was used for this study. A 1x10^-3M stock solution of lycopene was prepared fresh and diluted before use in the RPMI-1640 medium and added to the cultures at final concentrations of 10^-9M – 10^-4M. The corresponding vehicle cultures received the carrier solvent at the same dilutions as the lycopene.

Cell Proliferation

The cells were cultured for 48 hrs before treatment with lycopene. After incubation periods of 24, 48, 72 and 96 hrs, floating cells were washed off and the remaining cells were
detached from the wells with Trypsin-EDTA and collected for cell counting. Cell proliferation was measured by direct cell counts with an hemacytometer.

**Statistical Analysis**

For in-vitro growth assays, replicates of two experiments each in triplicate were performed. Results are expressed as mean ±SEM. Statistical differences were analyzed by using a one-way ANOVA followed by the Dunnet multiple comparison test and Student's t-tests (Instat, v. 2.02, GraphPad Software, San Diego, CA); *p*<0.05 was considered statistically significant.

**Results**

The figures illustrate the dose-dependent effect of lycopene concentrations (10^{-9} M – 10^{-5} M) on the growth of LNCaP cells after various times of incubation. After 24hrs of incubation, lycopene dose-dependently inhibited the proliferation of LNCaP cells [Fig. 3-1: One-way ANOVA; n=6, F=3.150, *p*<0.05]. This treatment with lycopene resulted in a significant decrease in LNCaP cell number of 20.4% at a lycopene concentration of 10^{-7} M (P<0.05). After 48hrs of treatment, lycopene dose-dependently inhibited the proliferation of LNCaP cells [Fig. 3-2: One-way ANOVA; n=6, F=11.27, *P*<0.001], and at lycopene concentrations of 10^{-9} M, 10^{-6} and 10^{-5} M, cell numbers were significantly reduced by 3.8, 24.4 and 25.6% (*P*<0.05), respectively. Similarly, the effect of lycopene for 72hrs incubation caused a dose-dependent inhibition of LNCaP cell growth [Fig. 3-3: One-way ANOVA; n=6, F=54.51, *P*<0.0001] and also, lycopene concentrations of 10^{-8}, 10^{-6} and 10^{-5} M significantly reduced the growth of LNCaP cells by 15.2, 31.9 and 25.5% (*P*<0.05), respectively. After an incubation of 96hrs, lycopene was
Figure 3-1. Dose-response effect of lycopene on LNCaP cell number after 24 hr incubation. Values are means±SEM, n=6
**Figure 3-2.** Dose-response effect of lycopene on LNCaP cell number after 48hr incubation. Values are means±SEM, n=6. * P<0.05; significantly different from vehicle-control.
Figure 3-3. Dose-response effect of lycopene on LNCaP cell number after 72hr incubation. Values are means±SEM, n=6. * P<0.05; significantly different from vehicle-control.
observed to significantly decrease cell numbers by 42.8% at $10^{-6}$ M and 30.8% at $10^{-3}$ M (P<0.05) and in addition, the effect of lycopene was also dose-dependent in inhibiting LNCaP cell proliferation after 96hrs of treatment [Fig. 3-4: One-way ANOVA; n=6, F=297.5, p<0.0001].

The inhibitory effect of lycopene at each of these concentrations was significantly (p<0.05) greater than their respective vehicle controls. Cell growth was completely inhibited with $10^{-4}$ M lycopene concentrations at all incubation periods (data not shown).

**Discussion**

Prostate cancer has become an important public health problem in the Western world. Recent interest in lycopene has focused on its antioxidant properties and its association with decreased risk of chronic diseases such as cancer and cardiovascular diseases. Lycopene has been found to be concentrated in the prostate and other body tissues such as liver, adrenals and adipose tissue (27, 151). The presence of lycopene in the prostate (27) has suggested the hypothesis that lycopene may have direct effects within the prostate and contribute to the reduced prostate cancer risk observed in individuals who consume high amounts of tomato-based, lycopene rich foods (58).

The results from this experiment provide evidence that lycopene can inhibit the growth of human prostate cancer cells and that the growth inhibition and cytotoxic effects of lycopene depends on the concentration, as well as on the duration of exposure to lycopene. In this study, when cells were exposed to lycopene for extended periods of time, cell growth and viability were significantly decreased. The effect of lycopene was dose-dependent and was most significant, having the greatest effect at higher concentrations. With lycopene concentrations of $10^{-4}$ M, LNCaP cell proliferation was completely stopped at all incubation periods (data not shown).
Figure 3-4. Dose-response effect of lycopene on LNCaP cell number after 96hr incubation. Values are means±SEM, n=6. * P<0.05; significantly different from vehicle-control.
However, this effect was also seen when cells were treated with the vehicle-control at the same lycopene concentration. Thus, the toxic effect may not be a direct result of the lycopene itself.

Although the exact mechanism by which lycopene reduces the growth of human prostate cancer cells is not fully understood, it can be suggested that the decrease in LNCaP cell proliferation by lycopene may in part be due to direct effects of lycopene on cellular processes controlling cell growth and protection against oxidative damage caused by highly reactive oxygen species (ROS). ROS are thought to be involved in stimulating the activity of cell-cycling genes and in particular, in enhancing cancer cell proliferation. In turn, there is also evidence that cancer cells produce more ROS therefore, providing for a greater continuous proliferation of cancer cells (30). In addition, because ROS react with the cell membrane, they are likely to cause damage to normal, healthy cells causing cell death. This leads to an environment where cancer cells proliferate without competition as they may acquire resistance to the effect of ROS. Yet, lycopene is able to capture these free radicals caused by ROS and thus, reduce the effects caused by them (Fig. 3-5). However, hypothesized mechanisms specifically on the reduction of prostate cancer cell growth are limited and remain speculative. Therefore, additional studies are needed to determine the mechanism of action of lycopene on LNCaP cells.

Lycopene, which is present in high amount in tomatoes and tomato-derived products, has been shown in several in-vitro culture studies to have a growth inhibition on mammary, lung and endometrial cancer cell proliferation (89). Additional roles of lycopene such as the induction of the gap-junction communication between cells through the increased synthesis of connexin 43 were found (194). This effect has been associated with the ability of lycopene to restore the loss of gap junctions occurring in malignant processes. To date, much of the research involving tissue culture work that has investigated the anti-carcinogenic effects of lycopene have been on
Figure 3-5. Working Hypothesis for Human Prostate Cancer Cells
various forms of cancer, other than the prostate. One in-vitro study has demonstrated a strong inhibitory effect of prostate carcinoma PC-3 cell proliferation by the simultaneous addition of lycopene together with α-tocopherol at physiologic concentrations (126). A more recent study by Xu et al. (191) reported that lycopene contained in micelles was taken up by LNCaP cells and was stable up to 96 hrs under standard cell culture conditions. Neither the micelles themselves nor lycopene solubilized in micelles at concentrations up to 10ug/mL in the cell culture media produced inhibition of cell proliferation. The significant results obtained from this study differ from previous reported studies possibly as a result of a more precise and sensitive approach to counting cell number and more importantly, because of a new form of lycopene treatment. Due to its extreme hydrophobicity, lycopene is insoluble in tissue culture media. As well, lycopene is highly unstable and degrades rapidly when dissolved in organic solvents or aqueous systems containing organic co-solvents and exposed to light and/or air. Therefore, this study was the first to use a water-dispersible form of lycopene as a convenient method for delivering lycopene to the cells.

In conclusion, the observed results suggest that the effect of lycopene could be due to its antioxidant properties and is responsible for the inhibition of human prostate LNCaP cancer cell growth. These results have implications with respect to the use of lycopene in the chemoprevention of human prostate cancer.
Chapter Four

THE EFFECTS OF LYCOPENE ON THE GROWTH AND DIFFERENTIATION OF SaOS-2 CELLS
Introduction

Bone remodeling is a continuous process that occurs throughout life. Osteoblasts and osteoclasts are the two major types of cells that are primarily responsible for bone formation and bone resorption, respectively. The excessive bone loss that characterizes the pathogenesis of bone diseases such as osteoporosis results from abnormalities in this bone remodeling cycle (22, 109, 134). Oxidative stress induced by ROS has been suggested to play an important role in affecting osteoblast and osteoclast activity. A recent study by Varanasi et al. (176) speculated about the involvement of free radical production and oxidative stress in bone loss and in the pathogenesis of osteoporosis. They suggest that this was indirectly due to the fact that most anti-resorptive agents have antioxidant activity and destroy superoxide anions. Several epidemiological studies have suggested that antioxidants including vitamins C, E and beta-carotene may play a role in the prevention of osteoporosis (102, 106, 154). Very few studies have been carried out in-vitro. However, the study by Park et al. (122) showed that retinol and beta-carotene dose-dependently inhibited the proliferation of the osteoblastic cell line MC3T3-E1 cells, as well as DNA synthesis. Retinol induced differentiation of the MC3T3-E1 cells by increasing alkaline phosphatase activity dose dependently, in a range from 1 to 100nm while beta-carotene increased alkaline phosphatase activity in a dose-related manner in a range from 0.1 to 5μM. However, the exact cellular and molecular mechanisms involved are virtually unknown.

Lycopene is one of the most potent carotenoid antioxidants naturally present in many plant foods and abundant in tomatoes. It is not synthesized in humans but is obtained through strict dietary consumption. Numerous studies have proven that lycopene is an effective
antioxidant and a potent free radical scavenger. Although there are a large number of studies correlating the antioxidative properties of lycopene in the prevention of cancers, there are presently no data that correlates the health benefits of lycopene in relation to prevention of diseases resulting from the disturbances of bone remodeling equilibrium such as osteoporosis. The aim of the study was to investigate the effect of lycopene on human osteoblastic cell line SaOS-2 proliferation, as well as the effect of cell differentiation on the effect of lycopene.

Materials and Methods

Cells

The human cell line, SaOS-2, was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were maintained in a CO₂ incubator at 37°C with a 5% CO₂ atmosphere and subcultured weekly in a 75cm² flask in Ham's F-12 medium supplemented with 28mM HEPES buffer, pH 7.4, 1.1mM CaCl₂, 2mM glutamine, 1% antibiotic-antimycotic solution, and 10% fetal calf serum. For experimental use, the cells were plated into 12-well culture dishes at a density of 5 x 10⁵ cells/ml. Twenty-four hours after plating, different concentrations of lycopene were added and the cells were further cultured for 24, 48, 72, 96 and 144 hrs and processed for assay as described below. Respective vehicle control of similar dilution for each concentration of lycopene was also evaluated.

Lycopene Sample Preparation

Lycopene in an appropriate vehicle was used for this study (water-dispersible lycopene, LycoRed Natural Products Industries Ltd., Israel) in an appropriate vehicle was used for this study. A 1x10⁻³M stock solution of lycopene was prepared fresh before use in supplemented
Ham's F-12 medium, diluted further and added to the cultures at final concentrations of $10^{-8}$M – $10^{-4}$M. The corresponding control cultures received the vehicle at the same dilutions as the lycopene.

**Cell Proliferation**

The cells were cultured for 24 hrs before treatment with lycopene. After a further incubation period of 24, 48, 72, 96 and 144 hrs, the cells were washed with PBS and the remaining cells detached with Trypsin-EDTA and collected for cell counting. Cell proliferation was measured by direct cell counts with an hemacytometer.

**Alkaline Phosphatase (ALPase) Assay**

SaOS-2 cells were plated in 12-well dishes at a density of $5 \times 10^5$ cells per well in medium containing vehicle (alcohol) in the absence of dexamethasone (less differentiated SaOS-DEX cells) or in medium containing 10nmol/L dexamethasone (more differentiated SaOS+DEX cells), as was previously described (140). The medium was changed every 2-3 days. The cells were maintained under these culture conditions for 1, 3 or 6 days. The medium was then replaced with the respective medium containing various concentrations of lycopene or the vehicle-control. The incubation was then continued for a further 24, 48 and 72hrs. At the end of incubation, the reaction was stopped by removing the medium, followed by washing, scraping, and sonicating the cells in 0.5ml of Triton X-100. The samples were kept frozen until needed for ALPase assays. ALPase activity was measured by a modified method of Lowry (95). The release of $p$-nitrophenol from 10mM $p$-nitrophenyl phosphate in a buffer containing 1.0 mM MgCl$_2$-6H$_2$O in 2-amino-2-methyl-1-propanol, pH 10.3, was measured at 30°C. The reaction was stopped after 5
min with 0.5N NaOH and the absorbance determined at 405nM using a microtiter plate reader (Multiscan; Flow Laboratories, McLean, VA). The ALPase activity was expressed as nanomoles per min/mg protein.

Protein

Protein was determined using a commercially available Coomassie Blue dye reagent (Biorad, Mississauga, Ontario) of Bradford (18) and bovine serum albumin as the standard.

Statistical Analyses

For in-vitro growth assays, replicates of two experiments each in triplicate were performed. Results are expressed as mean ±SEM. The graphs were plotted using Prism version 4. For statistical analysis, a one-way ANOVA followed by the Dunnet multiple comparison test and Student's t-tests (Instat, v.2.02, GraphPad Software, San Diego, CA) were used; p<0.05 was considered statistically significant.

Results

The data show that lycopene treatment of SaOS-2 cells resulted in a significantly higher cell number than the corresponding vehicle treatment as follows: after 24, 48, 96 and 144 hrs, by 57.4% (P<0.05) (Fig. 4-1a), 50.0% (P<0.01) (Fig. 4-1b), 25.4% (P<0.05) (Fig. 4-1d) and 21.01% (P<0.05) (Fig. 4-1e) respectively, all at a lycopene concentration of 1x10^-6 M. Lycopene at 1x10^-5 M caused a significant increase in SaOS-2 cell proliferation by 35.4% (P<0.05) (Fig. 4-1a) after 24hrs, 20.3% (P<0.001) (Fig. 4-1d) after 96 hrs of incubation and by 32.4% (P<0.05) (Fig. 4-1e) after 144 hrs of incubation. Cell growth was completely inhibited at 10^-4 M lycopene.
Figure 4-1a. Dose-response effect of lycopene on SaOS-2 cell number after 24hr incubation. Values are means±SEM, n=6. * P<0.05; significantly different from vehicle-control.
Figure 4-1b. Dose-response effect of lycopene on SaOS-2 cell number after 48hr incubation. Values are means±SEM, n=6. * P<0.05; significantly different from vehicle-control.
Figure 4-1c. Dose-response effect of lycopene on SaOS-2 cell number after 72hr incubation. Values are means±SEM, n=6
Figure 4-1d. Dose-response effect of lycopene on SaOS-2 cell number after 96hr incubation. Values are means±SEM, n=6. * P<0.05; significantly different from vehicle-control
Figure 4-1e. Dose-response effect of lycopene on SaOS-2 cell number after 144hr incubation. Values are means±SEM, n=6. * P<0.05; significantly different from vehicle-control
concentrations at all incubation periods. However, this effect was also seen when cells were treated with the vehicle-control at the same lycopene concentration indicating possible toxicity caused by the vehicle rather than by lycopene. At lower concentrations of 1x10^{-8}M and 1x10^{-7}M, it was observed that lycopene did not have any significant effect on SaOS-2 cell proliferation after incubation times of 24, 48 and 72hrs (Figs. 4-1a-c).

SaOS-2 cell differentiation was determined by measuring the differentiation marker, ALPase, in the presence and absence of both Dex and lycopene. Figures 4-2 to 4-7 show the dose-response effects of lycopene on ALPase activity. Results showed that Dex, an inducer for osteoblastic differentiation (140, 145), enhanced alkaline phosphatase activity (ALPase) in a time-course manner indicating differentiation of the cells. SaOS-2 cells cultured in the absence of Dex (SaOS-Dex) showed low ALPase activity and lycopene had an overall inhibitory effect on ALPase. However, one exception was lycopene at a concentration of 1x10^{-6}M after 3 days which increased ALPase after 72 hrs by 36.1 % (P<0.05) (Fig. 4-3c) and after 3 days with 1x10^{-7}M after 24 and 72 hrs (data not significant) (Fig. 4-3a, c). Addition of lycopene at other times did not have any effect on SaOS-2 cells (Figs. 4-2a,b,c to 4-4a,b). In the presence of Dex (SaOS+Dex), there were no significant effects observed when lycopene was added after 1d and further incubated for 24, 48 and 72hrs (Figs. 4-5a,b,c). At the highest concentration of 1x10^{-5}M, lycopene was shown to significantly increase ALPase by more than 90% after 3d addition and 24hrs incubation (P<0.05) (Fig. 4-6a) and by 85.4% after 6d and 24hrs incubation (P<0.0001) (Fig. 4-7a). There was also a slight but not significant increase after 3d and 72hrs of incubation. Although lycopene at 1x10^{-6}M showed that a significant ALPase was not detected after 24 hrs exposure, when lycopene was added after 3d, there appeared to be a significant increase in ALPase after the 48 and 72 hr incubation periods by 55.8% and 50.2%, respectively (P<0.05).
Figure 4-2a. Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 1d) - 24hrs incubation. Values are mean ±SEM, n=6
Figure 4-2b. Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 1d) - 48hrs incubation. Values are mean ±SEM, n=6
Figure 4-2c. Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 1d) - 72hrs incubation. Values are mean ±SEM, n=6
**Figure 4-3a.** Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 3d) - 24hrs incubation. Values are mean ±SEM, n=6.
Figure 4-3b. Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 3d) - 48hrs incubation. Values are mean ±SEM, n=6
Figure 4-3c. Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 3d) - 72hrs incubation. Values are mean ±SEM, n=6
Figure 4-4a. Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 6d) - 24hrs incubation. Values are mean ±SEM, n=6
Figure 4-4b. Effect of lycopene on ALPase activity on SaOS-Dex cells (addition of lycopene after 6d) - 48hrs incubation. Values are mean ±SEM, n=6
**Figure 4-5a.** Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 1d) - 24hrs incubation. Values are mean ±SEM, n=6
Figure 4-5b. Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 1d) - 48hrs incubation. Values are mean ±SEM, n=6
Figure 4-5c. Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 1d) - 72hrs incubation.
Values are mean ±SEM, n=6
Similarly, after 6d addition, lycopene significantly stimulated ALPase by 39.7% (P<0.05) (Fig. 4-7a) when incubated for 24 hrs. In the same way, there was an increase after 1d (24 and 48 hrs incubation); after 3d and 24 hrs incubation and after 6d and 48 hrs incubation (data not significant). Finally, lycopene at the lowest concentration of 1x10^{-7}M showed a significant increase in ALPase when added after 3d by more than 90% (P<0.001) and incubated for 72 hrs and when added after 6d and incubated for 24 and 48 hrs by 39.1% (P<0.001) and 25.6% (P<0.05), respectively (Figs. 4-7a, b). When lycopene was added after 3d and incubated for 24 and 48 hrs, there was a non-significant increase at the same concentration. On the other hand, similar to the SaOS-Dex cells, lycopene concentration at 1x10^{-7}M was also inhibitory when added at day 1 for 48 hrs and at 1x10^{-5}M when added after 1d and 72 hrs and after 3d for 48 hrs. The effect of lycopene on ALPase activity was found to be dependent upon the concentration of lycopene, time of incubation and the stage of cell differentiation.

**Discussion**

The results of this study show that cell growth and ALPase in SaOS-2 cells are significantly affected by lycopene. In particular, lycopene was found to stimulate proliferation in SaOS-2 cells. A lycopene concentration of 10^{-6}M was found to be the optimum effective concentration in stimulating cell proliferation at all incubation periods. SaOS-2 cells did not show any response to 10^{-5}M concentrations of lycopene after 24 and 48 hrs of incubation. However, after 96 and 144 hrs, there was a significant increase in cell number, suggesting that the lycopene may have needed a longer incubation period in order for an effect to be observed. Lycopene at concentrations of 10^{-8} and 10^{-7}M did not have any significant effects on ALPase activity and cell number in SaOS-2 cells added at all time points studied. It can be concluded
Figure 4-6a. Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 3d) - 24hrs incubation. Values are mean ±SEM, n=6.
* P<0.05; significantly different from vehicle-control
Figure 4-6b. Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 3d) - 48hrs incubation. Values are mean ±SEM, n=6. * P<0.05; significantly different from vehicle-control.
Figure 4-6c. Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 3d) - 72hrs incubation. Values are mean ±SEM, n=6. * P<0.05; significantly different from vehicle-control
Figure 4-7a. Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 6d) - 24hrs incubation. Values are mean ±SEM, n=6.
* P<0.05; significantly different from vehicle-control
Figure 4-7b. Effect of lycopene on ALPase activity on SaOS+Dex cells (addition of lycopene after 6d) - 48hrs incubation. Values are mean ±SEM, n=6.
* P<0.05; significantly different from vehicle-control
that lycopene at low concentrations does not have an effect on the growth and differentiation of human osteoblast cells. Results indicated that at a lycopene concentration of $10^{-4}$ M, SaOS-2 cell proliferation was completely inhibited at all incubation periods (data not shown). However, this effect was also seen when cells were treated with the vehicle-control at the same lycopene concentration. Thus, the toxic effect may not be a direct result of the lycopene itself.

From the results of this study, we can speculate a working hypothesis depicted in Figure 4-8. From this figure, it can be thought that ROS produced by oxidative stress acts to stimulate bone resorption. At the same time, this would decrease bone formation by osteoblasts and would eventually lead to bone loss. However, a potent antioxidant carotenoid such as lycopene, can be thought to mitigate the oxidative effects of these ROS and therefore inhibit the formation of ROS-secreting osteoclasts, decreasing bone resorption and in turn, stimulate bone formation. Our results are supported by findings of Cortizo et al. (31), indicating a possible role of oxidative stress in the acceleration of bone loss. Thus, Cortizo et al. showed that both MC3T3-E1 mouse osteoblast and UMR106 osteosarcoma cell lines induced by vanadate, resulted in an incremental increase in ROS and TBARS, which was then inhibited by vitamin E. Similarly, another study using cortical bone cells of elderly patients (176) suggested free radical mediated damage to proteins and DNA caused by mitochondrial DNA deletions (dmtDNA). As a result of this DNA damage, cells experienced delays in certain phases of the cell cycle to allow time for DNA repair, a diminished rate of mitosis or apoptosis. Osteoblasts possessing substantial dmtDNA were likely to be eliminated at a faster rate than cells without such deletions leading to the loss of osteoblasts, which in turn, is also representative of a diminished rate of bone formation eventually leading to osteoporosis.
Figure 4-8. Working Hypothesis for SaOS-2 Cells
Although the exact mechanism by which lycopene stimulates the growth of human osteoblast-like cells is not fully understood, it can be suggested that the increase in SaOS-2 cell proliferation by lycopene may be a result of its inhibition of oxidative damage caused by highly reactive oxygen species. Indeed the studies of Cortizo et al. showed that osteoblasts do produce ROS. Because ROS react with the cell membrane, they are likely to cause damage to normal, healthy cells causing cell death or apoptosis. Hence, lycopene acting as an antioxidant, will counteract the effect of ROS, which could ultimately lead to the prevention of osteoporosis. However, additional studies are needed to determine the mechanism of action of lycopene on SaOS-2 cell proliferation.

In this study, it also appeared that lycopene, in general, had an overall inhibitory effect on the ALPase of human osteoblastic SaOS-Dex cells. Without the addition of Dex, the osteoblasts were less likely to show high levels of ALPase activity and when compared to the vehicle, lycopene indicated a greater reduction in ALPase at all concentrations. However, in the SaOS+Dex cells, at higher concentrations of lycopene and after longer periods of incubation, there was a greater opportunity for the lycopene to reverse the inhibitory effect seen with SaOS-Dex cells and thus increase ALPase. These results suggest that the effect of lycopene is differentiation-stage dependent with an inhibition of ALPase at less-differentiated stages (-Dex) and early time points of differentiated stages (+Dex), and with a stimulation at more differentiated stages (at days 3 and day 6) of SaOS+Dex cells. Thus, exposure of SaOS-2 cells to lycopene after they have undergone primary cell differentiation, stimulate ALPase. One possible explanation could be that as the cells differentiate, a specific population of cells emerges, which are receptive to lycopene. Moreover, the more differentiated cells (SaOS+Dex)
were observed to respond more effectively to the effects of lycopene, especially at a concentration of $10^{-6}$M.

Osteoporosis is one of the most common chronic diseases affecting people of all nations, primarily due to the aging of the world’s population (100). Bone mass is influenced by many factors such as genetics, nutrition, exercise, hormonal state and lifestyle. Among these, nutrition is gaining attention as an important factor for normal bone growth and preventing osteoporosis. Oxidative stress is also recognized now as an important factor influencing bone health. Recent research has focussed on the use of antioxidants including lycopene to mitigate the damaging effects of oxidative stress. Furthermore, other non-oxidative mechanisms such as cell cycling genes and gap junction communication may also be used to explain lycopene action in-vitro. In this study, lycopene, a carotenoid antioxidant, was evaluated for its effect on the growth and differentiation of human SaOS-2 cells and therefore, the results are important as they may have implications in the pathogenesis, treatment and prevention of osteoporosis.

It is thought that periods of osteoblast phenotype development are defined by the existence of a reciprocal and functionally coupled relationship between proliferation and differentiation and through the sequential and strictly regulated expression of genes (90). The extent to which this expression of genes is sequential and mutually exclusive rather than concomitant is in part dependent upon their position in the osteoblast lineage. Therefore, with the addition of lycopene, proliferation and differentiation of human osteoblast cells had an inverse effect. Lycopene acted by stimulating the growth of SaOS-2 cells and causing cell numbers to increase significantly. In response, ALPase was also initially decreased when lycopene was incorporated into the cells as compared to the vehicle-control. Moreover, when DEX was added to the cells to induce cell differentiation, a similar effect was observed with a
decrease in ALPase activity, except at higher concentrations and longer incubation periods when the opposite effect was observed. However, the differences were that the ALPase concentrations were much higher and in some cases, almost twice than that of the SaOS-DEX cells.

Glucocorticoids such as dexamethasone, promote osteoblast differentiation, reflected both by increased numbers and the size of the bone nodules (90, 145). The phenotypic properties are accompanied by changes in the expression of growth, extracellular matrix and bone-related genes at the level of transcription or mRNA stabilization. Increased alkaline phosphatase levels occur reproducibly in these progenitor cell populations. In this investigation, the possibility of lycopene functioning as a stimulator for cell proliferation in human osteoblast-like cells is most evident, yet the mechanism of induction of differentiation by lycopene remains to be clarified in future experiments.

More specifically, the study of osteoblasts is essential for understanding bone formation and regulation. It has recently been hypothesized that the osteoblast may be the central cell through which bone resorption as well as bone formation is mediated (75). In support of this, one study showed that culture medium that was recovered from isolated osteoblasts and exposed to bone resorbing agents, was also stimulatory for isolated osteoclasts (145). Thus, osteoblasts are thought to mediate the systemic signals for the recruitment and activity of osteoclasts and so it is essential for these ‘bone-forming’ cells to be tightly coupled with the ‘bone-resorbing’ cells so that a balance is maintained.

Only a limited number of studies have been reported in the literature examining the effect of lycopene on osteoblast cells. In 1995, Park et al. (122) showed that lycopene inhibited cell proliferation and increased alkaline phosphatase activity in a clonal mouse osteoblastic
MC3T3-E1 cell line. However, the inhibitory effect of lycopene was less than for β-carotene. They also showed that retinol and β-carotene inhibited the proliferation of MC3T3-E1 cells and induced differentiation of the cells by increasing ALPase activity. Another study demonstrated the effect of ascorbic acid 2-phosphate on the HuO-3N1 human osteoblast cell line and found that alkaline phosphatase activity was significantly enhanced with the addition of this antioxidant (68). A more recent study by Lochter et al. (92), also showed that parathyroid hormone (PTH) triggered retraction of osteoblast cells was inhibited by antibodies of PTH and oxygen radical scavengers. Further studies are therefore required to understand the role of oxidative stress and antioxidants, in particular, lycopene on the activity of human osteoblast-like cells.

In conclusion, the observed results suggest that the antioxidant properties of lycopene could be responsible for the stimulation of human SaOS-2 cell growth and decrease in alkaline phosphatase activity. Yet it appeared that depending on the time of incubation and concentration of lycopene, these results were reversed, especially for the SaOS+Dex cells. The concentration of lycopene used in this study was within the range of physiological concentration seen in humans. Consequently, these data provide a possible role underlying the beneficial effects of lycopene in bone formation and may have potential implications with respect to its use in the chemoprevention of chronic diseases including osteoporosis.
Chapter Five

GENERAL DISCUSSION
5. GENERAL DISCUSSION

As the concern for a relationship between environmental factors and the development of chronic diseases continues to grow, the role of dietary components in the causation and prevention of chronic diseases is becoming a major area of research. To develop the link between diet and chronic diseases, several types of research efforts have been established. Firstly, numerous epidemiological studies including case-control, cohort and correlation studies have been conducted, which provide evidence to a possible link between certain components of the diet and the development of cancer/cardiovascular diseases (CVDs) at different sites. Secondly, based on these epidemiological findings, human and animal experiments have been further conducted to confirm the hypotheses generated by epidemiological studies and to possibly explain the mechanisms of action of various components in the diet. Doll and Peto (42) concluded that dietary modification may reduce death due to all forms of cancer up to 35% in North America.

While several dietary components such as total energy, fat and animal protein have been shown to have a positive correlation with the development of major forms of cancer, others such as dietary fibers, vitamin E, selenium and beta-carotene have resulted in negative correlations. Based on epidemiological and experimental evidence, dietary guidelines have been recommended to combat chronic diseases. These suggest an increased consumption of plant foods, including fruits, vegetables, cereals and legumes and decreased consumption of meat and meat products. In addition to the traditional nutrients, plant foods also contain a variety of phytonutrients including antioxidant phytochemicals such as the carotenoid. Oxidative stress is now recognized as one of the major etiological factors responsible for the causation of cancer.
Focus of recent chemopreventive strategies is to decrease the damaging effect of oxidative stress by increasing the consumption of antioxidants such as the carotenoid. Epidemiological studies provide evidence in support of carotenoids in the prevention and treatment of cancer and other chronic diseases.

Of the more than 600 known carotenoids in nature, only about 20 are found in human plasma and tissues (8). The principal carotenoids found in humans are α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene.

Tomatoes and tomato products are the major sources of dietary lycopene in North America. Recent studies have indicated that consuming tomatoes and tomato-based products reduces the risk of several chronic diseases including cancer and cardiovascular diseases (162). Lycopene has been identified as being responsible for this beneficial effect. Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals. The anticancer properties of lycopene are thought to be related to: i) singlet oxygen quenching ability and protection against membrane damage caused by free radicals (14, 41), ii) selective blockage of the cell cycle (89), and iii) enhancing gap junction communication in neoplastic cells (158).

More specifically, prostate cancer is becoming of great concern as it is the 2nd most common cause of cancer deaths in North American men (40, 124). There is increasing evidence-epidemiologic, experimental and metabolic- suggesting that oxidative stress may play an important role in the development and progression of prostate cancer. It is also theorized that lycopene may deter prostate cancer by means of its antioxidant function and its ability to snuff out free radicals and DNA. Another chronic disease of major public health importance is osteoporosis. Oxidative stress has likewise been implicated in this disease and an interest in the use of antioxidants for its prevention. Despite the growing evidence for the effect of lycopene in
disease prevention, further studies are still needed to address the exact role and mechanism of its action.

The overall objective of this study was to investigate the role of lycopene on the proliferation of human prostate LNCaP cancer cells and human osteoblast-like SaOS-2 cells in-vitro. To carry out this objective, a series of four experiments were conducted. The specific objective for each experiment was as follows: i) to establish that both the LNCaP and SaOS-2 cells grew under standard cell culture conditions. This study was carried out by collecting and counting cell numbers and establishing a standard cell growth curve; ii) to study the effect of lycopene on human prostate cancer LNCaP cell growth. Initially, a concentration range of $10^{-6} - 10^{-4} \text{M}$ was used, which was then expanded to a wider range of lycopene concentrations ($10^{-9} - 10^{-7} \text{M}$); iii) to evaluate the effect of a wide range of lycopene concentrations ($10^{-8} - 10^{-4} \text{M}$) on human osteoblast-like SaOS-2 cell proliferation; and iv) to measure the ALPase activity, a marker of osteoblast differentiation in the SaOS-2 cells treated with or without Dex and with lycopene concentrations of $10^{-7} - 10^{-5} \text{M}$. In all the experiments, a water-dispersible lycopene was used that facilitated its incorporation in the tissue culture media.

Throughout this investigation, lycopene was shown to possess biological effects on human prostate cancer and osteoblast-like cells. The results of this study indicated that lycopene acts by 1) inhibiting the growth of LNCaP cells and 2) stimulating the proliferation of SaOS-2 cells and decreasing ALPase activity. These results provide new insight into the possible contribution of lycopene in cell culture studies, which, in the past, have not yet been studied in-depth.

A lycopene concentration of $1 \times 10^{-5} \text{M}$ had the greatest effect on the proliferation of LNCaP prostate cancer cells demonstrating a significant inhibition in cell number after 48, 72
and 96hrs of incubation. The overall effect of lycopene was dose-dependent with lower concentrations of lycopene showing little or no effect. In human SaOS-2 osteoblast cells, lycopene was shown to have a stimulatory effect on cell growth with the most ideal results seen with lycopene at $1 \times 10^{-6}$M. In addition, longer exposure time to lycopene showed significantly greater effects on the stimulation of SaOS-2 cells.

Oxidative stress has been widely postulated to be involved in the causation and progression of several chronic diseases (5, 6, 63, 64, 130, 157, 187). Some endogenous or exogenous activity has been implicated to cause the generation of reactive oxygen species (ROS). In turn, dietary antioxidants, which inactivate ROS and provide protection from oxidative damage are being considered as important preventive strategic molecules.

In this particular study, lycopene, acting as a dietary antioxidant, was suggested to play a role in the chronic disease process and affect the growth of human prostate cancer and osteoblast-like cells. For human prostate cancer LNCaP cells, the working hypothesis (Fig. 3-5) is that certain forms of oxygen species are linked to the development of prostate cancer (143) and thus, an increase in cell proliferation of prostate cancer cells. However, antioxidants such as lycopene could reduce prostate cancer cell growth by modulating the effects of cellular processes by its ability to trap peroxyl radicals and to quench $O_2^-$, which can be seen by the reduction in LNCaP cell proliferation with the addition of lycopene to these cells. In the case of the human osteoblast-like SaOS-2 cells (Fig. 4-8), oxidative stress is hypothesized to cause a decrease in osteoblast cells (31), which are the bone-forming cells necessary for the bone remodeling process. Similarly in this process, lycopene, acting as a potent peroxyl radical scavenger, is thought to inhibit the free radical chain reaction and therefore, protect cell loss by regulating
osteoblast activity, which may be important in the prevention of osteoporosis due to oxidative stress.

In the first experiment, lycopene inhibited the growth of LNCaP prostate cancer cells in a time (24, 48, 72 and 96hrs) and dose-dependent manner ($10^{-9} - 10^{-5}$M). There was a significant reduction in cell growth, especially when cells were incubated for longer periods and at higher concentrations. This decrease in cell proliferation observed in lycopene treated cells may be due to induction of $G_1$ cell cycle arrest as a result of multiple genes. The loss of cell cycle control in $G_1$ has been implicated in tumor development and proliferation (33). Hence, it can be speculated that in this study, lycopene may be acting to suppress cell-cycling genes and in turn, may be affecting cell growth, particularly in the $G_1/S$ stages of the cell cycle. On the other hand, LNCaP cell inhibition could also be due to induction of apoptosis. Apoptosis is one of the important pathways through which chemopreventive and chemotherapeutic agents inhibit the growth of cancer cells (26). However, the exact mechanisms of cell number reduction in human prostate cancer cells are not yet fully understood and therefore require future studies.

Lycopene stimulated SaOS-2 cell proliferation most significantly after 24, 48, 96 and 144hrs incubation with $1 \times 10^{-6}$ and after 96 and 144hrs with $1 \times 10^{-5}$M lycopene. Lycopene concentrations of $1 \times 10^{-7}$ and $1 \times 10^{-8}$M did not show any effect on SaOS-2 cell growth. On the other hand, alkaline phosphatase activity was compared using two different stages of osteoblastic differentiation, SaOS+DEX and SaOS-DEX. Cells were grown for 1, 3 and 6 days before lycopene concentrations of $10^{-7}$, $10^{-6}$, and $10^{-5}$M were added and then incubated for a further 24, 48 and 72hrs. SaOS+DEX enhanced the level of alkaline phosphatase activity with or without the addition of lycopene compared to the SaOS-DEX cells. In general, in the presence of lycopene, there was an inhibition of ALPase activity on the SaOS-DEX cells, particularly after
24 and 48hrs of incubation (after 1d addition). After lycopene addition after 3 days, lycopene had no effect on ALPase activity and further, after 6 days, there were even less differences observed. However, after longer incubation times, ALPase activity was seen to increase, especially with $1 \times 10^{-6}$M lycopene concentrations.

The results of the second experiment indicate a possible role of lycopene in bone formation. It has been hypothesized that osteoblast cells contain basal levels of oxidative stress (31), resulting in the accumulation of ROS and causing lipid and DNA damage. Moreover, this leads to the speculation about the involvement of free radical production and oxidative stress in bone loss and also in the pathogenesis of osteoporosis, which is indirectly supported by the fact that most anti-resorptive agents including nitric oxide donors, have antioxidant activity and destroy superoxide anions (29, 98). Hence, the effect of lycopene on human SaOS-2 cells can be thought to be involved in mitigating ROS formation by inhibiting bone resorption and promoting bone growth, which would then lead to an overall decrease in bone loss.

Although a previous study has looked at the effect of lycopene in mouse osteoblast-like cells and reported that lycopene induced osteoblastic differentiation, these findings are inconsistent with our study. However, so far, previous literature has emphasized in-vitro studies of cellular and molecular biology concentrating on rodent cells and various other animal systems. Yet it has been noted that species differences need to be accounted for (140) and therefore, analyses of the skeletal action in human systems are necessary, in particular, the effect of lycopene in osteoblasts of human origin.

The effect of dexamethasone on the differentiation of osteoblast cells was investigated and it was observed that SaOS+DEX cells expressed higher ALPase activity compared to the corresponding less differentiated SaOS-DEX cells. Furthermore, the effect of lycopene on both
the differentiated and undifferentiated SaOS-2 cells showed that lycopene decreased or had no effect on ALPase activity in the SaOS-DEX cells and in the SaOS+DEX cells, lycopene initially decreased ALPase activity and then increased, depending on the concentration of lycopene and time of incubation. This suggests that lycopene effects on markers of osteoblast differentiation such as ALPase depend on the stage of differentiation, culture conditions, as well as the concentration of lycopene used.
Chapter Six

SUMMARY AND CONCLUSIONS
6. Summary and Conclusions

6.1. Summary of the research

Research was undertaken with the overall objective of studying the effect of lycopene on the growth of human prostate cancer and osteoblast cells in tissue culture. Water dispersible lycopene sample was used throughout the study. Cell proliferation was measured by counting the number of cells after specific periods of incubation. Cell differentiation was determined by measuring ALPase activity. A series of three experiments were performed. Based on the results, the following conclusions were made:

1. Growth of LNCaP cells was significantly reduced in the presence of water-dispersible lycopene solutions compared to their vehicle controls. These effects were dependent on the dose of lycopene and time of incubation.

2. Lycopene had stimulatory effects on the growth of SaOS-2 cells in a dose-dependent manner; and

3. The stage of cell differentiation and maturity were observed to influence the effect of lycopene.

6.2. Implications of the Study and Future Research

This study suggests that lycopene may be acting at a cellular level to play a role in human prostate cancer and osteoblast-like cells in-vitro. Inhibition of human prostate cancer cells by lycopene may have important implications in the dietary management of prostate cancer and likewise, in view of the evidence that ROS may be important in osteoporosis, lycopene
seems to have a significant role in regulating osteoblast activity and eventually, the incidence of osteoporosis. This is the first report of the effects of a water-dispersible lycopene on human prostate cancer LNCaP cells and the first in osteoblasts of human origin.

Suggested future studies include: 1) investigations on specific mechanisms including the effect of lycopene at different stages of the cell cycle, 2) measuring the uptake of lycopene by the cells, 3) comparison of other dietary antioxidants such as Vitamin C and E alone and in addition with lycopene, and 4) further investigations on the effects of feeding lycopene to tumor bearing mice (in-vivo).
Chapter Seven

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7. REFERENCES


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