Prostate Cancer, Phytochemicals, Long-Chain Omega-3 Fatty Acids and Oxidative Stress

Master Thesis in Clinical Nutrition

by

Magnhild Kverneland

Supervisors:

Rune Blomhoff

Anette Karlsen

Department of Nutrition

Institute of Basic Medical Sciences, Faculty of Medicine

UNIVERSITY OF OSLO

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Magnhild Kverneland

Summary

Oxidative stress may be connected to cancer. The present work has addressed several biomarkers of oxidative stress and antioxidant status in a group of prostate cancer (PC) patients compared to a group of healthy men. Results indicate higher oxidative stress among the PC patients than the reference population.

Epidemiological studies have suggested that dietary factors modulate risk of PC. Administration of dietary compounds, with minimal side effects, to prevent, slow or reverse PC, could be attractive as an addition to conventional PC treatment.

The current work presents interim results (27 patients of 102) of a clinical study investigating whether diagnosed, localised PC may benefit from a food intervention. The study is the "Prostate cancer, phytochemical and PUFA intervention study" (PFPI). PC patients were randomized in tomato, multidiet and control groups. The tomato group patients received 200 – 250 grams tomato products daily, while the multidiet group participants were given pomegranate and grape juices, green and black tea, selenium, omega-3 and soy in addition to the same amount of tomatoes as the tomato group, for a period of three weeks. Prostate specific antigene (PSA) and biomarkers of oxidative stress were determined in blood and urine before and after the intervention.

The intervention compliance was excellent. Biomarkers of oxidative stress determined in plasma and urine before and after the intervention, revealed no effects on oxidative stress. The tomato group experienced a non-significant trend of PSA reduction compared to the control group ($p=0.072$). The multidiet group showed a slightly weaker trend of reduction ($p=0.114$), while statistically significant PSA reduction ($p=0.045$) was detected over the three weeks' intervention when comparing the intervention groups to the control group.

Contents

List of abbreviations

AA	Ascorbic acid
AMACR (P504S)	alpha-Methylacyl CoA Racemase (P504S)
BER	Base exision repair
EPA	Eicosapentaenoic acid
CRP	C-reactive protein
CYP	Cytochrome P450
DHA	Docosahexaenoic acid
DHAA	Dehydroascorbic acid
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
D-ROMs test	Diacron reactive oxygen metabolites test
COX-2	Cyclooxygenase 2
ELISA	Enzyme-Linked ImmunoSorbent Assay
FRAP	Ferric reducing ability of plasma
GGT	Gamma glutamyl transferase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione or Glutathione disulfide
GST	Glutathione–S- transferase
HCl	Hydrocloric acid
HEL	Hexanoyl-epsilon-lysine
HGPIN	High-grade PIN
HNE	4-hydroxy-2-trans-nonenal
H_2O_2	Hydrogen peroxide

HPLC	High performance liquid chromatography
IGF-1	Insulin-like growth factor 1
mFRAP	Modified FRAP
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
$OH^{\bullet}$	Hydroxyl radical
8OHdG	8-hydroxy-2'-deoxyguanosine
p53	Protein 53 or Tumor protein 53
PC	Prostate cancer
PFPI	Prostate cancer, phytochemical and PUFA intervention study
PIN	Prostatic intraepithelial neoplacia
PSA	Prostate specific antigene
PUFA	Poly unsaturated fatty acids
RBC	Red blood cells
RNA	Ribonucleic acid
RNASEL	Ribonuclease L (also known as hereditary PC 1, HP1)
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RS	Reactive species
SELECT	Selenium and Vitamin E Cancer Prevention Trial
SOD	Superoxide dismutase
TAA	Total ascorbic acid
TRX	Thioredoxin
XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1

1. Introduction and background

1.1 Prostate cancer

1.1.1 Epidemiology, incidence, mortality and trends

Prostate cancer (PC) is the most common cancer among Norwegian men (1). The Norwegian rate of mortality from PC is among the highest in the world. In 2004 there were 1074 deaths while in 2006 1042 Norwegian men died from PC. There is some evidence that recent age-adjusted mortality trends are plateauing or beginning to decline (2). Internationally, PC is the second highest cancer related death cause in men in the western countries (3).

Due to the ageing male population, the disease is estimated to increase by 40% towards 2020. In the absence of competing causes of death, approximately one in eight men currently develop PC in their lifetime (defined as ages 0-74) (2).

PC incidence and mortality rates are much lower in Japan (8 deaths per 100000 in 2002) and other Asian countries than in Western countries (32 deaths per 100000 in 2002 in Norway) (4). However, focal non–invasive PC has been shown to be as common in Japan as in the Western countries, and migration from Japan to California shows 4-fold increase in risk of clinically diagnosed PC (5). This indicates that lifestyle and especially diet influence the development of aggressive disease. Recently, a rising trend in incidence and mortality has been seen in Asia. Adaptation of Western lifestyle and diet may explain this development (6).

Due to the availability of prostate specific antigen (PSA) measurement there was a considerable increase in age-adjusted incidence of PC from 1988 to 1992, in symptomatic and asymptomatic PC. In Norway PSA testing was available from 1989 (7), but systematic screening has not been introduced to date. In 2007 4391 new cases of PC were diagnosed in Norway, an increase of 15% from 2006. The increase is

explained by PSA testing. PC screening by use of PSA is controversial because it detects small cancers (0.4 mL) which are clinically insignificant in otherwise healthy non-symptomatically men. The effect of PSA screening on PC mortality is not clear (8).

1.1.2 Risk according to age, race and family

Age is the most profound risk factor of all cancers, and this is also true for PC. Seventy percent of men above eighty years have asymptomatic PC, and are likely to die with it rather than from it. Men of African etnicity have higher risk of developing PC than men of other races (9).

Family history, including first degree relatives with PC has been recognised as a risk factor, especially for those who are diagnosed with PC before 60 years of age. Genetic factors have been saught, but no strong single gene mutations like BRCA in breast cancer have been found. However, polymorphisms in the Ribonuclease L (RNASEL) (also known as hereditary PC 1, HPC1) gene have been connected to hereditary PC and to disease severity. Other PC susceptibility genes have been identified and the functions of the gene products are related to inflammation and oxidative stress (9). Recently, a Swedish research team documented a cumulative effect of five different SNPs (single nucleotide polymorphisms) on risk of PC, and this result has been confirmed in other populations (10).

1.1.3 Alcohol, tobacco, overweight and inflammation

Intake of alcoholic beverages is not proven to influence on the risk of PC. Tobacco use is not an established cause of PC (11), but there are indications of a worse prognosis for smokers with PC (12).

Obesity, diabetes type 2 and metabolic syndrome increase worldwide. Low grade inflammation, oxidative stress and hormonal changes accompany these conditions, and these are factors that may influence on the pathogenesis of PC. Obesity in early

life (< 30 yrs) seems to have a strong inverse association with PC risk in middle or old age (13). There are some indications that diabetes protect against low-grade PC, while obesity seems to increase the risk of aggressive disease (13;14). Obesity is associated with increased levels of Insulin-like growth factor 1 (IGF-1), and elevated IGF-1 may increase the risk of PC. IGF-1 is a growth factor associated with inflammation. Chronic low grade inflammation may be a consequence of obesity, and has been associated with enhanced tumor growth. However, how the epidemic of obesity influences the risk of PC needs to be investigated further (15).

Chronic inflammation is one probable factor in the development of PC. Chronic inflammation may lead to cell death. Furthermore, accumulation of inflammatory cells and release of pro-inflammatory mediators including cytokines, chemokines, prostaglandines and reactive oxygen species (ROS) are related to cancer development (16). In addition to stimulating the inflammation, many of these mediators may stimulate cell growth, angiogenesis and mutations. The cytokine production is often disturbed or changed in malign conditions and this imbalance may induce disease progression (17).

Assessing biomarkers of inflammation in relation to PC, such as C-reactive protein (CRP), interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF alpha) would be of great interest, but it is beyond the scope of this master thesis.

1.1.4 Physical activity

Proposed mechanisms regarding physical activity and its possible effect on PC are modulation of hormone levels, obesity prevention, enhanced immune function and oxidative stress reduction. Physical activity may increase the production of sex hormone-binding globulin, resulting in lower levels of free testosterone (18). When androgen levels are severely depressed, as in castration, PC is not observed. Athletes have been shown to have lowered levels of testosterone (19). Most likely, exercise has an indirect effect on PC risk by preventing obesity. The evidence for the

association between physical activity and PC risk is however inconsistent and too week to draw conclusions (11).

Most research has focused on physical activity in cancer prevention, but exercise could be of importance in detection, quality of life, rehabilitation and survival after diagnosis (18).

1.1.5 The prostate gland

The walnut sized prostate gland is a part of the male reproductive system and lies just below the urine bladder **(Figure 1.1).** The urethra and the seminal ducts fuse while passing through the gland. The prostate produces an alkaline liquid which constitutes 10-30% of the seminal fluid. During ejaculation, both smooth muscle in the gland and muscles of the pelvic floor contract to expel semen. Benign prostate hyperplasia is a condition in elderly men where enlargement of the prostate may affect urination.

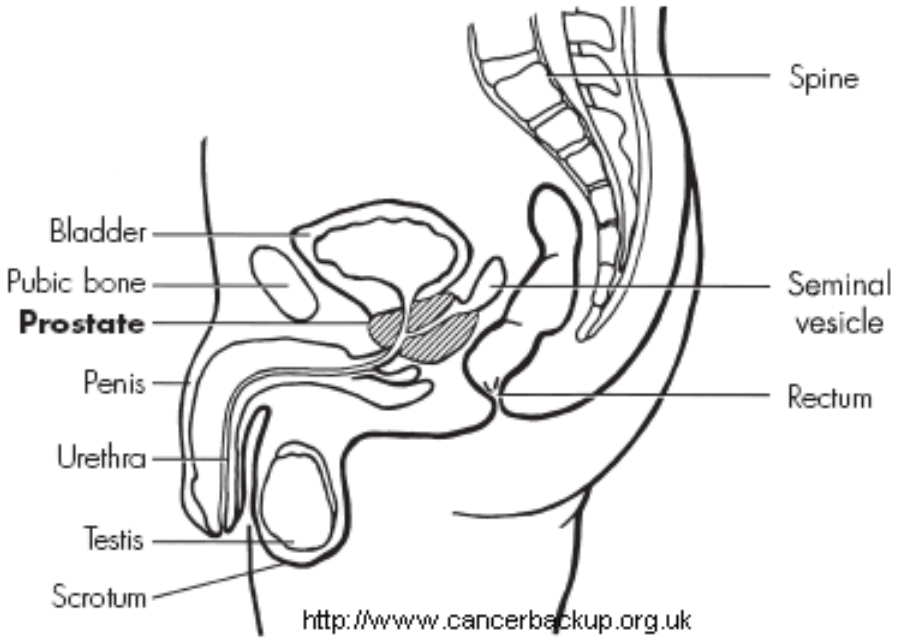


Figure 1.1 The prostate gland and male abdomen (20)

Free testosterone is reduced to 5-alpha-dihydrotestosterone (DHT) in the prostate tissue by the cytoplasmic enzyme 5-alpha reductase. The growth of prostate is regulated by testosterone and the more potent DHT. High levels of male sex

hormones in plasma are known to increase the risk of PC. But from recent findings, DHT level within the prostate gland seems to be of greater importance (21).

The 5-alpha reductase inhibitors are drugs that block the convertion of testosterone to DHT. Results from the Prostate Cancer Prevention Trial suggest that Finasteride, a 5-alpha reductase inhibitor is capable of slowing the development of early PC. Reduce, a study examining effects of the more effective 5-alpha reductase inhibitor Dutasteride, will terminate this year and it is expected that the results from this study may change the current practice of early PC treatment (22).

1.1.6 PC pathology

PC is a heterogeneous disease with multiple causes and variable progression. It takes 10 – 30 years to develop, and the sequence of events is only partly understood. Cancer is often divided into four stages: initiation, clonal expansion, progression and metastasising.

Cancer may be initiated by unrepaired damage to deoxyribonucleic acids (DNA) that switch on a proto-oncogene or switch off a tumor suppressor gene. Damaging mutations to genes that control the cell cycle, growth, apoptosis and survival make the cell able to divide with a damaged DNA and the cell is more likely to acquire more mutations.

Mutations to receptors of paracrine signaling molecules increase the independence of the transformed cells. Mutated cells will divide faster than their healthy neighbors, resulting in a clonal expansion.

The progression of PC may be ongoing for many years from the initiation until the tumor is recognized by the host. The neoplastic cells grow in number and size, and mutations accumulate. Factors that increase cell proliferation, decrease cell apoptosis and stimulate to cell growth are factors that may aggravate and accelerate the development of PC. Growth factors such as IGF and androgens may be important for progression.

For the cancer cells to metastasise, ie migrate out of the origin tissue, angiogenesis is required, which is yet another gained property of the cancer cells.

1.1.7 PC initiation and development

The initiation of PC is not understood in detail. Inflammation of the prostate has been suggested to increase the risk of later malignancy **(Figure 1.2)**. Inflammation of prostate includes bacterial acute and chronic inflammation and conditions of extensive pelvic pain with no known infection. Inflammatory cells and excretion of cytokines, chemochines, prostaglandins and reactive oxygen and nitrogen species (ROS/RNS) may be related to increased risk and progression of PC (23). A condition of cellular growth, PIA (Proliferative inflammatory atrophy) frequently accompanies the inflammation.

An early stage of PC is frequently associated with the loss of glutathione-S-transferase (GST) P1 and has been termed prostatic intraepithelial neoplacia (PIN) **(Figure 1.2)**. PIN is a neoplastic transformation of the secretory epithelium lining prostatic ducts and acini, with no extension through the basal lamina. High-grade PIN (HGPIN) is characterized by cells with more neoplastic traits than low-grade PIN, and has several features similar to PC. There is much evidence pointing to HGPIN as a frequent precursor of PC. The incidence and extent of PIN seems to increase with age (24).

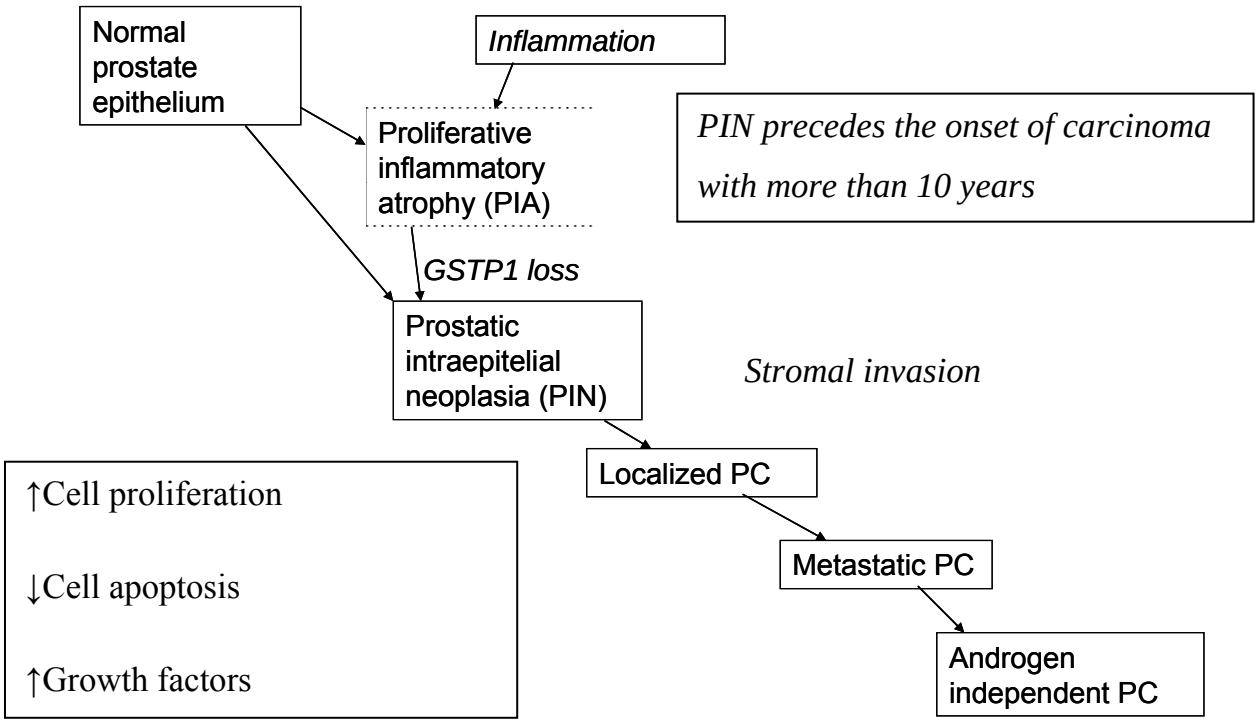


Figure 1.2 Stages in PC development

1.1.8 PC classification and treatment

Risk assessment of PC is based on three categories, TNM classification, Gleason score and PSA level.

The TNM system (Classification of Malignant Tumours), is a system of classifying all cancers, and is routinely used for PC. The T refers to the extent of primary tumor; the N describes the status of regional lymph nodes and the M presence or not of distant metastases. An adenocarcinoma of the prostatic gland is classified as T1c when carcinoma cells are present on needle biopsy, while T3a describes a carcinoma with unilateral local extra prostatic extension.

The Gleason system, defined in the 1960s and thus an established grading system of PC, is based on histological patterns of carcinoma cells in H&E-staining. The histological scores range from 2 to 10, and the score has been related to tumor size, margin status, and pathologic stage. It has also been linked to clinical end-points such as clinical stage, progression to metastatic disease, and survival (25).

Based on this system patients with localised disease are categorised in low, intermediate and high risk patients. In general radiotherapy is the preferred option for aggressive disease and surgery for less aggressive tumours. For patients in the low risk group active surveillance is an alternative to radical treatment

PSA is a small glycoprotein that is produced in the epithelial cells of the prostate gland and is a constituent of semen which functions as a protease to make the semen more viscose, improving the sperm mobility and nutrient absorption. Small amounts of PSA are normally present in the blood. Damage to the basal layer and membrane separating the ducts from blood and lymph in the prostate may produce a leakage of PSA into the circulation. Increased PSA in blood is not specific for PC, but can be due to other diseases involving the epithelial cells of the prostate gland, but PSA in diagnosed PC is recognised as a good indicator of disease progression.

Howver, PSA is not an opimal cancer specific biomarker, and although sensitive for PC it is also a biomarker of other prostate conditions. Therefore there has been a search for more specific biomarkers, and one promising may be the alpha-Methylacyl CoA Racemase (P504S) or AMACR (26). PSA increase indicates disease progression, while PSA decrease is interpreted as disease retreatment.

In patients with elevated PSA levels biopsy is mandatory for diagnosis. In many patients PC is a slowly progressive disease while in others the clinical behaviour is very aggressive with early metastatic spread and eventually leading to death. It remains a challenge to distinguish between mild and aggressive cases at time of diagnosis. If radical local treatment is recommended, computer-assisted prostatectomy or radiotheraphy (including brackytheraphy) are the two options. Radical prostatectomy seems to improve survival compared to a ‘wait and see’ strategy. Addition of brackytheraphy to standard external radiotheraphy with installation of radioactive sources in the prostate gland, a higher dose is delivered to the tumour with no increased side-effects (27).

Some patients experience increasing PSA levels after the initial treatment which indicates progressive, metastatic disease **(Figure 1.2)**. Taxan-based chemotheraphy may be the next treatment which has documented effect on survival in patients with verified metastatic disease (28).

Androgen deprivation therapy, drugs that block androgen receptors in the whole body is used in advanced or metastatic disease, and 70-80 % responds initially (29). Unfortunately, advanced PC always develops into androgen independent disease **(Figure 1.2)** and the patients usually progress within 2 – 3 years.

1.2 Oxidative stress

Oxygen is vital for all aerobe organisms on earth. It is the electron acceptor in the electron transport chain of the mitochondria, and thus indispensable in energy production of aerobes. Its ability to accept and donate electrons gives oxygen its special properties (12).

We often speak of oxygen radicals or reactive oxygen species (ROS). These are oxygen derivatives that react more or less spontaneous with other biomolecules, setting up a chain reaction where new reactive products are generated. The most reactive molecule is the hydroxyl radical, $OH^{\bullet}$. It reacts at high rate constants with sugars, amino acids, lipids and nucleotides. Other less reactive ROS are superoxide O_2^- and hydrogen peroxide H_2O_2 (30).

Oxidative phosphorylation is taking place over the mitochondria inner membrane to generate ATP (adenosine triphosphate). It has been estimated that 3-5% of the oxygen utilized in the mitochondria of a human is incompletely oxidized and thus potentially transformed to ROS (31).

Free radicals are not always based on oxygen. Reactive nitrogen species (RNS) such as nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$) are also free radicals.

The term oxidative stress was introduced by Sies in 1985 and defined by the same author in 1991 as a “disturbance in the prooxidant – antioxidant balance in favor of the former, leading to potential damage” (12). Later, oxidative stress has been described as “disturbed intracellular redox equilibrium” (32).

The creation of RS (Reactive Species including both ROS and RNS) is caused by normal oxidative metabolism, various diseases, cigarette smoking, pollution, drugs, alcohol and radiation.

RS need to be eliminated, and in healthy individuals one task of the antioxidant defence system is to scavenge the RS. However, in a condition of elevated RS generation the antioxidant defence system may become insufficient. RS will accumulate and subsequently impose damage to molecules such as nucleotides in RNA and DNA, lipids, sugars and proteins, and may result in a situation of oxidative stress. Oxidative stress is recognized in the pathogenesis of many chronic diseases such as inflammatory diseases, ischemic diseases, cancer, hemochromatosis, HIV (human immunodeficiency virus), emphysema, hypertension, preeclampsia, and neurodegenerative diseases (33).

1.2.1 Defence system against oxidative damage

Over the time of evolution, advanced systems of protection against oxygen toxicity has developed in bacteria, plants and animals, making up the antioxidant defences of living species. These systems are enzymes and scavenging compounds acting solely or interactively, and they may be endogenously derived or supplied by the diet.

Halliwell and Gutteridge define the term antioxidant as “any substance that delays, prevents or removes oxidative damage to a target molecule” (12).

To present a brief overview of the defence system against oxidative damage, this section is divided into two sections: endogenous antioxidants and dietary antioxidants.

Endogenous antioxidants

The endogenous antioxidant defence system includes all endogenously derived substances that delay, prevent or remove oxidative damage to a molecule (12).

Antioxidant enzymes

The major antoxidant enzymes in eukaryotes are superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), peroxiredoxins and thioredoxin reductase.

SOD catalyses the conversion of superoxide anion to hydrogen peroxide and O_2:

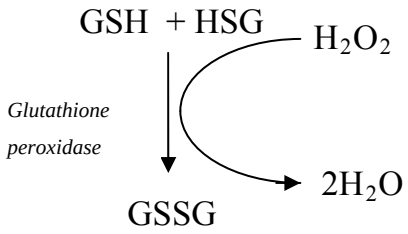
$$2\ O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

There are three isoenzyme forms of SOD; a cytosolic Cu^+-Zn^{2+} form (Cu-Zn-SOD), a Mn^{2+} form present in the mitochondria, manganese-containing superoxide dismutase (MnSOD) and an extra cellular Cu^+-Zn^{2+} form (EC-SOD) (30).

Catalase catalyses the reduction of hydrogen peroxide to water, preventing it to forming the hydroxyl radical in the Fenton/Haber-Weiss reactions. Catalase is abundant within in the peroxisomes:

$$2\ H_2O_2 \xrightarrow{catalase} 2\ H_2O + O_2$$

GPx and GR are superfamilies of enzymes in the glutathione metabolism. Reduced glutathione (GSH) acts as a substrate in the GPx catalysed conversion of hydrogen peroxide to water. Spontaneous or catalysed by GPx, two GSH molecules are oxidized (donating electrons) to form a single molecule: Glutathione disulfide or oxidized glutathione (GSSG). In this scavenging reaction, H_2O_2 will be reduced to water as illustrated below; lipid peroxides will be reduced to nontoxic alcohols (12).



Glutathione reductase (GR) (requiring NADPH) regenerates GSSG to produce GSH, making up the glutathione redox cycle.

Peroxiredoxins are a family of nonseleno-peroxidases that reduce hydrogen peroxide and organic peroxides. Thioredoxin reductase (TR) regenerates thioredoxin as described below.

Non-enzymatic, endogenous antioxidants

Low-molecular weight antioxidants

Glutathione (GSH) is an abundant endogenous compound in the cellular protection against oxidative damage. GSH, gamma-glutamylcysteinylglycine, is a tripeptide composed of glutamate, cysteine and glycine. Glutathione is synthesized within all tissues, but the liver is the main site of synthesis and excretion of GSH to the bloodstream for supply to other tissues (34). Gamma-glutamyl-cysteine synthetase (GCS) catalyses the rate-limiting step in the GSH synthesis. GCS activity and substrate availability are the main determinants of the GSH synthesis within the cell. The homeostasis of GSH is maintained by GSH consumption, production and regeneration from the oxidized form (35).

GSH and its oxidized form GSSG is a dominating redox pair within the cell. The glutathione redox cycle has been mentioned above. GSH also plays an important role in detoxification by conjugation within the xenobiotic metabolism (the mercapturate pathway). The family of GSTs catalyses xenobiotic compounds' conjugation with GSH (12). GSH reacts non-enzymatically with the hydroxyl radical ($OH^{\bullet}$) which is the highly reactive product of the Fenton reaction. It also reacts non- enzymatically with various cytotoxic nitric oxide products (35).

Uric acid is a powerful antioxidant abundant in human plasma. At physiological pH almost all uric acid is ionized to urate. Most species other than primates produce urate oxidase that break down the uric acid. In humans urate accumulate in plasma to a concentration of 0.2 – 0.4 mM (12). The loss of urate oxidase function may have been beneficial to primates, and it has been suggested that the increase in life span during human evolution could be due to the protective antioxidative action of urate in human plasma caused by this mutation (30). Urate has been shown to increase vastly after consumption of apples, possibly by fructose-mediated urate production (36).

Polypeptides

Thioredoxins (TRX) is a member of the thioredoxin protein superfamily and possesses strong reducing properties. It is able to reduce oxidized protein. TRX is upregulated in response to oxidative stress. TRX is itself regenerated by the enzyme thioredoxin reductase (TrxR) and NADPH (37). In addition to its ability to scavenge RS and thus influence the cellular redox status, TRX reduce cysteine residues that interact in cellular signalling pathways. In oxidative stress TRX translocate to the nucleus and modulate gene transcription (38). Other polypeptides acting as antioxidants are glutaredoxins and sulfiredoxins.

Metal binding proteins

Metals like Cu, Zn and Fe are essential in eukaryotes for the synthesis of various proteins involved in respiration, O_2 transport and antioxidant defence. However, these metals also act as prooxidants and convert less reactive to more reactive species. Metallothioneins are metal binding low molecular weight thiol proteins that sequestrate metal ions like Cu and Zn. Metallothioneins are found in the cytosol and nucleus of eukaryote cells, especially in liver, kidney and gut. Metallothioneins might also be antioxidants due to the high –SH content. Caeruloplasmin binds Cu and also exhibits ferroxidase activity; it oxidizes Fe(II) to Fe (III) and may facilitate iron loading on to transferrin and possibly ferritin (12).

Albumin is a small, highly soluble plasma protein at a concentration of about 40 mg/ml. It contains an exposed –SH group at position 34 and contributes up to 500

μM to total plasma thiols. Albumin has multiple roles and could be an important extracellular antioxidant. It binds Cu tighly and Fe weakly. It also binds heam thereby protecting lipoproteins against haemdependent oxidation (12).

Dietary antioxidants

Fruits and vegetables are the major sources of dietary antioxidants. The antioxidants are produced by plants for protection against sun exposure during photosynthesis and other stress factors (12). The antioxidants vary in their structure, physical and chemical properties and are thus divided into groups. The most commonly known groups of dietary antioxidants are carotenoids, tocopherols, ascorbic acid (AA) and polyphenols (39).

The carotenoid content in human bodies varies with the diet. Many carotenoids are colourful pigments of fruits and vegetables. Around 600 carotenoids have been identified in nature. Of these are 20 found in human blood and tissues (40). The carotenoids are insoluble in water, stored in fatty compartments within the cells and transported in the lipoproteins in blood. The carotenoids are all very good singlet O_2 quenchers *in vitro*, lycopene being the most efficient (12).

Lycopene is the carotenoid that gives tomatoes their red colour. It is the most abundant antioxidant in tomatoes, and tomatoes are the best source to lycopene. In the intestine, lycopene is absorbed with fat, and transported to the circulation with fatty acids in chylomicrons (41). In raw tomatoes, lycopene is mostly present in the trans-form. During processing and heating, some of the lycopene is converted to cis-isomers, which seem to be better absorbed. Processing during preparation may also increase the availability of lycopene from the tomato food matrix, where lycopene is tightly bound to macromolecules (41).

The lipid soluble antioxidants scavenge RS in membranes and lipoproteins. Vitamin E plays an important antioxidant role in the lipofilic compartments of the cell. Two major related metabolite groups, the tocopherols (alpha-, beta-, gamma- and delta-) and the tocotrienols (alpha-, beta-, gamma- and delta-), constitute what is called

vitamin E. Of the eight isoforms with variable biologic activity, alpha-tocopherol is the most abundant in plasma, while tissue concentrations of alpha-tocopherol and gamma-tocopherol are comparable (42).

Sources to Vitamin E in the diet are vegetable oils, vegetable-based spreads, nuts and seeds, certain fatty fish, egg yolk and whole grain cereals (43). Alpha-tocopherol content is high in sunflower seed and corn oil, while gamma-tocopherol is abundant in rapeseed oil (43). Gamma-tocopherol is the major form of vitamin E in the US diet (44). Gamma-tocopherol may act as a more effective scavenger of free radicals than alpha-tocopherol, while alpha-tocopherol is the metabolite primarily used in dietary supplements (44). Increased intake of alpha-tocopherol may suppress bioavailability of gamma-tocopherol (42).

Prolonging shelf life of many foods, vitamin E comes naturally with plant oils, nuts and seeds. These products contain polyunsaturated fatty acids, and vitamin E protects them from going rancid. In a similar manner that vitamin E protects the food it may protect against oxidative damage in the human body by breaking the peroxyl chain reaction (30).

Vitamin C or AA is a potent antioxidant and it acts as an electron donor. It is the most abundant chain breaking antioxidant in the water soluble compartments of the body and it readily scavenges the most potent ROS and RNS and thereby protecting lipids, proteins and DNA from oxidative damage. The ascorbyl radical that is produced in these reactions has low reactivity under physiological conditions, and this makes AA an ideal antioxidant. The ascorbyl radical may degenerate to AA and dehydroascorbic acid (DHAA) (12;39).

Plant phenols comprise an enormous number of compounds, having in common an -OH group attached to a benzene ring. The number of -OH groups and their position are determinants of the antioxidant properties. Polyphenols are made up of more than two monophenols (12). Bioavailability of polyphenols differs greatly; the absorption

is accompanied by extensive conjugation and metabolism such that the forms appearing in the blood are usually different from the forms found in food (39).

1.2.2 The antioxidant network hypothesis

Scavenging antioxidants may neutralize and stop the reactive chain by donating or accepting a single electron. Efficient antioxidants, like vitamins E and C, carotenoids and various plant phenols are believed to exert properties that impose a direct effect to prevent oxidative damage. *In vitro*, these substances are able to break the chain reaction initiated by oxidation. The scavengers can be divided into lipid phase and aqueous phase antioxidants, indicating in which macromolecules and compartments they may exert their effect.

As presented by Packer et al. (45), it is likely that the chain breaking antioxidants interact *in vivo* (45) **(Figure 1.3)**. A chain of antioxidants could reduce and oxidize each other according to the redox potentials of the substances. Each successive redox reaction could transfer the radical challenge to a molecule with lower oxidation potential, thereby being less reactive. Subsequently the radical challenge would be transferred to molecules that are easily eliminated from the body, like for instance AA and phenols. AOX-n and AOX-n radical denotes arbitrary antioxidant redox pairs with adequate redox potentials.

As an example, ascorbate may regenerate the tocopheryl radical at the aqueous-lipid interface and thus producing alpha-tocopherol. In a similar way, glutathione may recycle ascorbate from dehydroascorbate (30).

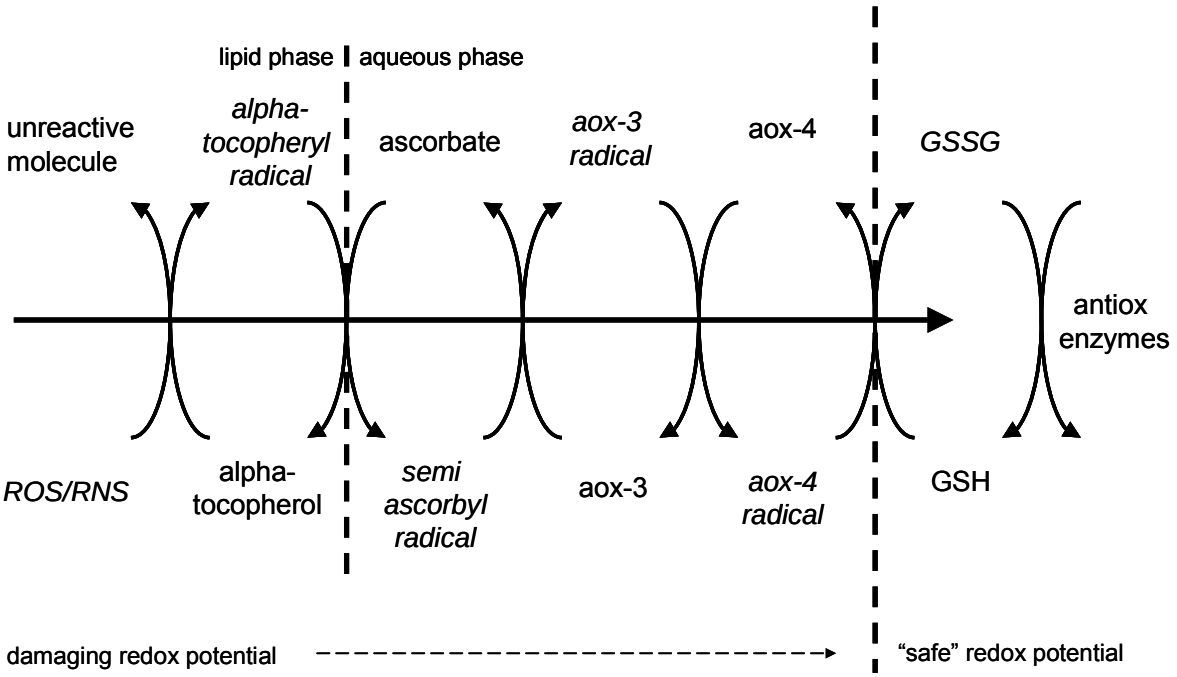


Figure 1.3 The antioxidant defence network, modified after Packer et al. (45)

1.2.3 Oxidative molecular damage

Oxidation may damage molecules such as DNA, lipids and proteins.

There are approximately $5x10^{13}$ cells in a human body. It has been estimated that there are a few hundred oxidations in the DNA of each cell per day. Direct damage to DNA by RS can affect the purine (adenine, guanine) or pyrimidine (thymine, cytosine) bases and the deoxyribose sugar. DNA attacked by for example hydroxyl radical ($OH^{\bullet}$) generates a whole range of base and sugar modification products. Guanine has the lowest oxidative potential; therefore the highest rate of oxidation is on this base. The cells own repair mechanisms are of vital importance to avoid damaging mutations to DNA. Base exision repair (BER) is the most common method to remove oxidative damage (12).

Lipid peroxidation has been described as "oxidative deterioration of polyunsaturated lipids". Fatty acids, and especially polyunsaturated fatty acids (PUFAs) going rancid have been a problem as long as foods containing these fatty acids (dairies, oils and fatty fish) have been stored (12).

PUFAs with double bindings on either side of the carbon atom, are more vulnerable to oxidation than others, thus the oxidizability of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) is much greater than that of linolenic acid (12). Lipid peroxidation may be initiated by adding a hydroxyl radical or nitrogen oxide to a methylene group or by abstracting a hydrogen atom from a methylene group. The carbon radical will react with O_2 to give a peroxyl radical:

$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$

Peroxyl radicals may abstract an hydrogen atom ($H^{\bullet}$) from an adjacent fatty acid, and thus a propagation of lipid peroxidation may continue to generate multiple peroxide molecules. A series of damaging actions on the cell membranes, increasing leakiness and reducing function of membrane proteins may result. Membranes may even disintegrate (12).

Oxidative protein damage may occur as a direct attack of RS or as secondary damage caused by end-products of lipid peroxidation. Damage to cellular proteins may be serious and irreversible. For example damaged DNA repair enzymes could increase the mutation frequency. Accumulation of damaged proteins could lead to cell death. Damaged proteins are therefore taken care of by the cell, removed by lysosymes or proteasomes (12).

1.3 Oxidative stress and carcinogenesis

There are strong indications that carcinogenesis is related to oxidative stress. Both elevated RS (by cigarette smoking) and impaired antioxidant defence, (eg CuZnSOD knockout mice) show increased risk of cancer development (46).

Increase in oxidative stress is associated with ageing, and the largest single risk factor of cancer is age. Interestingly, the cancer risk is not clearly elevated in diseases with chronic oxidative stress like rheumatoid arthritis and diabetes (12).

In the past decade awareness has grown around the importance of the balance between oxidation and reduction within cells. The oxidative state of the cells is of vital importance not only in disease but also in health.

In cell culture, one may characterize cell growth, proliferation, apoptosis and necrosis after exposing cells to increasing levels of RS. When exposing the cells to increasing levels of RS, the cellular GSH/GSSG ratio may also be measured as an indicator of oxidative stress. With increasing levels of RS, the GSH/GSSG ratio decreases due to lower GSH and higher GSSG.

How cells cope with increasing oxidative stress was characterised by Halliwell and Gutteridge (12) in five levels:

1) In a healthy, resting cell, the GSH/GSSG ratio is high due to a low quantity of GSSG.

2) Mild oxidative stress increase intracellular Ca^{2+}, protein phosphorylation and proliferation.

3) Further increase in oxidation give an adaptive response featured by increased transcription of protective enzymes and proteins, and slowing down cell cycle to allow DNA repair.

4) Exposed to even greater oxidation, the cell may undergo apoptosis due to DNA injury and other non repairable damage.

5) Severe oxidative stress result in necrosis, and thus transition metals and other oxidizing factors may be released to the neighboring cells and increase the oxidation even further (12).

According to Halliwell and Gutteridge (12), carcinogenesis and oxidative stress may interact in many ways, but “the balance of evidence supports the view that oxidative DNA damage is involved in the carcinogenesis, whatever carcinogen started the process” (12).

Oxidative stress may modulate all the stages of carcinogenesis. In cancer initiation, oxidative stress may increase the rate of oxidation in DNA and RNA leading to base modifications and mutations. In smokers, who have increased risk of some cancers, higher rates of urinary 8-hydroxy-2-deoxyguanosine (8OHdG) excretion have been measured. Elevated levels of 8OHdG which may result from oxidative stress have been found in human cancer tissue. Cause and effect is however not possible to distinguish (47). Persistent mild oxidative stress may stimulate cell growth and proliferation, perhaps contributing to clonal expansion and proliferation of tumor cells (12).

Role of oxidative stress in PC

Oxidative stress is assumed to play a role in PC. Some examples that indicate a connection are given below.

The loss of expression (silencing) of the GSTP1 gene by hyper methylation is an early and the most common (>90%) genetic alteration reported to date in PC (48). PC cells with loss of GSTP1 function seem to survive and proliferate better than cells that express GSTP1 properties when exposed to oxidative stress. The family of glutathione –S- transferases is important in the endogenous defence against oxidative damage (49).

In a group of thirtytwo PC patients, Chen et al. (50) measured reduction of 8OHdG in leucocytes and prostate tissue after a three weeks intervention with tomato-based pasta dishes. Compared to pre-intervention, leukocyte 8OHdG was reduced by 21.3 % after the intervention. 8OHdG in prostate tissue was 28.3% lower in the patients after intervention compared to a randomly selected control group (50).

An enzyme having a key function upon peroxisomal oksidation of fatty acids, alfa-methyl-acyl-CoA-racemase (AMACR), is up-regulated in tumor tissue compared to normal tissue. Elevated AMACR activity may lead to increased release of hydogenperoxide that again may induce DNA damage (51).

1.4 Dietary factors that may influence PC incidence and progression

The risk of PC varies between populations, and the variation may be attributed to dietary differences. The population of men from Crete, as documented by Ancel Keys in the classic Seven Countries Study in the 1960s, had the lowest rate of cardiovascular disease and cancer in this multi center study, even lower than the Japanese participants. The diet of Crete has a omega-6 to omega-3 fatty acid ratio of 2:1, while western countries are estimated at ratios around 15:1. Tomatoes, onion, grape, garlic, grains, seeds, wild plants and herbs are staples in this diet, in addition to large amounts of olive oil (52). However, from epidemiology, fruits and vegetable intake in general seems to have a weak or no effect on PC prevention (12).

Furthermore, Japanese and Eskimo men with high intakes of marine omega -3 fatty acids have a low risk of PC (53).

There are vast dietary differences between populations of low (East Asia) and high (Western) PC risk. The East Asian diets are traditionally low in energy and dietary fat. The diets include isoflavones (phytoestrogens) from soy beans, polyphenols from green tea, and are low in meat, milk and dairy products. The Japanese diet is rich in marine fatty acids, and traditional Asian diets are prepared by boiling or steaming, not grilling or smoking (54).

The amount of literature on nutrition and cancer prevention has increased since the mid – 1990s. Single food items and individual nutrients have been investigated. The report “Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective” was issued in November 2007 (11), also known as The Second Expert Report, is the most comprehensive report of the connection between diet and cancer risk published to date. The report is a joint effort by the World Cancer Research Fund and American Institute for Cancer Research. Through what is called Systematic Literature Reviews a structured methodology was built that combine the findings from individual studies into classification of each food or nutrient and its possible

prevention of cancer at various sites. The nutrients' protective or causative properties are classified into "convincing", "probable", "limited-suggestive" and "limited –no conclusion" (11).

	Decreases risk	Increases risk
Convincing		
Probable	Foods containing lycopene Foods containing selenium Selenium	Diets high in calcium
Limited-suggestive	Pulses, legumes Foods containing vitamin E Alpha-tocopherol	Processed meat Milk and dairy products
Substantial effect on risk unlikely	Foods containing beta-carotene Beta-carotene	

Figure 1.4 Matrix displaying the strength of evidence causally relating food and risk of PC, as judged by the panel of the Second Expert Report by November 2007 (11)

To date, no nutrient regarding PC has been classified as convincing. The Second Expert Report stated that foods containing lycopene, foods containing selenium and selenium supplements probably reduce the risk of PC **(Figure 1.4)**. Diets high in calcium probably increase the risk, while there is limited suggestive evidence that pulses (soya), foods containing vitamin E and alpha-tocopherol (50 mg per day) reduce the risk of PC. Processed meat (meats preserved by smoking, curing, salting or chemicals) and milk and dairy products are limited suggestive in increasing the risk. **Figure 1.4** displays a graphical summary of the most studied foods and nutrients and their proposed effects on PC.

The list of foods classified as "limited – no conclusion" (not shown) is very long and includes tea, fruits, non-starchy vegetables, fish and vitamin D (11). The grading of the uncertainty with respect to effect reflects the amount and quality of documentation available for each food and nutrient.

The following sections about nutrients and PC risk are arranged according to The Second Expert Report's classification by November 2007, with emphasis on protection.

1.4.1 Probable and suggestive decreased risk

Tomatoes and lycopene

The Second Expert Report states that "Foods containing lycopene probably protect against PC" (11).

From epidemiology we know that consumption of tomato products is inversely correlated to PC risk. Consumption of tomato products over time result in accumulation of lycopene in prostate tissue and significantly increased levels of lycopene in serum. Further investigations have shown that lycopene blood levels are inversely correlated to PC risk (55). Lycopene half-life in human plasma has been reported to vary between 5 to 9 days (56).

Studies of PC cell lines have shown that lycopene possess several anti-carcinogenic effects. Areas of interest include: 1) Antioxidant function 2) Inhibition of cell cycle progression 3) Induction of apoptosis 4) Increase in gap-junctional communication 5) Reduced IGF-1 signal transduction 6) Up regulation of phase 2 enzymes 7) Reduce androgen effect (55).

Human PC interventions

Eating tomatoes may prevent men from contracting PC. Tomatoes, given to PC patients, could also dampen the disease or slow down the carcinogenesis.

Kucuk et al. (57) found that 30 mg lycopene daily for 3 weeks was sufficient to modulate PC, and significantly increase connexin 43 (connexins are gap juntional proteins that are essential in cell-to-cell communication) and decrease IGF-1. The study by Chen et al. (50) has already been mentioned above. In addition to the effect on 8OHdG, PSA was reduced significantly in this trial (50).

More clinical PC trials are discussed in Chapter 6, the section "Tomato effect on PSA". **Table 6.1** presents a list of six human PC tomato/lycopene trials.

Selenium

Selenium (Se) is an essential component in the glutathione family of enzymes, and low dietary levels are connected to increased risk of some cancers (11). According to the 2007 Second Expert Report (11), both foods containing selenium and selenium supplementation may protect against PC.

The main dietary sources of selenium in the Norwegian diet are fish, wheat and dairy products (58-60). Wheat imported from the North America has high selenium content, whereas wheat grown in the Nordic countries, with the exception of Finland, has low selenium content. In Finland, agricultural fertilizers have been enriched with selenium from 1984, and the average serum selenium concentration (men and women) in Finland is 100-120µg/L compared to Sweden and Denmark where it is 70-80µg/L (43). Interestingly, the age adjusted PC incidence and death numbers in Finland are low compared to the other Nordic countries (4).

Selenium may inhibit carinogenesis via many possible mechanisms. Cell models of PC have shown that Se inhibit growth, apoptosis and PSA excretion in a dose dependent manner (61). In experimental animal models supplementation with Se has been shown to reduce oxidative damage and induce apoptosis in PC cells (62). Studies in healthy men have shown that supplementation with 200 µg Se per day significantly increase Se levels and have a significant effect on oxidative damage in prostate tissue (63).

In Norway, the daily recommended intake of Se for men is 50 µg (64). Selenium supplementation dosageing must be careful, because the maximum safe intake is thought to be around 15µg/kg body weight, but could be lower in some sensitive individuals (65).

Supplementation with selenium and vitamin E has been thoroughly investigated in the study called Selenium and Vitamin E Cancer Prevention Trial (SELECT), where 35533 men were recruited and randomly assigned to 4 groups (selenium, vitamin E, selenium + vitamin E, and placebo) in a double-blind fashion. The selenium dose was 200µg daily. Selenium supplementation revealed no effect on PC, while a small increase in diabetes type 2 among those who received selenium was observed (66).

Soy beans and isoflavones

Few and contradictory results have been published on soy and PC. Soy contains large amounts of isoflavones like genistein and daidzein and the observed protective effect has been attributed to this group of substances. A meta-analysis consisting of 2 cohort studies and 6 case-control studies showed an inverse relation between soy intake as food and risk of developing PC (67).

Dalais et al. (68) observed PSA reduction of 12.7 % after 3-4 weeks of soy and a 10 % PSA reduction by soy and linseed enriched diet versus wheatbread diet in PC patients. Kumar et al. (69) observed a trend towards a reduction in PSA after soy supplementation in early stage PC patients (69). A significant reduction in PSA percentage increase was observed in PC patients supplemented with soy for 5-6 months (70). Long time supplementation with soy in healty elderly men or men with increasing PSA has been effective to reduce PSA (71-73).

Vitamin E

There are several studies investigating the relationship between alpha-tocopherol and incidence of PC.

Results from the ATBC Alpha-Tocopherol, Beta-Carotene Cancer prevention study with 29133 Finnish participants; male smokers aged 50 to 69 years; showed that a daily supplement of 50 mg vit E (alpha-tocopherol) reduced the risk of PC. In the same group, however, more deaths from hemorrhagic stroke were observed (74).

In the United States Health Professional Study cohort (observational study of 47780 healthy males) no general association between dietary vitamin E supplementation and risk of PC was found. However, a decreased risk of aggressive PC was found among smokers taking vitamin E supplements (75), which was consistent with the Finnish trial. In a nested case-control study of blood donors, 117 PC patients and 233 controls, an inverse association was found between increased serum levels of alpha-tocopherol and selenium, but this was connected to high plasma concentration of gamma-tocopherol.

The Physicians Health Study II, a Randomised Controlled Trial including 14641 participants showed no effect of long term vitamin E supplementation in prevention of PC (76).

The SELECT study (described in the selenium chapter above) recently discontinued. No evidence of benefit from vitamin E (alpha-tocopherol) was demonstrated but rather a small but not statistically significant increase in PC among the patients who received vitamin E was detected (66). Yearly PSA testing by the participants on their own intiative probably removed diagnosed cases from the study at an early stage of disease and because of this, effect from the intervention on disease progression was probably not seen. However, more information from the SELECT and Physicians health study randomized controlled trials are expected, and results from subgroup analyses like smokers, genetic factors and baseline serum levels may reveal modifications to these initial results (77).

1.4.2 Probable and suggestive increased risk

Processed meat

Processed meat as denoted in The Second Expert Report includes preserved meat (salted and smoked), meat products, hamburgers, ham, salami, bacon, cured meat and sausage as an overall category. There is limited evidence suggesting that processed meat is a cause of PC. Nitrates added as preservatives and produced in gastric acid

may contribute to N-nitroso componds that are suspected mutagens and carcinogens. High levels of salt and nitrite may accompany meat, while meats cooked at high temperatures may contain heterocyclic amines and polycyclic aromatic hydrocarbons (11).

Calcium

Many epidemiologic studies have reported a positive association between dairy products and PC. Calcium or saturated fatty acids in dairy products have been suspected as the causative agent (78). However, pooled data from 45 observational studies did not support an association between dairy product use and an increased risk of PC (79).

In Japan, where the intake of dairy products, calcium, and saturated fatty acids are low and the incidence of PC is low, a population-based prospective study was conducted enrolling 43435 Japanese men ages 45 to 74 years. Dairy products were associated with a dose-dependent increase in the risk of PC. A statistically significant increase in risk was observed for both calcium and saturated fatty acid, but the associations for these were attenuated after controlling for potential confounding factors (80).

The European Prospective Investigation into Cancer and Nutrition examined consumption of animal foods, protein and calcium in relation to risk of PC among 142251 men. After an average follow-up time of 8.7 years, there were 2727 incident cases of PC. A high intake of dairy protein was associated with an increased risk, with a hazard ratio for the top versus the bottom fifth of intake of 1.22 (95% confidence interval (CI): 1.07 - 1.41, P (trend) = 0.02). Calcium from dairy products was also positively associated with risk, but not calcium from other foods. The results supported the hypothesis that a high intake of protein or calcium from dairy products may increase the risk for PC (81).

1.4.3 Inconclusive

Grape juice

Important polyphenols in grapes and grape juice are resveratrol, quercetin, catechin and anthocyanins.

Resveratrol has been shown improve health and survival of mice on a high-calorie diet (82). Resveratrol is a stilbene found in grapes and one of the most important antioxidants in red wine. Resveratrol may be an important contributor to "the french paradox" ie that a moderate red wine consumption reduce the mortality from cardiovascular disease and some cancers in spite of high intake of saturated fat and red meat in the French population. Resveratrol may regulate proteines involved in DNA syntesis and cell cycle like p53 (tumour protein 53), Rb/E2F, cyclins, CDKs (cyclin-dependent kinases) and inhibitors of these. Resveratrol also may reduce the activity of transcription factors involved in proliferation and stress response, such as NF-kB (Nuclear factor kappaB), AP1 (Activator Protein 1) and Egr1 (Early Growth Response Protein 1). In cell models for PC, resveratrol has shown favourable effects in relation to PSA excretion, cell cycle- and proliferation specific genes (83). Resveratrol may also induce apoptosis (84).

Pomegranate juice

Pomegranates contain very high levels of antioxidants (85;86), and in recent years several studies have been published that indicate favourable effects of this fruit. Pomegranate (Punica granatum) is a good source of at least two polyphenols: anthocyanines, giving the red colour, and ellagitannins, both being active antioxidants (87). The ellagitannin abundant in pomegranates is punicalagin which is the largest molecular weight polyphenol known. According to Heber (88) ellagitannins are hydrolysed to ellagic acid before absorption in the gut. Heber claims that ellagitannins and not antocyanidins account for the anticarcinogenic properties of pomegranates. Being a denominator of inflammation, the NF-kB transcription factor

is found to be constitutively upregulated in PC cells. Ellagitannins are seen to inhibit the activation of NF-kB and other inflammatory agents (88).

Malik et al. (89) have shown that favourable effects from pomegranate extracts on PC both *in vivo* and *in vitro*. In an experimental mouse model the animals were divided into groups where one group received pure water and the two other groups were served water enriched with pomegranate extract of 0.1% and 0.2% respectively. The pomegranate animals had significantly smaller tumorsizes and the strongest concentration was most effective. One month after the tumorinoculation the size of the tumors in the 0.2% group was approximately half as big as in the controls. The PSA level was significantly reduced in both pomegranate groups (89).

Recent reports have indicated that pomegranate juice may interfere with the cytochrome p450 system of xenobiotic detoxification and excretion. Experiments on human hepatocytes and rodents have shown that CYP3A4 may be inhibited by pomegranate juice. It was found that pomegranate juice consumption decreased total hepatic cytochrome P450 (CYP) content as well as the expression of CYP1A2 and CYP3A. It was suggested that prevention of procarcinogen activation through CYP activity/expression inhibition may be involved in pomegranate juices' effect on tumor initiation, promotion, and progression (90-92). A study on healthy volunteers did however not produce the same effect on humans (93).

One clinical trial has been conducted on PC patients to date (94). Results from this trial were published in 2007. Eligible patients had a detectable PSA > 0.2 and < 5 ng/mL and Gleason score < 7, which may be characteristic of localised PC. The patients received 8 oz (237 ml) of pomegranate juice daily over two years or more. It showed a significant increase in PSA doubling time from a mean of 15 months at baseline to 54 months post-treatment ($p < 0.001$) (94).

Tea

Some epidemiological studies have indicated that people who regularly consume tea have a decreased risk of PC (95-97). But in a retrospective cohort study using

participants in the 1970-1972 Nutrition Canada Survey, no association was observed between tea (predominantly black tea) intake and PC (98). The Second Expert Report indicates that black tea suggestively prevent PC, but according to this comprehensive report there was no evidence of association between green tea intake measured in cups per day and PC (11). However, Jian et al. (95) conducting a retrospective case-control study of tea intake in China showed strong protection from (mainly) green tea consumption over many years, and high daily intake (> 1 litre) (95).

Bettuzzi et al. (99) supplied men, with HGPIN, of whom 30% were expected to develop PC during one year with 600 mg/d of green tea cathechins in capsules. The trial lasted for one year, and in the control group 30% of the patients developed cancer while in the intervention group only 3% developed cancer (99).

The active substance that may impose an anticarcinogenic effect is possibly epigallocatechin-3-gallate, a polyphenol constituting around 1/3 of the total polyphenol content in green tea and up to 10 % of the total polyphenols in black tea. Oral infusion of green tea polyphenols analogous to 6 cups daily have been shown to inhibit PC in a transgenic mouse model of PC (TRAMP), and numerous mechanisms for green tea effect on PC have been suggested from cell culture studies: influence on androgen metabolism, PSA decrease, induction of apoptosis (via p53 and NF-kB) and improved cell cycle control (100;101).

High dose administration of green tea polyphenols to patients with advanced androgen independent PC showed minimal effect (102;103).

Fatty fish and omega-3

Long chained omega-3 fatty acids are unique nutrients in fish. Epidemiological studies and case-control studies have shown an inverse relationship between omega-3 fatty acids (EPA and DHA) intake and risk of total and aggressive PC (104-107). Freeman et al. (105) have showed an inverse connection between omega-3 fatty acids measured in prostate tissue and spreading to adjacent organs. Few randomized trials with omega-3 supplement have studied the effect on patients with PC. One study

(108) report that intervention with omega-3 fatty acids have no effect on serum PSA, however a reduction in cyclooxygenase 2 (COX-2) expression and an increase in the omega-3:omega-6 ratio was observed (108). Inhibition of COX-2 expression blocks inflammatory effects and may be useful for prevention or theraphy of PC (101).

1.4.4 Additive or synergistic effect

Assuming that the various anticarcinogenic food substances act differently, it could be favourable to combine substances to achieve a greater effect than one could obtain from one substance alone. An additive effect would be the sum of two effects, but one could also imagine a synergistic effect, ie that two or more substances together give a greater effect than just the sum of the individual effects. Such effects could be present in natural diets.

1.5 Foods versus supplements

According to Parcelsus, “All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.” Considerable documentation has shown that supplementing well nourished human populations with high concentrations of single antioxidants give no cancer prevention, rather the opposite (109). Interventions with synthetic antioxidant supplements have failed to show a protective effect, and some have even increased mortality. In October 2008, the SELECT study terminated the selenium and vitamin E trial due to no effect on PC prevention and possible adverse effects (110). However, the baseline serum selenium average of all groups in the US study population was approx 135 μg/L. This is much higher than the Nordic values (65), indicating that the population baseline serum level should be taken into account when the results are interpreted for Norwegian or Nordic populations.

Antioxidant supplements are not subjected to the same rigorous toxicity studies as other pharmaceutical agents (109). Like other chemical substances, vitamins and

minerals cause adverse and toxic effects if consumed in excessive amounts (43). However, adverse effects from supplementation studies with essential vitamins and minerals have occurred at doses well below established upper intake level. **Figure 1.5** below displays possible relationships between antioxidant dose and risk of disease.

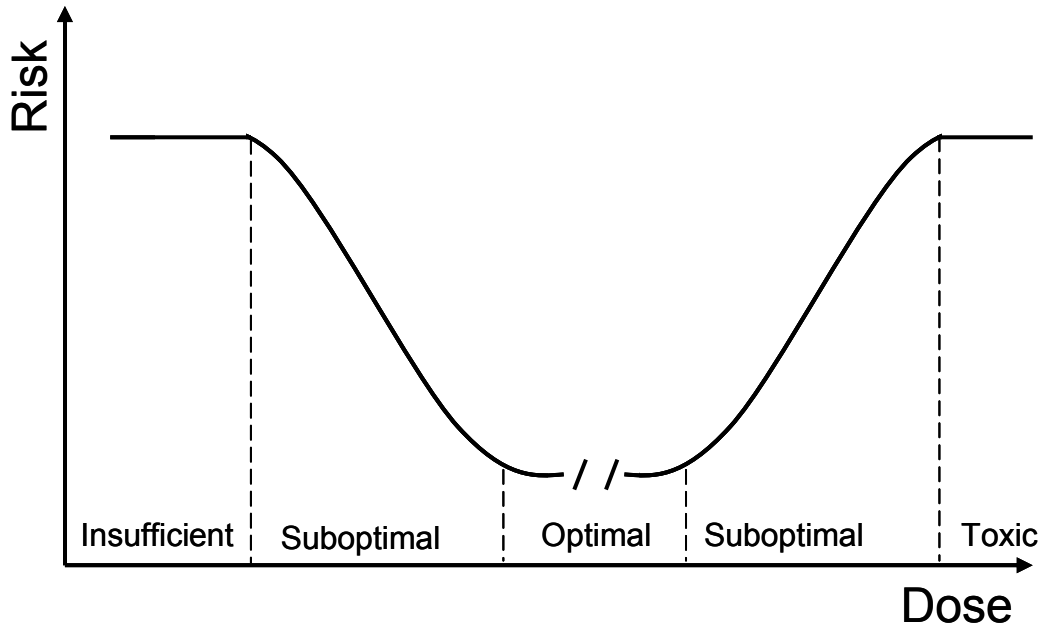


Figure 1.5 Possible relationships between antioxidant dose and risk of disease

The failure of the antioxidant supplementation interventions does not necessarily lead to the conclusion that the dietary antioxidants have no effect on carcinogenesis. However, there seem to be important differences between (1) natural plant foods containing a variety of substances and (2) capsules with a high concentration of a single antioxidant metabolite.

(1) Unprocessed plant foods supply tissues with a blend of substances whereof many possess antioxidant properties. Such a combination is only obtainable from natural foods, fruits and vegetables and not from synthetic supplements. According to the hypothesis of the antioxidant defence network, the substances interact due to diverse chemical properties and reduction potentials. These networks could be very delicate and well balanced.

(2) Capsules with a high dose of a single antioxidant metabolite may increase the tissue level of this single substance to a level that could bring the antioxidant network out of balance. Great individual variation could be expected and adverse effects may only affect susceptible individuals.

1.6 Diet and gene interaction

Studies of relationships between dietary components and cancer risk often give contradictory results. This may be due to the weakness of the dietary assessment methods, but could also be caused by unrecognized heterogeneity of the study populations, such as genetic polymorphisms. Interaction between specific polymorphisms and individual food components or nutrients could mask associations (111). In recent years many reports of such connection between nutrients and genes have been reported, and below are some examples that could indicate the importance of allele variants in nutrition research:

Goodman et al. (112) reported an effect modulation by plasma antioxidants, the XRCC1 (X-ray repair complementing defective repair in Chinese hamster cells 1) genotype, lycopene intake and PC risk. The XRCC1 gene is involved in the BER mechanisms and their results indicate that the association between lycopene and PC is complex and may involve other antioxidants (112).

A Swedish study (113) reported that frequent consumption of fatty fish and marine fatty acids appears to reduce the risk of PC, and this association seems to be modified by genetic variation in the COX-2 gene. A strong protective effect of fatty fish intake was shown in the carriers of the variant allele but not among those carrying the common allele (113).

There have been inconsistent results from the role of trans-fatty acid intake on risk of PC. Liu et al. (114) suggest that RNASEL genotype may modulate the disease risk associated with total trans-fatty acid intake. Their study indicated that for men with the R462Q RR genotype, total trans-fatty acid intake was not associated with disease

risk, while among men with the QQ/RQ genotype, the association between total trans-fatty acid intake and PC was substantially stronger (114).

Much research has addressed polymorphisms in genes related to antioxidant defence systems and the xenobiotic metabolism, but inconsistent results have been reported with respect to susceptibility for PC. There are many possible confounding factors to such studies, and the genetic makeup differs between study populations. Katoh et al. (115) conclude that GSTM1, GSTT1 and GSTP1 are unlikely to be major determinants of PC susceptibility. However, according to Traka et al. (116), broccoli consumption seems to be protective in men that are GSTM1 positive compared to those who are GSTM1 null.

1.7 Oxidative stress biomarkers

Numerous methods for measurement of oxidative stress have been developed and tested. Jones (117) classifies five general approaches to potential biomarkers: 1) antioxidant enzymes, 2) low molecular weight antioxidants, 3) the balance between pro-oxidants and anti-oxidants, 4) oxidants, 5) products of oxidative damage (117). A sixth class is to measure 6) the total antioxidant capacity (118).

At present, there are no golden standards for measuring oxidative stress in clinical practice, and reference values lacking. There are, however, many potential biomarkers. The following selection will be studied in this master thesis:

- Antioxidant related enzyme: Gamma glutamyl transferase (GGT)
- Low molecular weight antioxidants: Glutathione (GSH), Ascorbic acid (AA)
    - Body thiol pool: Cysteine
- Products of oxidative damage to
    - DNA: 8-hydroxy-2'-deoxyguanosine (8OHdG)
    - Lipids: Hexanoyl-epsilon-lysine (HEL), Diacrons reactive oxygen metabolites test (d-ROMs test)

- Total antioxidant capacity: ferric reducing ability of plasma (FRAP), modified FRAP (mFRAP).

1.7.1 GGT

Cellbound gamma-glutamyl transferase or gamma-glutamyltranspeptidase, GGT, is an enzyme facing outwards on the plasma membrane of most cell types. GGT also exists as free fraction in plasma. A major task of GGT is to catalyse the breakdown of free GSH and GSSG which is excreted from the cell. The metabolites are then ready to be transported back into the cell to serve as substrates for new GSH synthesis. GGT is especially numerous in kidney cells and in the lungs (12).

In the clinic, serum GGT is established as a measure of liver disease and liver toxicity induced by alcohol and drugs. Moderate increase of serum GGT is seen in hepatitis, liver metastases, pancreatitis and renal diseases. Epidemiologic studies show that serum GGT are correlated with biomarkers of inflammation and oxidative stress, such as fibrinogen, CRP and F_2-isoprostanes (119).

Epidemiological findings suggests that serum GGT within normal ranges could be a predictive biomarker of oxidative stress related disease, and thus could be used as an early biomarker of diseases such as cancer and cardiovascular disease (119).

A pro-oxidative effect of GGT is seen in model studies (120). The reactive thiol glycyl-cystein metabolite, resulting from the GGT catalysed reaction, is more reducing than GSH, and will thus reduce Fe^{3+} to Fe^{2+} more readily, generating hydrogen peroxide.

$$GSH \xrightarrow{GGT} \text{glutamic acid} + \text{GS-SH}; \quad (1)$$

$$GC\text{-}SH \quad (pH > 7.0) \rightarrow GC\text{-}S^- + H^+; \quad (2)$$

$$GC\text{-}S^- + Fe^{3+} \rightarrow GC\text{-}S^{\bullet} + Fe^{2+}; \quad (3)$$

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^-; \quad (4)$$

$$O_2^- + 2H_2O \rightarrow \tfrac{1}{2}O_2 + 2H^+ + H_2O_2. \quad (5)$$

Up regulation of GGT due to increased levels of extra cellular GSH/GSSG could thus be part of a vicious cycle where the oxidative stress originating from other causes is reinforced (120).

GGT seems to be a very promising biomarker of oxidative stress.

1.7.2 GSH

GSH and the GSH related enzymes were described in section 1.2.1 “Defence system against oxidative damage.”

Plasma GSH is rapidly metabolized by GGT, discussed in a separate section. Its half-life is a few minutes. The turnover of GSH and the transcription of glutathione reductase (GR) increases in response to oxidative and nitrosative stress. Much studied, 4-hydroxy-2-trans-nonenal (HNE), a lipid peroxidation end product is shown to up regulate GSH synthesis (12).

The main mechanisms for upregulation are **(figure 1.6)**: a) ROS/RNS, b) cysteine and glutamate availability, c) GSH depletion and d) food factors that inhibit NO synthase (not shown in the figure) (121).

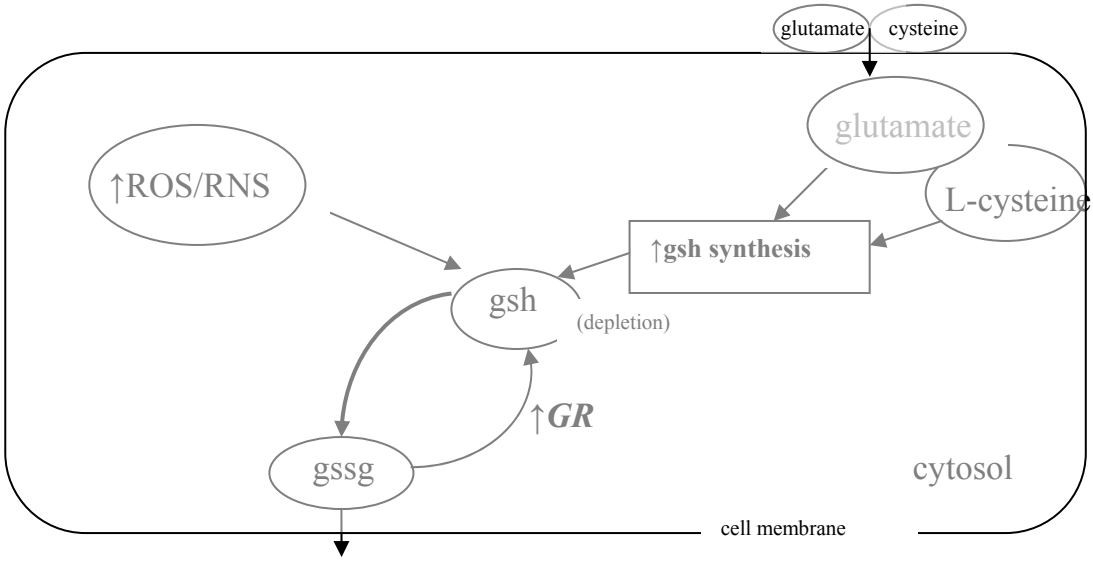


Figure 1.6 Regulation of glutation synthesis. The main mechanisms for upregulation are ROS/RNS, cysteine and glutamate availability, GSH depletion andfood factors that inhibit NO synthase (not illustrated)

The intracellular GSH content is known to be influenced by oxidative stress related diseases and conditions. Connection has been shown between the GSH levels and susceptibility and outcome in diseases such as cancer, neurodegenerative diseases, cystic fibrosis, and in ageing (122). Bøhn et al. (123) found that lowered extra cellular (plasma) GSH levels, as a measure of general oxidative stress in patients with head and neck squamous cell carcinoma, predicted the disease outcome (123).

The intracellular ratio GSH:GSSG is normally >100 under normal physiologic conditions. Within cells, the glutathione concentration varies from 0.5 to 10 mmol/L while the extra cellular concentration is much lower: 2 – 20 µmol/L in plasma. GSH is metabolized before excretion in urine, so urine may not be a good medium for GSH measurement. Plasma or blood cells could be of interest. Virtually all GSH in full blood is contained within cells. Thus, the plasma level of GSH is low and variable (12).

1.7.3 Cysteine

Cysteine is part of the GSH tripeptide, and the thiol group of cysteine is what gives GSH its unique property in the oxidative stress defence.

Cysteine is together with methionine the sulphur-containing amino acids normally consumed in the diet. Methionine is considered an essential amino acid and is a precursor of homocysteine and cysteine.

Absorption of the amino acids from the diet is highly efficient, and after absorption from the gut, the amino acids enter the portal vein and a substantial proportion of the sulphur amino acids are removed by the first pass through the liver for protein and GSH synthesis. Cysteine is present as CySH (thiol or reduced form), disulfide (cystine or CySSCy) and protein bound (PSSCy). Mean total cysteine plasma levels in healthy individuals range from 220 to 320 μmol/L (124).

1.7.4 Total ascorbic acid and dehydroascorbic acid

Ascorbic acid (AA) or vitamin C was isolated in 1928. Deficiency of AA with plasma levels below 11 μmol/L leads to scurvy which is characterized by spongy, bleeding gums, tooth loss, poor wound healing and swollen weakened limbs. Prospective studies of AA have shown increased cardiovascular, cancer and all cause mortality in subjects of the lowest plasma concentrations. The mean cut-off point for clearly lowered risk has been found to be a plasma concentration of 32 μmol/L. This corresponds to a daily intake of approximately 60 mg/d in men. Adding 25% for inter-individual variation gives the Nordic recommended intake of 75 mg, corresponding to a plasma concentration of 40 μmol/L. Also according to the Nordic Council of ministers' recommendations of 2004, 100 mg intake shows a saturation of blood cells and the plasma level will be 50 – 60 μmol/L. Very large doses (2.5 grams) are seen to increase the plasma levels up to 80 mmol/L (43).

Most primates are able to synthesise AA from glucose exept for humans, fruit-eating bats and guinea pig.

The ratio between DHAA and total AA has been suggested as a predictor of oxidative stress (125). Smokers have almost double turn-over of non-smokers, and this may be related to oxidative stress (124).

1.7.5 D-ROMs test

The company Diacron has developed a test called the "Diacron reactive oxygen metabolites test" (D-ROMs test) in order to assess systemic oxidative stress. The D-ROMs test is a spectrophotmetric test that measures the total concentration of all types of hydroperoxides (ROOH) in biological samples. ROOH are generated in cells by oxidative attack of ROS on a number of organic substrates (e.g. carbohydrates, lipids, amino acids proteins and nucleotides) (126).

The D-ROMs test essentially assesses plasma lipid peroxidation. Several publications indicate that D-ROM is a valuable biomarker for systemic oxidative stress showing higher levels in diseased (39;127;128) than in healthy.

Table 1.1 below is supplied by the manufacturer. It gives ranges of values as determined by the D-ROMs test, and suggests a corresponding oxidative stress level.

CARR U	**Oxidative stress level**
< 250	Below normal
250 - 300	Normal
300 - 320	Border line
321 - 340	Low
341 - 400	Middle
401 - 500	High
> 500	Very high

Table 1.1 Oxidative stress ranges of D-ROMs test

1.7.6 8OHdG

The most common way of assessing DNA ROS damage is by measurement of 8OHdG (129). 8OHdG is an abundant DNA lesion resulting from ROS. It is the result of a hydroxyl radical adduction to a guanine base.

If not repaired, this modification may result in a carcinogenic DNA mutation, and thus 8OHdG may be a biomarker of cancer risk (130;131). Base excision repair (BER), nucleotide excision repair and mismatch repair are mechanisms involved in the cellular defence against this specific DNA damage. When excised, the oxidized guanosine and adjacent nucleosides are being cut out of the DNA strand. The excised oxidized guanine nucleoside is subsequently excreted into urine and may be measured as 8OHdG.

The oxidized lesions are stable and fairly water soluble. In order to quantify the 8OHdG metabolite in other tissue, cleaving by various enzymes is required (130). Hydroxylated guanosines in the cellular pool are also excreted in urine and may contribute considerably to the measured amounts (132).

High performance liquid chromatography (HPLC) is the most widely used method for measurement of 8OHdG in tissue, lymphocyte and plasma.

One simple Enzyme-Linked ImmunoSorbent Assay (ELISA) method is commercially available from Japan Institute for the Control of Ageing; using a monoclonal antibody specific for 8OHdG. Wu et al. (130) describe another ELISA which correlate well with the Japanese method. Wu et al. report normal reference values in healthy men as 29.6 +/- 24.5 ng/mg creatinine. Cigarette smoking does not appear to influence the reference values (130).

The urinary excretion of 8OHdG is dependent on the rate of ROS damage on DNA in tissues. Increased levels of urinary 8OHdG may be a result of increased ROS attack or decreased antioxidant protection. Urinary 8OHdG does not directly reflect the

steady state level oxidative DNA damage, but it is an indicator of oxidative stress (132). 8OHdG from diet is believed not to affect the urinary measures (129).

There is good reason to believe that oxidative DNA damage, including hydroxylation of guanine bases, being the most frequent and most mutagenic lesion in nuclear DNA, contributes to the overall cancer risk in humans. Wu et al. (130) report some studies showing elevated 8OHdG in tissues and urine indicating increased cancer risk. Some connection with hepatocellular carcinoma and elevated 8OHdG due to increased iron load in liver cells has been reported. Cancer breast and prostate tissue have higher levels of 8OHdG compared to normal tissue (130), though this may be a consequence rather than a cause of the disease.

Quantification of oxidized guanosine, 8OHdG, in tissues or excreted in urine could in principle be a good indicator of oxidative stress. However, Halliwell raises a number of critical comments to the validity of 8OHdG as a general biomarker of oxidative stress: 1) There is no consensus to the true levels in humans, and 2) there are great problems of artifactual oxidation in sample preparation. 3) Measuring 8OHdG does not take into account the relation between the DNA ROS damage and the rate of DNA repair, which may be decisive for the overall disease risk (129;129).

1.7.7 HEL

Lipid peroxidation was briefly described in chapter 1.2.3 “Oxidative molecular damage”. Some important biomarkers of lipid oxidation are F_2-isoprostanes, malondialdehyde (MDA), acrolein, HNE and 2 – propenal (12). The lipid oxidative damage product examined in this thesis is hexanoyl epsilon lysine (HEL).

HEL is formed by a reaction between a peroxidated fatty acid and the essential amino acid lysine. The name “hexanoyl” indicates that there are six C-atoms in the fatty acid part of the HEL molecule. The side group of lysine is negatively charged (basic) at physiologic pH. The unstable fatty acid remnant reacts with the epsilon part of the lysine residue in a free or protein bound amino acid. HEL is then formed by

enzymatic decomposition of the protein. HEL is a stable molecule that is excreted in urine (133).

Based on existing documentation (133;134) and assumptions of the excretion pathway, plasma and urine could be good media for determination of the constant turnover of HEL in the body. HEL has been measured in some diseases, and correlation has been documented between the level of HEL, oxidative damage and disease indicators (133-135).

HEL has been explored as a biomarker of oxidative stress in plants, animals and humans (133;136;137). It seems to correlate very well with 8OHdG. It is a promising biomarker of oxidative stress, but more and larger studies are required to confirm this.

1.7.8 Ferric reducing ability of plasma (FRAP, modified FRAP)

The FRAP method is a way to measure the total antioxidant capacity. But in plasma, there are endogenous antioxidants that originate from uric acid and proteins. Benzie and Strain estimated that uric acid accounts for 60% of FRAP, AA contributes with 15%, proteins with 10% and alpha-tocopherol and bilirubin with 5% each (138). Yeum et al. claim that FRAP and other similar assays mainly measure the watersoluble antioxidants (139). The mFRAP method remove uric acid and proteins by adding uricase and ethanol before the FRAP analysis is performed. The mFRAP result is expected to reflect the levels of dietary antioxidants more closely than FRAP, both the water soluble and the lipid soluble fractions. However, antioxidants that act by H transfer (thiols and carotenoids) will not be determined in the FRAP assay (140). FRAP is analysed using a spectrophotometric method.

1.8 Compliance biomarkers

Compliance biomarkers are used in order to verify that the subjects of the study actually have ingested and absorbed the intervention foods and supplements.

1.8.1 Omega-3 fatty acids

Red blood cells (RBCs) are easily collected and analysed; and they have a more stable day-to-day fatty acid composition than plasma. It has been shown that omega-3 levels in RBC reflect omega-3 supplementation (141).

1.8.2 Lycopene

Plasma lycopene is recognised and well documented as a very good biomarker for intake of tomato and tomato products in individuals (142).

2. Aims and research questions

This master thesis addresses oxidative stress biomarkers in a group of PC patients compared to an age matched healthy reference population.

The present work also analyses interim results (27 patients of 102) of a clinical study where the overall aim is to investigate whether patients diagnosed with localised PC may benefit from a food intervention. The study is the "Prostate cancer, phytochemical and PUFA intervention study" (PFPI). The PC patients were given tomatoes and other phytochemical rich foods for a period of three weeks and their compliance was recorded. The food items were selected based on literature and knowledge of antioxidants and phytochemicals in plant foods from research conducted by Rune Blomhoff and his research team, and they were believed to give an additive or synergistic effect.

The specific aims of this thesis are:

- To analyse and compare biological oxidative stress biomarkers in PC patients compared to a group of healthy references.
- To study the compliance to the PC intervention.
- To analyse and compare the biomarkers of oxidative stress measured in plasma and urine samples from the cancer patients, before and after the intervention.
- To analyse and compare the surrogate biomarker of PC, PSA, as measured in the PC patients before and after the intervention.

3. Materials

This chapter provides a list of materials and equipment used for sample handling and analyses in the laboratories of the University of Oslo. Also purchasing information and other details about the intervention food products are listed here.

3.1 Intervention food items

Food	**Manufacturer**	**Location**
Barilla Pasta sauce with basil	Ideal Wasa	Hamar, Norway
Dolmio Pasta sauce with garlic	Mars Food	Melton Mowbray, England
Dolmio Pasta sauce classico	Mars Food	Melton Mowbray, England
Knorr pasta sauce with oregano and onion	Unilever Norge	Billingstad, Norway
Tomato juice 1 L	Cadiso Food	Frederikssund, Danmark
Crushed tomatoes, 500g	ICA AB	Solna, Sweden
Twinings Java green tea	Twinings	London, England
Twinings Earl grey tea	Twinings	London, England
Pomegranate juice, 330 mL	Tine	Oslo, Norway
Grape juice, 330 mL	Tine	Oslo, Norway
Solaray Selenium supplement	Nutraceutical Corp for Solaray Inc	Utah, USA
Soy supplement	Natures`s sunshine Super soy extra	Utah, USA
Omega-3 supplements	Nycoplus omega-3	Oslo, Norway

3.2 Lycopene content of tomato products

Manu-facturer	Product	Packsize	Lycopene concentration mg/100 g	Daily portion size	Lycopene content per daily portion (mg)
Barilla	Pastasauce with basil	400 g glass	16.7	½ glass	33.4
Dolmio	Pastasauce with garlic	500 g glass	11.6	½ glass	29.0
Dolmio	Pastasauce classico	500 g glass	14.9	½ glass	37.2
Knorr	Pastasauce oregano and onion	400 mL glass	15.8	½ glass	31.6
Cadiso	Tomato juice	1 L carton	11.4	¼ carton	28.5
ICA	Crushed tomatoes	500 g carton	12.4	½ carton	31.0

3.3 Equipment

CPT with sodium Citrate	BD Vacutainer	Franklin Lakes, USA
Collection tube with Sodium Heparin	BD Vacutainer	Franklin Lakes, USA
Collection tube with K_2EDTA	BD Vacutainer	Franklin Lakes, USA
Stabilyte blood sampling tubes	Biopool (Trinity Biotech plc)	Irland
Urine containers Uriset 24	Sarstedt	Nürnberg, Germany
Urine containers 500 ml	Sarstedt	Nürnberg, Germany
Microtubes 1,5 ml	Sarstedt	Nürnberg, Germany
96 well ELISA microplates	Greiner BIO-ONE	Frichenhausen, Germany

3.4 Chemicals

Chemical/compound	Manufacturer	Location
Acetic acid	Merck	Darmstadt, Germany
Acetone (CH_3COCH_3)	Merck	Darmstadt, Germany
Acetonitrile	VWR International	Norway

Ascorbic acid	Sigma-Aldrich	Germany
Argon	AGA, 100325	Oslo, Norway
Ethanol	Arcus Kjemi	Oslo, Norway
$FeSO_4 \cdot 7H2O$	Riedel-deHaën AG	Seelze, Germany
$FeCl_3 \cdot 6H_2O$	BDH Laboratory Supplies	Dorset, England
Hydrocloric acid (HCl)	Merck	Darmstadt, Germany
Meta-phosphoric acid (MPA)	Riedel de Haën	Germany
2,4,6-tripyrdyl-s-triazine (TPTZ)	Fluka Chemie AG	Deisenhofen, Switzerland
tris[2-carboxyethyl]phosphine hydrochloride (TCEP)	BioChemika Fluka	Switzerland
Trizma buffer	Sigma-Aldrich	Germany
Triz buffer	Sigma-Aldrich	Germany
DULBECCO´S Phosphate Buffered Saline (PBS)	Sigma-Aldrich	Germany
Urate oxidase	Sigma-Aldrich	Germany
NaH_2PO_4	Sigma-Aldrich	Germany
Dodecyltrimethylammonium chloride	Fluka Chemie	Switzerland
Na_2EDTA	Sigma-Aldrich	Germany
Milli-Q water	Millipore	Bedford, MA
NIST 970 SRM	(National Institute of Standards and Technology - Standard Reference Materials)	USA

3.5 Kits

Kit name	**Manufacturer**	**Location**
Homocysteine by HPLC-kit, Kit no 195-4073	BIORAD	California, USA

Diacrons reactive oxygen metabolites (D-ROMs test) Kit	Diacron International	Grosetto, Italy
New 8OHdG Kit	Japan Institute for the Control of Aging (JaICA)	Shizuoka, Japan
HEL Kit	Japan Institute for the Control of Aging (JaICA)	Shizuoka, Japan

3.6 Instruments

Instrument	**Manufacturer**	**Location**
Biofuge Fresco	Heraeus Instruments	Osterode, Germany
Technican RA 1000 system	Technicon Instruments Corp	New York, Ny
L46 Vortex Mixer	Labinco	the Netherlands
KS 125 basic microtiter shaker	IKA Labortechnik	Staufen, Germany
Termaks Laboratory Incubator	Termaks	Bergen, Norway
HP1100 (HPLC)	Hewlett Packard	USA
GS-6R Sentrifuge	Beckman	USA
Titertec Multiscan PLUS	EFLAB	Finland

3.7 Software

Software package	**Manufacturer**	**Location**
Microsoft Office XP	Microsoft Corporation	Redmont, WA
Reference manager 11	ISI Research soft	Carsbad CA
SPSS 16 for Windows	SPSS Inc	Chicago, Il
HP Chemstation version 4 (HPLC software)	Hewlett Packard	USA

4. Subjects and Methods

4.1 Subjects

4.1.1 The study population (PC patients)

The PC patients that constitute the study population were recruited from the Norwegian Radium Hospital, Oslo University Hospital in the period from July 2007 to March 2008 in the PFPI clinical trial. Eligible patients were enrolled for prostatectomy or brachy theraphy (regular therapy) at the hospital. Together with an appointment letter for clinical examination, the patients received an invitation letter and a letter of informed consent, and were phoned a week later. The inclusion criteria included histological diagnosed adenocarcinoma of the prostate gland, and low or moderate risk of recurrence (T1c-T3a, PSA < 20 ng/mL or Gleason score of at least 6) without androgen deprivation therapy. All the patients had expected lifetime > 5 years and some patients suffered what was described as “not important comorbidity”, such as hypertension and skeletal diseases. The inclusion criteria are listed in Appendix 3 (143).

62 patients that met the inclusion criteria were invited to participate, 27 accepted to participate and were included and randomized and 26 patients completed the study. **Figure 4.1** displays inclusion of participants in the PFPI study. Age distribution of the study population by five-year age groups is illustrated in **Figure 4.2.**

The study was approved by the Regional Ethics Committee for medical Research (REK Sør-Øst), and all men gave informed written consent. One participant left the study because he was treated prior to original schedule.

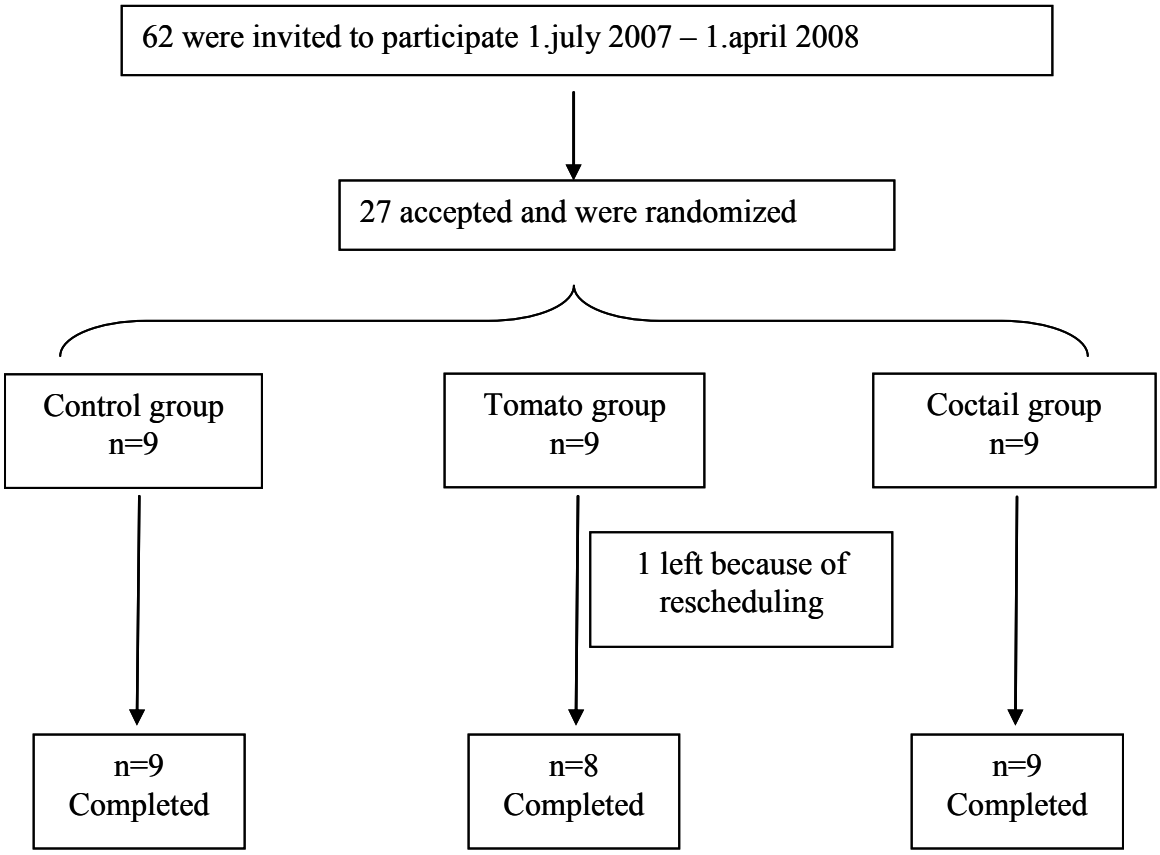


Figure 4.1 Inclusion and randomisation of participants in the PFPI study, constituting the study population

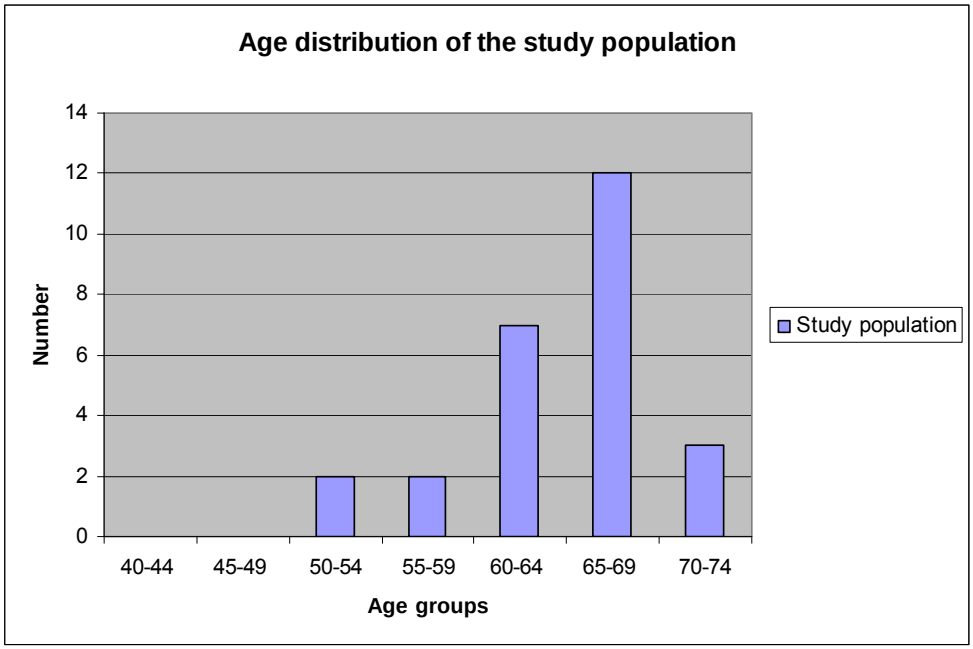


Figure 4.2. The age distribution by five-year age group of the study population

4.1.2 The reference population

The reference population was drawn from a population of 347 (162 men) apparently healthy individuals, random selected by the Statistics Norway, living in Oslo and Akershus. For the master thesis, a sub group of 32 were selected based on sex and age (male, 40 – 70 years), in order to match the Norwegian age-specific PC incidence rates (144). However, five participants had reported chronic inflammatory diseases and were left out, leaving 27.

Figure 4.3 shows the reference population divided by five-year age groups, using the same year intervals as for the study population.

Blood samples were available for all, while urine samples were available for 25 of the 27. In the reference population, 24 hour urine was collected in a pre-prepared can containing 9 ml 20% HCl and 240 mg Vitamin C.

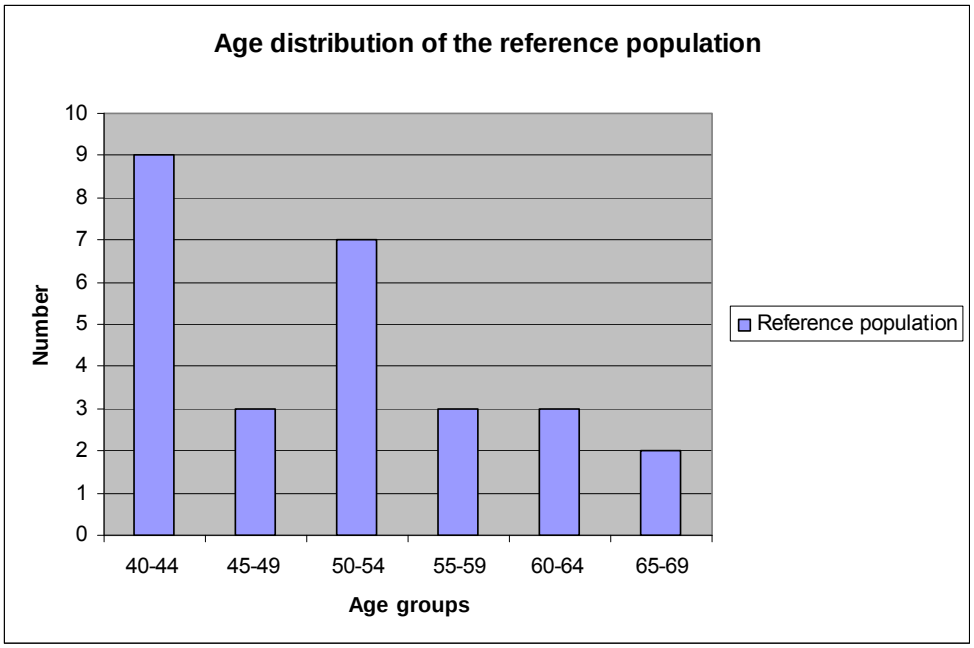


Figure 4.3. The age distribution by five-year age group of the reference population.

4.2 Study design and intervention

The PFPI study followed a randomized parallel design with a 3 week intervention period. The randomization was computer based and carried out by the Registry of Clinical Research at the Norwegian Radium Hospital, Oslo University Hospital. The subjects were randomized to 1 of 3 groups: one group that consumed a portion of various tomato products daily (tomato group, n=9) and one group that consumed tomato products and additional foods and supplements (multidiet group, n=9), and a control group who would continue their habitual diet (n=9). The intervention was given immediately preceding regular therapy. The patients visited the hospital three times: visit 1 for inclusion and randomization, visit 2 prior to the intervention period and visit 3 the day of regular theraphy. At all visits, overnight fasting blood samples and morning urine were collected.

Before visit 2, the participants filled in a food frequency questionnaire (FFQ). In this sheet the patients reported their dietary intake, antropometric measures such as height and weight, smoking, alcohol, comorbidity and supplement intake. The questionnaire

was filled in at home by the patient and returned in a prepaid envelope to the University of Oslo before visit 2. **Figure 4.4** gives an overview of the timing, visits and data collection.

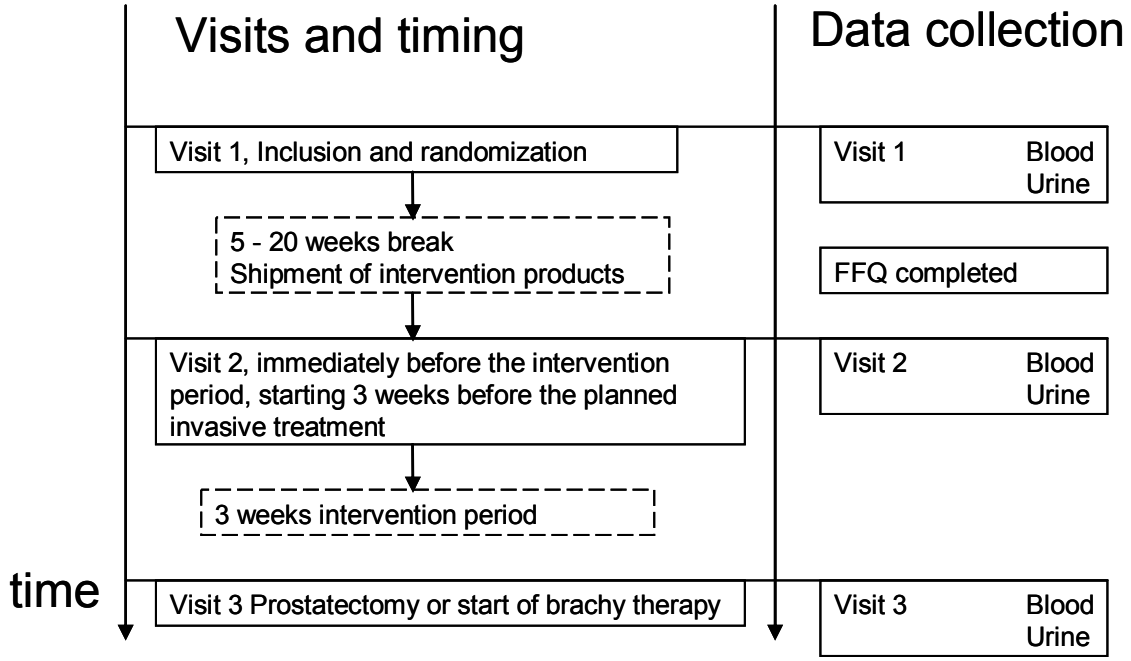


Figure 4.4 Timing, visits and data collection of the PFPI study

Blood and urine samples from visit 2 were missing for 1 subject in the control group and one in the tomato group, and visit 2 urine samples were missing for two control group patients.

4.2.1 Intervention foods and supplements

All patients were asked to continue their habitual diet during the intervention period. The control group did not receive any additional food. For the tomato and multidiet groups, the intervention products were intended as a supplement to their current diet.

The tomato group intervention

The patients randomized to the tomato group were supplied with various tomato sauces and canned tomatoes amounting to 200 – 250 grams per day. The tomato products had been analysed in our laboratory, and the products with the highest

lycopene content were chosen. The tomato group participants received at least 30 mg lycopene a day.

The multidiet group intervention

The multidiet group received juices, teas and tomato products for 24 days of intervention. They also received prepacked supplements from the chemists at the Norwegian Radium Hospital, before returning home after visit 1. The foods supplied to the multidiet group are listed in **Table 4.1**.

Supplement	**Morning**	**Evening**
Nycoplus omega-3, fish oil	3 × 1000 mg	2 × 1000 mg
Natures`s sunshine Super soy extra	2 × 40 mg	3 × 40 mg
Solaray selenium	1 ×100 µg	1 ×100 µg
Food	**Daily**	
Grape juice	330 ml	
Pomegranate juice	330 ml	
Tomatoes	200 – 250 grams, containing at least 30 mg lycopene	
Green tea	300 ml	
Black tea	300 ml	

Table 4.1 Multidiet group intervention products with daily portions.

The multidiet group received supplements of selenium, omega-3 fatty acids and soy. The daily dose of 200 µg selenium was given as two tablets of organic 1-selenomethionin. The omega-3 supplement was given as 5 capsules each containing 1000 mg fish oil, of which 65% is EPA and DHA, adding up to a daily dose of those two essential long chained omega-3 fatty acids of 3.25 grams. The patients were also supplemented with 200 mg isoflavones as soy bean extract. The daily supplement consisted of 5 tablets of ”Super soy ekstra” from Natures` Sunshine. The supplements were divided into morning and evening doses as shown above.

The multidiet group received the same amount of tomatoes as the tomato group.

The multidiet group received one cup daily of both Earl Grey green tea and Earl Grey black tea. Instructions for the preparation of the teas were as follows: “Heat 3 dl

water to the boiling point, pour the water over the tea bag and let it brew for 5 minutes before removing the tea bag. The tea may then be enjoyed hot or cold and sweetened with artificial sweeteners according to taste."

Juice from pomegranate and grape were prepared by TINE. The grape juice was made of the Merlot grape. The juices were supplied in boxes of 330 ml, constituting the daily portion.

All the intervention products are commercially available, except the pomegranate and grape juices which were prepared by Tine for research purposes.

All the participants were followed up weekly by telephone. Additionally they were supplied with telephone numbers and invited to contact the staff at the university or at the hospital for further information.

4.3 Laboratory analyses of biomarkers

Overnight fasting blood samples were collected by venous puncture in heparin, EDTA and citrate CPT (Cell Preparation Tube) tubes. Plasma samples were prepared by immediate centrifugation on 1500 g at 4 °C for 10 min. All samples were stored at -70 ºC if not immediately analysed.

For vitamin C analyses, to preserve vitamin C, heparin plasma was immediately acidified using an equal volume of 10% meta-phosphoric acid (MPA).

To determine d-ROMs test, FRAP and mFRAP, heparin plasma was used. EDTA plasma was used for determination of lycopene, GSH and cysteine.

Determination of d-ROMs test, FRAP, mFRAP, lycopene, GSH and cysteine was carried out in the Blomhoff laboratory at Department of Nutrition, UiO. For fatty acid analysis, RBCs were isolated from citrate CPT tubes. The analysis was performed by Vitas laboratories, Oslo. GGT and PSA were assayed by routine laboratory methods at the Department of Clinical Chemistry, Norwegian Radium Hospital.

HEL, 8OHdG and creatinine were determined in urine. Creatinine was analysed at Ullevål Hospital laboratory, while HEL and 8OHdG were tested in the Blomhoff laboratory.

The analyses of the samples from the PC study were performed from August to October 2008. The analyses of the reference population samples were performed during 2007 and 2008.

4.3.1 GGT

GGT were analysed in serum using routine laboratory methods at the clinical chemical department at the Norwegian Radium Hospital, Oslo University Hospital. This assay is based on VITROS GGT slides and VITROS Chemistry Products kalibratorkit 3 on VITROS Chemistry Systems, manufactured by Ortho-Clinical Diagnostics, Johnson & Johnson, United Kingdom.

Briefly, one drop patient serum is evenly distributed on the Vitros Slide. VITROS GGT slide is a multi layered analytical element coated on a polyester base. GGT present in the sample catalyses the transfer of the gamma-glutamyl portion of L-gamma-glutamyl-p-nitroanilide to glycylglycine, simultaneously producing p-nitroaniline. The kinetics of change in reflection densities is measured. Based on sequential readings of the slide reflectance at 400 nm over a fixed incubation period, the kinetics of change in reflection density is determined. By applying a prepared software multipoint calibration model, enzyme activity is calculated, and when a calibration has been carried out for each slide-slot, the GGT activity in unknown samples can be determined. Adult male reference interval as presented by the manufacturer for GGT is 15 – 73 U/L (145).

4.3.2 Reduced thiols, glutathione and cysteine

Our analysis method includes the pool of mixed and protein-bound disulfides in plasma. This measure is achieved by reduction of disulfides prior to analysis (123).

The reduced thiols glutathione (GSH) and cysteine were determined in EDTA plasma using the "Homocysteine by HPLC-kit", according to the manufacturer's instructions. In brief, 50 µL internal standard and 25 µL reduction reagent (trialcylphospide) was added to 25 µL plasma. To this solution, 50 µL derivatization reagent (ABD-F) was added. The sample was vortex mixed and incubated for 5 minutes at 50 Cº. The samples were then refrigerated for 5 minutes, before proteins were precipitated by the addition of 50 µL precipitation reagent (tricloroacetic acid). The samples were then vortex mixed and centrifuged for 15 min at 4000 g +4 ºC. From the clear supernatant, 20 µL were used for chromatographic analysis. As mobile phase the "Homocystein by HPLC" mobile phase (cat 195-4077) was used at 0.7 ml/min at 45 ºC with a "Homocysteine analytical cartridge" (Catalog no 195-4076, BioRad). For detection, a fluorescence detector (FLD) was used (385nm (ex) and 515 nm (em)).

The healthy population plasma samples for GSH analysis were pre-treated with serine borate, in order to avoid GGT to continue its enzymatic activity *in vitro* (146). The plasma samples from the cancer patients were not pre-treated in this way.

4.3.3 Dehydroascorbic acid: total ascorbic acid

Total ascorbic acid (TAA) is constituted by AA and DHAA.

A recently published HPLC method for rapid and accurate determination of the vitamin C isoforms DHAA and AA was used (147). In brief, heparin whole blood was immediately centrifuged at 2000 g at +4 ºC for 10 min for generation of plasma. To preserve vitamin C, the plasma was immediately acidified using an equal volume of 10% meta-phosphoric acid (MPA). Ideally, the analysis should be performed within 3 months of sample collection to avoid degradation of AA to DHAA.

The mobile phase consisted of 2% acetonitrile in an aqueous buffer containing 2.5 mmol/L NaH_2PO_4, 1.875 mmol/L dodecyltrimethyl ammonium chloride and 1.25 mmol/L Na_2EDTA in milli-q water. The flow rate was 6.0 mL/min. Samples were pretreated to reduce DHAA to AA before chromatographic determination. For

separation of AA from interfering plasma constituents, a Chromolith Performance RP18-e, 4.6x100mm column was used. For detection, a variable wavelength UV detector was used at 264 nm. Plasma calibrators quantified against the NIST 970 SRM (National Institute of Standards and Technology - Standard Reference Materials) was used as standards.

Direct determination of AA

At time of analysis, the acidified samples were centrifuged at 3000 g and +4 ºC for 15 min. 100 µL of the clear supernatant was diluted with 400 µL of the aqueous buffer in an auto sampler vial. The sample was centrifuged at 3500g for 10 min at +4 ºC and 5µL was used for chromatographic analysis and determination of AA.

Reduction of DHAA to determine TAA

At time of analysis, the acidified samples were centrifuged 3000 g at +4 ºC for 15 min. 50 µL reduction agent (2.3 mmol/L tris [2-carboxyethyl] phosphine hydrochloride (TCEP) in 800 mmol/L trizma buffer, pH 9.0) were added to 100 µL of the clear supernatant. After vortex mixing, the sample was incubated at room temperature for 7 min for complete reduction of DHAA, before the sample was diluted with 350 µL of the aqueous buffer. In this step, DHAA was reduced to AA, and the TAA can be determined in the sample. The sample was centrifuged at 3500g for 10 min at +4 ºC and 5 µL was used for chromatographic analysis and determination of TAA.

Concentration of DHAA

The concentration of DHAA was calculated by subtracting the concentration of AA from the TAA concentration. TAA and the ratio between DHAA and TAA is used for statistical analysis.

4.3.4 D-ROMs test

Diacrons reactive oxygen metabolites (D-ROMs) test was performed according to the information provided by the manufacturer (Diacron International, Grosetto, Italy). In brief, heparin plasma was diluted in an acidic buffer solution (pH 4.8). Iron present in the sample catalysed the breakdown of plasma hydroperoxides to alkoxyl and peroxyl radicals, which generate a coloured complex with the chromogen, N, N,-diethylparaphenylendiamine. The coloured complex was quantified in a photometer under the following conditions: filter of 500 nm wavelength, optical path 1 cm, temperature 37ºC and kinetic mode. The hydroperoxide concentration was correlated with detected colour intensity. The results are denominated in arbitrary units, Carratelli Units (CARR U) and 1 CARR U corresponds to 0.08mg H_2O_2 /100mL (126).

The reaction of this system (simplified):

$R\ OOH + Fe^{3+} \rightarrow R\ OO\bullet + Fe^{2+} + H^+$

$R\ OO\bullet + A\text{-}NH_2 \rightarrow R\text{-}OO^- + [A\ NH_2\bullet]^+$ (coloured)

Where:

- R OOH is a generic peroxide.
- R O• is the alkoxyl radical of a generic peroxide
- R OO• is the peroxyl radical of a generic peroxide
- $A\text{-}NH_2$ is N,Ndiethyl-para-phenylendiamine (chromogenic substrate).
- $[A\text{-}NH_2\bullet]^+$ is the coloured radical cation of the chromogenic substrate (148).

4.3.5 The Ferric Reducing Ability of Plasma (FRAP) Assay

The FRAP assay was used to measure the content of total redox active compounds, antioxidants in plasma. The assay is based on the method by Benzie and Strain, 1996.

In brief, 300 µL of FRAP reagent **(Table 4.2)** was added to 10 µL heparin plasma. Reducing agents (antioxidants) in the sample reduces the ferric ions to ferrous ions, which forms a highly coloured complex with the chromogen, tripyrdyltriazine TPTZ, and an intense blue colour with an absorption maximum of 593nm will appear, proportional to the reducing ability of the sample. The reduction takes place at an acidic pH. The reaction is non-specific, and any half-reaction which has less positive redox potential than the Fe^{3+}/Fe^{2+} half reaction will drive the reduction. A Technican RA 1000 system was used to determine triplicates of absorption at 600nm. An aqueous solution of 500 µmol $FeSO_4$ reagent (Riedel de Haen) was used for instrument calibration.

Reagents	**Components**	**Volume in FRAP reagent**
Acetate buffer	300 mmol/l acetate buffer, pH 3.6 + $C_2H_4O_2$ 16 ml buffer solution	25 ml
HCl	40 mmol/l	2.4 ml
$FeCl_3$	20 mmol/l	2.5 ml
TPTZ solution	250 mg in 5 ml methanol	155 µl

Table 4.2 Frap reagents

4.3.6 mFRAP, FRAP after removing uric acid and proteins

For preparation of samples free of uric acid and proteins, 10 µL uricase (0.1 units/10 µL) in triz buffer (pH 8.5, 400 mmol/L) were added to 20 µL heparin plasma. After incubation for 6 min at room temperature, 80 µL ethanol was added to precipitate proteins. The samples were incubated at 4 ºC for 6 min before centrifugation at 13000g at 4 ºC for 10 min. The clear supernatant was used for FRAP analysis as described above.

4.3.7 Hexanoyl Epsilon Lysine (HEL) in urine

The amount of HEL in urine was determined by ELISA according to the manufacturer's instructions. The urine samples were sentrifuged at 4000 g and +4 ºC for 20 minutes, before 100 µL urine were diluted with 400 µL PBS. 50 µL of the diluted urine samples were placed in the wells of the microtiterplate before 50 µL of the primary antibody (anti HEL monoclonal antibody) was added. The wells were precoated with a hexanoyl-Lys adduct (HEL) from the manufacturer and the HEL in the samples would compete with HEL on the well surface for the added anti HEL antibody. The plate was sealed and incubated at 250 rpm/min at 4 – 7 ºC for 18-20 hours. The plate was washed 3 times, removing all HEL in the sample bound to anti HEL antibody, before the addition of the secondary antibody. The secondary antibody would bind to the anti HEL antibody that was left. The plate was sealed and incubated at 500 rpm/min at room temperature for 1 hour. The plate was washed 3 times to remove unbound secondary antibody, before the addition of 100 µL chromogen solute and the plate sealed with light proof aluminium foil and incubated at 500 rpm/min at room temperature for 15 minutes. Addition of the chromogen results in the development of colour in proportion to the amount of antibody bound to the well. The stop solute was added and the absorption was measured at 450 nm within 3 minutes. Diluting the wells 1:5 with MilliQ water was necessary to obtain absorbance within valid limits. The results were calculated using the standard curve as suggested by the manufacturer, with a linear approximation to the logarithmic

graph. Standard A and Standard F were removed from the linear curve to get a better approximation for the measured values.

4.3.8 8OHdG in urine

The amount of 8OHdG in urine was determined by the "New 8-OHdG Check", ELISA Kit according to the manufacturer's instructions. The kit is utilizing anti 8-OHdG monoclonal antibody (clone N45.1) which is highly specific for 8-OHdG. "New 8-OHdG Check" ELISA is suitable for urine and serum sample from animal and human (149). The urine samples were sentrifuged at 4000 g +4 ºC for 20 minutes, before 100 µL urine were diluted with 300 µL PBS. 50 µL of the diluted urine samples were placed in the wells of the microtiterplate and then 50 µL of the primary antibody was added. The plate was sealed and left to incubate at 37 ºC for 1 hour. The plate was emptied and dried with clean cell-tissue and then thoroughly washed. The secondary antibody was mixed with secondary antibody buffer and 100 µL added to the wells. The plate was again sealed and left to incubate at 37 ºC for 1 hour. The plate was again thoroughly washed. Then 100 µL chromogen solute was added, the plate sealed with aluminium foil (light proof) and incubated (shaken at 500 rpm) at room temperature (20 - 25 ºC) for 15 minutes. The stop solute was added and the absorption was measured in the spectrometer at an absorbance of 450 nm. Dilution of the samples 1:5 was required for valid measurement.

4.3.9 Creatinine

Determination of creatinine was conducted by the clinical chemical department at Ullevål University Hospital using the "DRI Creatinine-Detect Test", according to the manufacturer's instructions. The analysis was performed using an Olympus AU400 clinical chemistry analyser. BIORAD Liquicheck Urine Chemistry Control 1 lot nr 62831 with mean of 6.89 mmol/L and Range 5.86 – 7.92 mmol/L was used for instrument calibration.

The method is based on the Jaffe reaction:

Creatinine + Picric Acid -> Janovski Complex (Red)

The Janovski Complex gives a red colour. The colour intensity is directly proportional to the creatinine concentration and is measured spectrophotometrically at 505nm.

Urine creatinine is used as a simple measure of concentration, for instance in connection with drug abuse testing. It normally varies a lot during the day, greatly influenced by e.g. fluid intake.

Urine male reference values for 24 hours collection are 4 – 17 mmol/d, while the reference value for spot urine samples is > 3 mmol/L (150).

4.3.10 PSA

PSA were analysed in serum using routine laboratory methods at the clinical chemical department at the Norwegian Radium Hospital, Oslo University Hospital, using the AutoDELFIA Prostatus™ PSA Free/Total assay (Perkin Elmer, Inc., Finland). This assay determines the total and free amounts of human PSA in serum using the 1235 AutoDELFIA immunoassay system. Only the total PSA results are reported here.

The assay is a solid phase time-resolved fluoroimmunometric assay based on the direct sandwich technique in which three monoclonal antibodies (derived from mice) are used (151). In serum, PSA detectable by immunohistochemical methods is predominantly found as complexed to alpha-1-antichymotrypsin (PSA-ACT). Small amounts of PSA complexed to other serine protease inhibitors are also found. The free or uncomplexed fraction of PSA represents 5 – 30 % of the total immuno accessible PSA in serum. Total-PSA consists of free PSA and PSA-ACT (152).

4.3.11 Lycopene

Lycopene was determined in plasma by HPLC. Proteins were precipitated and removed by the addition of a 4.5 volume of isopropanol followed by centrifugation at 3000 g and 4 °C for 15 minutes. The internal standard astaxanthin was added with the isopropanol. 25 µL of the clear supernatant were used for analysis. The mobile phases consisted of A: 20 % water and 24 % acetone in ethanol and B: acetone. The gradient conditions were as follows: From 2 to 100 % B within 20 minutes, followed by 100 % B for 15 minutes. Detection was performed at 453 nm using a variable wavelength detector. Plasma calibrators quantified against the NIST 968c SRM served as standards.

4.3.12 Fatty acids in RBC

Determination of fatty acids in red blood cells was performed at AS Vitas, Oslo. 100µL of whole blood was prepared with a direct transmethylation and hexan extraction of fatty acid methyl esters (FAME) which were subsequently analysed with a GC – FID. The results were reported as g fatty acid/100g FAME (Fatty Acid Methyl Esters). The reported fatty acids were C20:5, n-3 (EPA); C22:5, n-3 (DPA); C22:6, n-3 (DHA) and sum of omega-3 fatty acids. The reported fatty acids, including the omega-3 fatty acids, constitute 94 – 97 % of the total fatty acids in the sample.

4.4 Intervention compliance

When included, and during weekly telephone interviews, the patients were encouraged to consume the intervention foods and supplements as prescribed. Compliance was recorded in a custom-made questionnaire for daily intake. The sheets ”Skjema for registrering av tomatprodukter i intervensjonsperioden” and ”Skjema for registrering av multi-diett gruppe produkter i intervensjonsperioden” are

included in Appendices 6 and 8. The sheet was supplied with a prepaid envelope and the patients returned these to the university immediately after the intervention period.

Lycopene in plasma and fatty acids in red blood cells were analysed as biomarkers of compliance. Lycopene in plasma is known to be a very good biomarker for intake of tomato products, and omega-3 fatty acids in red blood cells reflect the intake of these fatty acids from food and supplements.

Compliance of the individual participant was evaluated based on the self reported sheets, and participants with less than 50% would be classified as non-compliant and excluded.

4.5 Combining biomarkers into indexes

Measuring a range of biomarkers gives an opportunity to combine them into indexes. We suggested that these indexes would reflect the status better that the individual biomarkers. The biomarkers were combined into four indexes:

- Endogenous index (GSH + Cysteine) – these biomarkers may reflect the status of the endogenous oxidative stress defence system.
- Damage index (8OHdG + HEL + d-ROMs test + DHAA:TAA), combining the biomarkers that are associated with oxidative stress damage.
- Antioxidant index (Lycopene + TAA + mFRAP) – these biomarkers measure dietary antioxidants.
- Combined index (DHAA:TAA + GGT + d-ROMs test – GSH – Cysteine). The selection of biomarkers for the combined index was based on comparison of the study population with the reference population. The selected biomarkers were those that turned out significantly different between the two populations and GGT which showed a strong trend. The levels of DHAA:TAA, GGT and d-ROMs test were higher in the study group than in the reference population,

while GSH and Cysteine were lower. Accordingly, we decided to add the first three biomarkers and subtract the last two.

4.5.1 Calculating indexes

The indexes were calculated in two steps, (1) normalization and (2) adding or subtracting selected biomarkers.

(1) The differences from baseline to after the intervention (Δbiomarker) were normalized by the following formula:

$$\text{normalized } \Delta\text{biomarker A (patient a)} = \frac{(\Delta\text{biomarker A (patient a)} - \text{group mean } \Delta \text{ biomarker A}}{\text{group standard deviation } \Delta\text{biomarker A}}$$

(2) The indexes were calculated by addition or subtraction of the normalized Δbiomarkers:

index 1(patient a) =

normalized Δbiomarker A (patient a) +

normalized Δbiomarker B (patient a) + … + normalized Δbiomarker N (patient a)

4.6 Statistical analysis

Student`s t-test (for normally distributed data) and Mann-Whitney non-parametric test (for non-normally distributed data) were performed to analyse the mean and median baseline values between the reference population and the PC patients and to compare changes that were observed during the intervention period between the multidiet, tomato and control groups in the intervention study.

Spearman's and Pearson's correlation coefficients were used for correlation between parameters.

Visit 2 samples for some of the patients were lacking, as mentioned earlier. Due to the small number of participants we used visit 1 samples instead of visit 2 samples as baseline values. The statistical analyses were performed with and without these values, without substantial differences in the level of statistical significance.

All statistics were performed using SPSS version 16.0. A p-value below 0.050 was considered statistical significant. Results are expressed as mean (range) for normally distributed and median (range) for non-normally distributed data.

4.7 Contribution of the master candidate to the clinical study (PFPI)

Telephone based patient communication including information, asking for participation and weekly follow up during the intervention period was carried out by the candidate. Coordination and collaboration with the hospital staff and packing and shipping the intervention products (juices, teas and tomato products) was handled by the candidate.

Analyses of the biological samples were planned, prepared and coordinated by the candidate. HPLC analyses (vitamin C, GSH and cysteine) were carried out by Anette Karlsen and observed by the candidate. FRAP, mFRAP and d-ROMs test was carried out by the candidate with assistance by Kari Holte. ELISA determination of HEL and 8OHdG was carried out by the candidate.

All statistical analyses of all parameters were prepared, executed and interpreted by the candidate.

5. Results

5.1 Comparing the study population at baseline to the reference population

Descriptive data of the study population and the reference population were compared (**Table 5.1)**. Biomarkers related to oxidative stress and antioxidant status were analysed for the two populations and statistical comparisons were performed. From the study population, baseline data (from visit 2) were used.

Table 5.1: Differences between the study population and the reference population

Parameter	**Reference population n = 27[a]**	**Study population n = 25**	**p**
Descriptive data			
Age [b], years	52 (41 - 67)	64 (52 - 74)	< 0.001
BMI [a, b], kg/m^2	27 (22 - 40)	27 (21 - 36)	n.s.
Nr of smokers	2	3	
Antioxidant biomarkers in plasma			
TAA [c] µmol/L	n = 27 52.0 (7.3 – 84.0)	n = 24 73.2 (34.5 – 140.1)	< 0.001
GSH [c] µmol/L	5.3 (2.8 – 14.10)	4.2 (1.7 – 9.1)	0.009
FRAP [c] µmol/L	1297 (793–1604)	1322 (861-1765)	0.383
Cysteine [c] µmol/L	293 (226 – 386)	253 (202 – 366)	0.003
mFRAP [c] µmol/L	374 (294–443)	421 (297 – 701)	0.003
Oxidative stress related biomarkers in plasma			
DHAA:TAA [c] ratio	n=27 0.071 (0.058 – 0.111)	n=24 0.147 (0.025 – 0.280)	< 0.001
GGT [c] U/L	25 (12–92)	30 (13 – 145)	0.160
d-ROMs test [c] Carr U	166 (105–243)	221 (29 – 295)	< 0.001

Parameter	Reference population n = 27[a]	Study population n = 25	p
Oxidative damage biomarkers in urine			
HEL [c] nmol/L	n =25 127 (43 – 255)	n=26 111 (57 – 281)	0.429
HEL [c] µmol/mol creatinine	14.6 (3.6 – 34.4)	8.5 (3.2–20.2)	0.004
8OHdG [c, d], ng/mL adjusted	9.8 (3.8 – 30.5)	10.0 (4.1 – 32.6)	0.865
8OHdG [c, d] ng/mmol creatinine adjusted	1057 (318 – 2291)	745 (320 –2029)	0.025
Urine excretion correction factor			
Creatinine [c] mmol/L	9 (5 – 20)	14 (5 – 22)	< 0.001

[a] Body weight was self reported in the study population, while it was measured by medical personnel in the reference population.

[b] Normally distributed parameter, presented as mean (range). Students T-test was used to compare the groups.

[c] Non-normally distributed parameter, presented as median (range). Mann-Whitney test was performed to compare the groups.

[d] Adjustment of 8OHdG: The control urine was calculated against the standardized curve. The control urine was then used to calibrate the next kits. ELISA is a semi-quantitative measure, and small variations are expected between the kits. Ideally, all the samples would be analysed in one kit, but due to the kit capacity, this was not possible.

5.1.1 Descriptive data

The mean age of the 27 healthy men and the 25 PC patients included in the analyses was 52 years (41 – 67) and 64 years (52 – 74) respectively, thus the PC patients were on average 12 years older than the reference population.

BMI was 27 (22 – 40) in the reference population and 27 (21 – 36) in the study population.

The age distribution in the study population is displayed in **Figure 4.2** while the reference population is shown in **Figure 4.3**. There were two smokers in the reference population and three smokers in the study population.

5.1.2 Antioxidant biomarkers in plasma

Vitamin C was elevated in the study population compared to the reference population ($p < 0.001$). FRAP was not different between the groups, while mFRAP was higher among the cancer patients ($p = 0.003$). GSH and cysteine were lower in the study population compared to the reference population ($p = 0.009$ and $p= 0.003$ respectively).

5.1.3 Oxidative stress related biomarkers in plasma

The ratio between oxidised and reduced vitamin C, DHAA:TAA was elevated within the study population ($p< 0.001$) compared to the reference population. There was a trend indicating that GGT could be higher in the study population compared to the reference population, but this was not statistically significant ($p=0.160$). D-ROMs test was higher in the study population at baseline ($p<0.001$).

5.1.4 Oxidative damage biomarkers in urine

Urinary HEL was not different between the study population and the reference population, but when adjusted for creatinine, there appeared to be a significant difference ($p<0.004$) between the groups **(Table 5.1)**. The reference population presented higher HEL levels than the study population. Urinary 8-OHdG, when adjusted for creatinine, was lower among the study population at baseline than the reference population ($p<0.025$).

5.1.5 Urine excretion correction factor

Urinary creatinine was elevated in the study population at baseline compared to the reference population ($p < 0.001$).

5.1.6 Correlations between selected biomarkers

Statistical significant correlations were revealed between some of the biomarkers. **Figure 5.1** and **Table 5.2** illustrate a strong nonlinear correlation between GSH and GGT in the study population ($p<0.001$) and a much weaker trend in the reference population ($p= 0.087$). **Figure 5.2** and **Table 5.3** display a strong inverse linear correlation between d-ROMs test and TAA in the study population ($p=0.009$) but not in the reference population.

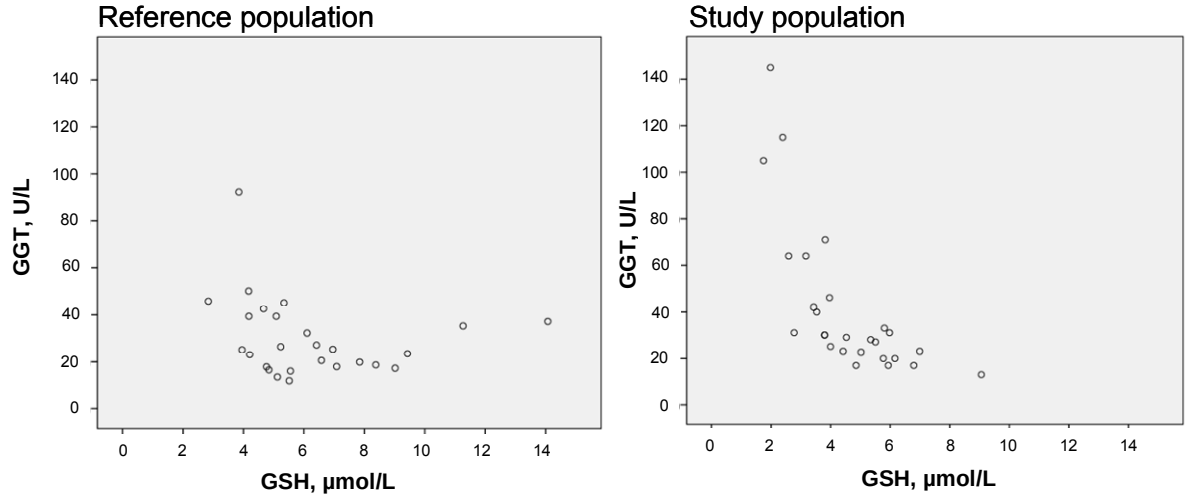


Figure 5.1 Scatter plot of GGT and GSH in the reference population (left) and the study population at baseline (right)

Spearman's rho	**GSH/GGT**	**p**
Reference population	-0.34	0.087
Study population, baseline	-0.82	< 0.001

Table 5.2 Spearman's rho correlation coefficient of GGT and GSH in the reference population and the study population at baseline

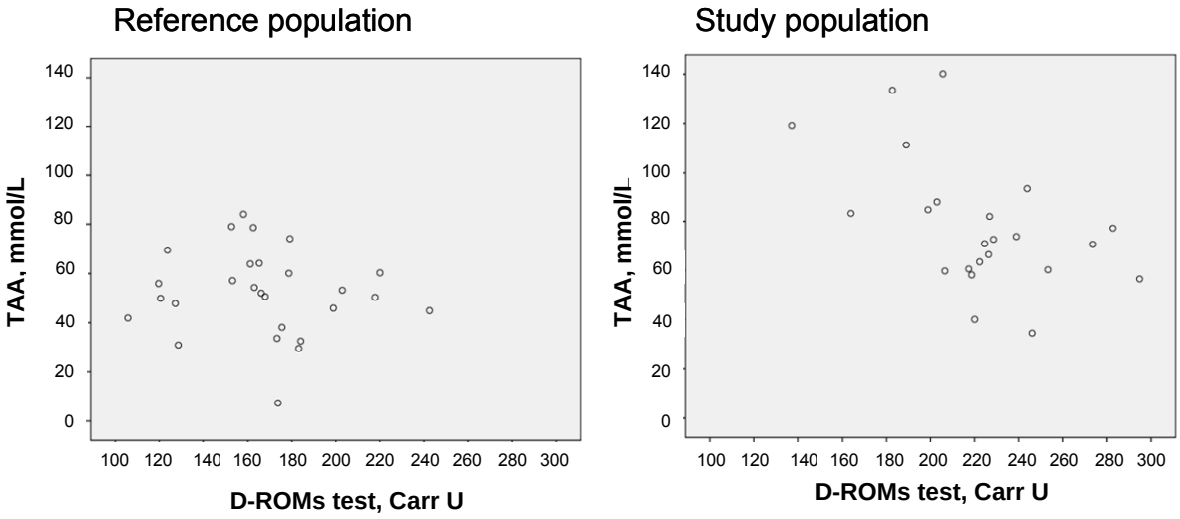


Figure 5.2 Scatter plot of TAA and d-ROMs test in the reference population (left) and the study population at baseline (right)

Pearson's correlation	**D-ROMs test/TAA**	**p**
Reference population	-0.096	0.632
Study population at baseline	-0.53	0.009

Table 5.3: Pearson's correlation coefficient of TAA and d-ROMs test in the reference population and the study population at baseline.

5.2 Effect of intervention

5.2.1 Descriptive parameters of the intervention groups

Table 5.4 shows the descriptive parameters of the intervention groups.

Parameter	**Tomato group**	**Control group**	**Multidiet group**
Age [b], years	64 (52 - 71)	64 (55 - 69)	65 (54 – 74)
BMI [a, b], kg/m^2	25 (21 - 33)	28 (22 - 36)	26 (22 - 29)
Nr of smokers	1	1	1

Table 5.4 Descriptive parameters of the intervention groups

[a]Body weight was self reported among the PC patients.

[b] Normally distributed parameter, presented as mean (range). Students T-test was used to compare the groups.

5.2.2 Compliance

17 patients, 8 in the tomato group and 9 in the multidiet group, completed the tomato intervention. The average intervention period lasted for 22.4 days, varying from 21 to 24 days. The intervention duration would vary to account for weekends, when the hospital is closed. The intervention started the day after visit 1 and lasted to include the day before treatment. All the patients received tomato products for 26 days of intervention. The compliance for the tomato products was 99%, ranging from 93% to 113%. The reason for compliance above 100% was that some participants consumed more than the prescribed daily intake.

Figure 5.3 shows what the patients preferred. Tomato juice seemed to be the most popular product, possibly because it did not require any preparations.

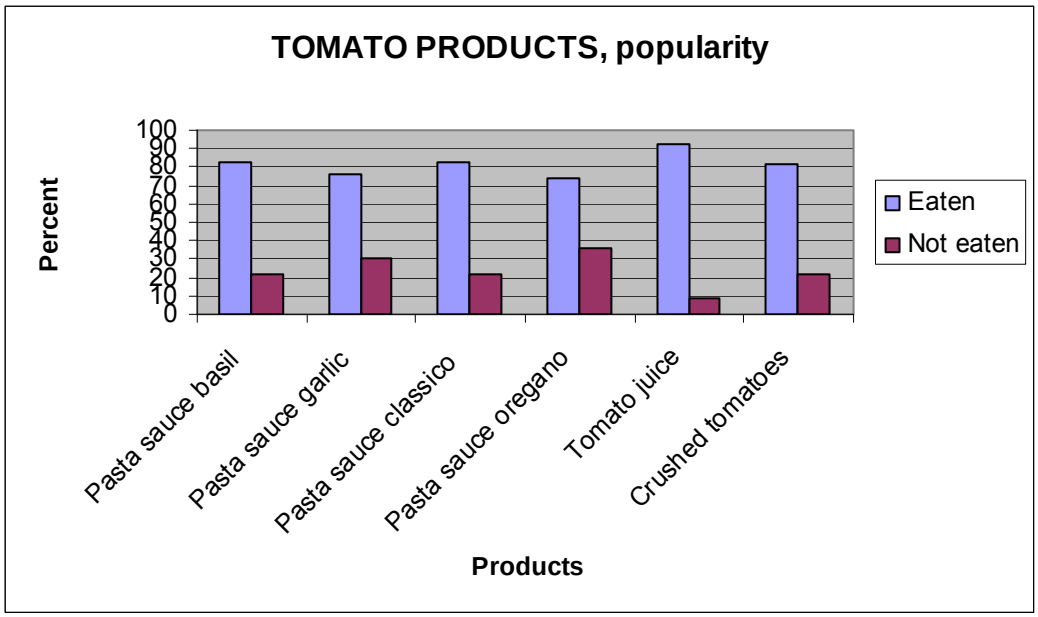


Figure 5.3 Consumption of the tomato products. The patients received tomato products enough for 26 daily portions. The bars indicate the self reported consumption.

The multidiet products were consumed by 9 patients. The days of intervention in this group varied from 21 to 24 days, with average length of 23 days. Compliance for the multidiet products is shown in **Table 5.5.**

Food item	Compliance % (range)
Green tea	96 (73 – 100)
Black tea	97 (91 – 100)
Pomegranate juice	98 (91 – 100)
Grape juice	99 (91 – 100)
Morning supplements	100 (96 – 100)
Evening supplements	99 (95 – 100)

Table 5.5 Consumption compliance of food and supplements in the multidiet group.

One patient reported problems with dyspepsia which resulted in reduced compliance. This patient was familiar with the symptoms. He managed to keep the problem at a minimum, and was able to complete the intervention. His digestion problem was mainly related to the concentrated juices.

5.2.3 Compliance biomarkers

Plasma lycopene and omega-3 fish fatty acids were analysed to verify the self reported compliance for tomatoes and omega-3 respectively. These are shown in **Table 5.6**. Plasma concentrations of lycopene increased significantly in the tomato group and the multidiet group ($p < 0.001$ in both groups) as compared to the control group. Plasma concentrations of total omega-3 fish fatty acids increased significantly in the multidiet group ($p < 0.001$) as compared to the control group and the tomato group.

Table 5.6 Compliance biomarkers baseline values and changes during the intervention period.

Parameter	Tomato group		Control group		*Multidiet group*		*Difference between groups*	
	n ***Baseline***	***Difference***	***n*** ***Baseline***	***Difference***	***n*** ***Baseline***	***n*** ***Difference***	***Tomato vs Control***	***Multidiet vs Control***
Lycopene [a], µmol/L	n=7 0.55 (0.062 – 0.992)	n=7 0.41 (0.209 – 1.109)	n=9 0.54 (0.21 – 0.82)	n=9 -0.10 (-0.55 – 0.05)	n=9 0.38 (0.25 – 0.73)	n=9 0.37 (0.18 – 1.07)	< 0.001	< 0.001
Total n-3 fatty acids [a], mg/100g (EPA, DHA, DPA)	n=8 8.7 (5.7 – 12.8)	n=8 -0.1 (-1.9 – 1.1)	n=9 9.1 (6.4 – 12.8)	n=9 -0.1 (-3.7 – 1.2)	n=9 10.5 (8.8 – 12.8)	n=9 1.1 (0.9 – 2.3)	0.963	0.001

[a] Non-normally distributed parameter, presented as median (range). Mann-Whitney test was performed to compare the groups.

5.2.4 Effects of the intervention on the biomarkers of oxidative stress

No effects of the intervention were observed in the biomarkers of antioxidant status and oxidative stress. Baseline values and changes after the intervention period for plasma and urine measures of antioxidants and oxidative stress status are listed in **Table 5.7**.

Table 5.7: PC intervention study. Comparison of baseline values and changes during the intervention period. Non-normally distributed parameters, presented as median (range). Mann-Whitney test was performed to compare the groups

Parameter	Tomato baseline	Tomato difference	Control baseline	Control difference	Multidiet baseline	Multidiet difference	P (difference) tomato vs control	P of (difference) multidiet vs control
Antioxidant biomarkers in plasma								
TAA , µmol/L	n=7 71 (57 – 111)	n=7 -4 (-36 – 26)	n=8 72 (35 – 140)	n=8 13 (-94 – 23)	n=9 82 (40 – 134)	n=9 -3 (-61 – 32)	0.536	0.277
GSH , µmol/L	n=8 4.5 (1.8 – 6.2)	n=8 1.1 (-0.9 – 2.0)	n=9 4.0 (3.4 – 9.1)	n=9 2.1 (0.1 – 3.3)	n=8 4.6 (2.0 – 6.0)	n=8 0.9 (-1.34 – 3.9)	0.167	0.815
Cysteine , µmol/L	n=8 246 (213 – 292)	n=8 61 (18 – 94)	n=9 255 (202 – 366)	n=9 63 (24 – 147)	n=8 282 (243 – 340)	n=8 45 (2 – 92)	0.606	0.200
FRAP, µmol/L	n=8 1286 (881 – 1675)	n=8 37 (-240 – 236)	n=9 1343 (966 – 1764)	n=9 21 (-158 – 213)	n=9 1328 (861 – 1706)	n=9 61 (-193 – 259)	0.888	1.000
mFRAP, µmol/L	n=8 427 (355 – 701)	n=8 -92 (-266 – 39)	n=9 417 (297 – 508)	n=9 -31 (-126 – 186)	n=9 407 (362 – 557)	n=9 -65 (-111 – 48)	0.167	0.387

Parameter	Tomato baseline	Tomato difference	Control baseline	Control difference	Multidiet baseline	Multidiet difference	P (difference) tomato vs control	P of (difference) multidiet vs control
Oxidative stress related biomarkers in plasma								
DHAA:TAA ratio	n=7 0.132 (0.079 – 0.201)	n=7 -0.031 (-0.119– 0.033)	n=8 0.172(0.025–0.233)	n=8 -0.068(-0.228– 0.010)	n=9 0.172 (0.058– 0.280)	n=9 -0.060 (-0.183– 0.014)	0.189	0.963
d-ROMs test, Carr U	n=6 248 (189 – 295)	n=6 -1 (-83 – 19)	n=9 225 (163 – 246)	n=9 1 (-18 – 28)	n=9 207 (137 – 253)	n=9 12 (-26 – 27)	0.776	0.340
GGT U/L	n=7 23 (17 – 105)	n=7 2 (0 – 9)	n=9 27 (13 – 46)	n=9 0 (-3 – 3)	n=9 33 (20 – 145)	n=9 0 (-48 – 39)	0.071	0.931
Oxidative damage biomarkers in urine								
U-HEL nmol/L	n=8 105 (57 – 124)	n=8 21 (-66 – 52)	n=9 128 (59 – 281)	n=9 -5 (-138 – 146)	n=9 150 (87 – 212)	n=9 17 (-55 – 87)	0.815	0.863

Parameter	Tomato baseline	Tomato difference	Control baseline	Control difference	Multidiet baseline	Multidiet difference	P (difference) tomato vs control	P of (difference) multidiet vs control
Oxidative damage biomarkers in urine								
U-HEL µmol/mol creatinine	n=8 7.0 (3.8 – 17.0)	n=8 2.7 (-1.8 – 11.4)	n=9 8.3 (3.2 – 20.2)	n=9 2.2 (-1.6 – 20.7)	n=9 9.1 (7.2 – 11.9)	n=9 2.9 (-5.4 – 24.2)	1.000	0.730
U-8OHdG [a] ng/mL adjusted	n=8 8.4 (4.1 – 32.6)	n=8 0.13 (-5.7 – 6.6)	n=9 11.9 (6.3 – 27.1)	n=9 -1.3 (-11.0 – 12.8)	n=9 12.4 (4.1 – 26.1)	n=9 -2.1 (-8.1 – 3.5)	0.200	0.863
U-8OHdG [a] ng/mmol creatinine, adjusted	n=8 761 (385 – 1906)	n=8 184 (-621 – 341)	n=9 752 (481 – 2029)	n=9 17 (-595 – 1574)	n=9 739 (320 – 1194)	n=9 65 (-190 – 396)	0.888	0.796
Urine excretion correction factor								
Creatinine mmol/L	n=8 13.8 (4.5 – 17.1)	n=8 -1.0 (-8.1 – 1.7)	n=9 13.9 (8.6 – 20.4)	n=9 -2.1 (-10.4 – 3.2)	n=9 15.0(9.6 – 22.0)	n=9 -3.6 (-12.8 – 12.2)	0.423	0.489

[a] Adjustment of 8-OHdG: The control urine was calculated against the standardized curve. The control urine was then used to calibrate the next kits. ELISA is a semi-quantitative method and small variations are expected between the kits. Ideally, all the samples would be analysed in one kit, but due to the kit capacity, this was not possible.

5.2.5 Combining the measures: indexes

To evaluate a combination of the biomarkers four indexes were calculated. These were Endogenous index, Damage index, Antioxidant index and Combined index. Explanation of the index calculation was presented in chapter 4.5

The results and analyses of the indexes are listed in Appendix 9. No effects of the intervention were observed in the combined biomarkers.

5.2.6 Prostate Specific Antigene

Plasma PSA was determined immediately before and after the intervention period and the results are presented in **Table 5.8**. Although not significant when compared to the control group ($p = 0.071$), a strong trend of PSA reduction was observed for the tomato group. There was also a trend for lower increase of PSA within the multidiet group ($p= 0.114$) when compared to the control group.

Table 5.9 shows that PSA developed significantly different between the intervention groups (tomato and multidiet groups, added together) and the control group ($p=0.045$).

Figure 5.4 presents bar charts of the PSA and lycopene differences over the three week's intervention. No controls presented PSA decline, while in the multidiet group four of nine experienced a reduction of PSA. In the tomato group three of seven showed total PSA reduction.

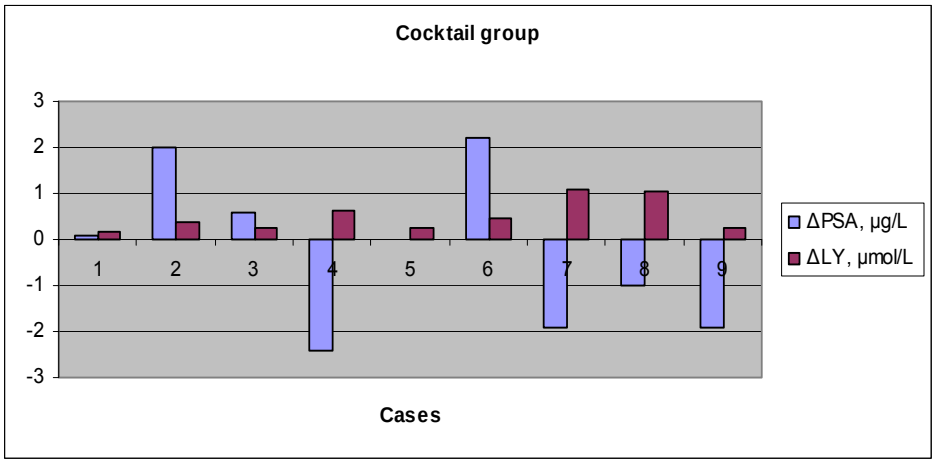
Table 5.8: PC patient intervention study, plasma PSA before and after the intervention period. Non-normally distributed parameter, presented as median (range). Mann-Whitney test was performed to compare the differences between the groups. The baseline values did not differ statistically significant between the groups.

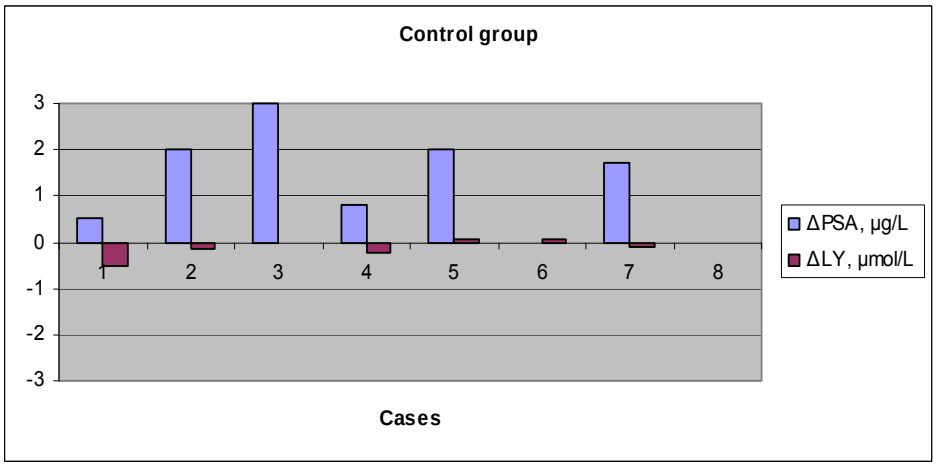
Parameter	Tomato group[a]	Tomato group[a]	Control group[a]	Control group[a]	Multidiet group[a]	Multidiet group[a]	P of difference	
	n ***Baseline***	***n*** ***Difference***	***n*** ***Baseline***	***n*** ***Difference***	***n*** ***Baseline***	***n*** ***Difference***	***Tomato vs Control***	***Multidiet vs Control***
P-PSA, µg/L	n=7 8.1 (4.7 – 19.0)	n=7 0.0 (-3.0 – 2.7)	n=8 7.7 (3.9 – 16.0)	n=8 1.3 (0.0 – 3.0)	n=9 10 (4.9 – 20.6)	n=9 0 (-2.4 – 2.2)	0.072	0.114

[a] Non-normally distributed parameter, presented as median (range). Mann-Whitney test was performed to compare the groups.

Table 5.9 PC patient intervention study. Plasma PSA before and after the intervention period for the patients receiving tomatoes (The tomato and multidiet groups are added). Non-normally distributed parameter, presented as median (range). Mann-Whitney test was performed to compare the differences between the groups. A p-value below 0.050 is considered statistically significant. The baseline values did not differ statistically significant between the groups.

Parameter	Intervention groups	Intervention groups	Control group	Control group	P of difference
	n ***Baseline***	***n*** ***Difference***	***n*** ***Baseline***	***n*** ***Difference***	**Intervention vs Control**
P-PSA, µg/L	n=16 9.2 (4.7 – 20.6)	n=16 0.0 (-3.0 – 2.7)	n=8 7.7 (3.9 – 16.0)	n=8 1.3 (0.0 – 3.0)	0.045





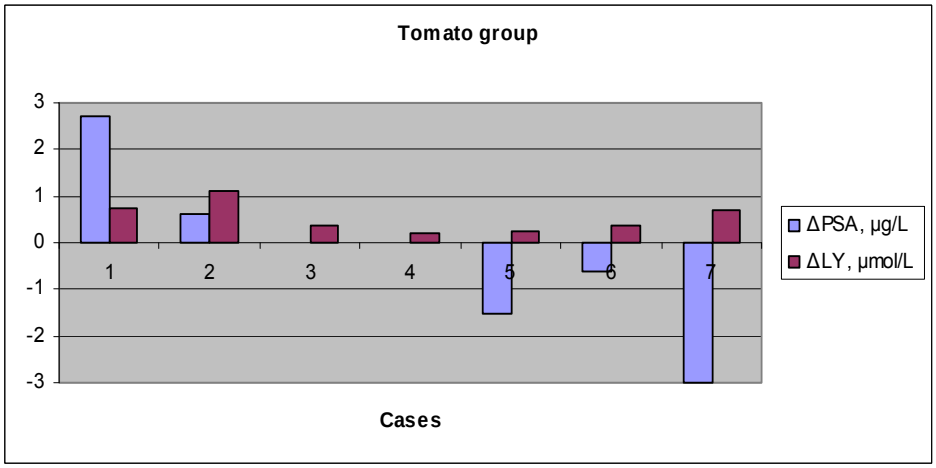


Figure 5.4 Bar charts showing the ΔPSA µg/L and Δlycopene µmol/L for each patient as a result of the 3 week intervention

6. General discussion

The health benefits of fruits and vegetables are widely believed to be due partly to the presence of phytochemicals. Several of these compounds act as antioxidants that may prevent oxidative damage to cells and molecules, thereby reducing oxidative stress in the human body.

Recent literature indicates that oxidative stress appears in connection with cancer, but it is not clear whether patients that contract cancer are characterised by initially lower antioxidant status and higher oxidative stress than others or whether the disease increases oxidative stress and diminishes antioxidant defence.

The following hypothesis is proposed:

PC is associated with elevated oxidative stress and with diminished antioxidant defences. This hypothesis leads to the following predictions, which can be examined in the light of our results:

1. Higher oxidative stress and lower antioxidant status in the study population compared to the healthy reference population
2. The food and supplement intervention will increase antioxidant status
3. The food and supplement intervention will decrease markers of oxidative stress
4. The food and supplement intervention will decrease markers of disease (PSA)
5. Additive or synergistic interaction between the foods and supplements in the multidiet intervention will impose stronger effects on antioxidant status, markers of oxidative stress, and PSA than tomato intervention alone.

The foods and supplements used in the intervention were selected because of their proposed PC preventive properties. Furthermore, the selected plant foods contain

high amounts of phytochemicals with strong antioxidant properties, and the tomato products were selected based on laboratory determination of lycopene contents.

The present results are interim, based on 27 patients, while the study was designed for 102 patients. The results must be interpreted accordingly.

6.1 COMPARING THE CANCER PATIENTS TO THE REFERENCE POPULATION

6.1.1 Populations and selection

The mean BMI (Body Mass Index) and the number of smokers were similar between the populations. However, the mean age of the study population was 12 years higher than the mean age of the reference population and the age distribution was slightly different as shown in **Figures 4.2** and **4.3**.

According to "The free radical theory of ageing", introduced in 1954 by Denham Harman (153), the continuous metabolism of O_2 in mitochondria produces free radicals (or rather ROS) that accumulate and cause damage, and this constitutes the inborn ageing process (153). Several hypotheses trying to elucidate the mechanisms of ageing have been proposed, but none are generally accepted. However, according to Voss and Sies (154), oxidative damage products such as lipid, protein and DNA damage may increase with age, while the plasma thiol concentration may decline and the amount of oxidized cysteine (cystine) and GSH (GSSG) may increase (154). Therefore it is possible that the age difference between the PC population and the healthy reference population could affect our results.

6.1.2 Antioxidants

Vitamin C and mFRAP

Plasma TAA and mFRAP medians were significantly higher ($p<0.001$ and $p=0.003$) in the study population (73 µmol/L and 421 µmol/L) than the reference population (52 µmol/L and 374 µmol/L). These results were in contrast to our prediction of diminished antioxidant defences in the study population compared to the reference population.

There are a number of case-control studies that have compared AA between cancer patients and healthy controls, and the majority of these do not support our findings. Surapaneni et al. (155) found a decrease in erythrocyte AA in a group of PC patients and suggested that this was due to higher antioxidant turnover. Furthermore, lower plasma vitamin C status has been measured in other groups of cancer patients compared to age and sex matched controls (39;156).

Differences were probably not due to sample preparation, storage and analysis as these were performed according to the description in Chapter 4.3.3, and the samples from the two populations were treated equally. With respect to TAA determination, due to acidification and excellent treatment before storage, higher levels of TAA may be measured using this method than other methods (157).

One plausible reason for the higher plasma TAA and mFRAP in the study population than the reference population is increased health awareness as a consequence of the serious cancer diagnosis. This may entail higher intake of fruits and vegetables among the cancer patients than the reference population. Furthermore, the subgroup of cancer patients who enrol for a study like this may also be interested in healthy eating and they may believe that this could improve their prognosis to a higher degree than those who decline to participate.

Ten PC patients presented plasma TTA above 80 µmol/L and this is higher than one would expect from food sources alone (43), indicating that these patients used vitamin supplements. Although the patients were requested not to use dietary supplements when enrolling in the study, they probably did.

Thus, the differences between the groups may be due to a combination of vitamin supplements and increased fruits and vegetable intake.

GSH and cysteine

The GSH and cysteine medians were significantly lower in the study population than in the reference population, (p=0.009) and (p=0.003) respectively, and this was in line with our predictions.

According to the antioxidant network hypothesis (45), this result could be anticipated in a condition of oxidative stress: Low levels of GSH could indicate elevated turnover of GSH. High GSH turnover would require increased GSH synthesis. Cysteine is a precursor of GSH, and high GSH synthesis may lead to cysteine depletion which eventually would impair GSH synthesis.

The sample collection was, however, performed differently between the groups. The samples from the reference population had serine borate added to inhibit GGT enzymatic activity *in vitro*, while the samples from the study population did not. GGT enzymatic activity in the sample during storage and analysis may break down GSH and thus affect the results. Possible interaction between GGT and GSH *in vitro* may be illustrated by the strong non-linear correlation detected between GSH and GGT in the study population. There was a trend towards a similar correlation in the reference population, but this was not significant. See **Figure 5.1** and **Table 5.2**.

Serine borate would, however, not influence levels of cysteine. The significant lower plasma cysteine in the study population compared to the reference population could be due to oxidative stress in the study population, and suggest that the difference in GSH may also be explained by a condition of oxidative stress.

There are limited data comparing GSH in cancer patients to healthy controls; however, the few data that are available do not suppoort our findings. In an unpublished work of Sakhi et al. (39), employing the same method for total GSH determination treated with serine borate, no significant difference in total GSH was observed between a group of untreated head and neck squamous cell carcinoma (HNSCC) patients compared to controls.

6.1.3 Oxidative stress biomarkers in plasma

In spite of extensive research in the area of oxidative stress biomarkers, there is currently no generally agreed gold standard of oxidative stress determination, and the levels of various biomarkers diverge and are not easily reproduced between laboratories and methods of analysis (158). A challenge for oxidative stress measurement is artifactual oxidation occurring at any stage during sample collection, storage, thawing and analysis and this may introduce substantial error (12). Of the analysis techniques employed, HPLC is a quantitative determination method while ELISA is semi quantitative. According to Yoshida et al. (159), results with the commercial ELISA kit for 8OHdG determination correlate well with a HPLC assay and could be used to compare groups, but other reports have concluded differently (159).

As both plasma and urine may reflect the whole body exposure, these may not be relevant media for determination of oxidative stress in PC. Assuming that the prostate weighs 30 grams in a male body of 70 kg, the oxidative stress produced

by the prostate gland alone probably would not be measurable in the systemic body fluids (H.E.Poulsen and A.R.Collins, manuscript in preparation).

The various biomarkers are influenced by factors such as nutrition, lifestyle, genetic background and smoking implying that large numbers of individuals are needed to get significant differences in studies monitoring the change of biomarkers with age (154).

GGT

Although not significant, a trend indicating higher GGT levels in plasma of the study population than the reference population was observed. As higher GGT in the study population may indicate a state of oxidative stress in this group, this corresponded to our predictions.

Higher GGT may be caused by biliary tract disease, liver disease or excessive alcohol consumption. However, the median values in the groups were 25 U/L (reference population) and 30 U/L (study population) respectively, and virtually all values were within normal range, suggesting that the difference may not be due to liver disease or alcohol intake.

The NORIP reference value (150) upper limit steps from 80 U/L before to 115 U/L after 40 years of age, suggesting an expected increase of GGT with age. Thus, the difference between the groups may be due to the observed age differences between the two populations.

Our findings are supported in the comprehensive literature of GGT and cancer. By data from cell and animal studies, increased GGT activity is associated with enhanced tumour growth (160) and tumour oxidative stress resistance (161). GGT has been shown to be up-regulated under conditions of oxidative stress in carcinoma cells (162).

Strasak et al. (163) demonstrated stronger associations between GGT and cancer risk in participants below 65 years. Several prospective studies have demonstrated GGT as predictor of cancer risk and mortality of various sites (163-166).

D-ROMs test

Our study population had significantly higher median d-ROMs results (221 Carr U) than the median of the reference population (166 Carr U), indicating higher oxidative stress among the cancer patients which is in line with our predicted expectations ($p<0.001$).

However, measures made by our laboratory seem to give lower values than the Diacron scale. According to the scale none of our cancer patients expressed oxidative stress. Our values do correspond to the results of Sakhi et al. (39), a project run in the same laboratory, where controls versus pre-radiotherapy HNSCC d-ROMs levels were 155.9 Carr U and 219.6 Carr U respectively.

A possible explanation to the low levels could be the Norwegian eating pattern being different from the Italian. But in a Norwegian study of preeclampsia, d-ROM median values in healthy pregnant women were 457 (411–514) Carr U and in preeclampsia 550 (425–586) Carr U (127).

According to Diacron, the d-ROMs test results are not influenced by age (126), suggesting that the measured difference between the two populations could be due to oxidative stress. Supporting the hypothesis of an antioxidant defence network, a strong inverse linear correlation between the d-ROMs test and TAA was observed in the study population though not in the reference population. See Figure 5.2 and **Table 5.3**. An interpretation of this observation could be that the cancer patients were experiencing oxidative stress and in this group AA was utilised to reduce oxidative stress implying that those with high TAA were able to scavenge more

ROS than those with lower TAA levels, while in the reference population the AA turnover was lower and therefore not correlated to d-ROMs test.

Disregarding the measurement scale, a difference in d-ROM values between the study population and the reference population has been confirmed in other cancer populations. Already mentioned were the HNSCC results, and in a group of lung cancer patients the levels of oxidative stress were higher than those in normal subjects (555.3+/-30.35 Carr U versus 360.1+/-17.46 Carr U) (128).

DHAA:TAA

The study population had significantly higher ratio of DHAA:TAA than the reference population ($p<0.001$). Applying the antioxidant network theory, high DHAA:TAA could indicate that oxidation of vitamin C occurs at a high rate and that the thiol cycle and other reducing agents would be unable to recycle DHAA back to AA at a corresponding rate. The high ratio of DHAA:TAA suggested a condition of systemic oxidative stress, which could be connected to the PC diagnosis, hence this results confirmed our prediction.

Lykkesfeldt found higher plasma DHAA in smokers than in non-smokers (167) and in a study of untreated chronic leukaemia patients, a significantly higher plasma DHAA:TAA ratio was measured compared to a control group (168).

6.1.4 Oxidative damage biomarkers in urine

The daily excretion of the biomarkers in urine is stable, but the urine biomarker concentration varies with urine volume, depending on fluid intake and fluid excretion. Urinary creatinine was determined to enable correction of the day-to-day variation in urine volume.

Creatinine

The creatinine levels were significantly higher ($p<0.001$) in the study group than in the reference population. Elevated creatinine is, however, not associated with PC and the difference was unexpected.

The urine samples from the cancer patients, both baseline and after the intervention, were collected and treated similarly, and analysed in the same batch at Ullevål University Hospital.

Urine retention was an exclusion criterion among the cancer patients, and could thus be ruled out as a cause of this difference.

Creatinine is supposed to be stable but may vary with meat intake, heavy exercise, urine volume and muscle mass; however such factors should cause effects in both groups, and were not expected to give significant differences between the groups.

The reference population had 24- hours' urine samples collected, while the study population collected overnight urine. This could explain the differences.

8OHdG

Before correcting for creatinine, 8OHdG was not different between the study population and the reference population, but the creatinine adjusted 8OHdG in urine was significantly higher in the reference population than in the study population. We predicted elevated oxidative stress among the cancer patients. As increased level of 8OHdG is an indicator of oxidative stress, we would expect higher levels of 8OHdG among the cancer patients than the reference population.

Possible explanations of the results could be methodological or due to the differences in creatinine concentration between the population. Difference in urine collection procedures may influence the creatinine concentration and hence the

creatinine adjusted 8OHdG. Furthermore, we experienced large 8OHdG inter-assay variation, and the control sample was used as calibrators.

There are limited data comparing urinary 8OHdG between cancer patients and healthy controls; but, the few that are available do not support our findings. Miyake et al. (169) showed significantly higher levels of urinary 8OHdG/creatinine in PC patients compared to age matched healthy controls. Miyake also demonstrated that age was significantly associated with urinary 8OHdG/creatinine among the cancer patients but not to disease stage or PSA (169). Chiou et al. (170) measured higher 8OHdG in 16 PC patients than in healthy controls, but no information about clinical stage or PSA was presented (170).

HEL

There was no difference in urinary HEL between the two populations, however creatinine adjusted HEL in urine was significantly higher in the reference population than in the study population. This was not in line with our prediction. Rather, being an indicator of oxidative stress, HEL levels were expected to be higher in the study group.

The unpredicted results may be explained by weaknesses in the analysis method and by differences in urinary creatinine concentration.

There are to date no published data concerning urinary HEL determination in human cancer patients.

Our results revealed no correlation between HEL and 8OHdG. This was in contrast to Kato et al. (133) who, using a chromatographic analysis method, documented correlation between HEL and 8OHdG in human urine of healthy

subjects. Furthermore, Kato et al. detected higher urinary HEL levels in diabetic subjects than in controls (133).

6.1.5 Summing up the comparison

There are indications of differences in levels of cysteine, DHAA:TAA, d-ROMs test, GGT (although not significant) and mFRAP between the populations. Cysteine was significantly lower in the study population than in the reference population, while DHAA:TAA, d-ROM, GGT and mFRAP were higher in the study population than in the reference population.

Relating these results to the antioxidant network hypothesis, a condition of oxidative stress could imply increased turnover of substrates in the AA cycle and the thiol cycle. Low cysteine, high DHAA:TAA ratio and high d-ROMs test in the study population support the antioxidant network hypothesis and are consistent with our predictions of higher oxidative stress and lower antioxidant status in the PC patients compared to the reference population.

6.2 EFFECT OF INTERVENTION

6.2.1 COMPLIANCE

The compliance of the intervention was excellent. The average tomato product consumption was 99% while the average intake of the multidiet products varied from 96% (green tea) to 100% (morning supplements). The reported compliance was confirmed by the two biomarkers: lycopene and omega-3 fatty acids.

The patients that accepted to join the study were very motivated, and few and mild adverse symptoms were reported. These were mainly related to dyspepsia. None of the patients reported adverse symptoms from the tomato products.

The patients in the multidiet group received grape juice, pomegranate juice, green tea and black tea, and in volume this added up to 1.3 litres of liquid per day. In addition to the liquid they would eat or drink 200 – 250 grams of tomato products daily and take capsules of omega-3, selenium and soy. As anticipated, the intervention foods did replace some of their usual intake of coffee and juice, and increased the pasta and tomato sauce intake for dinner. The patients in the multidiet group were generally happy to finish off after the three weeks of intervention.

Our observation of high tolerability was in contrast to Jatoi et al. (171) **(Table 6.1)** who reported gastrointestinal side effects and 43 of 46 discontinued the treatment. However, this intervention, conducted by the Mayo Clinic, lasted for years rather than months, and the disease stage was advanced (171).

6.2.2 BIOMARKERS OF OXIDATIVE STRESS

Comparing each of the two intervention groups to the control group revealed no significant effect of the intervention in any of the measured oxidative stress related biomarkers.

An effect from the tomato intervention to dampen oxidative stress and increase antioxidant status was expected according to our predictions. Furthermore, the multidiet combining tomatoes, grape juice, pomegranate juice, green tea, black tea, omega-3 PUFA, soy and selenium was proposed to induce additive or synergistic effects on the biomarkers.

Several factors may moderate our results:

- Results could be hampered by weaknesses in the sample handling and analysis methods.
- Oxidative stress biomarkers originating from the prostate gland may not be measurable in systemic fluids.
- The intervention study was designed to include 102 participants. This interim analysis, based on 27 patients may not display effects due to large inter individual variation in response.
- Three week's intervention may be too short duration for detectable differences to appear.
- Our hypothesis and predictions may be wrong.

The food items and supplements selected for the intervention have indicated possible preventive effects on PC in epidemiologic studies. In cell culture and animal studies they have shown anti-carcinogenic effects. Selenium is essential in the endogenous antioxidant defence system. Apart from selenium and omega-3 PUFAs the food items are also known for their high contents of polyphenols and antioxidants.

The literature indicates that tomatoes, grape juice, pomegranate juice and selenium, omega-3 PUFA and soy supplements inhibit oxidative damage and support the endogenous antioxidant defence system and potentially reduce oxidative damage. Chen et al. (50) documented reduction in leukocyte 8OHdG after three weeks intake of tomato-based pasta dishes in 32 PC patients (50). Similar effects on lymphocyte DNA damage in healthy volunteers from tomato extract and purified lycopene supplementation have been confirmed by others (172;173). Daily consumption of grape juice for 8 weeks reduced DNA damage in peripheral lymphocytes significantly (174). Selenium supplementation inhibited

8OHdG formation in rodents exposed to various carcinogens (175). Conflicting results were reported about effect of pomegranate juice on GSH in rodents (176;177). In a group of elderly subjects, supplementation with either fish or soy oil was related to an increase of Cu/Zn SOD activity and an increase in GSH plasma levels (178). Studies of animal models of skin, lung, colon, liver and pancreatic cancer have consistently indicated that tea and tea polyphenols administration inhibit carcinogen-induced increases of DNA damage (179).

6.2.3 PSA EFFECTS

There was a trend indicating an effect on PSA in the tomato (n = 7, p = 0.072) and multidiet groups (n = 9, p = 0.114) compared to the control group (n = 8), as shown in **Table 5.8**. The intervention effect on PSA in the joint tomato and multidiet groups (n = 16) compared to the control group (n = 8) was statistically significant (p = 0.045) as displayed in **Table 5.9**. **Figure 5.4** displays bar charts of all the patients' change in PSA over the three weeks intervention. Potential non-responders, i.e. individuals experiencing increased PSA values, are found in both intervention groups: four of nine in the multidiet group and two of seven in the tomato group were non-responding.

A reduction of PSA in both intervention groups compared to the controls was anticipated according to our predictions.

Tomato effect on PSA

Few participants may be one reason for non-statistically significant results from the tomato intervention. Stronger effects may be detected when including more patients in the study.

Non-responding could be due to one or more of several mechanisms; here are suggested three possibilities: 1) Diet gene interaction (see section 1.6); for

example Goodman et al. (112) documented that the Arg/Arg genotype of the XRCC1 gene combined with high lycopene and carotenoid plasma values could reduce PC risk dramatically (112). Other somatic genes may also interact with effective chemopreventive substances. 2) There may be subtypes of PC resistant to the tomato intervention. 3) Lycopene absorption seems to vary between individuals (180) and could be a reason for non-responding. However; in the current study there was no sign of correspondence between PSA non-responding and plasma lycopene levels as illustrated in **Figure 5.4**.

There is a large and growing literature about the effect of tomato products and other bioactive food constituents on diagnosed PC, and many clinical interventions are in progress. Of the clinical trials that have been published, few are randomized, blinded or controlled. The sample sizes of the studies are small, the duration is short and the clinical stages vary from a high-risk population to aggressive terminal disease.

Tomato extracts containing high amounts of lycopene are available as Lyc-O-Mato® capsules from LycoRed, an Israel based company. In addition to lycopene, Lyc-O-Mato contains various carotenoids, tocopherols, phospholipids and phytosterols (57;181). Four of the six trials in **Table 6.1** administrated extract capsules instead of whole food. Knowing the recent literature on adverse outcomes from antioxidant supplementation, there may be a good reason to be sceptical to many kinds of antioxidant supplements (109). However, there may be a difference between tomato extract capsules and synthetically manufactured lycopene.

Table 6.1 lists six recent clinical trials testing tomato or tomato extracts in PC patients. All six trials used PSA as a surrogate biomarker of PC progression. PSA was demonstrated as a valid biomarker linked to tumour response (182). Of the six trials, two addressed localised PC or stage t1-t2. Kucuk et al. (57) used Lyc-O-Mato, while Bowen et al. (50) used tomato sauce, both supplementing a daily

lycopene dose of 30 mg. The Lyc-O-Mato trial (57) had the same number of participants as the current study, duration of three weeks and did show a trend of difference in PSA between the groups, but not significant. Comparing our current significant result with the result of Kucuk et al. (50) may indicate that the tomato extract is less effective than whole food tomato products with known high lycopene content. The tomato sauce study (50) included 32 in intervention, only 7 controls and lasted for 3 weeks. The PSA reduction was highly significant. In a high-risk group of 81 Afro-Caribbean men supplied with Lyc-O-Mato capsules PSA decreased the first month but increased subsequently (183).

Clark et al. (184) treated a group of 36 advanced stage PC patients with Lyc-O-Mato for one year, with doses ranging from 15 to 120 mg lycopene. The conclusion from this trial was that there was no PSA response (184).

An Indian trial (185) treated 20 patients with androgen independent and metastatic disease. Administrating “Lycored softule” capsules of 10 mg lycopene daily, for three months Ansari and Gupta found one complete response, ie normalization of PSA and 16 patients had partial response or no progression (185).

Jatoi et al. (171) at the Mayo Clinic treated 46 androgen-independent and metastatic cases with tomato based juice and sauce and only one patient experienced a transient decline in PSA.

Multidiet effect on PSA

According to our predictions, the multidiet effect on PSA, where the patients received multidiet in addition to tomato products, would be greater than the effect of tomato products alone; however, no additional effect was seen.

Few participants and short duration may limit our findings. If there is an effect, the statistical power will be strengthened by including more participants. Potential

non-responding weakened the effect. Possible explanations for non-responding in the tomato group may be relevant also for the multidiet participants.

In addition to antioxidant effects, the many dietary constituents of the multidiet intervention food products that have been proposed to dampen PC may activate signalling pathways that lead to activation of response elements, upregulating gene transcription and translation of proteins involved in e.g. cell cycle control, apoptosis, cell growth and expression of detoxifying enzymes (89;90;186-189). Such adaptive effects may require more than three weeks of exposure to be fully effective. Thus, the intervention duration may have been too short to detect additional or synergistic effects on PSA in the multidiet group compared to PSA in the tomato group.

One study has shown promising effects of pomegranate juice on human PC (94); however this intervention lasted for 24 months and more. A few animal studies are published that show synergistic effects on cancer from combinations of foods. Zhou et al. (190) reported a synergistic effect on mice tumour weight and metastasis with a combination of soy concentrate and tea (190). A study in transgenic mice (191) showed a synergistic inhibitory effect of dietary vitamin E succinate, selenium, and lycopene on PC incidence. Clinical trials have yet to confirm this effect (191). A study of Chinese men (192) suggested that habitual tea drinking and intakes of vegetables and fruits rich in lycopene could lead to a reduced risk of PC. Interaction analysis showed that the protective effect from tea and lycopene consumption was synergistic ($p<0.01$). Together they had a stronger preventive effect than either component taken separately (192).

Table 6.1 Human PC tomato/lycopene trials

Food/extract from food	Lycopene dose	Study design	Duration	Disease stage	Number of Patients	Results	Pub year	Article/main author
Tomato oleoresin extract	30 mg	Randomized Controlled Trial	3 weeks	T1 and T2, scheduled for prostatectomy	15 intervention 11 controls	PSA increase in control group, decrease in intervention gr. P=0.25	2002	Omer Kucuk, Hawaii, USA (57)
Tomato sauce	30 mg	Clinical intervention	3 weeks	Localised prostate adeno carcinoma	32 on intervention 7 random controls	Average serum-PSA decreased p<0.001	2002	Phyllis Bowen, Illinois, USA (50)
Lycored softules	10 mg	Clinical intervention	3 months	Androgen independent metast. disease	20 patients, no controls	CR: 1 patient, PR: 6 patients, SD: 10 patients, Progr: 3 patients[b]	2004	Ansari and Gupta, India (185)
Lyc-O-Mato	15 – 90 mg	Clinical intervention	1 year	PSA increase after local therapy	36 on intervention No control group	No PSA response[a]	2006	Clark, North Carolina, USA (184)
Lyc-O-Mato and/or multivitamin	30 mg	Phase 1 clinical trial	4 months	High risk group	81 Afro-Caribbean men, randomized in 3 groups	PSA decreased first month but was back to baseline after 4 mnths. No diff between the groups	2007	Bunker, Pittsburgh, USA (183)
Tomato paste or tomato juice	30 mg	Phase 2 study	3-4 rep. cycl. of 28 days	Androgen independent	46 patients on intervention, no control group	PSA declined in one patient[a]	2007	Jatoi, Mayo Clinic, USA (171)

[a]Response defined as 50% decrease in serum PSA from baseline.
[b]A complete response (CR) was defined as a normalization of PSA (<4 ng/mL) and the disappearance of any sign of disease for at least 8 weeks. A partial response (PR) was defined as a >50% decrease in PSA level for at least 8 weeks associated with improvement (or no worsening) in ECOG PS and relief of bone pain if present. Stable disease (SD) was defined as a <50% decrease or <25% increase in the PSA level associated with no worsening of ECOG PS and/or bone pain for at least 8 weeks. (ECOG PS, Eastern Cooperative Oncology Group performance status.)

7. Conclusion

The hypothesis and predictions proposed in the discussion were not completely acknowledged in the present data; however predictions one and four may be confirmed. Furthermore, the effects were based on 27 patients and need to be reiterated upon the completion of the PFPI project when 102 patients have been included.

We have demonstrated that PC patients may have higher oxidative stress levels and diminished antioxidant defences than healthy controls. We suggest that the observed elevated biomarkers of oxidative stress may represent a plausible elevation of oxidative stress associated with PC. However, we cannot rule out the possibility that the age difference between the two groups influences our findings.

We observed that compliance and tolerability of the intervention diet were excellent, and only one patient reported dyspepsia but still managed to complete the intervention.

We suggest that tomato products or multidiet foods may be favourable for patients with localised PC. Increase in PSA accompanies the development of PC into an aggressive and terminal disease. Our findings indicate that the PSA increase may be delayed when PC patients eat tomato products with a high content of lycopene, possibly in combination with the other multidiet nutrients, over a three week period.

Our material did not reveal an additional effect from other phytochemicals than those found in tomatoes on PSA in localised PC, but the effect on PSA in the multidiet group was comparable to the effect in the tomato group.

We did not document any relationship between biomarkers of oxidative stress, antioxidant status and PSA development during the three weeks of intervention, but further research is required.

8. Future perspectives

PFPI has planned to include 102 patients in the study. Availability of these data may enable stronger PSA effects to be seen, from both intervention groups. Synergistic effect in the multidiet group may be revealed. Further PFPI analyses will examine oxidative stress in prostate tissue rather than in systemic body fluids as urine and blood.

Administration of dietary compounds, with minimal side effects, to prevent, slow or reverse cancer, could be attractive as a supplement to the conventional PC treatment. Tomato products or lycopene may be a prosperous candidate; however several issues need further evaluation before it may be recommended in clinic:

1) The effects on PSA may be transient and therefore longer lasting interventions are required.

2) Whether synthetic lycopene, Lyc-O-Mato capsules or whole food heat prepared tomatoes will give optimal effect needs to be addressed. If equally effective, it may be favourable to administrate capsules in a long term intervention.

3) Optimal lycopene dosage must be investigated.

4) Compliance for longer lasting intervention should be examined. It may be difficult to recruit participants to adhere to the multidiet intervention for a longer period. However, if the patients experience good effect and minimal adverse reactions, they may comply for a longer duration.

5) In which clinical stage such a treatment could be effective must be further studied.

6) Tomato products in combination with other foods could give valuable synergistic effects.

7) In future, an individual multidiet of foods or supplements may be tailored according to patient response and genetic factors.

DNA microarray is a technique for gene expression profiling that has become the standard research tool for high-throughput examination of genome wide expression changes in PC. In the search for better understanding of the disease pathogenesis and progression, the DNA microarray makes it possible to find and classify PC progression signatures like dysregulated genes, from cancer cells of groups of individuals (193). Investigating PC tissue using micro array analyses may reveal the genetic differences between cancers that do respond and do not respond to food and supplement treatment. However, such high-throughput techniques generate large amounts of data. Thus a great challenge in future research will be the interpretation and statistical analysis of information.

Systems biology will definitely influence the future research in nutrition and cancer. Building comprehensive models and quantifying all components and interactions probably give a better understanding of the antioxidant network and other cellular processes that may be of importance in this field.

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Appendices

Appendix 1 Invitation letter, PFPI study

Invitasjon til å delta i en vitenskapelig studie hvor vi undersøker om en kostintervensjon påvirker markører i blod, urin og prostata

Du mottar dette brevet i forbindelse med din behandling ved Rikshospitalet-Radiumhospitalet helseforetak (RR).

I samarbeid med Professor Rune Blomhoff ved Avdeling for Ernæringsvitenskap ved Universitetet i Oslo (UiO) ønsker vi å undersøke hvordan et endret kosthold over en kortere periode før strålebehandling eller prostatektomi påvirker kreftceller i vev, og hvilke forandringer dette fører til i blod og urin. De ulike matvarene er utvalgt med bakgrunn i at det er rapportert lavere forekomst av prostatakreft i befolkningsgrupper som spiser mye av disse.

Deltagelsen i undersøkelsen er frivillig, og dersom du ønsker å delta, vil dette på ingen måte påvirke den behandlingen du får i forbindelse med din sykdom.

Vedlagt finner du to eksemplarer av samtykkeinformasjonen ” Forespørsel om å delta i en vitenskapelig undersøkelse”. Denne informasjonen inneholder relevant informasjon om undersøkelsen, og vi vil be deg lese gjennom denne. Dersom du ønsker å delta er det viktig at du signerer et eksemplar av denne, og tar den med til den avtalte undersøkelsen.

I forbindelse med undersøkelsen ønsker vi også å kartlegge dine kostvaner. Dette gjør vi med et spørreskjema, ”Hva spiser du?”. Dette skjemaet vil du få ved først undersøkelse, og du kan ta det med hjem for utfylling før du returnerer det til oss per post. Du kan gjerne ta kontakt med oss underveis dersom du har spørsmål om utfylling av skjemaet.

Din deltagelse i denne undersøkelsen vil ikke forandre på behandlingen du skal få, men vil gi oss verdifull kunnskap til bruk i behandling av prostatakreft i fremtiden.

En forskningssykepleier vil i løpet av noen dager ta kontakt med deg for å gi eventuell utfyllende informasjon om undersøkelsen.

MVH

_____	_____
Rune Blomhoff	Sigbjørn Smeland
Professor, UiO	Klinikksjef, RR

Ta gjerne kontakt med oss dersom du har spørsmål om undersøkelsen!

Anette Karlsen, prosjektkoordinator, UiO Tlf 22 85 15 24

Prosjekttelefon: 41 00 56 89

e-mail: anette.karlsen@medisin.uio

Silje Kjelling, forskningssykepleier Tlf 22 93 58 02

e-mail: silje.kjelling@radiumhospitalet.no

Wolfgang Lilleby, behandlende lege Tlf 22 93 41 89

e-mail: wolfgang.lilleby@medisin.uio.no

Appendix 2 Letter of informed consent, PFPI study

Forespørsel om å delta i en vitenskapelig undersøkelse

Prostatakreft, fytokjemikalier og flerumettede fettsyrer (PUFA) intervensjonsstudie (PFPI)

Vi vil gjerne spørre deg om du kunne tenke deg å delta i en undersøkelse av hvordan et kostforsøk (bestående av tomatprodukter, grønn og sort te, granateple- og druejuice, soya, selen og omega-3-fettsyrer) før strålebehandling eller prostatektomi påvirker kreftceller i vev, og hvilke forandringer dette fører til i blod og urin.

En slik undersøkelse krever at vi må ta ekstra prostatabiopsi, samt ekstra blod- og urinprøver av deg.

Hensikten med undersøkelsen

Ved Rikshospitalet-Radiumhospitalet prøver vi å forbedre våre behandlingsmetoder.

Det er holdepukter for at prostatakreft kan påvirkes av ulike typer matvarer. Kostforsøk er en eksperimentell behandlingsform, hvor vi ønsker å undersøke hvorvidt enkelte pasienter kan ha nytte av en slik behandling. Overvåkning av kreftceller før og etter en forsøksperiode kan gi oss verdifull informasjon om kreftceller er følsomme for spesielle matvarer. De ulike matvarer er utvalgt med bakgrunn i observerte gunstige sammenhenger mellom inntak og forekomst av prostatakreft som har blitt rapportert.

Undersøkelsen gjøres i samarbeid med Professor Rune Blomhoff ved Avdeling for Ernæringsvitenskap ved Universitetet i Oslo.

Prøvetaking

Til undersøkelsen tas det blodprøver og urinprøver. I tillegg ønsker vi å ta to ekstra vevsprøver fra prostata. Den første vil tas før kostforsøket av urolog, utført med lokalbedøvelse på samme måte som da diagnosen ble stilt. Etter forsøket, og i forbindelse med innvendig strålebehandling eller operasjon taes den andre biopsien mens du ligger i

narkose. Av og til kan man få lettere ubehag ved prøvetaking fra prostata og observere noe blodtilblanding i avføring, urin og sæd. Det foreligger en viss risiko for urinveisinfeksjon, som reduseres ved bruk av forbyggende antibiotika. Vi vil også se hvordan nivået av

forskjellige varianter av gener involvert i oksidativt stress kan si noe om hvordan de markører vi måler påvirkes av undersøkelsen.

I tillegg til å avgi prøver til undersøkelsen, vil vi ved to tidspunkter (ved oppstart og ved ett års oppfølging) be deg fylle ut et skjema for registrering av dine kostvaner. Skjemaet kan du fylle ut hjemme og returnere til oss i ferdig frankert returkonvolutt.

Forsikring

Du er forsikret i henhold til reglene i Lov om produktansvar.

Registrering av data og opprettelse av forskningsbiobank

I forbindelse med undersøkelsen vil det bli laget et eget register for å kunne analysere resultatene på en rask og effektiv måte. Alle data vil bli behandlet konfidensielt, og under databehandlingen og publikasjon er alle data avidentifisert. Selve registeret vil bli slettet når studien er gjennomført og resultatene publisert, og senest i 2022.

Opplysninger i din journal er underlagt taushetsplikt i henhold til Helsepersonelloven, og bare de som trenger å se den i forbindelse med undersøkelse og behandling har tilgang til den. Statens legemiddelverk og annen kontrollmyndighet har også rett til innsyn i din journal ved deltagelse i denne type undersøkelse, dette for å sikre undersøkelsens kvalitet. Disse kontrollmyndighetene er også pålagt taushetsplikt.

Det biologiske materialet (blod-, urin-, vevsprøvene) vil bli oppbevart i en biobank ved Avdeling for Ernæringsvitenskap ved Universitetet i Oslo. Ansvarlig for biobanken er Professor Gunnar Nicolaysen ved Institutt for Medisinske Basalfag ved det Medisinske Fakultet, Universitetet i Oslo. Biobanken er godkjent av Sosial og Helsedirektoratet.

Du kan på et hvert tidspunkt før offentliggjøring av resultatene i internasjonale tidsskrift trekke tilbake ditt samtykke til at prøvene blir brukt til forskningsformål, og prøver og eventuelle personopplysninger vil da bli destruert og slettet

Studien er tilrådt av Regional komite for forskningsetikk (REK Sør) og av statens legemiddelverk (SLV).

Begrensninger ved deltagelse

Det er enkelte legemidler du ikke kan bruke mens du deltar i studien. Derfor er det viktig at du forteller legen hvilke legemidler du bruker eller ønsker å bruke. Det gjelder også legemidler som du får kjøpt uten resept og naturmedisiner.

Forsøksperioden

Ved innledning av studien vil deltagerne fordeles mellom tre grupper: tomatgruppe, multi-diett gruppe og kontrollgruppe. Hvilke gruppe du tildeles til er tilfeldig. Dersom du tildeles til tomatgruppen vil du bli bedt om å innta en rekke produkter som inneholder tomat over en periode av tre uker. For variasjonenes skyld er det ulike produkter å velge mellom. Dersom du tildeles til multi-diett gruppen vil du bli bedt om å innta tomatprodukter, grønn og sort te, granateple- og druejuice og kosttilskudd som inneholder omega-3 fettsyrer (PUFA), selen og soya over 3 uker. Også her kan du velge mellom ulike tomatprodukter. Dersom du tildeles til kontrollgruppen vil du kunne fortsette ditt vanlige kosthold.

Varighet av forsøket og oppfølging

Forsøksperioden varer i tre uker. Det er viktig for oss at du i denne perioden (..forsøksperioden) gjør ditt beste for å spise de matvarene du har fått utdelt, i den mengden vi ber deg om. For at vi skal registrere hvilke matvarer du har spist vil vi derfor be deg om å registrere dette i et skjema som du får hos oss.

I løpet av perioden vil du ukentlig bli kontaktet per telefon for å registrere eventuelle komplikasjoner som følge av forsøket. Dersom eventuelle komplikasjoner skulle oppstå, ber vi om at du rapporterer disse tilbake til oss i disse intervjuene, eller ved å kontakte ansvarshavende lege ved kontaktinformasjon som er gitt nedenfor.

Frivillighet

Din deltagelse i denne undersøkelsen vil ikke forandre på behandlingen du skal få, men vil gi oss verdifull kunnskap til bruk i behandling av prostatakreft i fremtiden. Din deltagelse er frivillig og du kan trekke deg når som helst. Dersom du ikke vil delta i studien eller trekker

deg, får du den behandlingen og oppfølgingen som vanligvis blir gitt til pasienter med samme diagnose som deg. Det er viktig at du informerer legen om du ønsker å avbryte behandlingen.

Vi gjør oppmerksom på at TINE BA bidrar med kr 400.000 til dekning av enkelte analysekostnader i tillegg til juicen som inngår i forsøket. Det økonomiske bidraget fra TINE utgjør kun en meget liten del av totalkostnadene til studien, og vil ikke påvirke den vitenskapelige publiseringen av resultatene fra studien.

Samtykke

Jeg samtykker i å delta i undersøkelsen. Jeg har mottatt en egen signert kopi av pasientinformasjonen.

(Dato) Navn (blokkbokstaver) (Underskrift)

Behandlende leges underskrift

Dersom du har spørsmål om behandlingen eller undersøkelse mens du er hjemme ta kontakt med ansvarlige lege for undersøkelsen ved Radiumhospitalet:

Dr. med. Wolfgang Lilleby Tlf. 22 93 41 89

Forskningsykepleier Guro Lindviksmoen Tlf. 22 93 57 74

Dersom du har spørsmål om kostforsøket, prosjektkoordinator Anette Karlsen:

Prosjektkoordinator Anette Karlsen Tlf: 22 85 15 24

Prosjektmedarbeider Magnhild Kverneland Tlf: 41 00 56 89

Dersom du har spørsmål om kostskjemaet, ta kontakt med ansvarlig for registrering av kosthold ved Avdeling for Ernæringsvitenskap:

Stipendiat Monica Carlsen Tlf. 22 85 15 13

Appendix 3 Inclusion criteria for the PFPI study

Pasient seleksjon og inklusjonskriteria

1. Histologisk bekreftet adenocarsinom
2. pN0/NXM0 (TNM/UICC 2002) og minst en negativ prognosefaktor for HDR-BT eller lav-intermediær risiko profil ved radikal prostatektomi.
3. PSA< 20 ng/mL+(Gleason score=>6) eller T1c- T3a, prostatavolum < 60mL
4. Performance status 0-1
5. Normale hvite og trombosytter, Hb >11g/dl
6. Ikke tidligere endokrin behandling
7. Livsforventing > 5 år
8. Ikke betydningsfull komorbiditet (hjerte-kar, KOLS, insulinkrevende diabetes mellitus, vaskulitt, inflammatorisk tarmsykdom etc. som kan ha innflytelse på livskvalitet eller strålebehandling.
9. Ikke urinretensjon, inkontinens, IPPS score <12 (gjelder HDR-BT pasienter).

Appendix 4 Informasjon til alle

Kjære deltager i undersøkelsen kostintervensjon ved prostatakreft

I denne forsendelsen har du fått de produkter du skal spise og drikke i forsøksperioden.

I tillegg har du fått to beholdere i klar plast. **Disse er til urinprøver**. Når du skal til blodprøvetakingen før du starter forsøket og etter at forsøket er avsluttet, samler du morgenurinen på beholderen og tar denne med til laboratoriet på Radiumhospitalet.

Lykke til!

Dersom du har spørsmål om undersøkelsen kan du når som helst kontakte oss.

Anette Karlsen, prosjektkoordinator, UiO Tlf 22 85 15 24

e-mail: anette.karlsen@medisin.uio

Magnhild Kverneland, prosjektmedarbeider

Prosjekttelefon: Tlf 41 00 56 89

e-mail: magnhild.kverneland@studmed.uio.no

Guro Lindviksmoen, forskningssykepleier Tlf 22 93 57 74

e-mail:guro.lindviksmoen@radiumhospitalet.no

Wolfgang Lilleby, behandlende lege Tlf 22 93 40 00

e-mail: wolfgang.lilleby@medisin.uio.no

Appendix 5 Informasjon til tomatgruppen

Du har fått følgende tomatprodukter for forsøksperioden:

1 glass Barillo Pastasaus basilikum

1 glass Dolmio Pastasaus hvitløk

1 glass Dolmio pastasaus classico

1 glass Knorr pastasaus oregano og løk

3 kartonger Cadisco tomatjuice 1 L

3 kartonger ICA tomater hakket

Du kan selv velge hvilke tomatprodukt du vil spise eller drikke en porsjon av hver dag.

Tomatproduktene skal konsumeres over hele forsøksperioden. Det er viktig at du spiser omtrent den rette daglige porsjonen av det enkelte produkt, som angitt nedenfor:

Barilla	Pastasaus med basilikum	½ glass/porsjon
Dolmio	Pastasaus hvitløk	½ glass/ porsjon
Dolmio	Pastasaus classico	½ glass/ porsjon
Knorr	Pastasaus med oregano og løk	½ glass/ porsjon
Cadisco	Tomatjuice	¼ kartong (2,5 dL)/ porsjon
ICA	Tomater, hakket	½ kartong/ porsjon

Vedlagt finner du noen forslag til anvendelse av de enkelte produktene.

Det er viktig at du så langt det er mulig ikke gjør store endringer i kostholdet i løpet av forsøksperioden. Det vil si at den ekstra porsjonen med tomat så langt det er mulig skal komme utenom det du spiser til vanlig.

Når du har spist ellet drukket den angitte porsjonen, krysser du dette av i skjemaet du har fått vedlagt "Skjema for registrering av produkter i forsøksperioden: Tomatgruppe".

Dersom du for eksempel har spist ½ glass Barilla Pastasaus med basilikum dag 1, 5/3-2007, og drukket ¼ kartong (2,5 dL) Cadisco tomatjuice dag 2, 6/3-2007, krysser du av som vist:

	Merke navn	**Produkt**	**Inntak**	**Dag 1**	**Dag 2**	**Dag 3**
			Dato:	**5/3**	**6/3**	
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass	×		
	Dolmio	Pastasaus extra hvitløk	½ glass			
	Dolmio	Pastasaus classico	½ glass			
	Knorr	Pastasaus med oregano og løk	½ glass			
	Cadisco	Tomatjuice	¼ kartong		×	
	ICA	Tomater, hakket	½ kartong			

Du har fått utdelt nok produkter til 25 dager. Dette er fordi det for noen kan være vanskelig å få til nøyaktig 21 dager. Dersom du har produkter til over etter avsluttet forsøksperiode, trenger du ikke levere disse tilbake til oss.

Skjemaet "Skjema for registrering av produkter i forsøksperioden: Tomat gruppe" som du har fylt ut underveis, sender du til oss i den frankerte konvolutten du har fått tilsendt, etter forsøksperioden.

Lykke til!

Dersom du har spørsmål om undersøkelsen kan du når som helst kontakte oss.

Anette Karlsen, prosjektkoordinator, UiO Tlf 22 85 15 24

e-mail: anette.karlsen@medisin.uio

Magnhild Kverneland, prosjektmedarbeider Tlf 41 00 56 89

e-mail: magnhild.kverneland@studmed.uio.no

Guro Lindviksmoen, forskningssykepleier Tlf 22 93 57 74

e-mail:guro.lindviksmoen@radiumhospitalet.no

Wolfgang Lilleby, behandlende lege Tlf 22 93 40 00

e-mail: wolfgang.lilleby@medisin.uio.no

Appendix 6 Skjema for registrering av daglig inntak for tomatgruppen

Skjema for registrering av produkter i forsøksperioden:

Tomatgruppe *ID nr:*

Skjemaet er på 4 sider (1 side for hver uke).

For hver dag skal du velge en porsjon av tomatprodukt gitt i tabellen. Den mengden som tilsvarere en porsjon som du skal spise eller drikke er gitt under inntak (kolonne 4). Det du spiser daglig skal registreres i tabellene.

Da det for mange kan være vanskelig å få til nøyaktig 21 dager, går skjemaene til og med 28 dager.

Skjemaene sendes inn til oss i den frankerte konvolutten du har fått tilsendt, etter forsøksperiodens slutt.

Uke 1:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 1**	**Dag 2**	**Dag 3**	**Dag 4**	**Dag 5**	**Dag 6**	**Dag 7**
			Dato:							
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass							
	Dolmio	Pastasaus extra hvitløk	½ glass							
	Dolmio	Pastasaus Classico	½ glass							
	Knorr	Pastasaus med oregano og løk	½ glass							
	Cadisco	Tomatjuice	¼ kartong							
	ICA	Tomater, hakket	½ kartong							

Uke 2:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 8**	**Dag 9**	**Dag 10**	**Dag 11**	**Dag 12**	**Dag 13**	**Dag 14**
			Dato:							
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass							
	Dolmio	Pastasaus extra hvitløk	½ glass							
	Dolmio	Pastasaus Classico	½ glass							
	Knorr	Pastasaus med oregano og løk	½ glass							
	Cadisco	Tomatjuice	¼ kartong							
	ICA	Tomater, hakket	½ kartong							

Uke 3:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 15**	**Dag 16**	**Dag 17**	**Dag 18**	**Dag 19**	**Dag 20**	**Dag 21**
			Dato:							
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass							
	Dolmio	Pastasaus extra hvitløk	½ glass							
	Dolmio	Pastasaus Classico	½ glass							
	Knorr	Pastasaus med oregano og løk	½ glass							
	Cadisco	Tomatjuice	¼ kartong							
	ICA	Tomater, hakket	½ kartong							

Uke 4:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 22**	**Dag 23**	**Dag 24**	**Dag 25**	**Dag 26**	**Dag 27**	**Dag 28**
			Dato:							
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass							
	Dolmio	Pastasaus extra hvitløk	½ glass							
	Dolmio	Pastasaus Classico	½ glass							
	Knorr	Pastasaus med oregano og løk	½ glass							
	Cadisco	Tomatjuice	¼ kartong							
	ICA	Tomater, hakket	½ kartong							

Dersom du har spørsmål underveis, må du gjerne ta konatkt med oss.

Lykke til!!

Anette Karlsen, prosjektkoordinator, UiO Tlf 22 85 15 24

e-mail: anette.karlsen@medisin.uio

Magnhild Kverneland, prosjektmedarbeider Tlf 41 00 56 89

e-mail: magnhild.kverneland@studmed.uio.no

Guro Lindviksmoen, forskningssykepleier Tlf 22 93 57 74

e-mail:guro.lindviksmoen@radiumhospitalet.no

Wolfgang Lilleby, behandlende lege Tlf 22 93 40 00

e-mail: wolfgang.lilleby@medisin.uio.no

Appendix 7 Informasjon til multi-diett gruppe

Tomatprodukter

Du har fått følgende tomatprodukter for forsøksperioden:

1 glass Barillo Pastasaus basilikum

1 glass Dolmio Pastasaus hvitløk

1 glass Dolmio pastasaus classico

1 glass Knorr pastasaus oregano og løk

3 kartonger Cadisco tomatjuice 1 L

3 kartonger ICA tomater hakket

Andre matvarer du også har fått:

1 pakke Twinings Java grønn te

1 pakke Twinings Earl grey te

24 kartonger a 330 mL granateplejuice

24 kartonger a 330 mL druejuice

I tilegg til disse matvarene kommer kosttilskuddene som du har fått ved apoteket på radiumhospitalet.

Tomatprodukter

Du kan selv velge hvilke tomatprodukt du vil spise eller drikke en porsjon av hver dag. Tomatproduktene skal konsumeres over hele forsøksperioden. Det er viktig at du spiser omtrent den rette daglige porsjonen av det enkelte produkt, som angitt nedenfor:

Barilla	Pastasaus med basilikum	½ glass/porsjon
Dolmio	Pastasaus hvitløk	½ glass/ porsjon
Dolmio	Pastasaus classico	½ glass/ porsjon
Knorr	Pastasaus med oregano og løk	½ glass/ porsjon
Cadisco	Tomatjuice	¼ kartong (2,5 dL)/ porsjon
ICA	Tomater, hakket	½ kartong/ porsjon

Vedlagt finner du noen forslag til anvendelse av de enkelte produktene.

Det er viktig at du så langt det er mulig ikke gjør store endringer i kostholdet i løpet av forsøksperioden. Det vil si at den ekstra porsjonen med tomat så langt det er mulig skal komme utenom det du spiser til vanlig.

Når du har spist ellet drukket den angitte porsjonen, krysser du dette av i skjemaet du har fått vedlagt ”Skjema for registrering av produkter i forsøksperioden: multi-diettgruppe”.

Dersom du for eksempel har spist ½ glass Barilla Pastasaus med basilikum dag 1, 5/3-2007, og drukket ¼ kartong (2,5 dL) Cadisco tomatjuice dag 2, 6/3-2007, krysser du av som vist:

	Merke navn	Produkt	Inntak	Dag 1	Dag 2	Dag 3
			Dato:	5/3	6/3	
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass	×		
	Dolmio	Pastasaus extra hvitløk	½ glass			
	Dolmio	Pastasaus classico	½ glass			
	Knorr	Pastasaus med oregano og løk	½ glass			
	Cadisco	Tomatjuice	¼ kartong		×	
	ICA	Tomater, hakket	½ kartong			

Andre matvarer

Ved siden av tomatproduktene skal du også drikke grønn og sort te, granateplejuice og druejuice. Av disse produktene skal du innta **samme mengde av alle produktene hver dag** i hele forsøksperioden. Dette vil si:

1 kopp grønn te daglig (Twinings green tea)

1 kopp sort te daglig (Twinings earl grey)

1 kartong granateplejuice daglig (0,33 L)

1 kartong druejuice daglig (0,33 L)

Grønn og sort te tilberedes på følgende måte: Bruk et krus som rommer tre dL. Varm 3 dL vann til kokepunktet. Hell vannet over teposen og la dette trekke i 5 minutter før du tar ut teposen. Teen kan deretter drikkes varm, eller avkjøles og drikkes kald. Dersom du foretrekker søt te kan du bruke suketter etter eget ønske.

Av juicen drikker du en kartong granateplejuice og en kartong druejuice hver dag.

Når du har drukket grønn te/sort te/granateplejuice/druejuice er det viktig at du registrerer dette i skjemaet ” Skjema for registrering av produkter i forsøksperioden: multi-diett gruppe”.

For dag 15/3-2007, og dag 2, 6/3-2007 vil dette se slik ut:

	Merke navn	**Produkt**	**Inntak**	**Dag 1**	**Dag 2**	**Dag 3**
			Dato:	**5/3**	**6/3**	
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass	×		
	Dolmio	Pastasaus extra hvitløk	½ glass			
	Dolmio	Pastasaus classico	½ glass			
	Knorr	Pastasaus med oregano og løk	½ glass			

	Cadisco	Tomatjuice	¼ kartong		×	
	ICA	Tomater, hakket	½ kartong			
Disse produktene skal inntas daglig	Twinings	Java grønn te	1 kopp	×	×	
	Twinings	Earl grey te	1 kopp	×	×	
		Granateplejuice	330 mL	×	×	
		Druejuice	330 mL	×	×	
	Dosett	Kosttilskudd 1	Alle			
		Kosttilskudd 2	Alle			

Kosttilskudd

Dose 1 (morgen) består av 3 × 1000 mg fiskeolje (Nycoplus omega-3), 2 × 40 mg soya (Natures`s sunshine Super soy extra), 1 ×100 µg selen (Solaray selen) og skal inntas på morgenen.

Dose 2 (kveld) inneholder 2 × 1000 mg fiskeolje(Nycoplus omega-3), 3 × 40 mg soya (Natures`s sunshine Super soy extra), 1 ×100 µg selen (Solaray selen) og skal inntas på kvelden.

Du har fått med Nycoplus omega-3, Natures`s sunshine Super soy extra og Solaray selen fra apoteket. Det står tydelig merket på boksene hvilke doser som skal tas av hvert enkelt preparat morgen og kveld. Ta kontakt med oss dersom du er i tvil!

Det er ikke nødvendig å ta kosttilskuddene til måltidene, og de kan tas med drikke etter eget ønske.

Når du har tatt en dose av kosttilskuddene er det viktig at du krysser av dette i skjemaet "Skjema for registrering av produkter i forsøksperioden: Multi-diett gruppe".

For dag 1, 5/3-2007, og dag 2, 6/3-2007, vil dette se slik ut:

	Merke navn	**Produkt**	**Inntak**	**Dag 1**	**Dag 2**	**Dag 3**
			Dato:	**5/3**	**6/3**	
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass	×		
	Dolmio	Pastasaus extra hvitløk	½ glass			
	Dolmio	Pastasaus classico	½ glass			
	Knorr	Pastasaus med oregano og løk	½ glass			
	Cadisco	Tomatjuice	¼ kartong		×	

	ICA	Tomater, hakket	½ kartong			
Disse produktene skal inntas daglig	Twinings	Java grønn te	1 kopp	×	×	
	Twinings	Earl grey te	1 kopp	×	×	
		Granateplejuice	330 mL	×	×	
		Druejuice	330 mL	×	×	
	Dosett	Kosttilskudd 1	Alle	×	×	
		Kosttilskudd 2	Alle	×	×	

Du har fått utdelt nok produkter til 25 dager. Dette er fordi det for noen kan være vanskelig å få til nøyaktig 21 dager. Dersom du har produkter til over etter avsluttet forsøksperiode, trenger du ikke levere disse tilbake til oss.

Skjemaet "Skjema for registrering av produkter i forsøksperioden: Multi-diett gruppe" som du har fylt ut underveis, sendes til oss etter forsøksperioden i den frankerte konvolutten du har fått tilsendt.

Lykke til!

Dersom du har spørsmål om undersøkelsen kan du når som helst kontakte oss.

Lykke til!!

Anette Karlsen, prosjektkoordinator, UiO Tlf 22 85 15 24

e-mail: anette.karlsen@medisin.uio

Magnhild Kverneland, prosjektmedarbeider Tlf 41 00 56 89

e-mail: magnhild.kverneland@studmed.uio.no

Guro Lindviksmoen, forskningssykepleier Tlf 22 93 57 74

e-mail:guro.lindviksmoen@radiumhospitalet.no

Wolfgang Lilleby, behandlende lege Tlf 22 93 40 00

e-mail: wolfgang.lilleby@medisin.uio.no

Appendix 8 Skjema for registrering av daglig inntak for cocktailgruppen

Skjema for registrering av produkter i forsøksperioden: multi-diett gruppe *ID nr:*

Skjemaet er på 4 sider (1 side for hver uke).

For hver dag skal du velge en porsjon av tomatprodukt gitt i tabellen. Den mengden som tilsvarere en porsjon som du skal spise eller drikke er gitt under inntak (kolonne 4). Det du spiser daglig skal registreres i tabellene.

Da det for mange kan være vanskelig å få til nøyaktig 21 dager, går skjemaene til og med 25 dager.

Skjemaene sendes til oss i den frankerte konvolutten du har fått, etter forsøksperiodens slutt.

Uke 1:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 1**	**Dag 2**	**Dag 3**	**Dag 4**	**Dag 5**	**Dag 6**	**Dag 7**
			Dato:							

Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass							
	Dolmio	Pastasaus extra hvitløk	½ glass							
	Dolmio	Pastasaus classico	½ glass							
	Knorr	Pastasaus med oregano og løk	½ glass							
	Cadisco	Tomatjuice	¼ kartong							
	ICA	Tomater, hakket	½ kartong							
Disse produktene skal inntas daglig	Twinings	Java grønn te	1 kopp							
	Twinings	Earl grey te	1 kopp							
		Granateplejuice	330 mL							
		Druejuice	330 mL							
	Dosett	Kosttilskudd 1	Alle							

		Kosttilskudd 2	Alle							

Uke 2:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 8**	**Dag 9**	**Dag 10**	**Dag 11**	**Dag 12**	**Dag 13**	**Dag 14**
			Dato:							
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass							
	Dolmio	Pastasaus extra hvitløk	½ glass							
	Dolmio	Pastasaus classico	½ glass							
	Knorr	Pastasaus med oregano og løk	½ glass							
	Cadisco	Tomatjuice	¼ kartong							
	ICA	Tomater, hakket	½ kartong							

Disse produktene skal inntas daglig	Twinings	Java grønn te	1 kopp							
	Twinings	Earl grey te	1 kopp							
		Granateplejuice	330 mL							
		Druejuice	330 mL							
	Dosett	Kosttilskudd 1	Alle							
		Kosttilskudd 2	Alle							

Uke 3:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 15**	**Dag 16**	**Dag 17**	**Dag 18**	**Dag 19**	**Dag 20**	**Dag 21**
			Dato:							
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass							
	Dolmio	Pastasaus extra hvitløk	½ glass							
	Dolmio	Pastasaus classico	½ glass							
	Knorr	Pastasaus med oregano og løk	½ glass							
	Cadisco	Tomatjuice	¼ kartong							
	ICA	Tomater, hakket	½ kartong							
Disse produkte	Twinings	Java grønn te	1 kopp							

	Twinings	Earl grey te	1 kopp							
		Granateplejuice	330 mL							
		Druejuice	330 mL							
	Dosett	Kosttilskudd 1	Alle							
		Kosttilskudd 2	Alle							

Uke 4:

Kryss av det du har spist eller drukket hver dag

	Merke navn	**Produkt**	**Inntak**	**Dag 22**	**Dag 23**	**Dag 24**	**Dag 25**
			Dato:				
Av disse produktene kan du velge en porsjon daglig	Barilla	Pastasaus med basilikum	½ glass				
	Dolmio	Pastasaus extra hvitløk	½ glass				
	Dolmio	Pastasaus classico	½ glass				

	Knorr	Pastasaus med oregano og løk	½ glass				
	Cadisco	Tomatjuice	¼ kartong				
	ICA	Tomater, hakket	½ kartong				
Disse produktene skal inntas daglig	Twinings	Java grønn te	1 kopp				
	Twinings	Earl grey te	1 kopp				
		Granateplejuice	330 mL				
		Druejuice	330 mL				
	Dosett	Kosttilskudd 1	Alle				
		Kosttilskudd 2	Alle				

Dersom du har spørsmål underveis, må du gjerne ta konatkt med oss.

Lykke til!!

Anette Karlsen, prosjektkoordinator, UiO Tlf 22 85 15 24

e-mail: anette.karlsen@medisin.uio

Magnhild Kverneland, prosjektmedarbeider Tlf 41 00 56 89

e-mail: magnhild.kverneland@studmed.uio.no

Guro Lindviksmoen, forskningssykepleier Tlf 22 93 57 74

e-mail:guro.lindviksmoen@radiumhospitalet.no

Wolfgang Lilleby, behandlende lege Tlf 22 93 40 00

e-mail: wolfgang.lilleby@medisin.uio.no

Appendix 9 Indexes results

Appendix 9: Oxidative stress indexes as the sum of normalized values from the prostate cancer intervention study. Comparison of baseline values and changes during the intervention period: Endogenous index (GSH+Cysteine), Damage index (8OHdG+HEL+d-ROMs test+DHAA:TAA), Antioxidant index (Lycopene, TAA, mod FRAP) and combined index (DHAA:TAA + GGT + d-ROMs test – GSH – Cysteine). A p-value below 0.050 is considered statistical significant. Non-normally distributed parameter, presented as median (range). Mann-Whitney test was performed to compare the differences between the groups.

Parameter	**Tomato group[a] baseline**	**Tomato group[a] difference**	**Control group[a] baseline**	**Control group[a] difference**	**Multidiet group[a] baseline**	**Multidiet group[a] difference**	**P (difference) tomato vs control**	**P (difference) multidiet vs control**
Endogenous index	n=8 -0.2 (-1.1 – 1.1)	n=8 0.0 (-0.8 – 0.5)	n=9 0.1 (-0.9–0.9)	n=9 0.3 (-0.8 – 0.8)	n=8 -0.0 (-0.5 – 1.3)	n=8 -0.3 (-1.5 – 1.5)	0.630	0.481
Damage index	n=7 -0.1 (-0.8 – 0.8)	n=7 -0.1 (-0.8 – 0.6)	n=8 -0.1(-0.7– 0.8)	n=8 0.0 (-0.6 – 0.5)	n=9 0.1 (-0.7 – 0.6)	n=9 -0.0 (-0.8 – 0.7)	0.699	0.700
Antioxidant index	n=7 0.1 (-0.8 – 1.4)	n=7 -0.0 (-0.9 – 1.0)	n=8 -0.2 (-0.5 – 0.6)	n=8 -0.0 (-1.2 – 0.8)	n=9 -0.0 (-0.6 – 1.0)	n=9 0.2 (-1.0 – 0.6)	0.817	0.773
Combined index	n = 5 -0.3 (-0.9 – 1.0)	n = 5 -0.0 (-0.5 – 0.3)	n = 8 -0.1 (-0.7 – 0.6)	n = 8 -0.3 (-0.6 – 0.5)	n = 8 -0.1 (-1.0 – 0.8)	n = 8 0.0 (-0.7 – 0.7)	0.661	0.529

[a] Non-normally distributed parameter, presented as median (range). Mann-Whitney test was performed to compare the groups.